UNITED STATES DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE

Petition for an Interpretive Rule Declaring all enterohemorrhagic Shiga Toxin-producing Serotypes of *Escherichia coli* (*E. coli*), Including Non-O157 Serotypes, to be Adulterants Within the Meaning of 21 U.S.C. § 601(m)(1)	)	Docket No. _____

CITIZEN PETITION

Submitted by:

Marler Clark LLP, PS

Outbreak, Inc.

The Family of June Dunning

Megan Richards

Shiloh Johnson

October 5, 2009

FSIS Docket Clerk
Department of Agriculture
Food Safety and Inspection Service
Room 2534 South Building
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Washington, DC 20250-3700

I. REQUESTED ACTIONS

A. Issuance of an Interpretive Rule

Pursuant to 5 U.S.C. § 553(e), 9 C.F.R. § 392, and 7 C.F.R. § 1.28, we submit this petition requesting the administrator of the Food Safety and Inspection Service (FSIS) to issue an interpretive rule declaring all enterohemorrhagic (EHEC) Shiga toxin-producing serotypes of *Escherichia coli* (*E. coli*), including non-O157 serotypes, to be adulterants within the meaning of the Federal Meat Inspection Act (FMIA).[1] The relevant FMIA provision, 21 U.S.C. § 601(m)(1), states in pertinent part that a carcass, part thereof, meat, or meat food product is adulterated "if it bears or contains any poisonous or deleterious substance which may render it injurious to health." FSIS interpreted this provision in 1994 to declare *E. coli* O157:H7 as an adulterant. It is respectfully submitted, however, that the 1994 interpretive rule, and its subsequent application and enforcement, ignores the grave dangers that current scientific and medical research demonstrates are not limited to *E. coli* O157:H7, but instead extend to all Shiga toxin-producing *E. coli* (STEC). As a result of the narrow scope of the 1994 interpretive rule, the safety of American consumers is at risk. Issuing a new interpretive rule that declares that all STEC are

[1] For ease of reference and to avoid an implicit redundancy, EHEC Shiga toxin-producing serotypes of *E. coli*, which are by definition pathogenic, will be referred to as non-O157 STEC or STEC.

adulterants within the meaning of the FMIA will encourage increased monitoring efforts and better ensure the safety of the general public, as is required by the FMIA.[2]

B. A Grant of Expedited Review

Because this petition requests action intended to enhance the public health by reducing food safety hazards, the petitioners ask for expedited review. As stated in the recently amended FSIS petition procedures, 9 CFR § 392.8(a):

> A petition will receive expedited review by FSIS if the requested action is intended to enhance the public health by removing or reducing foodborne pathogens or other potential food safety hazards that might be present in or on meat, poultry, or egg products.

This petition requests an interpretive rule that will prompt better monitoring of all enterohemorrhagic *E. coli*, thus decreasing foodborne contamination. In accordance with 9 CFR § 392.8(b), the requested action is supported by scientific information that demonstrates that such an interpretive rule will reduce foodborne pathogens that are likely to be present in meat products. For these reasons, the petitioners request FSIS to grant this petition expedited review.

II. ABOUT THE PETITIONERS

Marler Clark LLP, PS, located in Seattle, Washington, is the nation's foremost law firm representing victims of foodborne illness. The Marler Clark attorneys spend the majority of their time working on food-related cases, representing victims of *Campylobacter*, *E. coli* O157:H7, non-O157 STEC, Hepatitis A, *Listeria*, *Norovirus*, *Salmonella*, and *Shigella* outbreaks across the country.

[2] As stated in the FMIA, "It is essential in the public interest that the health and welfare of consumers be protected by assuring that meat and meat food products distributed to them are wholesome, not adulterated, and properly marked, labeled, and packaged." 21 U.S.C. § 602 (2004).

Outbreak, Inc. was formed in 1998 by Marler Clark's founding partners as the nonprofit consulting arm of the firm. Each lawyer travels several days a month on behalf of Outbreak, Inc., giving speeches to food-industry groups and health agencies, focusing on preventing foodborne illness.

June Dunning, represented here by her family, was a Hagerstown, Maryland woman whose *E. coli* O146:H21 infection led to her unfortunate and untimely death in 2006.

Megan Richards is a Millville, Utah woman who, due to an *E. coli* O121:H19 infection in 2006, suffered a protracted illness punctuated by a lengthy hospitalization with severe complications due to hemolytic uremic syndrome.

Shiloh Johnson is a young girl from Pryor, Oklahoma who developed hemolytic uremic syndrome after becoming infected with *E. coli* O111 in 2008. She endured a lengthy hospitalization and required numerous dialysis treatments.

III. SOME BACKGROUND

Although first isolated in 1975, and subsequently associated with foodborne illness in 1982, FSIS did not interpret *E. coli* O157:H7 to be an adulterant under the FMIA until 1994. The classification of *E. coli* O157:H7 as an adulterant came in the wake of a 1993 large-scale foodborne outbreak that left over six-hundred persons ill and four children dead.[3] In a FSIS policy statement, dated January 19, 1999, the agency emphasized the continuing risk of *E. coli* contamination:

> Exposure to *E. coli* O157:H7 has been linked with serious, life-threatening human illnesses (hemorrhagic colitis and hemolytic uremic syndrome). Raw ground beef

[3] This outbreak is commonly referred to as the "Jack in the Box outbreak." *See* Company News; Jack in the Box's Worst Nightmare, N.Y. Times, Feb. 6, 1993, *available at* http://query.nytimes.com/gst/fullpage.html?res=9F0CE7DB153CF935A35751C0A965958260&sec=&spon=. At the time, the outbreak, originating from tainted hamburger patties, was the largest *E. coli* O157:H7 outbreak to date.

> products present a significant public health risk because they are frequently consumed after preparation (*e.g.,* cooking hamburger to a rare or medium rare state) that does not destroy *E. coli* O157:H7 organisms that have been introduced below the product's surface by chopping or grinding (*e.g.,* ground beef, veal patties, and beef pattie mix).
>
> The public health risk presented by beef products contaminated with *E. coli* O157:H7 is not limited, however, to raw ground beef products. Given the low infectious dose of *E. coli* O157:H7 associated with foodborne disease outbreaks and the very severe consequences of an *E. coli* O157:H7 infection, the Agency believes that the status under the FMIA of beef products contaminated with *E. coli* O157:H7 must depend on whether there is adequate assurance that subsequent handling of the product will result in food that is not contaminated when consumed.[4]

Despite strong scientific evidence that many strains of non-O157 STEC are as pathogenic as *E. coli* O157:H7, FSIS has thus far failed to include all STEC as adulterants under the FMIA. Recent studies have repeatedly shown that non-O157 STEC is a serious food safety hazard. According to one study, non-O157 STEC are prevalent in beef production systems at rates as high as 70.1%.[5] A United States Department of Agriculture (USDA) study states that non-O157 STEC have been found in ground beef and on cattle hides and feces at levels comparable to *E. coli* O157:H7.[6] Furthermore, European studies indicate that non-O157 STEC infections occur more frequently than *E. coli* O157:H7 infections.[7] With such a ubiquitous presence, the potential risk for harm caused by non-O157 STEC may be on par with, or even greater than, the risk created by *E. coli* O157:H7. Indeed, another study concluded that "non-O157 STEC can cause severe illness that is comparable to the illness caused by STEC O157."[8]

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[4] Federal Register. January 19, 1999. [Docket No. 97-068N].

[5] Hussein, H. S. 2006. Prevalence and pathogenicity of shiga toxin-producing *Escherichia coli* in beef cattle and their products. J Anim Sci. 85:E65.

[6] Eblen, Denise. Public Health Importance of Non-O157 Shiga Toxin-Producing *Escherichia coli* (non-O157 STEC) in the US Food Supply. 2007. FSIS.

[7] Bareta, J. K. Edge, S. Lathrop. 2009. Shiga Toxin-producing *Escherichia coli*, New Mexico, USA, 2004-2007. 15 Emerging Infect Dis. (No. 8) (Aug. 2009).

[8] Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A.

On October 17, 2007, FSIS, along with the Food and Drug Administration's Center for Food Safety and Applied Nutrition (FDA CFSAN) and the National Centers for Disease Control and Prevention (CDC), co-sponsored a public meeting to consider the public health significance of non-O157 STEC. In the Notice of the meeting, published on October 9, 2007, FSIS stated:

> In the United States, there is growing awareness that STECs other than *E. coli* O157:H7 (non-O157:H7 STECs) cause sporadic and outbreak-associated illnesses. This awareness is attributable in part to the increasing availability of laboratory reagents that can be used to diagnose illnesses and to detect strains of STECs in food and other environmental samples. The number of non-O157:H7 STEC infections reported to the CDC from 2000 to 2005 increased from 171 to 501 cases, suggesting a higher burden of illness than previously thought.
>
> Outbreaks associated with non-O157:H7 STECs have been reported worldwide, including thirteen in the United States from 1990 to 2006. The 2006 data is still preliminary. Many outbreaks were attributed to consumption of fresh produce; none were attributed to ground beef consumption. However, in 2006, non-O157:H7 STEC illness was diagnosed in a patient in New York who had consumed ground beef shortly before illness onset. The same STEC strain, indistinguishable by pulsed field gel electrophoresis, was detected in the patient's stool and in leftover ground beef that the patient had consumed. In this case, FSIS was unable to take further action because the product could not be definitively traced to a production lot.[9]

The interpretive rule proposed in this Petition is consistent with FSIS' current policies and objectives. As stated in the FSIS 2008-2013 Strategic Plan, one of FSIS' current primary goals is to "enhance the development of science and risk-based policies and systems."[10] To that end, FSIS has created an objective seeking "reduced *E. coli* O157:H7 and *other Shiga toxin-producing E. coli (STEC)* consistent with Healthy People 2010 and Healthy People 2020 goals through development and implementation of policy."[11] The goal of this petition, and the

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Strockbine. 2005. Non-O157 shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. J Infect Dis. 192:1422-9.

[9] Federal Register. October 9, 2007. [Docket No. FSIS-2007-0041].

[10] FSIS. 2008. 2008-2013 Strategic Plan. 27.

[11] *Id.* (emphasis added).

interpretive rule it proposes, is to accomplish precisely what the FSIS Strategic Plan objective seeks: reduced *E. coli* O157:H7 and other STEC through better monitoring and prevention standards, which will be precipitated by the declaration that all STEC are adulterants.

What follows is divided into three sections. The first states the grounds—both legal and scientific—for issuing the proposed interpretive rule. The second describes the stories of three victims affected by non-O157 STEC. The third section concludes with a request for action to resolve the clear danger that *both E. coli* O157:H7 and non-O157 STEC represent to the United States food supply.

IV. STATEMENT OF GROUNDS

A. Pathogenesis of Shiga Toxin-producing *E. coli*

The virulence of *E. coli* is a result of the ability of certain strains to produce Shiga-like toxins.[12] It has been theorized that generic *E. coli* picked up this deadly ability through horizontal transfer of virulence genes from the *Shigella* bacteria.[13] STEC strains are known to cause diarrhea and hemolytic uremic syndrome (HUS).[14] The most common STEC that causes illness in the United States is *E. coli* O157:H7. As the CDC notes, however, non-O157 STEC strains are emerging pathogens that pose a significant health threat, with more strains reported every year.[15] Non-O157 STEC have caused multiple outbreaks in the United States. Furthermore, as documented by several studies, non-O157 STEC have been isolated from

[12] Patricia M. Griffin & Robert V. Tauxe, The Epidemiology of Infections Caused by *Escherichia coli* O157:H7, Other Enterohemorrhagic *E. coli*, and the Associated Hemolytic Uremic Syndrome, 13 Epidemiologic Reviews 60, 61-62 (1991) (noting that the nomenclature came about because of the resemblance to toxins produced by *Shigella dysenteriae*).

[13] *Id*. at 62 (using the more technical term "phage-mediated transfer").

[14] CDC. 2005. Bacterial Foodborne and Diarrheal Disease National Case Surveillance Annual Report, 2005. 16.

[15] *Id.*

diarrheal stools as frequently as *E. coli* O157:H7.[16]

After a susceptible individual ingests a sufficient quantity of *E. coli*, the bacteria attach to the inside surface of the large intestine and initiate an inflammatory reaction. The result is bloody diarrhea and intense abdominal cramps, both symptoms of severe infectious gastroenteritis.

HUS accounts for the majority of the chronic illness and death caused by *E. coli* bacteria.[17] It is the most common cause of renal failure in children.[18] Approximately half of the children who suffer HUS require dialysis, and at least 5% of those who survive have long term renal impairment.[19] The same number suffers severe brain damage.[20] While somewhat rare, serious injury to the pancreas, resulting in death or the development of diabetes, can also occur.[21] There is no cure or effective treatment for HUS.[22] And, tragically, as too many parents can attest, children with HUS often die.[23]

HUS develops when the Shiga toxins from the bacteria enter the body's circulation

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[16] *Id.*

[17] Richard L. Siegler, MD, The Hemolytic Uremic Syndrome, 42 Ped. Nephrology, 1505 (Dec. 1995). ("[HUS] is now recognized as the most frequent cause of acute renal failure in infants and young children.") *See also* Beth P. Bell, MD, MPH, *et al*., Predictors of Hemolytic Uremic Syndrome in Children During a Large Outbreak of *Escherichia coli* O157:H7 Infections, 100 Pediatrics 1, 1 (July 1, 1997), at http://www.pediatrics.org/cgi/content/full/100/1/e12.

[18] Chinyu Su, MD & Lawrence J. Brandt, MD, *Escherichia coli* O157:H7 Infection in Humans, 123 Annals Intern. Med. (Issue 9), 698-707.

[19] Nasia Safdar, MD, *et al*., Risk of Hemolytic Uremic Syndrome After Treatment of *Escherichia coli* O157:H7 Enteritis: A Meta-analysis, 288 JAMA (No. 8) 996, 996 (Aug. 28, 2002) (going on to conclude that administration of antibiotics to children with *E. coli* O157:H7 appeared to put them at higher risk for developing HUS).

[20] Richard L. Siegler, MD, Postdiarrheal Shiga Toxin-Mediated Hemolytic Uremic Syndrome, 290 JAMA (No. 10) 1379, 1379 (Sept. 10, 2003).

[21] Pierre Robitaille, *et al*., Pancreatic Injury in the Hemolytic Uremic Syndrome, 11 Pediatric Nephrology 631, 632 (1997) ("although mild pancreas involvement in the acute phase of HUS can be frequent").

[22] Safdar, *supra* note 19, at 996; *see also* Siegler, *supra* note 20, at 1379. ("There are no treatments of proven value, and care during the acute phase of the illness, which is merely supportive, has not changed substantially during the past 30 years.")

[23] Su & Brandt, *supra* note 18 ("the mortality rate is 5-10%"). *See also Kriefall v. Excel*, 265 Wis.2d 476, 483, 665 N.W.2d 417 (2003). ("three-year old Brianna Kriefall died from food that everyone party to this appeal…recognize was cross-contaminated by *E. coli* O157:H7 bacteria from meat sold by Excel.")

through the inflamed bowel wall.[24] Shiga toxins, and most likely other chemical mediators, attach to receptors on the inside surface of blood vessel cells (endothelial cells) and initiate a cascading chemical reaction that results in the formation of tiny thrombi (blood clots) within these vessels.[25] Some organs seem more susceptible, perhaps due to the presence of increased numbers of receptors; these include the kidneys, pancreas, and brain.[26] By definition, when fully expressed, HUS presents with a triad of conditions or diagnoses: hemolytic anemia (destruction of red blood cells), thrombocytopenia (low platelet count), and acute renal failure (loss of the filter function of the kidney).[27]

As already noted, there is no known therapy to halt the progression of infectious gastroenteritis to HUS. HUS is a frightening complication that even in the best American medical centers has a notable mortality rate.[28] Among survivors, at least five percent will suffer end stage renal disease ("ESRD") with the resultant need for dialysis or transplantation.[29] But, "[b]ecause renal failure can progress slowly over decades, the eventual incidence of ESRD cannot yet be determined."[30] Other long-term problems include the risk for hypertension, proteinuria (abnormal amounts of protein in the urine that can portend a decline in renal function), and reduced kidney filtration rate.[31] Because the longest available follow-up studies of HUS victims cover only 25 years, an accurate lifetime prognosis is not available and remains

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[24] Amit X. Garg, MD, MA, *et al*., Long-term Renal Prognosis of Diarrhea-Associated Hemolytic Uremic Syndrome: A Systematic Review, Meta-Analysis, and Meta-regression, 290 JAMA (No. 10) 1360, 1360 (Sept. 10, 2003).

[25] *Id*. Siegler, *supra* note 20, at 1509-11 (describing what Dr. Siegler refers to as the "pathogenic cascade" that results in the progression from colitis to HUS).

[26] Garg, *supra* note 24, at 1360; *see also* Su & Brandt, *supra* note 18, at 700.

[27] Garg, *supra* note 24, at 1360; Su & Brandt, *supra* note 18, at 700.

[28] Siegler, *supra* note 20, at 1519 (noting that in a "20-year Utah-based population study, 5% dies, and an equal number of survivors were left with end-stage renal disease (ESRD) or chronic brain damage.")

[29] Garg, *supra* note 24, at 1366-67.

[30] Siegler, *supra* note 20, at 1519.

[31] *Id*. at 1519-20; Garg, *supra* note 24, at 1366-67.

controversial.[32] All that can be said for certain is that HUS causes permanent injury, including loss of kidney function, and it requires a lifetime of close medical-monitoring.

B. Legal Basis for Declaring All STEC Adulterants Under the FMIA

The FMIA does not require the USDA to engage in substantive rulemaking as a predicate to interpreting the Act to deem a particular substance an adulterant.[33] Pursuant to the Administrative Procedures Act (APA), 5 U.S.C. § 553(b)(3)(A), agencies may issue "interpretive rules, general statements of policy, or rules of agency organization, procedure, or practice" without the notice and comment procedures required for proposed rule making. In 1994, several supermarket and meat industry organizations sought an injunction against the USDA, attempting to prevent the agency from declaring *E. coli* O157:H7 an adulterant, and barring it from implementing an *E. coli* sampling program.[34] Addressing the petitioners' claims, the court was careful to distinguish interpretive rules from substantive rules by stating that interpretive rules do not create new law, instead they are "statements as to what the administrative officer thinks the regulation means."[35]

To determine whether the 1994 declaration of *E. coli* O157:H7 as an adulterant was an interpretive rule, the *Espy* court relied on criteria established in *American Mining Congress v. Mine Safety & Health Administration*[36], which stated:

> Accordingly, insofar as our cases can be reconciled at all, we think it almost exclusively on the bases of whether the purported interpretive rule has "legal effect," which in turn is best ascertained by asking (1) whether in the absence of the rule there would not be an adequate legislative basis for enforcement action or

[32] Garg, *supra* note 24, at 1368.
[33] *Texas Food Industry Ass'n, et al. v. Espy* 870 F. Supp. 143, 147 (1994).
[34] *See Id.*
[35] *Id.* at 147.
[36] *American Mining Congress v. Mine Safety & Health Administration* 302 U.S. App. D.C. 38, 995 F.2d 1106 (D.C. Cir. 1993).

> other agency action to confer benefits or ensure the performance of duties, (2) whether the agency has published the rule in the Code of Federal Regulations, (3) whether the agency has explicitly invoked its general legislative authority, or (4) whether the rule effectively amends a prior legislative rule. If the answer to any of these questions is affirmative, we have a [substantive], not an interpretive rule.[37]

Applying these criteria, the court held that the declaration of *E. coli* O157:H7 as an adulterant was within the USDA's interpretive rulemaking powers, and thus did not require notice and comment procedures.

The legal process to issue an interpretive rule declaring all STEC to be adulterants under the FMIA is identical to the process utilized by the USDA in the 1994 *E. coli* O157:H7 declaration. As with the rule upheld in *Espy*, the interpretive rule proposed in this Petition fits well within the *American Mining Congress* criteria. First, as reaffirmed in *Espy*, because the FMIA does not require the USDA to engage in substantive rulemaking to determine whether a particular substance is an adulterant, the agency has "the discretion to proceed through case-by-case adjudication and interpretive orders, rather than through the rulemaking process."[38] Second, the request in this Petition does not require FSIS to publish the rule in the Code of Federal Regulations, or invoke its general legislative authority. Finally, the proposed interpretive rule does not amend a prior legislative rule. Thus, all of the *American Mining Congress* criteria are sufficiently met.

Other legal concerns raised by opponents in *Espy*, namely that the requested action would be arbitrary and capricious, and that the FMIA does not grant the USDA authority to declare non-O157 STEC adulterants, would also be unfounded. First, as stated in *Espy*, the USDA may properly declare substances to be adulterants with the intended purpose of spurring industry to

[37] *Id.* at 1112.
[38] *Texas Food Industry Ass'n, et al. v. Espy* 870 F. Supp. 143, 147 (1994).

create and implement preventative measures.[39] Similarly, the purpose here is to encourage the meat industry to engage in more effective oversight measures in order to prevent STEC outbreaks. Second, despite a court ruling over thirty years ago that *Salmonella* is not an adulterant *per se*[40] (as was conceded at the time by FSIS), certain Shiga toxin-producing *E. coli* strains are properly declared to be adulterants on account of the unique health risk they present. This is due to the fact that, as stated in FSIS policy documents,[41] low infectious doses of such *E. coli* often cause severe health consequences. Furthermore, products contaminated with such *E. coli* are often consumed after preparation that does not fully destroy the pathogens. Indeed, as is the case with *E. coli* O157:H7, "proper" cooking of meat will not necessarily protect consumers from infection from all STECs. As stated in *Espy*:

> [U]nlike other pathogens, it is not "proper" cooking but "thorough" cooking that is necessary to protect consumers from *E. coli*. The evidence submitted by Defendants indicates that many Americans consider ground beef to be properly cooked rare, medium rare, or medium. The evidence also indicated that *E. coli* contaminated ground beef cooked in such a manner may cause serious physical problems, including death. Therefore, *E. coli* is a substance that renders "injurious to health" what many Americans believe to be properly cooked ground beef. Based on this evidence, the Court finds that *E. coli* fits the definition of an adulterant under the FMIA.[42]

In sum, as established by both the USDA and prior judicial decisions, the interpretive rule proposed in this Petition has clear legal precedent and does not violate APA procedures.

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[39] *Id.* at 148.

[40] A ruling that, given the wealth of scientific data detailing the prevalence and toxicity of *Salmonella* (especially of the antibiotic resistant variety), is now controversial, to say the least.

[41] Federal Register. January 19, 1999. [Docket No. 97-068N].

[42] *Texas Food Industry Ass'n, et al. v. Espy* 870 F. Supp. 143, 149 (1994).

C. Scientific Basis for the Regulation of Shiga Toxin-Producing *E. coli*

1. Prevalence of Shiga Toxin Producing *E. coli*

Non-O157 STEC are the causative agents of zoonotic emerging infectious diseases, often of bovine origin. Below is a general review of non-O157 STEC prevalence studies in humans, cattle, and beef products.

a. Humans

Non-O157 STEC infections are under-recognized and under-reported due to inadequate epidemiological and laboratory surveillance. In the United States, *E. coli* O157:H7 became nationally notifiable in 1994, whereas non-O157 STEC infections were not reportable until 2000, following adoption of a position statement (2000 ID#1) by the Council for State and Territorial Epidemiologists (CSTE). At that time, the CSTE recognized that the threat to public health from STEC infections extended beyond just the *E. coli* O157:H7 serogroup.

In recent years, improved diagnostic assays for non-O157 STEC have contributed to an increased appreciation of the severity of disease caused by these strains including hemolytic uremic syndrome (HUS). Notably, the number of non-O157 STEC cases reported to CDC's FoodNet has risen steadily each year; from 2000-2006, there was an overall four-fold increase in incidence (0.12 cases per 100,000 to 0.42 cases per 100,000 population) at FoodNet sites. The most common serogroups reported to cause foodborne illness in the United States are O26, O111, O103, O121, O45, and O145.[43]

Johnson *et al* evaluated the emerging clinical importance of non-O157 STEC and concluded that these strains may account for up to 20 to 50% of all STEC infections in the

[43] Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Strockbine. 2005. Non-O157 shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. J Infect Dis. 192:1422-9.

United States.[44] Clearly, the prevalence of non-O157 STEC infections is placing an enormous burden on society and the health care system in the United States.

b. Cattle as Reservoirs

Beef and dairy cattle are known reservoirs of *E. coli* O157:H7 and non-O157 STEC strains.[45] In reviews of STEC occurrence in cattle worldwide, the prevalence of non-O157 STECs ranged from 4.6 to 55.9% in feedlot cattle, 4.7 to 44.8% in grazing cattle, and 0.4 to 74% in dairy cattle feces. The prevalence in beef cattle going to slaughter ranged from 2.1 to 70.1%.[46] While most dairy cattle-associated foodborne disease outbreaks are linked to milk products, dairy cattle still represent a potential source of contamination of beef products when they are sent to slaughter at the end of their useful production life (termed "cull" or "spent" dairy cows); this "dairy beef" is often ground and sold as hamburger.

The high prevalence of non-O157 STEC in some cattle populations, combined with the lack of effective on-farm control strategies to reduce carriage, represents a significant risk of contamination of the food supply and the environment.

c. Beef Products

Numerous non-O157 STEC serotypes known to cause human illness are from bovine origin, thus putting the beef supply at-risk. Both *E. coli* O157:H7 and non-O157 STEC may colonize the gastrointestinal tract of cattle, and potentially contaminate beef carcasses during processing. Although not as well studied, the risk factors for contamination of beef products

[44] Johnson, K. E., C. M. Thorpe, and C. L. Sears. 2006. The emerging clinical importance of non-O157 shiga toxin-producing *Escherichia coli*. Clin Infect Dis. 43:1587-95.

[45] Hussein, H. S. 2006. Prevalence and pathogenicity of shiga toxin-producing *Escherichia coli* in beef cattle and their products. J Anim Sci. 85:E63-72; Hussein, H. S. and T. Sakuma. 2005. Prevalence of shiga toxin-producing *Escherichia coli* in dairy cattle and their products. J Dairy Sci. 88:450-65.

[46] *Id.* at 465.

from cattle colonized with non-O157 STECs are likely the same or very similar to *E. coli* O157:H7. For example, cattle hides contaminated with *E. coli* O157:H7 during slaughter and processing are a known risk factor for subsequent *E. coli* O157:H7 contamination of beef products. One study showed that the prevalence of non-O157 STEC (56.6%) on hides is nearly as high as that found for *E. coli* O157:H7 (60.6%).[47]

Hussein and Bollinger evaluated published reports from over three decades and found that non-O157 STEC were more prevalent in beef products compared with *E. coli* O157. In their study, the prevalence of non-O157 STEC ranged from 1.7 to 58% in packing plants, from 3 to 62.5% in supermarkets, and an average of 3% in fast food restaurants. In a recent survey of retail ground beef products in the United States, 23 (1.9%) of 1,216 samples were contaminated with non-O157 STEC.[48] In another study, researchers found a 10 to 30% prevalence of non-O157 STEC in imported and domestic boneless beef trim used for ground beef.[49]

2. Non-*E. coli* O157:H7 Outbreaks

Worldwide, non-O157 STEC outbreaks emerged in the 1980s, and the first reported outbreaks in the United States occurred in the 1990s.[50] Although the number of reported outbreaks due to non-O157 STECs remains relatively low in the United States, most experts

[47] Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157:H7 serotypes, and Salmonella in commercial beef processing plants. J Food Prot. 66:1978-86.

[48] Samadpour, M., V. Beskhlebnaya, and W. Marler. 2009. Prevalence of non-O157 enterohaemmorrhagic *Escherichia coli* in retail ground beef in the United States. 7th International Symposium on Shiga Toxin (Verocytoxin)-producing Escherichia coli Infections. Buenos Aires, Argentina.

[49] Bosilevac J. M., M. N. Guerini, D. M. Brichta-Harhay, T. M. Arthur, and M. Koohmaraie. 2007. Microbiological characterization of imported and domestic boneless beef trim used for ground beef. J Food Prot. 70:440-9.

[50] Hussein, H. S. 2006. Prevalence and pathogenicity of shiga toxin-producing *Escherichia coli* in beef cattle and their products. J Anim Sci. 85:E63-72; Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Strockbine. 2005. Non-O157 shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. J Infect Dis. 192:1422-9.

agree that documented outbreaks only represent the "tip of the iceberg." From 1990 to 2007, twenty-two non-O157 STEC outbreaks were reported in the United States.[51] This number, however, pales in comparison to the estimated 36,700 illnesses, 1,100 hospitalizations, and 30 deaths the CDC annually attributes to non-O157 STEC.[52] If the past is any indication, the number of reported outbreaks will only increase as more laboratories test for non-O157 STEC.

3. Products Implicated in Previous Outbreaks

There is some lack of information identifying specific vehicles of transmission for human non-O157 STEC infections, nonetheless, contaminated raw dairy products, produce, and water have been implicated in the United States.[53] A review of non-O157 STEC in Connecticut showed that exposures, including ground beef, were similar in both non-O157 STEC and *E. coli* O157:H7 cases, suggesting that the routes of transmission are similar.[54] Considering the relatively high prevalence of both *E. coli* O157:H7 and non-O157 STEC in cattle populations, it is not surprising that ground beef and other beef products could be a common food vehicle.

Outbreaks of non-O157 STEC infection and illness attributed to ground beef and its sausage products have been documented outside the United States including Argentina, Australia, Germany, and Italy. These beef-related outbreaks involved eight STEC serogroups (O1, O2, O15, O25, O75, O86, O111, and O160). HUS cases were reported in five of the six outbreaks, predictably most often striking children and the elderly.

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[51] Gould, L. Hannah. September 14, 2009. Update on the Epidemiology of Shiga toxin-producing *E. coli* in the United States. Capital Area Food Protection Association Meeting.

[52] *Id.*

[53] Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Strockbine. 2005. Non-O157 shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. J Infect Dis. 192:1422-9.

[54] CDC. 2007. Laboratory-confirmed non-O157 shiga toxin-producing *Escherichia coli* – Connecticut, 2000-2005. MMWR. 56:29-31.

More rigorous investigation into the cause of non-O157 STEC outbreaks is needed to better understand the role of beef products and other foods in the contamination of the human food supply with these strains. Bettelheim described non-O157 STECs as "under-rated pathogens."[55] Indeed, the surveillance trends suggest that if left unchecked, it is only a matter of time before the United States experiences large non-O157-related outbreaks. Amending FMIA regulations to include pathogenic non-O157 STEC strains under the definition of "adulterated" is an urgently needed step in the prevention and control of these potentially deadly pathogens.

V. THE SUFFERING CAUSED BY NON-O157 *E. COLI* INFECTIONS

What follows are just a few of the personal stories associated with non-O157 STEC outbreaks. These stories are presented on behalf of the Petitioners to give a small insight into the significant harm that results when the STEC already present in the national food supply causes illness.

A. June Dunning, *E. coli* O146:H21, 2006, Death

Right up until the time of her death, June Dunning remained an active, self-aware, and outgoing woman. Her health had always been good. For the last seven years of her life, she lived in Hagerstown, Maryland with her daughter and son-in-law. On August 28, 2006, June consumed a small amount of Dole baby spinach from a bag her daughter had purchased at the local grocery store seven days earlier. The bag later tested positive for *E. coli* O146:H21.

June fell ill on September 2, 2006. Her illness quickly progressed and she was taken to the hospital the following day. She was first seen by a triage nurse, who noted that June had experienced a sudden onset of diarrhea the night before, which had progressed to bloody stools

[55] Bettelheim, K. A. 2007. The non-O157 shiga-toxigenic (verocytotoxigenic) *Escherichia coli*: under-rated pathogens. Crit Rev Microbiol. 33:67-97.

and severe abdominal pain in the morning. June rated her pain at "9" on a 10 point scale. Further examination and blood tests revealed a number of disturbing problems. A CT scan showed diffuse thickening and swelling of the colon, with severe, acute inflammatory colitis of the ascending and transverse colon. Her blood pressure was elevated and she was beginning to show signs of renal insufficiency. Concerned about her worsening condition, her physician admitted her to the hospital and started her on intravenous fluids.

Admission to the hospital did not slow the deterioration of June's condition. She began to lose her mental faculties. She spoke, but her words did not make sense. She often spoke of going to see her husband, who has passed away ten years prior. All the while, she continued to suffer from frequent, painful bloody diarrhea. Her renal failure worsened. Her doctors were concerned that the colitis would soon lead to systemic toxemia, and thus determined that she needed surgical removal of a portion of her colon.

June survived the surgery, but her overall health continued to deteriorate. She became anemic and was placed in the intensive care unit. She soon stopped producing urine, and progressed to a coma-like state. In the early morning hours of September 7, she suffered a grand mal seizure. On September 9, she suffered another seizure, followed by a drop in her oxygen levels. In reaction to her failing bodily functions, she was placed on mechanical ventilation. By this point in the hospitalization, her medical bills totaled nearly $50,000.

From this point forward, it was painfully clear what the unfortunate outcome of June's condition would be. An EEG on September 11 showed slowing brain activity. Her daughter and son-in-law stayed with her for the final hours. Late in the evening on September 11, the ventilator and all medical support except for morphine were disconnected. The doctors said they

expected June to pass within the hour. Instead, she persevered without life-support. For the majority of the next 36 hours, she appeared to be resting comfortably. In one frightening episode during the early hours of September 12, however, she experienced one final seizure. She gripped her daughter's hand, eyes wide open, moaning and sighing. Thankfully, the seizure passed. June clung to life until just after dawn on September 13, passing away at 6:45 AM.

B. Megan Richards, *E. coli* O121:H19, 2006

In 2006, Megan Richards, of Millville, Utah, was a young wife, mother, and educational conference coordinator with a bright future. On June 30 of that year, she consumed a seemingly safe take-out lunch from a Wendy's restaurant in Ogden, Utah. Three days later, Megan fell ill with significant painful diarrhea. Despite treatment by her regular physician, her condition did not improve and, on July 10, she developed persistent vomiting. That afternoon, she was rushed to an emergency room in Logan, Utah.

Blood tests in the emergency room indicated that Megan's kidneys were failing, and so she was admitted to the hospital. Her illness was later determined to be one of many illnesses in an outbreak of *E. coli* O121:H19 linked by public health officials to food served at Wendy's. Over the next day, her kidney function continued to slow, eventually halting altogether. She was transferred to McKay-Dee hospital in Ogden, Utah, to receive more specialized care. There, a diagnosis of hemolytic uremic syndrome (HUS) was confirmed. On July 14, Megan endured a kidney biopsy. The results were frightening: "necrosis of nearly the entire specimen [noted to be kidney cortex]." The renal cortex is where the kidney's filtering units are located and cortical necrosis indicates permanent loss of those filters—a finding typically found only in the most severe cases of HUS. The finding carried dire prognostic significance.

That same day, the nurses found Megan unresponsive and exhibiting seizure-like activity. A code was called. Dr. Pittman responded and arrived to find Megan with a heart rate of 160 beats per minute and tonic clonic seizures. Her oxygen saturation level was shockingly low at 71%. Fortunately, the physicians were able to get her seizures under control and her oxygen levels back up; it was clear, however, at this point that she was fighting for her life. On July 15, she began hemodialysis and plasmaphoresis to compensate for loss of kidney function. She remained hospitalized through July 28. Upon discharge, her kidneys were still not functioning normally, thus she continued treatment in an out-patient hemodialysis program.

Megan returned to the hospital three days a week for hemodialysis through September 7, at which point she was reduced to two sessions a week. Throughout this time, her kidney function remained abnormally low. She finally was able to discontinue regular dialysis in early October 2006. Her medical bills were over $350,000. Despite the extensive medical treatment, the damage to her kidneys was permanent and irreversible. Her prognosis as of 2008 was reported as follows:

> Based on the severity of her HUS, the evident extensive damage to her renal cortex, her markedly reduced estimated filtration rate of currently only 35 mls/min and the fact that Megan also now has evidence of significant proteinuria, it is my opinion, based on reasonable medical probability, that Megan will develop end stage renal disease (ESRD) and require renal replacement therapy in the form of chronic dialysis or kidney transplantation in the future.

It is estimated that Megan will require renal replacement therapy or a transplant by age 40 to 45. And after that, her future is still uncertain.

Megan will face many challenges once she undergoes a kidney transplant operation. She will need to take immunosuppressive medications for the rest of her life. Such medications are not only very costly, they also have significant side effects including high blood pressure,

diabetes, osteoporosis, altered appearance (such as moon faces due to steroids, and either hair loss or excessive hair growth with calcineurin inhibitors), and memory impairment. Immunosuppressive medications also significantly increase the risk for life-threatening infection or cancer.

C. Shiloh Johnson, *E. coli* O111, 2008

Shiloh Johnson was one of hundreds of persons sickened in the August 2008 *E. coli* O111 outbreak at the Country Cottage restaurant in Locust Grove, Oklahoma. Shiloh developed bloody diarrhea, and was hospitalized on August 22, 2008.

Once admitted, Shiloh's stool sample was tested and subsequently cultured positive for *E. coli* O111. Immediately after the start of the hospitalization, she began to suffer from hemolytic uremic syndrome (HUS). Her kidneys failed and her red blood cell and platelet counts plummeted. With a complete loss of kidney function, she required dialysis to survive. She was placed on continuous renal replacement therapy.

Forty-eight hours into the dialysis treatment, disaster struck. Shiloh developed a significant pericardial effusion (fluid around the heart) with tamponade (stoppage of blood flow caused by fluid). She went into cardiorespiratory arrest. She was endotrachoeally intubated and the pericardial fluid was drained. She was given a round of epinephrine, and the arrest was reversed. Shiloh remained on a ventilator through September 12. Soon, the area around her lungs also became inundated with fluid, necessitating the placement of chest tubes.

Throughout this time, Shiloh experienced full renal failure. She received dialysis treatment around the clock. On September 10, her doctors placed a periotoneal catheter and

switched her to peritoneal dialysis. The dialysis continued through September 27. She was finally discharged on October 3. By this point, her medical bills amounted to $450,000.

The severity of Shiloh Johnson's HUS, and in particular the length of her renal failure, puts her at serious risk of future complications including end stage renal disease. The extent of her long-term injury is still being assessed.

VI. CONCLUSION

In light of current scientific and medical research, the health hazards posed by STEC are undeniable. The CDC recognized these hazards in 2000 when the agency made all STEC nationally notifiable. Since reporting was implemented in 2001, instances of non-O157 STEC have steadily increased year by year. In 2005 alone, 501 cases of non-O157 STEC were reported through the National Notifiable Diseases Surveillance System.[56] This has become an issue that is too big to ignore any longer. Indeed, in a presentation given on September 14, 2009, L. Hannah Gould, MS, PhD from the CDC stated that non-O157 STEC causes an estimated 36,700 illnesses, 1,100 hospitalizations, and 30 deaths annually.[57]

Accordingly, the petitioners urge the administrator of FSIS to issue an interpretive rule declaring all STEC adulterants within the meaning of the FMIA in order to avoid the same kind of large-scale disaster that precipitated the 1994 declaration of *E. coli* O157:H7 as an adulterant. With this action, FSIS will take a significant leap forward in ensuring the safety of American consumers.

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[56] CDC. 2005. Bacterial Foodborne and Diarrheal Disease National Case Surveillance Annual Report, 2005. 16.

[57] Gould, L. Hannah. September 14, 2009. Update on the Epidemiology of Shiga toxin-producing *E. coli* in the United States. Capital Area Food Protection Association Meeting.

As the numbers of reported illnesses from non-O157 STEC steadily increase, immediate action on this issue is critical.

Very truly yours,

William Marler, Esq., on behalf of:

Marler Clark LLP, PS
Outbreak, Inc.
The Family of June Dunning
Megan Richards
Shiloh Johnson

Enclosures

ATTACHMENTS

Attachment No. 1. *American Mining Congress v. Mine Safety & Health Administration* 302 U.S. App. D.C. 38, 995 F.2d 1106 (D.C. Cir. 1993).

Attachment No. 2. Amit X. Garg, MD, MA, *et al*., Long-term Renal Prognosis of Diarrhea-Associated Hemolytic Uremic Syndrome: A Systematic Review, Meta-Analysis, and Meta-regression, 290 JAMA (No. 10) 1360 (Sept. 10, 2003).

Attachment No. 3. Bareta, J. K. Edge, S. Lathrop. 2009. Shiga Toxin-producing *Escherichia coli*, New Mexico, USA, 2004-2007. 15 Emerging Infect Dis. (No. 8) (Aug. 2009).

Attachment No. 4. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157:H7 serotypes, and Salmonella in commercial beef processing plants. J Food Prot. 66:1978-86.

Attachment No. 5. Beth P. Bell, MD, MPH, *et al*., Predictors of Hemolytic Uremic Syndrome in Children During a Large Outbreak of *Escherichia coli* O157:H7 Infections, 100 Pediatrics 1, 1 (July 1, 1997), at http://www.pediatrics.org/cgi/content/full/100/1/e12.

Attachment No. 6. Bettelheim, K. A. 2007. The non-O157 shiga-toxigenic (verocytotoxigenic) *Escherichia coli*: under-rated pathogens. Crit Rev Microbiol. 33:67-97.

Attachment No. 7. Bosilevac J. M., M. N. Guerini, D. M. Brichta-Harhay, T. M. Arthur, and M. Koohmaraie. 2007. Microbiological characterization of imported and domestic boneless beef trim used for ground beef. J Food Prot. 70:440-9.

Attachment No. 8. Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Strockbine. 2005. Non-O157 shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. J Infect Dis. 192:1422-9.

Attachment No. 9. CDC. 2005. Bacterial Foodborne and Diarrheal Disease National Case Surveillance Annual Report, 2005. 16.

Attachment No. 10. CDC. 2007. Laboratory-confirmed non-O157 shiga toxin-producing *Escherichia coli* – Connecticut, 2000-2005. MMWR. 56:29-31.

Attachment No. 11. Chinyu Su, MD & Lawrence J. Brandt, MD, *Escherichia coli* O157:H7 Infection in Humans, 123 Annals Intern. Med. (Issue 9), 698-707.

Attachment No. 12. Company News; Jack in the Box's Worst Nightmare, N.Y. Times, Feb. 6, 1993, *available at* http://query.nytimes.com/gst/fullpage.html?res=9F0CE7DB153CF935A35751C0A965958260&sec=&spon=.

Attachment No. 13. Eblen, Denise. Public Health Importance of Non-O157 Shiga Toxin-Producing *Escherichia coli* (non-O157 STEC) in the US Food Supply. 2007. FSIS.

Attachment No. 14. Federal Register. January 19, 1999. [Docket No. 97-068N].

Attachment No. 15. Federal Register. October 9, 2007. [Docket No. FSIS-2007-0041].

Attachment No. 16. FSIS. 2008. 2008-2013 Strategic Plan.

Attachment No. 17. Gould, L. Hannah. September 14, 2009. Update on the Epidemiology of Shiga toxin-producing *E. coli* in the United States. Capital Area Food Protection Association Meeting.

Attachment No. 18. Hussein, H. S. 2006. Prevalence and pathogenicity of shiga toxin-producing *Escherichia coli* in beef cattle and their products. J Anim Sci. 85.

Attachment No. 19. Hussein, H. S. and T. Sakuma. 2005. Prevalence of shiga toxin-producing *Escherichia coli* in dairy cattle and their products. J Dairy Sci. 88:450-65.

Attachment No. 20. Johnson, K. E., C. M. Thorpe, and C. L. Sears. 2006. The emerging clinical importance of non-O157 shiga toxin-producing *Escherichia coli*. Clin Infect Dis. 43:1587-95.

Attachment No. 21. *Kriefall v. Excel*, 265 Wis.2d 476, 665 N.W.2d 417 (2003).

Attachment No. 22. Nasia Safdar, MD, *et al*., Risk of Hemolytic Uremic Syndrome After Treatment of *Escherichia coli* O157:H7 Enteritis: A Meta-analysis, 288 JAMA (No. 8) 996, 996 (Aug. 28, 2002).

Attachment No. 23. Patricia M. Griffin & Robert V. Tauxe, The Epidemiology of Infections Caused by *Escherichia coli* O157:H7, Other Enterohemorrhagic *E. coli*, and the Associated Hemolytic Uremic Syndrome, 13 Epidemiologic Reviews 60 (1991).

Attachment No. 24. Pierre Robitaille, *et al*., Pancreatic Injury in the Hemolytic Uremic Syndrome, 11 Pediatric Nephrology 631, 632 (1997).

Attachment No. 25. Richard L. Siegler, MD, The Hemolytic Uremic Syndrome, 42 Ped. Nephrology, 1505 (Dec. 1995).

Attachment No. 26. Richard L. Siegler, MD, Postdiarrheal Shiga Toxin-Mediated Hemolytic Uremic Syndrome, 290 JAMA (No. 10) 1379, 1379 (Sept. 10, 2003).

Attachment No. 27. Samadpour, M., V. Beskhlebnaya, and W. Marler. 2009. Prevalence of non-O157 enterohaemmorrhagic *Escherichia coli* in retail ground beef in the United States. 7th International Symposium on Shiga Toxin (Verocytoxin)-producing Escherichia coli Infections. Buenos Aires, Argentina.

Attachment No. 28. *Texas Food Industry Ass'n, et al. v. Espy* 870 F. Supp. 143 (1994).

LEXSEE 995 F2D 1106

American Mining Congress and National Industrial Sand Association, Petitioners v. Mine Safety & Health Administration and U.S. Department of Labor American Mining Congress, and National Industrial Sand Association, Petitioners v. U.S. Department of Labor and William J. Tattersall, Assistant Secretary of Labor for Mine Safety and Health, and Mine Safety and Health Administration, Respondents

No. 91-1501, No. 92-1188, No. 92-1331

UNITED STATES COURT OF APPEALS FOR THE DISTRICT OF COLUMBIA CIRCUIT

995 F.2d 1106*; *302 U.S. App. D.C. 38*; *1993 U.S. App. LEXIS 13767*; *1993 OSHD (CCH) P30,096

November 10, 1992, Argued
June 15, 1993, Decided

PRIOR HISTORY: [**1] Petitions for Review of an Order of the Mine Safety and Health Administration.

COUNSEL: Thomas C. Means argued the cause for petitioner. With him on the briefs were Edward M. Green, Mark G. Ellis, Timothy M. Biddle and Robert Timothy McCrum.

Marshall J. Breger, Attorney, Department of Labor, argued the cause for respondent. With him on the brief were Allen H. Feldman, W. Christian Schumann and Jerald S. Feingold.

JUDGES: Before: Williams, Sentelle and Randolph, Circuit Judges. Opinion for the Court filed by Circuit Judge Williams.

OPINION BY: WILLIAMS

OPINION

[*1107] Williams, *Circuit Judge* : This case presents a single issue: whether Program Policy Letters of the Mine Safety and Health Administration, stating the agency's position that certain x-ray readings qualify as "diagnoses" of lung disease within the meaning of agency reporting regulations, are interpretive rules under the Administrative Procedure Act. We hold that they are.

* * *

The Federal Mine Safety and Health Act, *30 U.S.C. § 801 et seq.*, extensively regulates health and safety conditions in the nation's mines and empowers the Secretary of Labor to enforce the statute and relevant regulations. [**2] See *id*. at *§§ 811*, 813-14. In addition, the Act requires "every operator of a ... mine ... [to] establish and maintain such records, make such reports, and provide such information, as the Secretary ... may reasonably require from time to time to enable him to perform his functions." *Id*. at *§ 813(h)*. The Act makes a general grant of authority to the Secretary to issue "such regulations as ... [he] deems appropriate to carry out" any of its provisions. *Id*. at § 957.

Pursuant to its statutory authority, the Mine Safety and Health Administration (acting on behalf of the Secretary of Labor) maintains regulations known as "Part 50" regulations, which cover the "Notification, Investigation, Reports and Records of Accidents, Injuries, Illnesses, Employment, and Coal Production in Mines." See 30 CFR Part 50. These were adopted via notice-and-comment rulemaking. See 42 Fed. Reg. 55568 (1977) (notice of proposed rulemaking); 42 Fed. Reg. 65534 (1977) (adopted rules). [1] Subpart C deals with the "Reporting of Accidents, Injuries, and Illnesses" and requires mine operators to report to the MSHA within ten days "each accident, occupational [**3] injury, or occupational illness" that occurs at a mine. See *30 CFR § 50.20(a)*. Of central importance here, the regulation also says that whenever any of certain occupational illnesses are "*diagnosed*," the operator must similarly report the diagnosis within ten days. *Id*. (emphasis added). Among the occupational illnesses covered are "silicosis, asbestosis, coal worker's pneumoconiosis, and other pneumoconioses." *Id*. at § 50.20-6(b)(7)(ii). An operator's failure to

report may lead to citation and penalty. See *30 U.S.C. §§ 814(a), 815(a) & (d), 816(a).*

> 1 The Part 50 regulations were promulgated after passage of the Mine Act but before its effective date. The regulations were initially issued by the Mining Enforcement and Safety Administration of the Department of Interior, the predecessor to the MSHA, and the Mine Act provided that such regulations would continue in force after the Mine Act became effective. See *30 U.S.C. § 961(c)(2).*

[**4] As the statute and formal regulations contain ambiguities, the MSHA from time to time issues Program Policy Letters ("PPLs") intended to coordinate and convey agency policies, guidelines, and interpretations to agency employees and interested members of the public. See *MSHA Administrative Policy and Procedures Manual*, Volume II, paragraph 112 (July 17, 1990); *MSHA Program Information Bulletin No. 88-03* (August 19, 1988). One subject on which it has done so--apparently in response to inquiries from mine operators about whether certain x-ray [*1108] results needed to be reported as "diagnoses"--has been the meaning of the term diagnosis for purposes of Part 50.

The first of the PPLs at issue here, PPL No. 91-III-2 (effective September 6, 1991), stated that any chest x-ray of a miner who had a history of exposure to pneumonoconiosis-causing dust that rated 1/0 or higher on the International Labor Office (ILO) classification system would be considered a "diagnosis that the x-rayed miner has silicosis or one of the other pneumonoconioses" for the purposes of the Part 50 reporting requirements. (The ILO classification system uses a 12-step scale to measure the concentration of opacities [**5] (i.e., areas of darkness or shading) on chest x-rays. A 1/0 rating is the fourth most severe of the ratings.) The 1991 PPL also set up a procedure whereby, if a mine operator had a chest x-ray initially evaluated by a relatively unskilled reader, the operator could seek a reading by a more skilled one; if the latter rated the x-ray below 1/0, the MSHA would delete the "diagnosis" from its files. We explain the multiple-reader rules further in the context of the third PPL, where they took their final form (so far).

The second letter, PPL No. P92-III-2 (effective May 6, 1992), superseded the 1991 PPL but largely repeated its view about a Part 50 diagnosis. In addition, the May 1992 PPL stated the MHSA's position that mere diagnosis of an occupational disease or illness within the meaning of Part 50 did not automatically entitle a miner to benefits for disability or impairment under a workers' compensation scheme. The PPL also said that the MSHA did not intend for an operator's mandatory reporting of an x-ray reading to be equated with an admission of liability for the reported disease.

The final PPL under dispute, PPL No. P92-III-2 (effective August 1, 1992), replaced the May 1992 PPL [**6] and again restated the MSHA's basic view that a chest x-ray rating above 1/0 on the ILO scale constituted a "diagnosis" of silicosis or some other pneumoconiosis. The August 1992 PPL also modified the MSHA's position on additional readings. Specifically, when the first reader is not a "B" reader (i.e., one certified by the National Institute of Occupational Safety and Health to perform ILO ratings), and the operator seeks a reading from a "B" reader, the MSHA will stay enforcement for failure to report the first reading. If the "B" reader concurs with the initial determination that the x-ray should be scored a 1/0 or higher, the mine operator must report the "diagnosis". If the "B" reader scores the x-ray below 1/0, the MSHA will continue to stay enforcement if the operator gets a third reading, again from a "B" reader; the MSHA then will accept the majority opinion of the three readers.

The MSHA did not follow the notice and comment requirements of *5 U.S.C. § 553* in issuing any of the three PPLs. In defending its omission of notice and comment, the agency relies solely on the interpretive rule exemption of *§ 553(b)(3)(A).*

We note parenthetically [**7] that the agency also neglected to publish any of the PPLs in the Federal Register, but distributed them to all mine operators and independent contractors with MSHA identification numbers, as well as to interested operator associations and trade unions. Compare *5 U.S.C. § 552(a)(1)(D)* (requiring publication in the Federal Register of all "interpretations of general applicability") with *id*. at *§ 552(a)(2)(B)* (requiring agencies to make available for public inspection and copying "those statements of policy and interpretations which have been adopted by the agency and are not published in the Federal Register"). Petitioners here make no issue of the failure to publish in the Federal Register.

* * *

The distinction between those agency pronouncements subject to APA notice-and-comment requirements and those that are exempt has been aptly described as "enshrouded in considerable smog," *General Motors Corporation v. Ruckelshaus, 239 U.S. App. D.C. 408, 742 F.2d 1561, 1565 (D.C. Cir. 1984)* (en banc) (quoting *Noel v. Chapman, 508 F.2d 1023, 1030 (2d Cir. 1975)*); see also *American Hospital Association v. Bowen, 266 U.S. App. D.C. 190, 834 F.2d 1037, 1046 (D.C. Cir. 1987)* [**8] (calling the line between interpretive and legislative rules "fuzzy"); *Community Nutrition Institute v. Young, 260 U.S. App. D.C. 294, 818 F.2d 943, 946*

(D.C. Cir. 1987) (quoting authorities describing [*1109] the present distinction between legislative rules and policy statements as "tenuous," "blurred" and "baffling").

Given the confusion, it makes some sense to go back to the origins of the distinction in the legislative history of the Administrative Procedure Act. Here the key document is the *Attorney General's Manual on the Administrative Procedure Act* (1947), which offers "the following working definitions":

> *Substantive rules*--rules, other than organizational or procedural under section 3(a)(1) and (2), issued by an agency pursuant to statutory authority and which implement the statute, as, for example, the proxy rules issued by the Securities and Exchange Commission pursuant to section 14 of the Securities Exchange Act of 1934 (*15 U.S.C. 78n*). Such rules have the force and effect of law.
>
> *Interpretative rules*--rules or statements issued by an agency to advise the public of the agency's construction of the statutes and [**9] rules which it administers....
>
> *General statements of policy*--statements issued by an agency to advise the public prospectively of the manner in which the agency proposes to exercise a discretionary power.

Id. at 30 n.3. See also Michael Asimow, *Public Participation in the Adoption of Interpretive Rules and Policy Statements*, 75 Mich. L. Rev. 520, 542 & n.95 (1977) (reading legislative history of Administrative Procedure Act as "suggesting an intent to adopt the legal effect test" as marking the line between substantive and interpretive rules).

Our own decisions have often used similar language, inquiring whether the disputed rule has "the force of law". See, e.g., *National Latino Media Coalition v. FCC, 259 U.S. App. D.C. 481, 816 F.2d 785, 787-88 (D.C. Cir. 1987)*. We have said that a rule has such force only if Congress has delegated legislative power to the agency and if the agency intended to exercise that power in promulgating the rule. See, e.g., *American Postal Workers Union v. U.S. Postal Service, 227 U.S. App. D.C. 351, 707 F.2d 548, 558 (D.C. Cir. 1983)*.

On its face, the "intent to exercise" language may seem [**10] to lead only to more smog, but in fact there are a substantial number of instances where such "intent" can be found with some confidence. The first and clearest case is where, in the absence of a legislative rule by the agency, the legislative basis for agency enforcement would be inadequate. The example used by the Attorney General's Manual fits exactly--the SEC's proxy authority under § 14 of the Securities Exchange Act of 1934, *15 U.S.C. § 78n*. Section 14(b), for example, forbids certain persons, "to give, or to refrain from giving a proxy" "in contravention of such rules and regulations as the Commission may prescribe". *15 U.S.C. § 78n(b)*. The statute itself forbids *nothing* except acts or omissions to be spelled out by the Commission in "rules or regulations". The present case is similar, as to Part 50 itself, in that *§ 813(h)* merely requires an operator to maintain "such records ... as the Secretary ... may reasonably require from time to time". *30 U.S.C. § 813(h)*. Although the Secretary might conceivably create some "requirements" ad hoc, clearly some agency creation [**11] of a duty is a necessary predicate to any enforcement against an operator for failure to keep records. Analogous cases may exist in which an agency may offer a government benefit only after it formalizes the prerequisites.

Second, an agency presumably intends a rule to be legislative if it has the rule published in the Code of Federal Regulations; 44 U.S.C. § 1510 limits publication in that code to rules "having general applicability and legal effect". See *Brock v. Cathedral Bluffs Shale Oil Co., 254 U.S. App. D.C. 242, 796 F.2d 533, 539 (D.C. Cir. 1986)* (Scalia, J.).

Third, " "if a second rule repudiates or is irreconcilable with [a prior legislative rule], the second rule must be an amendment of the first; and, of course, an amendment to a legislative rule must itself be legislative.' " *National Family Planning & Reproductive Health Ass'n v. Sullivan, 298 U.S. App. D.C. 288, 979 F.2d 227, 235 (D.C. Cir. 1992)* (quoting Michael Asimow, *Nonlegislative Rulemaking and Regulatory Reform, 1985 Duke L.J. 381, 396*). See also *State of Alaska v. DOT, 276 U.S. App. D.C. 112, 868 F.2d 441, 446-47 (D.C. Cir. 1989)*; [**12] *Homemakers North Shore,* [*1110] *Inc. v. Bowen, 832 F.2d 408, 412 (7th Cir. 1987)*.

There are variations on these themes. For example, in *Chamber of Commerce v. OSHA, 204 U.S. App. D.C. 192, 636 F.2d 464 (D.C. Cir. 1980)*, the agency had on a prior occasion claimed that a certain statutory term, correctly understood, itself imposed a specific requirement

on affected businesses. We found that interpretation substantively invalid, but noted the agency's power to promulgate such a requirement on the basis of more general authority. *Leone v. Mobil Oil Corp., 173 U.S. App. D.C. 204, 523 F.2d 1153 (D.C. Cir. 1975)*. The agency then issued a purported interpretive rule to fill the gap (without notice and comment), and we struck it down as an invalid exercise of the agency's legislative powers. *Chamber of Commerce, 636 F.2d at 469*.

We reviewed a similar juxtaposition of different agency modes in *Fertilizer Institute v. EPA, 290 U.S. App. D.C. 184, 935 F.2d 1303, 1308 (D.C. Cir. 1991)*. There a statute created a duty to report any "release" of a "reportable quantity" or "RQ" of certain hazardous materials, specifying the [**13] RQs but authorizing the EPA to change them by regulation. See *42 U.S.C. § 9602(b)*. In the preamble to a legislative rule exercising its authority to amend the RQs, the EPA also expatiated on the meaning of the statutory term "release"--improperly broadening it, as petitioners claimed and as we ultimately found. *935 F.2d at 1309-10*. But we rejected a claim that the agency's attempted exposition of the term "release" was not an interpretation and therefore required notice and comment. *Id. at 1307-09*.

In *United States v. Picciotto, 277 U.S. App. D.C. 312, 875 F.2d 345 (D.C. Cir. 1989)*, the Park Service had issued an indisputably legislative rule containing an "open-ended" provision stating that a "permit may contain additional reasonable conditions". *Id. at 346*. Then, in a rule issued without notice and comment, it established some such conditions. We struck down the disputed condition, as it was not an interpretation of the prior regulation but an exercise of the legislative authority reserved by the prior legislative rule. *Id. at 348*. [**14]

This focus on whether the agency *needs* to exercise legislative power (to provide a basis for enforcement actions or agency decisions conferring benefits) helps explain some distinctions that may, out of context, appear rather metaphysical. For example, in *Fertilizer Institute* we drew a distinction between instances where an agency merely "declares its understanding of what a statute requires," (interpretive) and ones where an agency "goes beyond the text of a statute" (legislative). *Id. at 1308*. See also *Chamber of Commerce, 636 F.2d at 469* (distinguishing between "construing" a statutory provision and "supplementing" it). The difficulty with the distinction is that almost every rule may seem to do both. But if the dividing line is the necessity for agency legislative action, then a rule supplying that action will be legislative no matter how grounded in the agency's "understanding of what the statute requires", and an interpretation that spells out the scope of an agency's or regulated entity's pre-existing duty (such as EPA's interpretation of "release" in *Fertilizer Institute*), will be interpretive, even if, as in that case itself, [**15] it widens that duty even beyond the scope allowed to the agency under *Chevron U.S.A., Inc. v. NRDC, 467 U.S. 837, 104 S. Ct. 2778, 81 L. Ed. 2d 694 (1984)*. See *Fertilizer Institute, 935 F.2d at 1308*.

Similarly, we have distinguished between cases where a rule is "based on specific statutory provisions" (interpretive), and where one is instead "based on an agency's power to exercise its judgment as to how best to implement a general statutory mandate" (legislative). *United Technologies Corp. v. EPA, 261 U.S. App. D.C. 226, 821 F.2d 714, 719-20 (D.C. Cir. 1987)*. A statute or legislative rule that actually establishes a duty or a right is likely to be relatively specific (and the agency's refinement will be interpretive), whereas an agency's authority to create rights and duties will typically be relatively broad (and the agency's actual establishment of rights and duties will be legislative). But the legislative or interpretive status of the agency rules turns not in some general sense on the narrowness or breadth of the statutory (or regulatory) term in question, but on the prior existence or non-existence of legal duties and rights.

Of course an agency [**16] may for reasons of its own choose explicitly to invoke its general [*1111] legislating authority--perhaps, for example, out of concern that its proposed action might be invalid as an interpretation of some existing mandate, as was true in *Leone*, the case that set the legal landscape for *Chamber of Commerce*. In that event, even if a court believed that the agency had been unduly cautious about the legislative background, it would presumably treat the rule as an attempted exercise of legislative power.

In an occasional case we have appeared to stress whether the disputed rule is one with "binding effect"--"binding" in the sense that the rule does not " "genuinely leave[] the agency ... free to exercise discretion.' " *State of Alaska v. DOT, 868 F.2d at 445* (quoting *Community Nutrition Institute v. Young, 260 U.S. App. D.C. 294, 818 F.2d 943, 945-46 (D.C. Cir. 1987)*. That inquiry arose in a quite different context, that of distinguishing *policy statements*, rather than interpretive rules, from legislative norms. The classic application is *Pacific Gas & Electric Co. v. FPC, 164 U.S. App. D.C. 371, 506 F.2d 33, 38 (D.C. Cir. 1974)*; see [**17] also *American Bus Ass'n v. United States, 201 U.S. App. D.C. 66, 627 F.2d 525, 529 (D.C. Cir. 1980)* (following *PG&E*, again in policy statement context). Indeed, the *agency* 's theory in *Community Nutrition* was that its pronouncement had been a policy statement. See *818 F.2d at 945-46*.

But while a good rule of thumb is that a norm is less likely to be a general policy statement when it purports (or, even better, has proven) to restrict agency discretion, see, e.g., *McLouth Steel Products Corp. v. Thomas, 267 U.S. App. D.C. 367, 838 F.2d 1317, 1320-21 (D.C. Cir. 1988)*, restricting discretion tells one little about whether a rule is interpretive. See *Attorney General's Manual, supra*, at 30 n.3 (discussing exercise of discretion only in definition of policy statements). Nor is there much explanatory power in any distinction that looks to the use of mandatory as opposed to permissive language. While an agency's decision to use "will" instead of "may" may be of use when drawing a line between *policy statements* and legislative rules, see *Community Nutrition, 818 F.2d at 946-47*, the endeavor miscarries [**18] in the interpretive/legislative rule context. Interpretation is a chameleon that takes its color from its context; therefore, an interpretation will use imperative language--or at least have imperative meaning--if the interpreted term is part of a command; it will use permissive language--or at least have a permissive meaning--if the interpreted term is in a permissive provision.

A non-legislative rule's capacity to have a binding effect is limited in practice by the fact that agency personnel at every level act under the shadow of judicial review. If they believe that courts may fault them for brushing aside the arguments of persons who contest the rule or statement, they are obviously far more likely to entertain those arguments. And, as failure to provide notice-and-comment rulemaking will usually mean that affected parties have had no prior formal opportunity to present their contentions, judicial review for want of reasoned decisionmaking is likely, in effect, to take place in review of specific agency actions implementing the rule. Similarly, where the agency must defend its view as an application of *Chevron* "prong two" (i.e., where Congress has not "clearly" decided for [**19] or against the agency interpretation), so that only reasonableness is at issue, agency disregard of significant policy arguments will clearly count against it. As Donald Elliott has said, agency attentiveness to parties' arguments must come sooner or later. "As in the television commercial in which the automobile repairman intones ominously "pay me now, or pay me later,' the agency has a choice...." E. Donald Elliott, *Reinventing Rulemaking, 41 Duke L.J. 1490, 1491 (1992)*. Because the threat of judicial review provides a spur to the agency to pay attention to facts and arguments submitted in derogation of any rule not supported by notice and comment, even as late as the enforcement stage, *any* agency statement not subjected to notice-and-comment rulemaking will be more vulnerable to attack not only in court but also within the agency itself.

Not only does an agency have an incentive to entertain objections to an interpretive rule, but the ability to promulgate such rules, without notice and comment, does not appear more hazardous to affected parties than the likely alternative. Where a statute or legislative rule has created a legal basis for enforcement, [**20] an agency can simply let its interpretation [*1112] evolve ad hoc in the process of enforcement or other applications (e.g., grants). The protection that Congress sought to secure by requiring notice and comment for legislative rules is not advanced by reading the exemption for "interpretive rule" so narrowly as to drive agencies into pure ad hocery--an ad hocery, moreover, that affords less notice, or less convenient notice, to affected parties.

Accordingly, insofar as our cases can be reconciled at all, we think it almost exclusively on the basis of whether the purported interpretive rule has "legal effect", which in turn is best ascertained by asking (1) whether in the absence of the rule there would not be an adequate legislative basis for enforcement action or other agency action to confer benefits or ensure the performance of duties, (2) whether the agency has published the rule in the Code of Federal Regulations, (3) whether the agency has explicitly invoked its general legislative authority, or (4) whether the rule effectively amends a prior legislative rule. If the answer to any of these questions is affirmative, we have a legislative, not an interpretive rule.

Here we conclude that [**21] the August 1992 PPL is an interpretive rule. [2] The Part 50 regulations themselves require the reporting of diagnoses of the specified diseases, so there is no legislative gap that required the PPL as a predicate to enforcement action. Nor did the agency purport to act legislatively, either by including the letter in the Code of Federal Regulations, or by invoking its general legislative authority under *30 U.S.C. § 811(a)*. See *MSHA Program Information Bulletin No. 88-03* (August 19, 1988) (characterizing PPLs generally as "interpretation"). The remaining possibility therefore is that the August 1992 PPL is a de facto amendment of prior legislative rules, namely the Part 50 regulations. See *National Family Planning & Reproductive Health Ass'n v. Sullivan, 979 F.2d at 235*; *State of Alaska v. DOT, 868 F.2d at 446-47*; *Sentara-Hampton General Hospital v. Sullivan, 298 U.S. App. D.C. 372, 980 F.2d 749, 759 (D.C. Cir. 1992)*.

> 2 The respondents argue that the challenges as to the first two PPLs are moot, although they concede that whether the challenges are moot will have no bearing on the interpretive/legislative status of the third PPL and little, if any, practical effect otherwise. (During the period the first two PPLs were in effect no citations were issued for an operator's failure to report a "diagnosis" by

x-ray). The record does not indicate whether the MSHA could bring an enforcement action for an as yet undiscovered non-reporting of an x-ray "diagnosis" which took place when the first two PPLs were in effect. Because our ruling as to the third PPL will clearly cover the earlier two, we believe the mootness issue is itself moot.

[**22]

A rule does not, in this inquiry, become an amendment merely because it supplies crisper and more detailed lines than the authority being interpreted. If that were so, no rule could pass as an interpretation of a legislative rule unless it were confined to parroting the rule or replacing the original vagueness with another. See *American Postal Workers Union, 707 F.2d at 558-59* (interpretive rule establishes new formula for computation of retirement annuities for certain postal workers); see also *Fertilizer Institute, 935 F.2d at 1309-10* (rule found to be interpretive notwithstanding its brightline character); *General Motors Corporation, 742 F.2d at 1564* (same).

Although petitioners cite some definitions of "diagnosis" suggesting that with pneumoconiosis and silicosis, a diagnosis requires more than a chest x-ray--specifically, additional diagnostic tools as tissue examination or at least an occupational history, see *Current Medical Methods in Diagnosing Coal Workers' Pneumoconiosis, and a Review of the Medical and Legal Definitions of Related Impairment and Disability*, submission to Congress [**23] by United States Department of Labor, Secretary of Labor William E. Brock (1986) at 4, 19 & 46--MSHA points to some administrative rules that make x-rays at the level specified here the basis for a finding of pneumoconiosis. See, e.g., *42 CFR § 37.7(a)*; *20 CFR § 410.428(a)(1)*; *Garcia v. Director, OWCP, 869 F.2d 1413, 1415-16 (10th Cir. 1989)* (applying *§ 410.428(a)(1)* and other authority). See also ILO, *ILO U/C International Classification of Radiographs of Pneumoconioses* 16 (1972) (indicating that ILO score above 1/0 reflects evidence of pneumoconiosis); U.S. Department of Health and Human Services, *Occupational Respiratory* [*1113] *Diseases* 148-49 (James Merchant et al., eds., 1986) (ILO scores under 1/0 "are usually regarded as normal or as exhibiting essentially no evidence of pneumoconiosis," whereas those 1/0 and above "are generally regarded as positive for pneumoconiosis"). A finding of a disease is surely equivalent, in normal terminology, to a diagnosis, and thus the PPLs certainly offer no interpretation that repudiates or is irreconcilable with an existing legislative rule.

We stress that deciding whether an interpretation is an amendment of [**24] a legislative rule is different from deciding the substantive validity of that interpretation. An interpretive rule may be sufficiently within the language of a legislative rule to be a genuine interpretation and not an amendment, while at the same time being an incorrect interpretation of the agency's statutory authority. Cf. *Fertilizer Institute, 935 F.2d at 1308* (petitioners' argument "confuses the question whether the agency is interpreting a statute with the question whether the agency is thoroughly, or properly, interpreting the statute"). Here, petitioners have made no attack on the PPLs' substantive validity. Nothing that we say upholding the agency's decision to act without notice and comment bars any such substantive claims.

Accordingly, the petitions for review are

Dismissed.

Long-term Renal Prognosis of Diarrhea-Associated Hemolytic Uremic Syndrome: A Systematic Review, Meta-analysis, and Meta-regression

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REVIEW

Long-term Renal Prognosis of Diarrhea-Associated Hemolytic Uremic Syndrome

A Systematic Review, Meta-analysis, and Meta-regression

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Context The long-term renal prognosis of patients with diarrhea-associated hemolytic uremic syndrome (HUS) remains controversial.

Objectives To quantify the long-term renal prognosis of patients with diarrhea-associated HUS and to identify reasons for different estimates provided in the literature.

Data Sources We searched MEDLINE and Experta Medica (EMBASE) bibliographic databases and conference proceedings, and we contacted experts until February 2003. We also searched the Institute for Scientific Information index and reference lists of all studies that fulfilled our eligibility criteria. The search strategy included the terms *hemolytic-uremic syndrome*, *purpura*, *thrombotic thrombocytopenic*, *Escherichia coli O157*, *longitudinal studies*, *kidney diseases*, *hypertension*, and *proteinuria*

Study Selection Any study that followed up 10 or more patients with primary diarrhea-associated HUS for at least 1 year for renal sequelae.

Data Extraction Two authors independently abstracted data on study and patient characteristics, renal measures, outcomes, and prognostic features. Disagreements were resolved by a third author or by consensus.

Data Synthesis Forty-nine studies of 3476 patients with a mean follow-up of 4.4 years (range, 1-22 years at last follow-up) from 18 countries, 1950 to 2001, were summarized. At the time of recruitment, patients were aged 1 month to 18 years. In the different studies, death or permanent end-stage renal disease (ESRD) ranged from 0% to 30%, with a pooled incidence of 12% (95% confidence interval [CI], 10%-15%). A glomerular filtration rate lower than 80 mL/min per 1.73 m^2, hypertension, or proteinuria was extremely variable and ranged from 0% to 64%, with a pooled incidence of 25% (95% CI, 20%-30%). A higher severity of acute illness was strongly associated with worse long-term prognosis. Studies with a higher proportion of patients with central nervous system symptoms (coma, seizures, or stroke) had a higher proportion of patients who died or developed permanent ESRD at follow-up (explaining 44% of the between-study variability, $P=.01$). Studies with a greater proportion of patients lost to follow-up also described a worse prognosis ($P=.001$) because these patients were typically healthier than those followed up. One or more years after diarrhea-associated HUS, patients with a predicted creatinine clearance higher than 80 mL/min per 1.73 m^2, no overt proteinuria, and no hypertension appeared to have an excellent prognosis.

Conclusions Death or ESRD occurs in about 12% of patients with diarrhea-associated HUS, and 25% of survivors demonstrate long-term renal sequelae. Patients lost to follow-up contribute to worse estimates in some studies. The severity of acute illness, particularly central nervous system symptoms and the need for initial dialysis, is strongly associated with a worse long-term prognosis.

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THE HEMOLYTIC UREMIC SYNdrome (HUS) is a disorder characterized by acute hemolytic anemia, thrombocytopenia, and renal insufficiency. HUS, especially in children, is a main cause of acute renal failure worldwide. The incidence of HUS is increasing,[1] with current estimates of 1 case per 50 000 patient-years for those younger than 18 years.[2] Ninety percent of childhood cases of HUS are associated with diarrhea and gastroenteritis and are due to Shiga toxin–producing *Escherichia coli*.[3] This typical form of diarrhea-associated HUS causes toxin mediated vascular endothelial cell damage and in the kidney causes thrombotic occlusion of capillary lumens, glomerular endothelial cell swelling, apoptosis of glomerular and tubular cells, and extensive cortical necrosis.[4]

With improved recognition and supportive care, more patients are surviving the acute phase of diarrhea-

See also pp 1337 and 1379 and Patient Page.

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associated HUS. An accurate estimate of the long-term renal prognosis is critical for patient counseling, follow-up, and monitoring. It informs the feasibility of future clinical trials, and guides the necessity of screening after large population outbreaks of Shiga toxin–producing *E coli* gastroenteritis. However, the long-term renal prognosis of diarrhea-associated HUS remains controversial, with markedly different results reported in various studies. This review was conducted to better quantify prognosis and to identify reasons for different estimates provided in the world's literature.

METHODS

Research Questions

The primary questions of this review were: (1) What is the incidence of death or end-stage renal disease (ESRD) or a glomerular filtration rate (GFR) lower than 80 mL/min per 1.73m^2, hypertension, or proteinuria at least 1 year after diarrhea-associated HUS; (2) Which factors are associated with a worse long-term prognosis in individual studies; and (3) Which factors (such as secular differences, methodological quality, baseline patient characteristics, and initial treatments) are associated with a worse long-term prognosis at the study level. The last question was tested in meta-regression and the a priori hypotheses were a worse long-term prognosis would be evident in more recent studies (more children surviving severe disease), studies with longer follow-up, studies with more loss to follow-up,[5-8] and studies with more patients with severe acute illness. Secondary questions included (1) Are patients with a less severe initial infection still at appreciable risk of long-term renal disease, and (2) Do patients develop renal disease after apparent renal recovery from diarrhea-associated HUS.

Included Studies

Case series, cohort studies, and randomized controlled trials were included if 2 among the reviewing group (A.X.G, R.S.S., F.R., D.M., M.P.R., M.S., or W.F.C.) had agreed independently that an article (1) described a study population of 10 or more patients with primary diarrhea-associated HUS (idiopathic or infection associated), (2) followed up patients for at least 1 year (because the natural history of the disease is an improvement of GFR over the first year[9,10]), and (3) reported 1 or more renal outcomes (proteinuria, hypertension, renal insufficiency, or ESRD). A third reviewer (A.X.G., R.S.S., or D.M.) resolved disagreements about whether a study should be included.

Finding Relevant Studies

An independent review of citations from MEDLINE (OVID 1966 to February 2003) and Experta Medica (EMBASE, 1980 to February 2003) bibliographic databases was conducted (A.X.G. and F.R.). Full-text articles were retrieved if any of the authors considered any citation potentially relevant. The search strategy, developed with an experienced librarian, used terms most sensitive for identifying studies of prognosis[11] and was pilot tested and modified with known relevant articles. The search strategy included the terms *hemolytic-uremic syndrome*, *purpura*, *thrombotic thrombocytopenic*, *Escherichia coli O157*, *longitudinal studies*, *kidney diseases*, *hypertension*, and *proteinuria* (complete strategies available on request). Supplementary methods of finding studies included a review of relevant article bibliographies, a review of articles citing relevant articles in the Institute for Scientific Information index, a review of American Society of Nephrology meeting abstracts, and information provided by primary study authors.

Data Abstraction From Studies

Two of the reviewing group, using created forms, independently abstracted data on study and patient characteristics, renal measures, outcomes, and prognostic features. Disagreements were resolved by a third reviewer or by consensus. Non-English/non-French articles were an exception to this process, and a single reviewer (A.X.G.), with the help of a language translator, abstracted necessary data from Dutch, German, Japanese, Polish, Portuguese, and Spanish articles. Attempts were made to contact primary authors of all relevant studies to confirm the accuracy of abstracted data and to provide additional missing data.

Statistical Analysis

The primary outcomes of this review were death or permanent ESRD, and a GFR lower than 80 mL/min per 1.73 m^2, hypertension, or proteinuria at follow-up. For one study,[12] original data provided by the study author was categorized according to criteria for this review. Confidence intervals (CIs) for single proportions were derived using the Wilson score method.[13] We used χ^2 tests to assess between-study heterogeneity. An approach based on generalized estimating equations, which accounted for the within-study and between-study variability (random effects modeling), was used to derive pooled estimates of proportions and their variances.[14] Estimates were computed using Excel. Overall, each study contributed a weight between 1% and 9% for any estimate. For each study, we assessed 8 measures of quality. We selected a priori not to use an existing quality scale or to create our own scale. We assessed 2 of the most important measures in the meta-regression analyses—percentage lost to follow-up and whether the method of renal assessment was reported.

In meta-regression, the following prognostic factors were considered at the study level: the mean age of patients; duration of follow-up; the mid point year of the study; whether the method of renal assessment was reported; the proportion of patients who presented with central nervous system (CNS) symptoms (coma, seizures, or stroke); the proportion of patients who received acute dialysis, plasma infusion or exchange, or corticosteroids; and the proportion who were lost to follow-up. Additionally, for the outcomes of GFR lower than 80 mL/min per 1.73 m^2, hypertension, or proteinuria, the proportion of patients experiencing the competing event of death or permanent ESRD was considered. To examine the impact of these factors on study outcomes, exploratory meta-

regressions were conducted using logistic normal random effects models using SAS PROC NLMIXED (SAS Inc, Cary, NC). Values for some factors were not available for some studies. For each meta-regression, only studies for which all factors were available were included in the analysis. The explanatory ability of each meta-regression model was summarized as the proportion of between-study variability explained on the logit scale. Univariate meta-regression was used for each outcome and each factor. Factors found to be significant at the $P = .10$ level were included in multivariate meta-regression models. Factors found to be nonsignificant in a multivariate model were subsequently removed. To generate best-fit lines in meta-regression graphs, an approximate correction was used to convert conditional means from the logistic normal model into marginal mean curves.[15]

RESULTS

Study Selection

From screening more than 3384 citations, 124 full-text articles were retrieved, and 49 articles were included in this review. The agreement beyond chance between 2 independent reviewers in the reviewing group for citation screening, article inclusion, and data abstraction was good (κ range, 0.56-0.74 on 20 different measures). The reasons for article exclusion were the study's follow-up was less than 1 year, reporting of renal outcomes was unclear, selection of population for follow-up was nonrepresentative, inception of a cohort of patients with HUS was unclear, population included a large proportion of recognized secondary causes of HUS, or patients seemed to have been described in other articles already included in this review. (Excluded articles are available on request.) A single-adult study was excluded from this review.[16] Of the 49 articles, we contacted 45 primary authors, 25 of whom provided additional information and confirmed the accuracy of abstracted data. All included studies were considered representative of patients with typical diarrhea-associated HUS. Results of patients with recognized secondary forms of HUS were excluded when data were abstracted and analyzed. From solely reading the primary reports, we found occasional ambiguity about the reported cause of HUS. Exclusion of 9 studies with such ambiguity from our analyses did not change the summary estimates provided in this review.[10,17-24]

Study Description

The 49 studies included 3476 patients living in 18 different countries who were followed up for a mean (SD) of 4.4 (4.2) years. The mean range of follow-up was 1 to 22 years. The studies were conducted between 1950 and 2001 (TABLE 1, TABLE 2, and FIGURE 1).[5-10,12,17-58] Fifty-two percent of patients were female, and the mean age was 2.4 years (range, 0.1-18 years at recruitment into studies). Seven studies were associated with outbreaks of Shiga toxin-producing *E coli*, the source was uncertain for 2 studies,[28,38] and the others were associated with municipal water,[58] radish sprouts,[57] hamburgers,[8] raw ground beef,[12,59] and fermented sausage.[56] During the last 15 years, identification of Shiga toxin–producing *E coli* in study reports did not appreciably improve. With the exception of outbreaks, only 14% of all patients had *E coli* O157:H7 confirmed by either stool culture, antibody-to-stool verotoxin, or serological testing.

Methodological Quality Assessment

Three study designs were clinical trials of urokinase and heparin[47] and plasma exchange or infusions[50,51] with the remaining being case-series and cohort studies. (We considered nonrandomized trials with historical control groups to be cohort studies [Table 1].) Excluding the 3 clinical trials from this review did not change the main study results. When assessing quality, 43% were prospective, 73% described consecutive HUS patients at an institution, 56% defined cutoff values for the diagnosis of HUS, 71% described important baseline characteristics (such as demographic and exposure characteristics), 69% described treatments used during acute HUS, 16% described treatments used during follow-up, 61% used clear methods for defining and reporting renal outcomes, and 47% measured all patients at the same follow-up time or used statistical methods that adjusted for varying lengths of follow-up. On average, 21% of patients were lost to follow-up (range, 0% to 59%), and 64% of studies either had less than 10% lost to follow-up or described the characteristics of those lost to follow-up.

Renal Function Assessment

When described, proteinuria, hypertension, low GFR, and combinations of these were defined and measured in different ways in the primary studies. Different definitions of proteinuria used in the primary studies included a random urine dipstick (albustix, labstix, multistix) of at least 0.1 g/L (trace, 0.3 g/L [1+] or 1.0 g/L [≥2+]),[9,23] or an early-morning urine protein-to-creatinine ratio of at least 177 mg/g (≥20 mg/mmol) [5,38] or at least 265 mg/g (≥30 mg/mmol),[56] or a random urine albumin-to-creatinine ratio of at least 177 mg/g (≥20 mg/mmol),[58] a 24-hour urine protein of at least 100 mg/d, 150 mg/d,[18,20,24] 200 mg/d,[25,42] 250 mg/d, or 300 mg/d,[23,31,44] or a 24-hour urine protein of at least 100 mg/m^2 of body surface area.[33,35] Definitions of hypertension included the use of antihypertensive medications,[27,54] 1 blood pressure measurement higher than the 90th,[6] 95th,[9,12,25,33,42,50] 97th, or 99th[8] percentile, or 10 mm Hg above the 95th[23] or 97th percentile,[51] using various population norms defined by age,[17,39] sex,[56] weight and/or height.[5] Definitions of decreased GFR included an elevated serum creatinine level, GFR estimated from predictive equations,[60] 24-hour urine creatinine clearance, measured GFR using injected inulin,[23] iothalamate,[8] technetium diethylenetriamine pentaacetic acid,[7] or EDTA,[9,41] with usual cutoff points of abnormality of less than 80 mL/min per 1.73 m^2 of body surface area.[9] In this review, the expression *long-term renal sequelae* refers to a low GFR (usually <80 mL/min per 1.73m^2),

Table 1. Characteristics of Long-term Renal Prognosis Studies of Diarrhea-Associated Hemolytic Uremic Syndrome (HUS)*

Source	Primary Location	No. of Patients	Years of HUS Presentation	Study Design	Prospective Study	Patient Age, Mean (Range), y	Patients Requiring Acute Dialysis, %
Gagnadoux et al,[25] 1996	Paris, France	96	1950-1978	Cohort	No	1 (0.2-4)	67
Gianantonio et al,[22] 1968	Buenos Aires, Argentina	123	1957-1965	Cohort	Yes	1 (0.6-2)	. . .
Janssen et al,[19] 1974	Brussels, Belgium	20	1957-1972	Cohort	No	1 (0.1-7)	70
Dolislager and Tune,[26] 1978	Stanford, United States	45	1962-1975	Cohort	No	4 (0.2-14)	. . .
Donckerwolcke et al,[27] 1979	Utrecht, the Netherlands	72	1964-1977	Cohort	No	3 (0.5-11)	69
de Jong and Monnens,[20] 1988	Nijmegen, the Netherlands	96	1965-1977	Cohort	Yes	3 (0.1-8)	. . .
McLean et al,[28] 1966	Rhyl, Wales	10	1963	Case series	Yes	2 (0.1-8)	10
Fitzpatrick et al,[29] 1991	London, England	103	1966-1985	Cohort	No	2 (0.2-14)	85
Riella et al,[30] 1976	Seattle, United States	18	1967-1973	Cohort	No	5 (0.1-14)	17
Blahova et al,[31] 2002	Prague, Czech Republic	57	1967-1997	Cohort	No	3 (0.3-14)	65
Sorrenti and Lewy,[32] 1978	Chicago, United States	19	1968-1975	Cohort	No	4 (0.5-16)	84
Spizzirri et al,[33] 1997	La Plata, Argentina	312	1968-1984	Cohort	No	1 (0.2-6)	. . .
Cordero et al,[34] 1990	Santiago, Chile	154	1968-1989	Cohort	No	1 (0.2-9)	39
Donckerwolcke et al,[35] 1973	The Netherlands and Belgium	49	1970-1971	Cohort	Yes	3 (0.1-12)	. . .
Kelles et al,[36] 1994	Leuven, Belgium	95	1970-1982	Cohort	Yes	2 (0.1-14)	. . .
Gusmano et al,[37] 1987	Genoa, Italy	92	1970-1985	Cohort	Yes	2 (0.1-14)	86
Coad et al,[38] 1991	Birmingham, England	74	1970-1987	Cohort	No	3 (0.3-13)	59
Siegler et al,[39] 1996	Salt Lake City, United States	265	1970-1993	Cohort	No	3 (0.1-18)	47
Gillor et al,[21] 1986	Cologne, Germany	31	1971-1982	Cohort	Yes	4 (0.5-9)	100
Tonshoff et al,[23] 1994	Heidelberg, Germany	89	1971-1988	Cohort	Yes	3 (0.1-12)	69
Campos et al,[17] 1982	Minneapolis, United States	26	1972-1979	Cohort	No	5 (2-16)	92
Sheth et al,[40] 1988	Milwaukee, United States	43	1972-1985	Cohort	No	3 (0.5-9)	63
Hughes et al,[41] 1991	Glasgow, Scotland	79	1972-1988	Cohort	No	3 (0.2-14)	75
Zurowska et al,[42] 2000	Gdansk, Poland	196	1972-1999	Cohort	No	. . .	83
Monnens et al,[18] 1978	Rotterdam, the Netherlands	35	1973-1977	Cohort	Yes	3 (1-7)	. . .
Diekmann,[43] 1980	Munster, Germany	26	1973-1978	Cohort	Yes	4 (0.5-11)	81
Wende-Fischer et al,[10] 1996	Hannover, Germany	61	1973-1989	Cohort	No	5 (0.3-15)	80
Imoberdorf et al,[44] 1993	Bern, Switzerland	42	1973-1991	Cohort	No	1 (0.1-13)	38
O'Brien et al,[6] 1994	New Haven, United States	45	1974-1989	Cohort	No	. . .	. . .
Vermylen et al,[45] 1988	Brussels, Belgium	53	1975-1987	Cohort	Yes	2 (0.1-14)	47
Loirat et al,[46] 1993	Paris, France	147	1975-1991	Cohort	No	1 (0.1-16)	76
Huseman et al,[9] 1999	Berlin, Germany	165	1976-1995	Cohort	Yes	2 (0.2-11)	82
O'Regan et al,[7] 1989	Montreal, Canada	50	1977-1982	Cohort	No	. . .	. . .
Loirat et al,[47] 1984	Paris, France	33	1978-1980	Trial	Yes	3 (0.1-16)	91
Mizusawa et al,[48] 1996	Brisbane, Australia	55	1979-1995	Cohort	No	2 (0.2-13)	64
Guyot et al,[49] 1986	Nantes, France	37	1980-1985	Cohort	No	2 (0.2-7)	59
Rizzoni et al,[50] 1988	Padova, Italy	32	1981-1985	Trial	Yes	2 (0.3-6)	100
Loirat et al,[51] 1988	Paris, France	79	1983-1985	Trial	Yes	2 (0.2-13)	84
Al-Eisa and Al-Hajeri,[52] 2001	Safat, Kuwait	14	1985-2000	Cohort	No	2 (1-3)	79
Miyazaki,[53] 1994	36 centers, Japan	122	1986-1990	Cohort	No	4 (1-6)	19
Small et al,[5] 1999	Nottingham, England	114	1986-1996	Cohort	Yes	3 (0.2-15)	72
Brichard et al,[54] 1993	Brussels, Belgium	33	1987-1991	Cohort	No	3 (0.2-14)	61
Ramos et al,[55] 2001	Janeiro, Portugal	16	1989-2000	Cohort	No	3 (0.8-14)	50
Ogborn et al,[12] 1998	Arviat, Canada	21	1991	Cohort	No	6 (0.8-14)	19
Brandt et al,[8] 1998	Seattle, United States	37	1993	Cohort	Yes	5 (1-15)	57
Litalien et al,[24] 1999	Ottawa, Canada	38	1994-1996	Cohort	Yes	4 (0.5-7)	37
Henning et al,[56] 1998	Adelaide, Australia	20	1995	Cohort	Yes	5 (0.5-12)	90
Yoshioka et al,[57] 1999	Osaka, Japan	15	1996	Cohort	Yes	8 (6-10)	33
Salvadori et al,[58] 2002	Walkerton, Canada	22	2000	Cohort	Yes	5 (1-16)	36

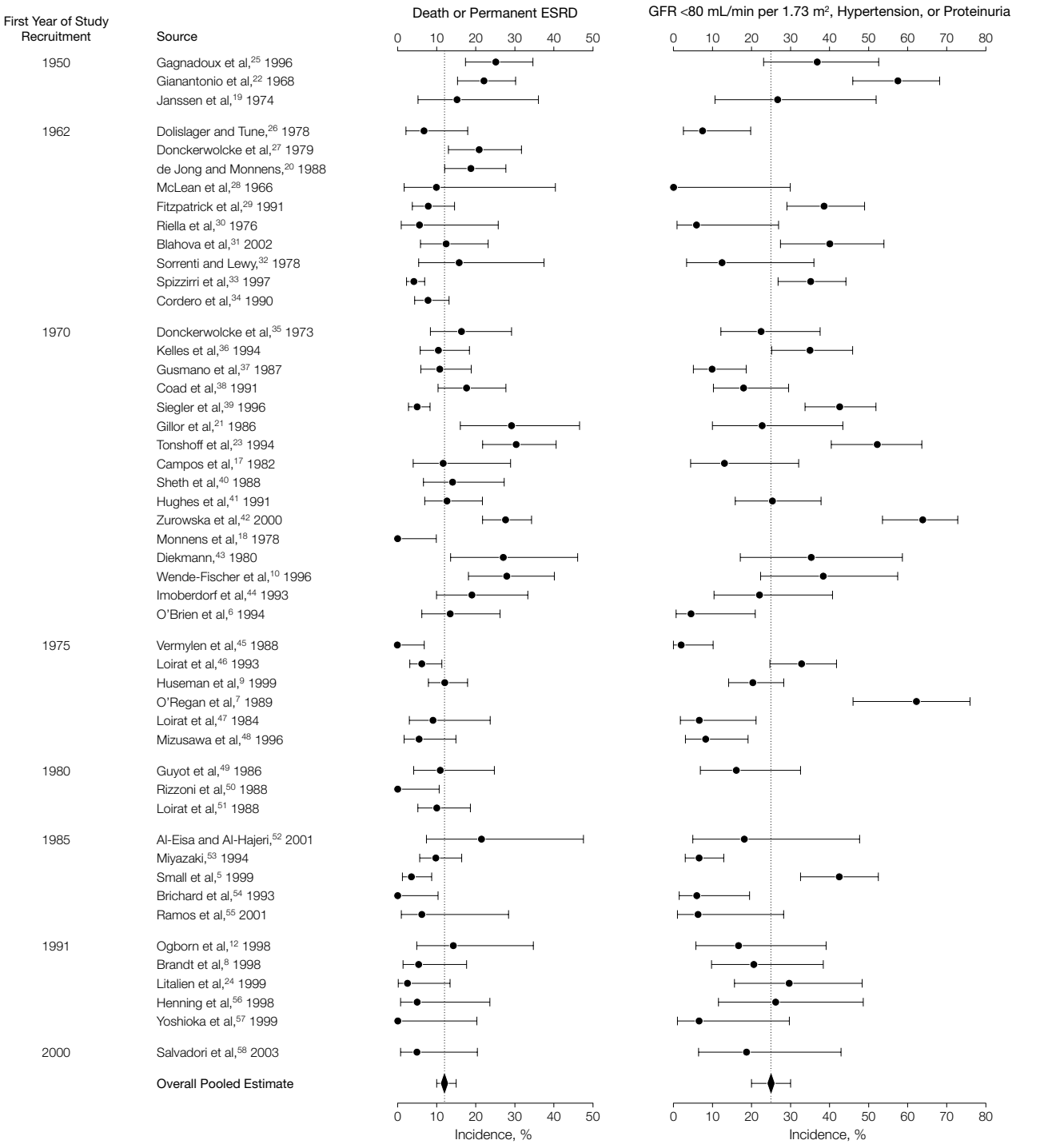
*Studies are arranged chronologically based on the initiation of study enrollment. Ellipses indicate not reported.

Table 2. Long-term Renal Prognosis Studies of Diarrhea-Associated Hemolytic Uremic Syndrome

Source*	Follow-up, Mean (Range), y	Lost to Follow-up, %	Died or Had ESRD at Follow-up, %	No. of Patients With Renal Testing	GFR <80 mL/min per 1.73 m², Hypertension, or Proteinuria, %†	Hypertension, %†	Proteinuria, %†	GFR, mL/min per 1.73 m², %†		
								60-80	30-59	5-29
Gagnadoux et al[25]	15 (10-25)	35	25	38	37	21	11	8	8	0
Gianantonio et al[22]	3 (1-8)	19	22	73	58	15	25	18	33	4
Janssen et al[19]	3 (0.2-12)	10	15	15	27	7	13	. . .	. . .	. . .
Dolislager and Tune[26]	4 (1-12)	4	7	40	8	5	8	5	0	0
Donckerwolcke et al[27]	1	3	21	55	. . .	11	7	. . .	. . .	. . .
de Jong et al[20]	10	5	19	73	. . .	14	6	4	4	1
McLean et al[28]	2	0	10	9	0	0	0	0	0	0
Fitzpatrick et al[29]	8 (5-21)	7	8	88	39	3	31	13	5	1
Riella et al[30]	3 (0.4-7)	0	6	17	6	6	. . .	0	6	0
Blahova et al[31]	7 (1-27)	0	12	50	40	8	40	26	2	12
Sorrenti and Lewy[32]	5 (1-8)	0	16	16	13	6	13	0	6	6
Spizzirri et al[33]	13 (10-20)	59	4	114	35	15	35	10	5	4
Cordero et al[34]	22	45	8	73	. . .	3	. . .	. . .	. . .	. . .
Donckerwolcke et al[35]	1	2	16	40	23	10	. . .	. . .	. . .	. . .
Kelles et al[36]	10	5	11	80	35	11	8	. . .	. . .	. . .
Gusmano et al[37]	1 (0.5-11)	3	11	79	10	. . .	4	5	3	3
Coad et al[38]	>1	0	18	61	18	8	8	0	10	0
Siegler et al[39]	4 (0.3-17)	52	5	115	43	11	17	17	. . .	. . .
Gillor et al[21]	1	0	29	22	23	23	. . .	. . .	. . .	. . .
Tonshoff et al[23]	2 (1-17)	15	30	67	52	. . .	. . .	12	. . .	. . .
Campos et al[17]	3 (0.5-7)	0	12	23	13	9	. . .	4	0	4
Sheth et al[40]	. . . (1-13)	0	14	37	. . .	. . .	. . .	. . .	. . .	. . .
Hughes et al[41]	4 (0.3-14)	16	13	59	25	7	10	. . .	. . .	2
Zurowska et al[42]	11 (2-27)	24	28	94	64	18	21	18	4	3
Monnens et al[18]	2	0	0	33	. . .	24	24	3	3	3
Diekmann[43]	2 (1-6)	8	27	17	35	6	38	. . .	. . .	. . .
Wende-Fischer et al[10]	5 (4-13)	30	28	26	38	19	12	. . .	. . .	. . .
Imoberdorf et al[44]	1	17	19	27	22	7	15	. . .	. . .	. . .
O'Brien et al[6]	6 (1-15)	36	13	23	4	0	4	0	0	0
Vermylen et al[45]	. . . (0.5-7)	4	0	51	2	0	2	0	0	0
Loirat et al[46]	>1	17	6	113	33	. . .	. . .	. . .	. . .	. . .
Huseman et al[9]	5 (2-13)	13	12	123	20	. . .	. . .	11	2	. . .
O'Regan et al[7]	7 (5-11)	26	. . .	37	62	0	19	27	30	0
Loirat et al[47]	2 (1-4)	0	9	30	7	7	27	3	3	0
Mizusawa et al[48]	5 (3-16)	7	5	49	8	6	8	0	8	0
Guyot et al[49]	2 (0.6-5)	5	11	31	16	10	6	3	0	0
Rizzoni et al[50]	2 (. . .)	0	0	32	. . .	6	6	. . .	. . .	. . .
Loirat et al[51]	1	13	10	61	. . .	10	20	. . .	. . .	. . .
Al-Eisa and Al-Hajeri[52]	5 (1-15)	0	21	11	18	18	18	0	0	18
Miyazaki[53]	3 (1-5)	4	10	105	7	6	7	. . .	. . .	. . .
Small et al[5]	1	18	4	92	42	16	22	21	5	1
Brichard et al[54]	3 (0.7-4)	0	0	33	6	0	6	0	0	0
Ramos et al[55]	5 (0.5-11)	0	6	16	6	0	. . .	6	0	0
Ogborn et al[12]	4	0	14	18	17	17	0	0	0	0
Brandt et al[8]	2 (1-3)	16	5	29	21	3	17	0	0	0
Litalien et al[24]	1	26	3	27	30	. . .	33	7	19	0
Henning et al[56]	2	0	5	19	26	26	26	11	16	0
Yoshioka et al[57]	1.5	0	0	15	7	0	0	7	0	0
Salvadori et al,[58] 2002	1	18	5	16	19	19	19	13	0	0

Abbreviations: ESRD, end-stage renal disease; GFR, estimated glomerular filtration rate. Ellipses indicate not reported.
*Studies are arranged chronologically, based on the initiation of study enrollment.
†A summary of the various study definitions for a GFR lower than 80 mL/min per 1.73 m², hypertension, and proteinuria are presented in the "Methods" section.

Figure 1. Proportion of Patients With Sequelae From Diarrhea-Associated Hemolytic Uremic Syndrome



Presented are the proportions of patients who died or developed permanent end-stage renal disease (ESRD), or developed a glomerular filtration rate (GFR) lower than 80 mL/min per 1.73 m², hypertension, or proteinuria, at an average last follow-up of 4 years (range, 1-22 years). Point estimates are provided with 95% confidence intervals and overall pooled estimates.

The 49 studies are arranged chronologically from first year of study recruitment. A total of 2372 patients without permanent ESRD had renal function assessed at last follow-up. One study of estimates of death or ESRD and 7 studies of estimates of renal function were excluded due to missing information. A summary of the various study definitions for a GFR lower than 80 mL/min per 1.73 m², hypertension, and proteinuria are presented in the "Methods" section.

Table 3. Pooled Analyses of 3476 Total Patients and 2372 Patients With Renal Testing at Follow-up*

Variable	Patients Who Died or Had ESRD at Follow-up (n = 3476)	GFR <80 mL/min per 1.73 m², Hypertension, or Proteinuria	Hypertension	Proteinuria	GFR, mL/min per 1.73 m²		
					60-80	30-59	5-29
Pooled average (95% CI), %	12 (10-15)	25 (20-30)	10 (8-12)	15 (10-20)	8 (5-11)	6 (3-8)	1.8 (0.8-3)
Range, %	0-30	0-64	0-26	0-40	0-27	0-33	0-18

Abbreviations: CI, confidence interval; ellipses, not reported; ESRD, end-stage renal disease; GFR, estimated glomerular filtration rate.
*Averages were pooled using a random-effects model. Most deaths or ESRD reported at last follow-up occurred during the acute stage of hemolytic uremic syndrome. A summary of the various study definitions for a GFR lower than 80 mL/min per 1.73 m², hypertension, and proteinuria are presented in the "Methods" section.

hypertension, or overt proteinuria at last follow-up, as assessed in the individual studies. Similarly, *renal recovery* refers to a normal GFR (usually ≥80 mL/min per 1.73m²) and no evidence of hypertension or overt proteinuria as assessed in the individual studies.

Long-term Renal Prognosis

For all outcomes, the variability between studies was larger than would be expected by chance (significant heterogeneity, $P<.001$). Given the large between-study variability with wide ranging heterogeneity (Figure 1), mathematically pooled results should be interpreted with caution. However such results are provided because they represent current best evidence for clinical care and guide sample-size calculations for future epidemiological studies. Death or permanent ESRD ranged from 0% to 30% (death, 0%-23%; permanent ESRD, 0%-17%; TABLE 3 and Figure 1). The mathematical pooled estimate of the incidence of death or permanent ESRD was 12% (95% confidence interval [CI], 10%-15%; death 9%, [95% CI, 7%-11%; permanent ESRD, 3% [95% CI, 2%-5%]), with the majority of cases occurring during the acute phase of HUS.

At a minimum of 1 year of follow-up, 2372 patients without permanent ESRD had an assessment of renal function. Renal sequelae ranged from 0% to 64% (Table 3 and Figure 1). The majority of studies did not differentiate whether these were persistent findings after acute HUS or whether they developed after apparent initial renal recovery. The pooled estimate of renal sequelae was 25% (95% CI, 20%-30%). The pooled estimate of the incidence of a GFR of 60 to 80 mL/min per 1.73 m² was 8% (95% CI, 5%-11%); of 30 to 59 was 6% (95% CI, 3%-8%); of 5 to 29 was 1.8% (95% CI, 0.8%-3%), hypertension was 10% (95% CI, 8%-12%), and proteinuria was 15% (95% CI, 10%-20%).

Prognostic Features

A number of prognostic features were described in the individual studies. Many of the primary study authors described an association between an increased severity of acute illness (greater infection or host response) and worse long-term prognosis, including an elevated white blood cell count higher than 20×10^3/μL with neutrophilia,[29,38] a high serum creatinine or urea concentration,[53] central nervous system symptoms (reduced consciousness, coma, stroke, or seizures),* ischemic colitis,[33,49] and hypertension.[9,10,23,36,44,48,53] Lower and prolonged levels of anemia and thrombocytopenia were inconsistently associated with both worse[8] and improved[38] outcomes. Compared with patients with oliguria of 8 days or less (an approximate urine output less than 300 mL/m² per day), those with oliguria of greater than 8 days or anuria of 1 to 8 days, and those with anuria of greater than 8 days had a step-wise worsening of prognosis.† A longer duration of dialysis was associated with worse prognosis, and no patient achieved full renal recovery when dialysis therapy exceeded 4 weeks.[5,23,29,41,42] Similarly after 3 weeks, the longer the duration of hospitalization, the worse the outcome.[56]

Certain studies examined the significance of renal biopsy near acute illness. Those with cortical necrosis or arterial thrombotic microangiopathy demonstrated a worse prognosis compared with patients with isolated glomerular lesions.[23,25,46] In patients with isolated glomerular lesions, those with more than 50% glomerular involvement had a worse prognosis.[46]

Younger ages (on average <2-5 years old)[10,24,31,33] and female sex[38] had an inconsistent effect on results. One study suggested a worse prognosis with HUS during winter.[38] Increased C-reactive protein[53] and cytokine levels (IL-6, IL-10, IL-1, Ra)[24] were associated with worse prognosis.

With respect to treatment, living no further than 100 km (62.5 miles) from a tertiary center,[42] and early recognition and treatment including dialysis[10,42] were associated with better prognosis. Better outcomes were not consistently achieved with streptokinase[18,43] or plasma exchange.[21,40,50,51]

Prognostic Features in Meta-regression

At the study level, severity of acute illness was associated with worse long-term prognosis in both univariate and multivariate analyses. Studies with a higher proportion of patients with CNS symptoms (coma, seizures, or stroke) had a higher proportion of patients who died or who developed permanent ESRD, explaining 44% of the between-study variability ($P=.01$; FIGURE 2). Similarly, studies with a higher proportion of patients requiring acute dialysis had a higher proportion of patients who died or developed permanent ESRD, explaining 10% of the between-study variability ($P=.02$), and had a higher proportion of patients with long-

*References 10, 23, 33, 36, 41, 44, 49, 53.
†References 9, 18, 20, 22, 23, 26, 33, 46, 48.

term renal sequelae, explaining 15% of the between study variability ($P<.001$; FIGURE 3).

Study level factors such as a higher proportion of patients of older age, female sex, receiving corticosteroids, and factors such as the years in which the study was conducted and whether the study reported careful methods of renal function assessment were not associated with worse outcomes. Similarly, studies with a lower proportion of patients with death or ESRD did not demonstrate a higher proportion of patients with renal sequelae at follow-up. In univariate but not multivariate analyses, studies with a longer follow-up time demonstrated a higher proportion of patients with long-term renal sequelae.

However, in both univariate and multivariate analyses, studies with a higher proportion of patients lost to follow-up demonstrated a higher proportion of patients with long-term renal sequelae, explaining 28% of the between-study variability ($P=.001$; FIGURE 4). When the analysis was limited to the 30 studies with better than 90% follow-up, with a mean follow-up of 3.6 years (range, 1-10 years), the pooled estimate of death or permanent ESRD was 11% (95% CI, 8%-14%) and renal sequelae was 17% (95% CI, 12%-22%). Studies with a higher proportion of patients receiving plasma infusion or exchange had a lower proportion of patients who died or developed permanent ESRD, explaining 8% of the between study variability ($P=.03$), and a lower proportion of patients with renal sequelae, explaining 28% of the between-study variability, $P=.03$; FIGURE 5). Visually this trend seemed to be influenced by a small number of studies.[45,52,54]

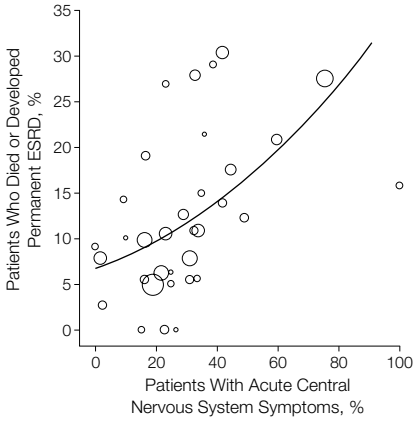
Sequelae After Milder Forms of Diarrhea-Associated HUS

A few studies have suggested that patients with less severe forms of HUS, including those with a preserved urine output, may still demonstrate renal sequelae at follow-up.[23,25] For example, 2 of 5 patients with acute diarrhea-associated HUS and mild renal impairment (normal urine output, no dialysis) demonstrated hypertension and proteinuria at long-term follow-up.[25] Similarly 5 of 18 patients with acute diarrhea-associated HUS and normal urine output later developed long-term renal sequelae including chronic renal failure.[23]

Renal Disease After Apparent Renal Recovery

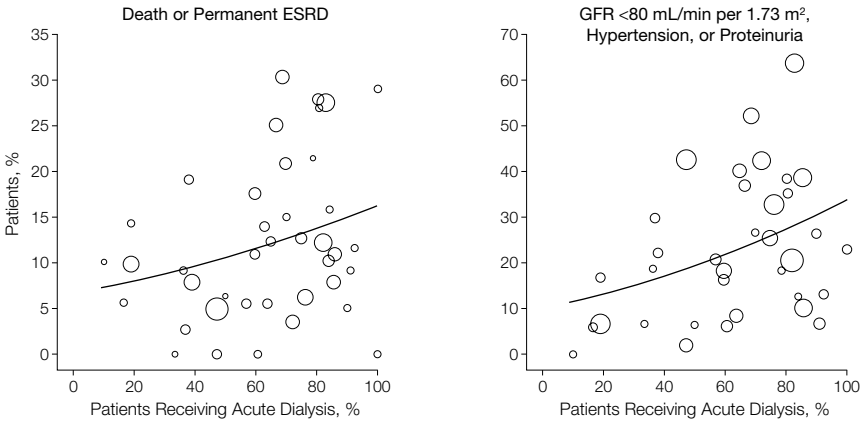
In studies of patients who develop long-term renal sequelae, the majority of studies did not differentiate patients who apparently completely recovered after the acute illness from those who demonstrated persistent renal abnormalities. Four studies that did differentiate these states using a single-screening test of clearance suggested that 8% to 61% of those who seemed to have a normal GFR after diarrhea-associated HUS (assessed either by a normal serum creatinine concentration[7] or a calculated[33] or measured GFR >80 mL/min per 1.73 m^2)[5,9] went on to develop a GFR lower than 80 mL/min per 1.73m^2, hypertension, or proteinuria during long-term follow-up. Similarly, a single study suggested that a quarter of those who recovered with an absence of proteinuria (<250 mg of protein per day) went on to develop renal sequelae during long-term follow-up.[33] On the other hand, 4 studies that considered 3 measures of renal health in combination suggested that no one who recovered with an absence of hypertension, an absence of dipstick proteinuria, and a calculated or measured GFR higher than 80 mL/min per 1.73m^2 developed future renal sequelae,[5,22,23,26] recognizing these studies are limited by follow-up times of less than 5 years.

Figure 2. Studies With a Higher Proportion of Patients With Central Nervous System Symptoms (Coma, Seizures, or Stroke)



These studies had a higher proportion of patients with death or permanent end-stage renal disease (ESRD) at follow-up, explaining 44% of the between-study variability ($P=.01$). The area of each circle is proportional to the number of patients in each study. Curve is best-fit line from meta-regression. See "Methods" section.

Figure 3. Studies With a Higher Proportion of Patients Who Required Acute Dialysis



These studies had a higher proportion of patients with death or permanent end-stage renal disease (ESRD) at follow-up, explaining 10% of the between-study variability ($P=.02$), and a higher proportion of patients with a glomerular filtration rate (GFR) lower than 80 mL/min per 1.73 m^2, hypertension, or proteinuria at last follow-up, explaining 15% of the between-study variability ($P<.001$). The area of each circle is proportional to the number of patients in each study. Curves are best-fit lines from meta-regression. See "Methods" section.

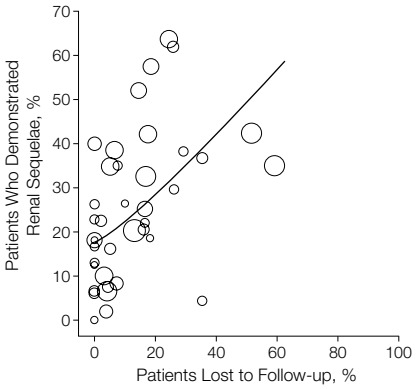
COMMENT

Studies of diarrhea-associated HUS are highly variable in their methodological rigor, methods of renal assessment, and estimates of long-term prognosis. For this reason, the long-term renal prognosis of diarrhea-associated HUS remains controversial. As highlighted in this review, the incidence of death or permanent ESRD ranged from 0% to 30% in the primary studies, and 0% to 65% of patients demonstrated long-term renal sequelae. Our mathematical pooled estimates based on existing studies suggest an average of 4 years after experiencing diarrhea-associated HUS, 9% of patients die (most during the acute phase of illness), an additional 3% develop permanent ESRD, and 25% demonstrate renal sequelae. Severity of the acute illness, especially the presence of CNS symptoms, is strongly associated with worse long-term outcome. These symptoms of coma, seizures, or stroke may be due to ischemic thrombotic microangiopathy, concurrent hypertension, or metabolic alterations of hyponatremia and hypocalcemia.[61] The need for initial dialysis was also strongly associated with worse outcome. No one achieved full renal recovery when dialysis therapy exceeded 4 weeks.

Some studies have confirmed long-term sequelae even after milder forms of diarrhea-associated HUS.[22,24] The natural history of diarrhea-associated HUS is often an improvement of GFR (approximately 25 mL/min per 1.73m^2 over the first year[9,10]). Thus it seems prudent to screen patients at least once for silent renal disease a year after acute diarrhea-associated HUS. As the sole method of characterizing patients free of disease, a nuclear GFR higher than 80 mL/min per 1.73m^2 after recovery is an inadequate screening test. However, those who demonstrate a predicted creatinine clearance higher than 80 mL/min per 1.73m^2, [60] no overt proteinuria ($\geq$1$^+$or 0.3 g/L on dipstick), and no hypertension (based on population reference values), a potentially easily implemented cost-effective screening strategy, appear to have an excellent prognosis during early follow-up.[5,22,23,26]

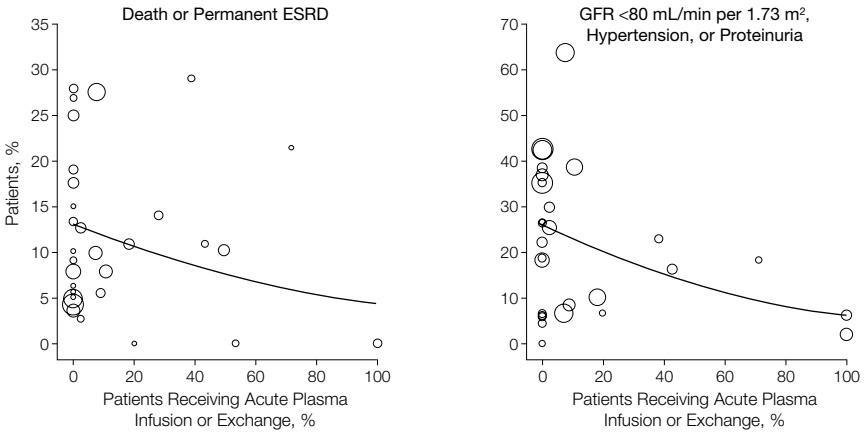
Similar to other renal insults, it is theorized that diarrhea-associated HUS can lead to a critical reduction in nephron number, with unsustainable remnant single-nephron hyperfiltration[62] and progressive renal disease.[63] However, these results confirm that the relationship between longer follow-up time and worse prognosis is in part complicated by a high number of patients lost to follow-up in some studies. Patients lost to follow-up contribute to worse estimates of long-term prognosis because they are typically healthier than those followed up. Further prognostic studies should adhere to the methodological quality criteria outlined in this review, and particularly should strive for less than 10% of patients lost to follow-up. Different definitions for surrogate outcomes of proteinuria, low GFR, and hypertension performed at different follow-up times complicate the interpretation and utility of results from many of the primary studies and the summary results of this review. A uniformly adopted time and method of measurement, with specified cutoff levels that have been associated with death or ESRD in prospective studies of children,[64] would be advantageous. Further characterization of prognostic factors and novel methods of screening patients after diarrhea-associated HUS, although critical for clarifying pathophysiology,[6,9,65,66] may prove difficult to apply clinically. Rather, improving the detection of Shiga toxin–producing *E coli*

Figure 4. Studies With a Higher Proportion of Patients Lost to Follow-up



These studies had a higher proportion of patients with renal sequelae at last follow-up, explaining 28% of the between-study variability (P=.001). The area of each circle is proportional to the number of patients in each study. Curve is best-fit line from meta-regression. See "Methods" section.

Figure 5. Studies With a Higher Proportion of Patients Who Received Acute Plasma Infusion or Exchange



These studies had a lower proportion of patients who died or developed permanent end-stage renal disease (ESRD), explaining 8% of the between-study variability (P=.03), and a lower proportion with renal sequelae (glomerular filtration rate [GFR] <80 mL/min per 1.73 m^2, hypertension, or proteinuria) at last follow-up, explaining 28% of the between-study variability (P=.03). Visually, this trend seemed to be influenced by a small number of studies.[45,52,54]

as the cause of diarrhea-associated HUS, which has been limited even in studies during the last 15 years, seems necessary for the proper characterization of future cohorts. In addition, given that long-term sequelae have been described after milder forms of diarrhea-associated HUS,[23,25] research examining the incidence of renal disease after toxigenic *E coli* gastroenteritis seems essential.

Future therapeutic studies may consider a preventive approach to the medical consequences of Shiga toxin–producing *E coli*, including increased sanitation, surveillance, assessment, reporting, and possibly bovine and human vaccination.[67,68] Avoidance of antibiotics and antimotility agents during gastroenteritis[69,70] and the role of protective antibodies[71] and oral synthetic verotoxin-receptor analogs attached to chromosorb [72] remain to be clarified. Early recognition and referral to a specialized medical care center experienced in the critical care of acute diarrhea-associated HUS seems to be a particularly important strategy.[42] Corticosteroids, antiplatelet agents, anticoagulant therapy, and thrombolytics during the acute phase of diarrhea-associated HUS are not clearly effective.[47,73] Careful blood pressure control and renin-angiotensin system blockade may be particularly beneficial for those who demonstrate renal sequelae after diarrhea-associated HUS. With an event rate of 12% death or permanent ESRD at 4 years, this review confirms that multicenter, multinational, clinical trials with thousands of patients are required for adequate statistical power to demonstrate the benefits of any therapy on these outcomes.

A therapy that may warrant this level of attention in future clinical trials is plasma exchange. Sixteen studies included in this review described a portion of patients treated with infusion or exchange. However smaller and possibly underpowered trials of plasma have not shown efficacy in diarrhea-associated HUS.[49,50] These meta-regression results of the potential benefits of plasma are intriguing, recognizing their interpretation is complicated by their observational nature, analytic limitations,[74] trends influenced by a small number of studies,[45,52,54] and an absence of risk benefit assessment. Publication biases (in which studies with larger efficacy are more likely to be reported) may also exist.

In summary, death or ESRD occurs in about 12% of patients 4 years after diarrhea-associated HUS, and 25% of survivors demonstrate long-term renal sequelae. The severity of acute illness, particularly CNS symptoms and the need for initial dialysis, is strongly associated with a worse long-term prognosis. Patients lost to follow-up contribute to worse estimates in some studies.

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REFERENCES

1. Tarr PI, Neill MA, Allen J, Siccardi CJ, Watkins SL, Hickman RO. The increasing incidence of the hemolytic-uremic syndrome in King County, Washington: lack of evidence for ascertainment bias. *Am J Epidemiol*. 1989;129:582-586.
2. Martin DL, MacDonald KL, White KE, Soler JT, Osterholm MT. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. *N Engl J Med*. 1990;323:1161-1167.
3. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J Infect Dis*. 1985;151:775-782.
4. Ray PE, Liu XH. Pathogenesis of Shiga toxin-induced hemolytic uremic syndrome. *Pediatr Nephrol*. 2001;16:823-839.
5. Small G, Watson AR, Evans JH, Gallagher J. Hemolytic uremic syndrome: defining the need for long-term follow-up. *Clin Nephrol*. 1999;52:352-356.
6. O'Brien JA, Van Why SK, Keller MS, Gaudio KM, Kennedy TL, Siegel NJ. Altered renovascular resistance after spontaneous recovery from hemolytic uremic syndrome. *Yale J Biol Med*. 1994;67:1-14.
7. O'Regan S, Blais N, Russo P, Pison CF, Rousseau E. Hemolytic uremic syndrome: glomerular filtration rate, 6 to 11 years later measured by 99mTc DTPA plasma slope clearance. *Clin Nephrol*. 1989; 32:217-220.
8. Brandt JR, Joseph MW, Fouser LS, et al. Cholelithiasis following *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Pediatr Nephrol*. 1998;12:222-225.
9. Huseman D, Gellermann J, Vollmer I, et al. Long-term prognosis of hemolytic uremic syndrome and effective renal plasma flow. *Pediatr Nephrol*. 1999;13:672-677.
10. Wende-Fischer R, Hoyer PF, Offner G, Brodehl J. Hamolytisch-uramisches Syndrom im Kindesalter: Haemolytic uraemic syndrome. *Monatsschr Kinderheilkd*. 1996;144:526-533.
11. Haynes RB, Wilczynski N, McKibbon KA, Walker CJ, Sinclair JC. Developing optimal search strategies for detecting clinically sound studies in MEDLINE. *J Am Med Inform Assoc*. 1994;1:447-458.
12. Ogborn MR, Hamiwka L, Orrbine E, et al. Renal function in Inuit survivors of epidemic hemolytic-uremic syndrome. *Pediatr Nephrol*. 1998;12:485-488.
13. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med*. 1998;17:857-872.
14. Zhou XH, Brizendine EJ, Pritz MB. Methods for combining rates from several studies. *Stat Med*. 1999;18:557-566.
15. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. *Biometrics*. 1988;44:1049-1060.
16. Schieppati A, Ruggenenti P, Cornejo RP et al. Renal function at hospital admission as a prognostic factor in adult hemolytic uremic syndrome: the Italian Registry of Haemolytic Uremic Syndrome. *J Am Soc Nephrol*. 1992;2:1640-1644.
17. Campos A, Kim Y, Miller K, Mauer SM, Loken MK, Michael AF. Radionuclide studies of the kidney in children with hemolytic-uremic syndrome. *Radiology*. 1982;145:811-813.
18. Monnens L, van Collenburg J, de Jong M, Zoethout H, Van Wieringen P. Treatment of the hemolytic-uremic syndrome: comparison of the results of heparin treatment with the results of streptokinase treatment. *Helv Paediatr Acta*. 1978; 33:321-328.
19. Janssen F, Potvliege PR, Vainsel M, Vis HL. Short- and long-term prognosis of the hemolytic-uremic syndrome. *Arch Fr Pediatr*. 1974;31:59-69.
20. de Jong M, Monnens L. Haemolytic-uraemic syndrome: a 10-year follow-up study of 73 patients. *Nephrol Dial Transplant*. 1988;3:379-382.
21. Gillor A, Roth B, Bulla M, Gunther H, Gladtke E. Plasmapherese bei der Behandlung von Kindern mit hamolytisch-uramischem Syndrom. *Nieren-und Hochdruckkrankheiten*. 1986;15:118-123.

22. Gianantonio CA, Vitacco M, Mendilaharzu F, Gallo G. The hemolytic-uremic syndrome: renal status of 76 patients at long-term follow-up. *J Pediatr*. 1968;72:757-765.
23. Tonshoff B, Sammet A, Sanden I, Mehls O, Waldherr R, Scharer K. Outcome and prognostic determinants in the hemolytic uremic syndrome of children. *Nephron*. 1994;68:63-70.
24. Litalien C, Proulx F, Mariscalco MM, et al. Circulating inflammatory cytokine levels in hemolytic uremic syndrome. *Pediatr Nephrol*. 1999;13:840-845.
25. Gagnadoux MF, Habib R, Gubler MC, Bacri JL, Broyer M. Long-term (15-25 years) outcome of childhood hemolytic-uremic syndrome. *Clin Nephrol*. 1996;46:39-41.
26. Dolislager D, Tune B. The hemolytic-uremic syndrome: spectrum of severity and significance of prodrome. *Am J Dis Child*. 1978;132:55-58.
27. Donckerwolcke RA, Kuijten RH, Tiddens HA, van GJ. Haemolytic uraemic syndrome. *Paediatrician*. 1979;8:378-393.
28. McLean MM, Jones CH, Sutherland DA. Haemolytic-uraemic syndrome: a report of an outbreak. *Arch Dis Child*. 1966;41:76-81.
29. Fitzpatrick MM, Shah V, Trompeter RS, Dillon MJ, Barratt TM. Long term renal outcome of childhood haemolytic uraemic syndrome. *BMJ*. 1991;303:489-492.
30. Riella MC, George CR, Hickman RO, et al. Renal microangiopathy of the hemolytic-uremic syndrome in childhood. *Nephron*. 1976;17:188-203.
31. Blahova K, Janda J, Kreisinger J, Matejkova E, Sediva A. Long-term follow-up of Czech children with D+ hemolytic uremic syndrome. *Pediatr Nephrol*. 2002;17:400-403.
32. Sorrenti LY, Lewy PR. The hemolytic-uremic syndrome: experience at a center in the Midwest. *Am J Dis Child*. 1978;132:59-62.
33. Spizzirri FD, Rahman RC, Bibiloni N, Ruscasso JD, Amoreo OR. Childhood hemolytic uremic syndrome in Argentina: long-term follow-up and prognostic features. *Pediatr Nephrol*. 1997;11:156-160.
34. Cordero J, Baeza J, Fielbaum O, et al. Hemolytic-uremic syndrome: experience with 154 cases. *Rev Chil Pediatr*. 1990;61:235-242.
35. Donckerwolcke RA, Tiddens HA, Kuijten RH. A prospective study of the haemolytic-uremic syndrome [author's translation]. *Acta Paediatr Belg*. 1973;27:284-298.
36. Kelles A, Van Dyck M, Proesmans W. Childhood haemolytic uraemic syndrome: long-term outcome and prognostic features. *Eur J Pediatr*. 1994;153:38-42.
37. Gusmano R, Perfumo F, Ciardi MR, Sarperi M. Long-term prognosis of haemolytic-uraemic syndrome in children. *Adv Exp Med Biol*. 1987;212:199-204.
38. Coad NA, Marshall T, Rowe B, Taylor CM. Changes in the postenteropathic form of the hemolytic uremic syndrome in children. *Clin Nephrol*. 1991;35:10-16.
39. Siegler RL, Pavia AT, Hansen FL, Christofferson RD, Cook JB. Atypical hemolytic-uremic syndrome: a comparison with postdiarrheal disease. *J Pediatr*. 1996;128:505-511.
40. Sheth KJ, Gill JC, Hanna J, Leichter HE. Failure of fresh frozen plasma infusions to alter the course of hemolytic uremic syndrome. *Child Nephrol Urol*. 1988;9:38-41.
41. Hughes DA, Beattie TJ, Murphy AV. Haemolytic uraemic syndrome: 17 years' experience in a Scottish paediatric renal unit. *Scott Med J*. 1991;36:9-12.
42. Zurowska A, Gockowska Z, Czarniak P, Marczak E. Changing clinical course of hemolytic uremic syndrome in children. *Pol Merkuriusz Lek*. 2000;8:234-235.
43. Diekmann L. Treatment of the hemolytic-uremic syndrome with streptokinase and heparin [author's translation]. *Klin Padiatr*. 1980;192:430-435.
44. Imoberdorf G, Bianchetti MG, Rossi E, Gugler E, Oetliker OH. Hemolytic-uremic syndrome in children: a 19-year retrospective study. *Schweiz Med Wochenschr*. 1993;123:1439-1444.
45. Vermylen C, Bodart E, Ninane J, Moulin D, Cornu G. The haemolytic uraemic syndrome of childhood: is living in Belgium a factor of good prognosis? *Acta Clin Belg*. 1988;43:101-108.
46. Loirat C, Baudouin V, Sonsino E, Mariani-Kurdjian P, Elion J. Hemolytic-uremic syndrome in the child. *Adv Nephrol Necker Hosp*. 1993;22:141-168.
47. Loirat C, Beaufils F, Sonsino E, et al. Treatment of childhood hemolytic-uremic syndrome with urokinase: cooperative controlled trial. *Arch Fr Pediatr*. 1984;41:15-19.
48. Mizusawa Y, Pitcher LA, Burke JR, Falk MC, Mizushima W. Survey of haemolytic-uraemic syndrome in Queensland 1979-1995. *Med J* Aust. 1996;165:188-191.
49. Guyot C, Daniel MD, Roze JC, Renoult E, Dubigeon P, Buzelin F. Prognosis of hemolytic uremic syndrome in children: importance of extrarenal involvement. *Arch Fr Pediatr*. 1986;43:253-258.
50. Rizzoni G, Claris-Appiani A, Edefonti A, et al. Plasma infusion for hemolytic-uremic syndrome in children: results of a multicenter controlled trial. *J Pediatr*. 1988;112:284-290.
51. Loirat C, Sonsino E, Hinglais N, Jais JP, Landais P, Fermanian J. Treatment of the childhood haemolytic uraemic syndrome with plasma: a multicentre randomized controlled trial. *Pediatr Nephrol*. 1988;2:279-285.
52. Al-Eisa A, Al-Hajeri M. Hemolytic uremic syndrome in Kuwaiti Arab children. *Pediatr Nephrol*. 2001;16:1093-1098.
53. Miyazaki S. The etiology and clinical features of hemolytic uremic syndrome. *Rinsho Ketsueki*. 1994;35:341-345.
54. Brichard B, Vermylen C, Heremans MT, Ninane J, Cornu G. Plasma infusion as treatment for 33 children with haemolytic uraemic syndrome: a good therapy? *Acta Clin Belg*. 1993;48:156-163.
55. Ramos S, Gomes L, Sarmento A, et al. Sindrome Hemolitico-Uremico Casuistica de uma decada. *Nascer e Crescer*. 2001;10:82-87.
56. Henning PH, Tham EB, Martin AA, Beare TH, Jureidini KF. Haemolytic-uraemic syndrome outbreak caused by *Escherichia coli* O111:H-: clinical outcomes. *Med J Aust*. 1998;168:552-555.
57. Yoshioka K, Yagi K, Moriguchi N. Clinical features and treatment of children with hemolytic uremic syndrome caused by enterohemorrhagic *Escherichia coli* O157:H7 infection: experience of an outbreak in Sakai City, 1996. *Pediatr Int*. 1999;41:223-227.
58. Salvadori M, Garg A, Howard J, Clark W, Matsell D. An outbreak of E. coli O157:H7 infections due to a contaminated municipal water supply in rural Ontaro. Short-term outcome of children developing hemolytic uremic syndrome. [abstract], *J Am Soc Nephrol* 2002;13(suppl S):699.
59. Orr P, Dong V, Lorencz B, et al. Hemolytic uremic syndrome secondary to an outbreak of verotoxin producing E. coli in the Canadian Arctic. *Artic Med Res*. 1994;53(suppl 2):630-634.
60. Schwartz GJ, Haycock GB, Edelmann CMJ, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics*. 1976;58:259-263.
61. Bale JF Jr, Brasher C, Siegler RL. CNS manifestations of the hemolytic-uremic syndrome: relationship to metabolic alterations and prognosis. *Am J Dis Child*. 1980;134:869-872.
62. Moghal NE, Ferreira MA, Howie AJ, Milford DV, Raafat E, Taylor CM. The late histologic findings in diarrhea-associated hemolytic uremic syndrome. *J Pediatr*. 1998;133:220-223.
63. Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. *Kidney Int*. 1996;49:1774-1777.
64. Wingen AM, Fabian-Bach C, Schaefer F, Mehls O, for the European Study Group of Nutritional Treatment of Chronic Renal Failure in Childhood. Randomised multicentre study of a low-protein diet on the progression of chronic renal failure in children. *Lancet*. 1997;349:1117-1123.
65. Tufro A, Arrizurieta EE, Repetto H. Renal functional reserve in children with a previous episode of haemolytic-uraemic syndrome. *Pediatr Nephrol*. 1991;5:184-188.
66. Perelstein EM, Grunfield BG, Simsolo RB, Gimenez MI, Gianantonio CA. Renal functional reserve compared in haemolytic uraemic syndrome and single kidney. *Arch Dis Child*. 1990;65:728-731.
67. Todd WT. Prospects for the prevention of haemolytic-uraemic syndrome. *Lancet*. 2001;357:1636-1638.
68. Bell BP, Goldoft M, Griffin PM, et al. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: the Washington experience. *JAMA*. 1994;272:1349-1353.
69. Kawamura N, Yamazaki T, Tamai H. Risk factors for the development of *Escherichia coli* O157:H7 associated with hemolytic uremic syndrome. *Pediatr Int*. 1999;41:218-222.
70. Safdar N, Said A, Gangnon RE, Maki DG. Risk of hemolytic uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 enteritis: a meta-analysis. *JAMA*. 2002;288:996-1001.
71. Matise I, Cornick NA, Booher SL, Samuel JE, Bosworth BT, Moon HW. Intervention with Shiga toxin (Stx) antibody after infection by Stx-producing *Escherichia coli*. *J Infect Dis*. 2001;183:347-350.
72. Armstrong GD, Rowe PC, Goodyer P, et al. A phase I study of chemically synthesized verotoxin (Shiga-like toxin) Pk-trisaccharide receptors attached to chromosorb for preventing hemolytic-uremic syndrome. *J Infect Dis*. 1995;171:1042-1045.
73. Perez N, Spizzirri F, Rahman R, Suarez A, Larrubia C, Lasarte P. Steroids in the hemolytic uremic syndrome. *Pediatr Nephrol*. 1998;12:101-104.
74. Thompson SG, Higgins JP. How should meta-regression analyses be undertaken and interpreted? *Stat Med*. 2002;21:1559-1573.

Shiga Toxin–producing *Escherichia coli*, New Mexico, USA, 2004–2007

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Sporadic infection with Shiga toxin–producing *Escherichia coli* (STEC) in New Mexico increased from 0.9 cases per 100,000 population (95% confidence interval [CI] 0.5–1.36) in 2004 to 1.7 (95% CI 1.14–2.26) in 2007. Non-O157 STEC was more common in nonwhite residents, children <5 years of age, and urban residents.

The epidemiology of infections and hemolytic uremic syndrome (HUS) caused by Shiga toxin–producing *Escherichia coli* (STEC) O157:H7 are well described (*1*–*4*). Non-O157 STEC infection also is associated with severe illness and HUS but often is underdiagnosed and less well understood (*3*–*7*). Studies in Europe indicate that non-O157 STEC infections occur more frequently than do STEC O157 infections (*3*). STEC O157 infection has been a notifiable disease in the United States since 1994, but non-O157 STEC infection became reportable only in 2000 (*8*). To understand trends in STEC O157 and non-O157 infections and their epidemiology in New Mexico, we analyzed population-based data from the active surveillance of clinical laboratories. This surveillance was performed as part of the Centers for Disease Control and Prevention's (CDC's) Foodborne Diseases Active Surveillance Network (FoodNet), to which New Mexico began contributing data in 2004.

The Study

All STEC isolates or broths were sent for confirmation to the Scientific Laboratory Division of the New Mexico Department of Health, where Shiga toxin expression was confirmed by enzyme immunoassay (EIA); the broth was then cultured and preliminarily serotyped. Pulsed-field gel electrophoresis was performed on all isolates. The laboratory submitted non-O157 STEC isolates to CDC for additional serotyping and PCR testing for toxin genes.

This analysis comprised only sporadic cases of STEC. Negative binomial regression models were used to calculate incidence rates and assess differences in risks for STEC O157 and non-O157 infections. Variables were reporting year, patient age, race/ethnicity, sex, and rural versus urban residence (*8*); $p \leq 0.05$ was considered statistically significant.

During 2004–2007, New Mexico FoodNet identified 111 cases of laboratory-confirmed sporadic STEC infection; 40 (36%) were STEC O157, and 71 (64%) were non-O157 STEC (Table 1). Six additional cases were outbreak associated. Incidence increased from 0.93 cases per 100,000 population (95% confidence interval [CI] 0.5–1.36) in 2004 to 1.07 per 100,000 (95% CI 0.61–1.52) in 2005 and 1.84 per 100,000 (95% CI 1.25–2.44) in 2006. The rate fell slightly in 2007, to 1.70 per 100,000 (95% CI 1.14–2.26) population, resulting in a test of trend that approached statistical significance ($p = 0.09$). From 2004 through 2007, sporadic STEC infections increased 94%. A total of 18 STEC serotypes were identified during this time. The primary se-

Table 1. Demographic characteristics of case-patients who had laboratory-confirmed STEC infections, New Mexico, USA, 2004–2007*

Characteristic	No. case-patients		
	O157 STEC	Non-O157 STEC	Total
Age group, y†			
<1	3	3	6
1–4	3	26	29
5–10	10	7	17
11–18	4	12	16
19–29	5	7	12
30–39	4	2	6
40 –49	1	2	3
50–59	1	5	6
≥60	9	7	16
Sex			
Male	18	34	52
Female	22	37	59
Race/ethnicity			
White non-Hispanic	17	17	34
White Hispanic	11	12	23
White, unknown ethnicity	5	4	9
Native American	3	10	13
African American	1	0	1
Other	0	5	5
Unknown	3	23	26
Type of county			
Urban	29	64	93
Rural	11	7	18
Year of diagnosis			
2004	7	11	18
2005	10	11	21
2006	14	23	37
2007	9	26	35
Total no. case-patients	40	71	111

*STEC, Shiga toxin–producing *Escherichia coli*; O157, serotype O157:H7.
†Median age (range) of patients: STEC O157–infected, 18 y (4 mo–78 y); non-O157 STEC–infected, 10 y (5 mo–70 y); total, 13.3 y (4 mo–78 y).

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rotypes responsible for the increase were STEC O157, O26, O111, and O103, constituting 75% of all STEC cases reported. Incidences of STEC serotypes O26, O111, and O103 combined increased 300% from 2004 through 2007. The proportion of non-O157 STEC ranged from 52% in 2005 to 74% in 2007.

Although STEC O157 was the 1 serotype most often identified, infections caused by non-O157 STEC (all serotypes) were diagnosed more frequently. STEC O26 (18%) and O111 (13%) were the most commonly identified non-O157 STEC serotypes. Other STEC O serotypes (O103, O121, O46, O177, and O91) were responsible for 33% of all STEC infections. Most isolates were positive for toxin gene stx_1 (86%), intimin (66%), and enterohemolysin A (81%).

STEC O157 infection was significantly more likely to be diagnosed in adults (half of all cases, one third of non-O157 STEC cases) than in children (<18 years of age) ($p = 0.01$). Non-O157 STEC serotypes were most commonly identified in children 1–4 years of age. STEC O157 infections occurred most commonly in children 5–10 years of age, followed by adults >60 years. Sex distributions were similar for patients with STEC O157 and non-O157 infections (55% female vs. 45% male and 52% vs. 48%, respectively). White non-Hispanics, which constitute 43% of New Mexico's population, made up 31% of all confirmed STEC infections in New Mexico; white Hispanics (42%) made up 21% of confirmed STEC cases; and Native Americans (10%) made up 12% of cases (Table 1).

More laboratory-confirmed STEC infections were diagnosed during the summer months than during the rest of the year. STEC O157 cases were diagnosed more frequently in September (10 cases); non-O157 STEC cases were most frequently diagnosed in June and July (9 cases each).

Although STEC O157 infections was diagnosed in more persons than were non-O157 STEC infections (28% vs. 16%), the difference was not significant. Patients with STEC O157 infections stayed in the hospital a mean of 6 days (median 4), compared with a mean of 4.5 days (median 3) for patients with non-O157 STEC infections, also not significant. All 5 reported cases of HUS were caused by STEC O157.

International travel was related to STEC infection for 12 (11%) patients. Two (5%) STEC O157 cases were travel related, as were 4 (10%) cases each of STEC O111 and O26; and 1 (1%) case each of STEC O128 and O103. Travel to Mexico was documented for 10 of the 12 travel-associated cases.

Most (99 [89%]) persons with STEC were from New Mexico's urban counties. We calculated incidence rate ratios between STEC O157 and non-O157 infections during 2004–2007 (Table 2), while adjusting for variables that were significant in negative binomial models, including race (white non-Hispanic vs. nonwhite), age (<5 years vs. ≥5 years), and county (urban vs. rural). More patients with non-O157 STEC infection during this time were nonwhite, <5 years of age, and residents of urban counties. Patients infected with STEC O157 were more likely to be white non-Hispanic, ≥5 years of age, and residents of rural counties.

Conclusions

The data collected by New Mexico's FoodNet surveillance network indicate that sporadic STEC cases increased substantially from 2004 through 2007. Reports of STEC O157 infection doubled from 7 to 14 from 2004 to 2006 but dropped to 9 in 2007. However, the number of non-O157 STEC cases continued to climb and accounted for most of the increase in overall STEC rates in New Mexico during this time, similar to rates in Connecticut and other FoodNet sites (*4*,*9*).

Non-O157 STEC infections ranged from 52% to 74% of all Shiga toxin–positive cases diagnosed each year and 64% of all identified STEC cases. New Mexico was second only to Colorado (2.12 cases per 100,000 population) among FoodNet sites for non-O157 STEC incidence in 2007 (*10*). Similar to serotypes reported from other locations, STEC serotypes O26, O111, and O103 made up most non-O157 infections in New Mexico (*1*), especially O26 and O111 (18% and 13% of all cases, respectively).

Table 2. Comparisons of risk for infection with STEC O157 versus non-O157 STEC types, by demographic characteristic, averaged population, New Mexico, USA, 2004–2007*

Characteristic	Unadjusted incidence rate ratio† (95% CI)	p value	Adjusted incidence rate ratio† (95% CI)	p value
Race				
Other	1		1	
White	3.21 (1.42–7.27)	0.01	3.03 (1.34–6.90)	0.008
Age, y				
<5	1		1	
≥5	2.61 (1.09–6.22)	0.03	2.74 (1.15–6.54)	0.031
County				
Urban	1		1	
Rural	1.96 (0.98–3.92)	0.05	1.84 (0.91–3.69)	0.089

*STEC, Shiga toxin–producing *Escherichia coli*; O157, serotype O157:H7; CI, confidence interval.
†Corrected for overdispersion by using negative binomial distribution.

As previously reported (*1*), non-O157 STEC infections occurred commonly in young children in this study. They also occurred at a higher rate for non-white New Mexico residents. Another recent study similarly found higher shigellosis rates in counties with higher proportions of Hispanics (*11*). This study concurs with others that have reported finding the highest rates of STEC O157 rates in rural communities (with increased opportunities for animal contact) and in the West (*12*,*13*).

Year-to-year increases in numbers of non-O157 STEC infections, both nationally and in New Mexico, must be interpreted with caution because of changes in laboratory testing practices. Although the state's largest clinical laboratory performed EIAs before active surveillance was implemented in 2004, other laboratories throughout the state might have changed their testing practices.

Risk factors for non-O157 STEC infections may be similar to those for STEC O157 infections (*9*), but additional studies are needed to elucidate differences and similarities between them, as well as among non-O157 STEC serotypes (*14*). Clinical laboratories should simultaneously screen for Shiga toxin and culture all positive isolates to determine the true incidence of non-O157 STEC infections.

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References

1. Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, et al. Non O157 Shiga toxin–producing *Escherichia coli* infections in the United States, 1983–2002. J Infect Dis. 2005;192:1422–9. DOI: 10.1086/466536
2. Fey PD, Wickert RS, Rupp ME, Safranek TJ, Hinrichs SH. Prevalence of non-O157:H7 Shiga toxin–producing *Escherichia coli* in diarrheal stool samples from Nebraska. Emerg Infect Dis. 2000;6:530–3.
3. Nielsen EM, Scheutz F, Torpdahl M. Continuous surveillance of Shiga toxin–producing *Escherichia coli* infections by pulsed-field gel electrophoresis shows that most infections are sporadic. Foodborne Pathog Dis. 2006;3:81–7. DOI: 10.1089/fpd.2006.3.81
4. Voetsch AC, Kennedy MH, Keene WE, Smith KE, Rabatsky-Her T, Zansky S, et al. Risk factors for Shiga toxin–producing *Escherichia coli* O157 infections in FoodNet sites, 1999–2000. Epidemiol Infect. 2007;135:993–1000. DOI: 10.1017/S0950268806007564
5. Werber D, Fruth A, Liesegang A, Littman M, Buchholz U, Prager R, et al. A multistate outbreak of Shiga toxin–producing *Escherichia coli* O26:H11 infections in Germany, detected by molecular subtyping surveillance. J Infect Dis. 2002;186:419–22. DOI: 10.1086/341457
6. Brooks JT, Bergmire-Sweat D, Kennedy M, Hendricks K, Garcia M, Marengo L, et al. Outbreak of Shiga toxin–producing *Escherichia coli* O111:H8 infections among attendees of a high school cheerleading camp. Clin Infect Dis. 2004;38:190–8. DOI: 10.1086/380634
7. Misselwitz J, Karch H, Bielazewska M, John U, Ringelmann F, Ronnefarth G, et al. Cluster of hemolytic-uremic syndrome caused by Shiga toxin–producing *Escherichia coli* O26:H11. Pediatr Infect Dis J. 2003;22:349–54.
8. US Department of Agriculture, Economic Research Services. Data sets. Rural–urban continuum codes [cited 2009 May 15]. Available from http://www.ers.usda.gov/data/ruralurbancontinuumcodes
9. Centers for Disease Control and Prevention. Laboratory-confirmed non-O157 Shiga toxin–producing *Escherichia coli*—Connecticut, 2000–2005. MMWR Morb Mortal Wkly Rep. 2007;56:29–31.
10. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2007. MMWR Morb Mortal Wkly Rep. 2008;57:366–70.
11. Chang M, Groseclose SL, Zaidi AA, Braden CR. An ecological analysis of sociodemographic factors associated with the incidence of salmonellosis, shigellosis, and *E. coli* O157:H7 infections in US counties. Epidemiol Infect. 2009;137:810–20. DOI: 10.1017/S0950268808001477
12. Haack JP, Jelacic S, Besser T, Weinberger E, Kirk D, McKee G, et al. *Escherichia coli* O157 exposure in Wyoming and Seattle: serologic evidence of rural risk. Emerg Infect Dis. 2003;9:1226–31.
13. Kistemann T, Zimmer S, Vagsholm I, Andersson Y. GIS-supported investigation of human EHEC and cattle VTEC O157 infections in Sweden: geographical distribution, spatial variation and possible risk factors. Epidemiol Infect. 2004;132:495–505. DOI: 10.1017/S0950268803001729
14. Centers for Disease Control and Prevention. Importance of culture confirmation of Shiga toxin–producing *Escherichia coli* infection as illustrated by outbreaks of gastroenteritis—New York and North Carolina, 2005. MMWR Morb Mortal Wkly Rep. 2006;55:1042–5.

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Seasonal Prevalence of Shiga Toxin–Producing *Escherichia coli*, Including O157:H7 and Non-O157 Serotypes, and *Salmonella* in Commercial Beef Processing Plants†

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ABSTRACT

The seasonal prevalence of *Escherichia coli* O157:H7, *Salmonella*, non-O157 *E. coli* (STEC), and *stx*-harboring cells was monitored at three Midwestern fed-beef processing plants. Overall, *E. coli* O157:H7 was recovered from 5.9% of fecal samples, 60.6% of hide samples, and 26.7% of carcasses sampled before the preevisceration wash. This pathogen also was recovered from 1.2% (15 of 1,232) of carcasses sampled at chilling (postintervention) at approximate levels of <3.0 cells per 100 cm^2. In one case, the *E. coli* O157:H7 concentration dropped from ca. 1,100 cells per 320 cm^2 at the preevisceration stage to a level that was undetectable on ca. 2,500 cm^2 at the postintervention stage. The prevalence of *E. coli* O157:H7 in feces peaked in the summer, whereas its prevalence on hide was high from the spring through the fall. Overall, *Salmonella* was recovered from 4.4, 71.0, and 12.7% of fecal, hide, and preevisceration carcass samples, respectively. *Salmonella* was recovered from one postintervention carcass (of 1,016 sampled). *Salmonella* prevalence peaked in feces in the summer and was highest on hide and preevisceration carcasses in the summer and the fall. Non-O157 STEC prevalence also appeared to vary by season, but the efficiency in the recovery of isolates from *stx*-positive samples ranged from 37.5 to 83.8% and could have influenced these results. Cells harboring *stx* genes were detected by PCR in 34.3, 92.0, 96.6, and 16.2% of fecal, hide, preevisceration carcass, and postintervention carcass samples, respectively. The approximate level of non-O157 STEC and *stx*-harboring cells on postintervention carcasses was ≥3.0 cells per 100 cm^2 for only 8 of 199 carcasses (4.0%). Overall, the prevalence of *E. coli* O157:H7, *Salmonella,* and non-O157 STEC varied by season, was higher on hides than in feces, and decreased dramatically, along with pathogen levels, during processing and during the application of antimicrobial interventions. These results demonstrate the effectiveness of the current interventions used by the industry and highlight the significance of hides as a major source of pathogens on beef carcasses.

Escherichia coli O157:H7 was first recognized in 1982 as a foodborne pathogen associated with the consumption of ground beef *(43)* and achieved general notoriety following a multistate outbreak in 1993 that resulted in four fatalities *(20)*. However, the organism was found on only 0.2% (4 of 2,081) of dressed (postintervention) fed-beef carcasses sampled during 1992 and 1993, and most probable number (MPN) analyses suggested that it was present at very low levels *(29)*. In 1994, the U.S. Food Safety and Inspection Service (FSIS) declared *E. coli* O157:H7 to be an adulterant in ground beef, making it the first microorganism given such status under the Federal Meat Inspection Act. The FSIS has now extended that declaration to include "non-intact" beef, such as mechanically tenderized or reconstructed products *(5)*.

Salmonella contamination of raw beef has also garnered attention recently, primarily because of the establishment of *Salmonella* performance standards by the FSIS *(4)*. These standards were set up as a measure of sanitation and describe limits for the frequency of *Salmonella* contamination of raw meats, including beef carcasses and ground beef. The data available at the time the standards were established suggested that, like *E. coli* O157:H7, *Salmonella* was present infrequently and at low levels on fed-beef carcasses: 1.0% (20 of 2,081) of postintervention carcasses tested positive at levels of <0.3 cells per cm^2 *(29)*. A subsequent study demonstrated a drop in the prevalence of *Salmonella* on postintervention carcasses at large processing plants to 0.1% *(44)*.

Non-O157:H7 Shiga toxin–producing *E. coli* (STEC) strains have been associated with human disease, cattle, and beef production *(7, 16)*. In the only study of non-O157 STEC on postintervention carcasses that has been carried out in the United States to date, a summertime prevalence of 8% (27 of 326) was found *(6)*, but it is not clear whether all of these STEC strains could cause disease. Not all strains of STEC have been associated with human disease *(1, 16, 36)*. Although Shiga toxin production is a key virulence factor, other factors also appear to affect pathogenicity.

Human clinical cases of *E. coli* O157:H7, non-O157 STEC, and *Salmonella* tend to peak during the warmer

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of factors. For example, a 1999 study conducted by the Animal and Plant Health Inspection Service demonstrated that the prevalence of *E. coli* O157:H7 and *Salmonella* in feedlot cattle feces also increases during the warmer months *(2, 3)*. However, no studies have demonstrated seasonal variation in pathogen prevalence during beef processing. The primary objective of this study, therefore, was to measure both the preharvest and the postharvest seasonal prevalence levels of *E. coli* O157:H7, *Salmonella,* and non-O157 STEC during beef processing. In addition, the levels of these pathogens on carcasses were estimated.

MATERIALS AND METHODS

Sample collection. Samples were collected during two visits per season to three large Midwestern beef processing plants. The sampling seasons were spring (late April to early May 2001), summer (August 2001), fall (late October to mid-November 2001), and winter (late January to mid-February 2002). To facilitate sampling and to ensure the sampling of several lots, 50 to 65 carcasses (every 8th to 10th carcass) were sampled during each visit.

For individual tagged carcasses, samples were obtained from the hide, from the feces, from the carcass prior to the preevisceration wash, and from the dressed carcass upon entry into the cooler (postintervention). Samples were obtained as previously described, with some modifications *(12, 25)*. Hides were sampled by swabbing a ca. 1,700-cm^2 area just to the right of the midline over the brisket. Ten-gram fecal samples were obtained from the distal portion of the colon after evisceration. Preevisceration carcass samples were obtained by swabbing the round and surrounding areas immediately after complete hide removal. Postintervention carcass samples were obtained by swabbing one side immediately prior to or within 2 h of entry into the cooler; the sampled area encompassed the inside round and rump plus a swath approximately 15 cm wide down the midline from the flank-plate juncture to the neck.

Salmonella was not recovered from samples taken during the first four fall visits owing to technical difficulties, so a supplementary visit was made to one of the processing plants. One hundred carcasses were tested for *Salmonella* only. Also, *Salmonella* was not recovered during the first two winter visits.

Enrichment and recovery of *E. coli* O157:H7 and *Salmonella*. Samples were analyzed for the presence of *E. coli* O157:H7 and *Salmonella* by the previously described Meats Research Unit (MRU) and MRU-tetrathionate (MRU-TT) methods *(12)*. These methods involve enrichment in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.), immunomagnetic separation (IMS), and selective plating. TSB was added within 3 h of sampling. After enrichment, two 1-ml aliquots were removed for storage at −70°C after 0.5 ml of 50% glycerol (Sigma Chemical Co., St. Louis, Mo.) was added.

The standard MRU method was used to recover *Salmonella* from spring fecal samples and from all hide and carcass samples. The MRU-TT method, which incorporates a TT (Difco) enrichment after the TSB enrichment and prior to IMS, was developed to improve recovery of *Salmonella* from fecal samples *(12)*. This method was used to analyze fecal samples collected in summer, fall, and winter.

Confirmation of *E. coli* O157:H7 and *Salmonella*. For *E. coli* O157:H7, up to three suspect colonies (based on colony phenotype on either or both agars) were tested for each sample with DrySpot latex agglutination tests (Oxoid, Ogdensburg, N.Y.). Growth from colonies identified as potentially positive was streaked for isolation on sorbitol MacConkey agar containing cefixime and potassium tellurite *(12)* and stored in nutrient agar stabs *(45)* for further testing. Broth cultures of each isolate were subsequently stored as glycerol stocks at −70°C. *E. coli* O157:H7 confirmation included detection of O157 and H7 antigens by indirect enzyme-linked immunosorbent assay *(25)*, multiplex PCR to detect stx_1, stx_2, *eae,* and *hlyA (6, 25, 37)*, and verification of *E. coli* with the use of the Sensititre Gram-negative Auto-Identification (AP80) system (Accumed International, Westlake, Ohio) and/or API 20E strips (BioMérieux, Hazelwood, Mo.). As necessary for confirmation, some isolates (e.g., nonmotile isolates) were also analyzed by multiplex PCR for the presence of the $fliC_{H7}$ and $rfbE_{O157}$ genes *(32)*. Samples were considered positive if at least one isolate (i) carried at least one *stx* gene, (ii) expressed the O157 antigen or carried the $rfbE_{O157}$ gene, (iii) expressed the H7 antigen or carried the $fliC_{H7}$ gene, and (iv) was identified as *E. coli*. Overall, 91% of samples testing positive by colony screening with DrySpot were positive on the basis of isolate characterization.

For *Salmonella,* up to three suspect (on the basis of colony phenotype on either or both agars) colonies were selected for each sample and streaked for isolation on Hektoen enteric agar containing novobiocin *(12)*. Colonies identified as potentially positive on the basis of phenotype were stored in nutrient agar stabs *(45)* for further testing. Broth cultures of each isolate were subsequently stored as glycerol stocks at −70°C. Confirmation included verification of *Salmonella* with the Sensititre AP80 system and/or API 20E strips. Serological identification by agglutination was carried out for positive isolates with the use of poly-group antisera as described by the manufacturer (Difco). Samples were considered positive if at least one isolate (i) was identified as *Salmonella* and (ii) provided a positive agglutination reaction.

PCR detection of *stx*-positive enrichments and recovery and confirmation of non-O157 STEC. *E. coli* O157:H7–negative enrichments were tested with the use of a previously described multiplex PCR to detect the presence of stx_1, stx_2, *eae,* and *hlyA (37)*. Samples were prepared for PCR as follows. (i) For postintervention carcass samples, cells were recovered from 100 μl of enrichment culture by centrifugation for 1 min at top speed, washed in 100 μl of 0.9% NaCl, and resuspended in 50 μl of sterile distilled, deionized water. The cell suspension was heated at 100°C for 10 min and was then centrifuged for 30 to 60 s to remove cellular debris. Two-microliter portions of these lysates were used in 30-μl PCR reactions. (ii) For preevisceration carcass samples, 150-μl aliquots were removed from enrichment glycerol stocks (see above) and treated similarly to postintervention samples. (ii) For fecal samples, the TSB enrichments were frozen at −20°C, without glycerol, prior to PCR. Cells recovered by centrifugation from 20 μl of these enrichments were washed in 400 μl of 0.9% NaCl and were then resuspended in 200 μl of InstaGene matrix (Bio-Rad Laboratories, Richmond, Calif.). The DNA was extracted according to the manufacturer's instructions. Forty microliters of the DNA supernatant was carefully removed and used in a 100-μl PCR reaction. (iv) Hide sample enrichments were initially evaluated as described for preevisceration carcass sample enrichments. If no PCR products were detected, then cells from 30 μl of the frozen enrichment (glycerol stocks at −70°C) were treated as described above for fecal sample enrichments. Samples were considered positive for *stx* genes if stx_1, stx_2, or both were detected. *E. coli* O157:H7–positive samples were presumed to be *stx*-positive.

Enrichments that tested positive for *E. coli* O157:H7 or *stx* genes were subjected to colony blotting for the recovery of non-

O157 STEC as previously described *(6)*, with the following modifications. Briefly, frozen enrichments (glycerol stocks at −70°C) were diluted and plated to yield 2,000 to 10,000 colonies on 182-mm plates containing EC agar (Difco) supplemented with 1% glucose. Colonies were transferred to Hybond-N+ membranes (Amersham Pharmacia Biotech, Piscataway, N.J.) and treated with a mixed probe to detect stx_1 and/or stx_2. Up to three presumptive positive colonies per sample were streaked for isolation on sorbitol MacConkey agar. Two colonies from each streak then were picked and tested by multiplex PCR *(37)*. This assay was carried out strictly to recover non-O157 STEC, so *stx*-positive colonies were screened by the DrySpot O157 latex test and discarded if they tested positive for O157. (Potential *E. coli* O157 *stx*+ colonies were identified for <0.3% of samples that had not been recognized as *E. coli* O157:H7 positive by the MRU method.) Presumptive non-O157 STEC isolates were stored in stabs and subsequently as glycerol stocks at −70°C. Isolates carrying *stx* genes were confirmed to be *E. coli* isolates with the Sensititre AP80 system and/or API 20E strips. Samples were considered positive for non-O157 STEC if at least one isolate (i) carried at least one *stx* gene and (ii) was identified as *E. coli*.

MPN assays. Three-tube MPN assays were used to estimate the levels of *E. coli* O157:H7, *Salmonella*, and *stx*-carrying cells on postintervention carcasses that were identified as presumptive positive. After observing the high prevalence of *E. coli* O157:H7 on preevisceration carcasses in the spring and summer, we also chose to estimate *E. coli* O157:H7 levels for these samples during the fall. Prior to enrichment, three 100-μl aliquots of each sponge sample were transferred to deep-well microtiter trays with each well containing 900 μl of buffered peptone water (Difco) supplemented with 50 μg of ferrioxamine E per ml (BPW-Fe *(41)*). To prepare ferrioxamine E, desferal (Sigma) was mixed with ferric ammonium sulfate (Sigma) at a 1:1 molar ratio and sterilized by filtration. Three serial 1:10 dilutions were prepared from each of the three inoculated wells, for a total of 12 wells per sample. (An extra 1:10 dilution was prepared in case unusual results were obtained *(28)*). The inoculated trays were incubated at 25°C for 2 h, were then transferred to 37°C for 16 to 18 h, and were subsequently stored at 4°C. Wells for samples identified as presumptive positive by the MRU method were processed as described below. Results are reported only for samples that were positive by the isolate criteria outlined above. The approximate number of cells per carcass area and the 95% confidence interval (CI) were determined with the use of an MPN index chart *(28)*. For postintervention carcasses, ca. 2,500 cm^2 of each carcass was sampled in 25 ml of BPW–0.05% Tween 20, so the approximate number of cells per 100 cm^2 was determined. For preevisceration carcasses, ca. 8,000 cm^2 of each carcass was sampled, so the approximate number of cells per 320 cm^2 was determined.

If at least one isolate recovered from a sample was presumed to be *E. coli* O157:H7 on the basis of DrySpot screening during the MRU method, then the corresponding BPW-Fe culture wells were analyzed with Meridian ImmunoCard STAT! devices (Meridian Bioscience, Cincinnati, Ohio). The manufacturer's recommended method was adapted by inoculating 350 μl of the BPW-Fe enrichments into 4.5 ml of MacConkey broth (Difco) and incubating the broth at 42°C for 16 to 24 h before testing for the presence of *E. coli* O157:H7.

If at least one isolate recovered from a sample was presumed to be *Salmonella* on the basis of colony phenotype during the MRU method, then the corresponding BPW-Fe cultures were analyzed with VIP for *Salmonella* devices (BioControl Systems, Inc., Bellevue, Wash.). The manufacturer's recommended method was adapted by transferring 350-μl portions of the BPW-Fe enrichments to 10 ml of Rappaport-Vassiliadis broth (Oxoid) for the selective enrichment and transferring 0.5 ml of the Rappaport-Vassiliadis enrichment to 4.5 ml of TSB supplemented with 2,4-dinitrophenol and novobiocin (Sigma) for the postenrichment prior to testing for the presence of *Salmonella*.

If TSB enrichments tested positive for *stx*-carrying cells by PCR, 100-μl portions of the corresponding BPW-Fe cultures were subjected to the multiplex PCR assay described above *(37)*.

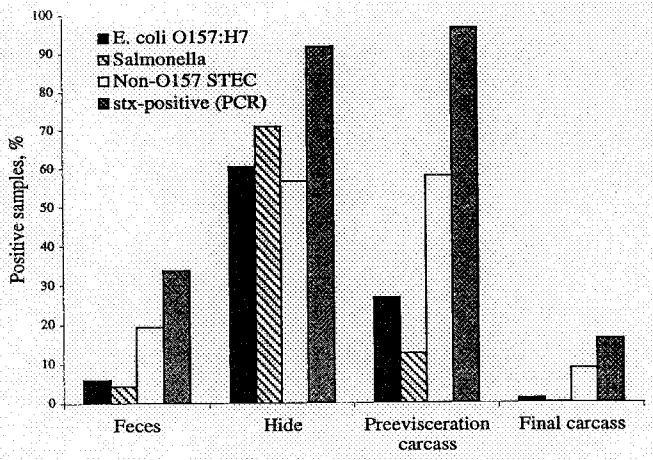

FIGURE 1. *Overall prevalence of* E. coli *O157:H7,* Salmonella, *non-O157 STEC, and* stx*-positive cells by sampling site.*

Statistical analyses. For each pathogen, prevalence was estimated for each type of sample (feces, hide, preevisceration carcass, and postintervention carcass) by season; data were pooled across all three processing plants. (The number of positive samples was divided by the total number of samples tested.) The exact binomial 95% CIs were calculated for each prevalence point estimate with PEPI software (version 2; USD, Inc., Stone Mountain, Ga. *(27)*). In order to test for sample type–specific prevalence differences between seasons for each pathogen, PEPI was used to calculate the pairwise difference in prevalence (spring versus summer, spring versus fall, spring versus winter, summer versus fall, summer versus winter, and fall versus winter; $P < 0.05$). To avoid inflated type I error rates due to multiple comparisons, the pairwise P values were adjusted with the use of Hommel's modification of the Bonferroni procedure *(31)*.

RESULTS

Pathogen prevalence in feces. Feces were sampled to measure pathogen prevalence for individual animals. Overall and in each season, the prevalence of each pathogen was lower in feces than on hides or on preevisceration carcasses (Fig. 1 and Table 1). *E. coli* O157:H7 prevalence in fecal samples was highest in the summer (12.9%) and lowest in the winter (0.3%) ($P < 0.05$). *Salmonella* prevalence in fecal samples also was highest in the summer ($P < 0.05$). However, the prevalence of *Salmonella* as detected in the spring could have been lower by a factor of up to one-half relative to the prevalence in the other seasons because of a change in methodology to improve recovery (see "Materials and Methods") *(12)*. Unlike *E. coli* O157:H7 and *Salmonella*, non-O157 STEC and *stx* genes were found in more fecal samples in the spring and fall than in the summer and winter ($P < 0.05$).

TABLE 1. *Seasonal variation in the prevalence of* E. coli *O157:H7,* Salmonella, *non-O157 STEC, and* stx*-positive cells in feces, on hides, and on preevisceration and postintervention carcasses*[a]

Season	Prevalence of *E. coli* O157:H7			Prevalence of *Salmonella*			Prevalence of non-O157:H7 STEC			Prevalence of *stx* (PCR)			Non-O157:H7 STEC isolation efficiency[b]	
	n	% positive	95% CI	*n*	% positive	95% CI	*n*	% positive	95% CI	*n*	% positive	95% CI	% isolated	95% CI
Feces														
Spring	285	3.9 B	1.9–6.8	285	2.1 B[c]	0.8–4.5	285	22.5 A	17.7–27.8	285	36.8 A	31.2–42.4	61.0 A	51–70
Summer	287	12.9 A	9.2–17.3	287	9.1 A	6.0–13.0	287	13.9 B	10.2–18.5	287	26.1 B	21.1–31.6	53.3 A	41–65
Fall	310	6.8 B	4.2–10.2	218	2.8 B	1.0–5.9	310	27.1 A	22.2–32.4	310	45.8 A	40.2–51.5	59.2 A	51–67
Winter	307	0.3 C	0.0–1.8	197	2.5 B	0.8–5.8	307	14.0 B	10.3–18.4	307	26.1 B	21.2–31.3	53.8 A	42–65
Hides														
Spring	305	73.8 A	68.5–78.6	306	61.4 C	55.7–66.9	305	43.0 C	37.3–48.7	305	88.5 B	84.4–91.9	48.5 C	42–55
Summer	321	73.5 A	68.3–78.3	321	91.6 B	88.0–94.4	321	56.1 B	50.5–61.6	321	97.2 A	94.7–98.7	57.7 B	52–63
Fall	332	67.2 A	61.8–72.2	219	97.7 A	94.8–99.3	332	77.7 A	72.9–82.1	332	92.8 AB	89.4–95.3	83.8 A	79–88
Winter	330	29.4 B	24.5–34.6	220	27.7 D	21.9–34.1	330	48.5 BC	43.0–54.0	330	88.2 B	84.2–91.5	55.0 B	49–61
Preevisceration carcasses														
Spring	303	38.9 A	33.4–44.7	305	3.0 B	1.4–5.5	304	60.2 A	54.5–65.7	304	99.7 A	98.2–99.9	60.4 A	55–66
Summer	319	40.8 A	35.3–46.4	319	19.7 A	15.5–24.6	319	64.9 A	59.4–70.1	319	98.7 A	96.8–99.7	65.7 A	60–71
Fall	330	27.3 B	22.5–32.4	217	24.9 A	19.3–31.1	330	65.5 A	60.0–70.6	330	97.6 A	95.3–99.0	67.1 A	62–72
Winter	329	1.2 C	0.3–3.1	219	4.1 B	1.9–7.7	329	41.6 B	36.3–47.2	329	90.3 B	86.6–93.3	46.1 B	40–52
Postintervention carcasses														
Spring	294	3.1 A	1.4–5.7	295	0.0 A	0.0–1.2	294	13.6 A	9.9–18.1	294	23.5 A	18.7–28.7	58.0 A	45–70
Summer	301	1.0 AB	0.2–2.9	301	0.3 A	0.0–1.8	301	4.0 B	2.1–6.9	301	10.6 B	7.4–14.7	37.5 A	21–56
Fall	312	1.0 AB	0.2–2.8	205	0.0 A	0.0–1.8	312	12.2 A	8.8–16.3	312	21.8 A	17.3–26.8	55.9 A	43–68
Winter	325	0.0 B	0.0–1.1	215	0.0 A	0.0–1.7	325	6.2 B	3.8–9.3	325	9.5 B	6.6–13.3	64.5 A	45–81

[a] Within a sample type, values with no common letter that are in the same column are significantly different ($P \geq 0.05$).

[b] Recovery of non-O157 STEC expressed as a percentage of *stx*-positive samples.

[c] In the spring, *Salmonella* was recovered from feces without the secondary TT broth enrichment used in the other seasons (see "Materials and Methods").

Pathogen prevalence on hides. Pathogen prevalence on hides may reflect several sources of contamination. Feces from one animal can contaminate multiple hides, and hides can be contaminated with feces from multiple animals, so these samples may reflect both lot and individual contamination. It has also been suggested that lairage may be a source of pathogens on hides *(8, 46)*. Overall, *E. coli* O157:H7 was isolated from 781 (60.6%) of 1,288 hides sampled throughout all four seasons (Fig. 1). *E. coli* O157:H7 prevalence in the spring did not differ significantly from that in the summer ($P > 0.05$) but fell slightly in the fall ($P > 0.05$) and dropped to its lowest point (29.4%) in the winter ($P < 0.05$; Table 1). *Salmonella* was recovered from 757 (71.0%) of 1,066 hides sampled throughout the four seasons. *Salmonella* prevalence was highest in the summer and fall ($P < 0.05$) and lowest in the winter ($P < 0.05$). The prevalence of non-O157 STEC on hides appeared to peak at 77.7% (258 of 332 samples) in the fall ($P < 0.05$). However, non-O157 STEC isolates were recovered from more of the *stx*-positive hide samples (83.8%) in the fall than in any of the other three seasons ($P < 0.05$). The prevalence of *stx*-positive hide samples was highest in the summer ($P < 0.05$) and fall ($P > 0.05$) and remained above 85% throughout all four seasons.

Pathogen prevalence and levels on preevisceration carcasses. Preevisceration carcasses were sampled to measure the transfer of pathogens from hides to the carcasses. At this point, immediately after hide removal and before the preevisceration wash, carcasses had been subjected to minimal antimicrobial interventions. The seasonal variation in the prevalence of *E. coli* O157:H7 and *Salmonella* on preevisceration carcasses mirrored that of hides but at much lower levels (Table 1). The prevalence of *E. coli* O157:H7 was highest in the spring and summer, lower in the fall ($P < 0.05$), and lowest in the winter (1.2%; $P < 0.05$). The prevalence of *Salmonella* was higher in the summer and fall than in the spring or winter ($P < 0.05$). The prevalence of non-O157 STEC did not differ significantly across the spring, summer, and fall ($P > 0.05$) but appeared to be lower in the winter ($P < 0.05$). A small but significant ($P < 0.05$) decrease in the prevalence of *stx*-positive samples in the winter was also observed. At least 90% of the preevisceration carcass samples were *stx* positive in all four seasons, with an overall prevalence of 96.5% (Fig. 1).

MPN assays were used to estimate levels of *E. coli* O157:H7 on preevisceration carcasses found to be culture positive during the fall sampling period (Table 2). Sixty-two (68.9%) of the 90 carcasses identified as positive had an MPN index of <3.0 *E. coli* O157:H7 cells per 320 cm^2. MPN indices for the other 28 positive carcasses ranged from 3.0 to 1,100 cells per 320 cm^2, with a mean and median of 54.3 and 3.6 cells per 320 cm^2, respectively. Eighteen (64.3%) of these 28 carcasses were estimated to carry fewer than 4 cells per 320 cm^2. With one exception, all preevisceration carcasses with MPN indices of >3.6 cells per 320 cm^2 were identified during one sampling visit, and *E. coli* O157:H7 was not detected on any of these nine carcasses at the end of processing (postintervention samples; see Table 3).

TABLE 2. *MPN indices and 95% CIs for* E. coli *O157:H7 recovered from preevisceration carcasses sampled during the fall*

Visit no.	No. of samples[a]	Results[b]	No. of cells per 320 cm^2	
			MPN index	95% CI
13	9	0, 0, 0	<3.0	0.0–9.5
	2	0, 1, 0	3.1	0.2–10.7
14	10	0, 0, 0	<3.0	0.0–9.5
	2	0, 0, 1	3.0	0.2–9.6
	1	0, 1, 0	3.1	0.2–10.7
	1	1, 0, 0	3.6	0.2–18.1
15	7	0, 0, 0	<3.0	0.0–9.5
	2	0, 0, 1	3.0	0.2–9.6
	1	1, 2, 0	11.4	3.6–42.0
16	16[c]	0, 0, 0	<3.0	0.0–9.5
	1	0, 0, 1	3.0	0.2–9.6
17	9	0, 0, 0	<3.0	0.0–9.5
18	11	0, 0, 0	<3.0	0.0–9.5
	3	0, 1, 0	3.1	0.2–10.7
	6	1, 0, 0	3.6	0.2–18.1
	1	0, 2, 0	6.2	1.2–18.1
	1	1, 0, 1	7.2	1.3–18.2
	1	1, 1, 0	7.4	1.3–20.3
	1	2, 0, 0	9.2	1.4–37.5
	1	2, 1, 0	14.7	3.7–42.0
	1	3, 0, 0	23.1	4.6–94.5
	1	3, 1, 0	42.7	9.0–183.0
	1	3, 3, 0	240	42.0–1,000.0
	1	3, 3, 2	1,100	180.0–4,100.0

[a] Number of samples per visit with the indicated MPN index.
[b] Numbers of positive wells (100 μl, 10 μl, 1 μl).
[c] Two of these carcasses were also positive at the postintervention sampling site. The third positive postintervention carcass was negative at the preevisceration sampling site.

Pathogen prevalence and levels on postintervention carcasses. Pathogen prevalence was determined for carcasses that had received a full complement of antimicrobial interventions. *E. coli* O157:H7 was recovered from 15 (1.2%) of the 1,232 postintervention carcasses sampled (Fig. 1). Nine of these carcasses were identified as positive in the spring (3.1% prevalence), three were identified as positive in the summer (1.0% prevalence), and three were identified as positive in the fall (1.0% prevalence; Table 1). The positive carcasses were detected during 5 of the 24 sampling visits (see Table 3); five of the positive carcasses were from the same lot. Seven of the 15 *E. coli* O157:H7–positive carcasses also carried non-O157 STEC (data not shown). For these 15 carcasses, *E. coli* O157:H7 was also recovered from 13 hide and 3 fecal samples; 8 of the 15 carcasses were *E. coli* O157:H7 positive prior to evisceration as well (data not shown). All 15 *E. coli* O157:H7–positive postintervention carcasses carried <3.0 cells per 100 cm^2 as determined by MPN assays (Table 3). *E. coli* O157:H7 levels were available for two of these carcasses at the preevisceration stage; these MPN indices were <3.0 cells per 320 cm^2.

TABLE 3. *MPN indices and 95% CIs for* E. coli *O157:H7,* Salmonella, stx, *and STEC recovered from postintervention carcasses*

Season	Visit no.	No. of samples[a]	Results[b]	No. of cells per 100 cm² MPN index	95% CI
E. coli O157:H7					
Spring	3	2	0, 0, 0	<3.0	0.0–9.5
	5	6	0, 0, 0	<3.0	0.0–9.5
	6	1	0, 0, 0	<3.0	0.0–9.5
Summer	12	3	0, 0, 0	<3.0	0.0–9.5
Fall	16	3	0, 0, 0	<3.0	0.0–9.5
Salmonella					
Summer	10	1	0, 0, 0	<3.0	0.0–9.5
stx (non-O157 STEC)					
Spring	1	1 (0)	0, 0, 0	<3.0	0.0–9.5
	2	12 (7)	0, 0, 0	<3.0	0.0–9.5
	2	1 (1)	2, 3, 2	38.2	17.7–82.6
	3	21 (10)	0, 0, 0	<3.0	0.0–9.5
	4	4 (2)	0, 0, 0	<3.0	0.0–9.5
	5	18 (13)	0, 0, 0	<3.0	0.0–9.5
	5	1 (0)	1, 1, 0	7.4	1.3–20.3
	6[c]	10 (7)	0, 0, 0	<3.0	0.0–9.5
	6	1 (0)	1, 0, 0	3.6	0.2–18.1
Summer	8	9 (4)	0, 0, 0	<3.0	0.0–9.5
	10	3 (3)	0, 0, 0	<3.0	0.0–9.5
	12	20 (5)	0, 0, 0	<3.0	0.0–9.5
Fall	13	1 (0)	0, 0, 0	<3.0	0.0–9.5
	13	1 (1)	0, 0, 1	3.0	0.2–9.6
	14	1 (0)	0, 0, 0	<3.0	0.0–9.5
	15	28 (24)	0, 0, 0	<3.0	0.0–9.5
	15	1 (1)	0, 0, 1	3.0	0.2–9.6
	15	1 (1)	1, 0, 0	3.6	0.2–18.1
	16	30 (10)	0, 0, 0	<3.0	0.0–9.5
	16	2 (0)	1, 0, 0	3.6	0.2–18.1
	17	1 (0)	0, 0, 0	<3.0	0.0–9.5
	18	2 (1)	0, 0, 0	<3.0	0.0–9.5
Winter	20	1 (1)	0, 0, 0	<3.0	0.0–9.5
	21	1 (1)	0, 0, 0	<3.0	0.0–9.5
	22	4 (3)	0, 0, 0	<3.0	0.0–9.5
	24	17 (11)	0, 0, 0	<3.0	0.0–9.5
	25	8 (4)	0, 0, 0	<3.0	0.0–9.5

[a] Number of samples per visit with the indicated MPN index; for *stx*-positive samples, the number in parentheses is the number of those samples from which a non-O157 STEC isolate was recovered.

[b] Numbers of positive wells (100 μl, 10 μl, 1 μl).

[c] The MPN index was not determined for one sample recovered during visit 6.

Salmonella was recovered from one postintervention carcass sampled in the summer, resulting in an overall prevalence of 0.1% (Fig. 1 and Table 1). This postintervention carcass tested negative for *E. coli* O157:H7, non-O157 STEC, and *stx* genes. Fewer than 3.0 *Salmonella* cells per 100 cm² of the sampled area were detected (Table 3). *Salmonella* was also recovered from the hide and feces of the same animal and from the same carcass prior to evisceration (data not shown).

Shiga toxin genes were detected in 200 (16.2%) of 1,232 postintervention carcass samples, and non-O157 STEC strains were recovered from 110 (8.9%) of these 200 carcasses (Fig. 1). The prevalence levels for both *stx*-carrying cells and non-O157 STEC on postintervention carcasses were highest in the spring and fall ($P < 0.05$), similar to the pattern observed for fecal prevalence (Table 1). The majority of the *stx*-positive postintervention carcasses (191 of 199; 96.0%) harbored fewer than 3.0 *stx*-carrying cells per 100 cm² (Table 3). The MPN indices for the remaining eight carcasses for which data were available ranged from 3.0 to 38.2 cells per 100 cm², with only two carcasses carrying more than 3.6 cells per 100 cm².

DISCUSSION

Cattle hides have been identified as a source of general microbial contamination on carcasses *(15, 30, 34, 42),* and it has been shown that *E. coli* O157:H7 can be transferred from hides to carcasses during processing *(17).* In the present study, *E. coli* O157:H7, *Salmonella,* and non-O157 STEC were found predominantly on hides, suggesting that hides are a more significant source of carcass contamination than feces. *E. coli* O157:H7 and *Salmonella* were recovered more frequently from preevisceration carcasses than from feces, and clear seasonal trends in the prevalence of these organisms on hides were mimicked on preevisceration carcasses. Pathogen prevalence on hides may reflect several sources of contamination, such as soils, feces from other animals, and possibly lairage *(8, 46).* For both *E. coli* O157:H7 and *Salmonella,* methodological differences could account for the discrepancies between the results of previous reports and the data presented here *(12, 23).* Previous reports have suggested substantially lower prevalence rates (ranging from 4.5 to 18.0%) for *E. coli* O157:H7 on hides in the United States at slaughter *(10, 25, 40).* In a summertime study of four feedlot pens, 51% (71 of 139) of ventral hides were found to test positive for *E. coli* O157:H7 *(33).* *Salmonella* prevalence rates of 15.4 to 86.9% have been found for hides in previous U.S. studies *(9, 10, 14, 40).* In addition, and in contrast to the results of the present study, Sofos et al. *(47)* reported a higher prevalence for *Salmonella* in the late fall and the winter (November to January) than in the spring (May to June).

In the present study, hides, not feces, appeared to be the primary source of the tested pathogens on carcasses. Previous studies had suggested that *E. coli* O157:H7 prevalence in feces was correlated with the pathogen's prevalence on carcasses *(11, 18, 24, 25),* and the prevalence levels of both *E. coli* O157:H7 and *Salmonella* in cattle feces have been investigated in depth. Although it is difficult to compare results from various studies because of differences in methodology, preliminary data have suggested that the MRU and MRU-TT methods are at least as sensitive as previously employed methods *(12).* Peak prevalence rates for *E. coli* O157:H7 in feces have been reported to occur in summer and to range from 17 to 37% *(2, 13, 35, 49),* comparable to the 12.9% peak summertime rate reported here. The results of the present study are similar to those of a 1999 feedlot study *(3)* demonstrating a peak in *Salmonella* prevalence in feces in the summer but contrast with

those of a study carried out at slaughter in 1995 and 1996 suggesting that *Salmonella* prevalence was higher from November to January (14.2%) than from May to June (8.3%) *(47)*. The overall fecal prevalence rate for *Salmonella* (4.4%) was in good agreement with reports of 5.5 to 6.7% for fed cattle in the United States *(3, 10, 26, 40)*.

Interestingly, the relative proportions of positive preevisceration carcasses versus those of positive hides were noticeably higher for *Salmonella* than for *E. coli* O157:H7 except in the winter. Assuming that hides are the major source of pathogens, this observation suggests that there may be a significant difference in carcass contamination rates for the two organisms. It is possible that the data reflect a disparity between the method's sensitivity for the recovery of *E. coli* O157:H7 and its sensitivity for the recovery of *Salmonella,* and this possibility should be investigated. Alternatively, levels of hide contamination may vary by site *(33),* so the data could reflect relative prevalence differences between the brisket area (hide sampling site) and the rump area (preevisceration carcass sampling site). It is also possible, but intuitively less likely, that *E. coli* O157:H7 is more successful at contaminating carcasses. Steam vacuuming was the only direct antimicrobial intervention administered prior to preevisceration carcass sampling, and *E. coli* O157:H7 and *Salmonella enterica* Typhimurium have been shown to be equally susceptible to this antimicrobial process *(38)*. An alternative explanation is that *E. coli* O157:H7 may be present on hides in larger numbers than *Salmonella,* making *E. coli* O157:H7 more likely to be transferred to the carcass.

Non-O157 STEC and *stx*-carrying cells were highly prevalent among all types of samples. To the best of our knowledge, this is the first study examining non-O157 STEC prevalence on hides. Previously, non-O157 STEC prevalence in fecal samples was examined for 23 range cattle in the United States, and one animal per season was found to carry these pathogens *(48)*. The reported fecal prevalence of non-O157 STEC for dairy cattle in the United States and other countries ranges from 5.8 to 19.0% (for a recent review, see Arthur et al. *(7)*). In the present study, the overall prevalence of non-O157 STEC in feces was found to be at the high end of this range (19.3%). Variations in seasonal and sample type prevalence should be interpreted with some caution, however. The relative sensitivity of the PCR protocols for each type of sample has not been established, and the efficiency of our protocols in isolating non-O157 STEC from *stx*-positive samples varied significantly by season ($P < 0.05$). Differences in the types and levels of background growth in the samples, among other things, could have affected both the PCR and the colony hybridization assays. As detection methods continue to improve, non-O157 STEC likely will be recovered increasingly more often from feces, hides, and other sources, judging from the number of samples testing positive for *stx* genes in this and other studies *(7, 39)*.

One primary indicator of beef-product contamination is the prevalence of carcass contamination after processing (postintervention). As was previously shown for *E. coli* O157:H7 *(25),* the number of carcasses contaminated with *E. coli* O157:H7, *Salmonella,* and non-O157 STEC dropped significantly from the preevisceration stage to the postintervention stage ($P < 0.0001$). The overall prevalence of *E. coli* O157:H7 on postintervention carcasses (1.2%) was similar to the prevalence reported in other studies that also used sensitive methods incorporating IMS *(13)*. Two studies have shown higher prevalence rates for *Salmonella* on dressed carcasses (1.0 and 1.3%, compared with the rate of 0.1% observed in this study). However, one of these studies was carried out during the summer, when prevalence rates may be higher *(9)*. The other study was completed prior to the introduction of hazard analysis critical control point regulations *(3),* and a follow-up report has suggested that *Salmonella* prevalence in large beef processing plants is now lower (0.1%) *(44)*. Non-O157 STEC prevalence rates (4.0%) on postintervention carcasses in the summer were lower than expected on the basis of previous data (8%) *(6),* possibly reflecting a lower efficiency of recovery, as noted above.

In addition to prevalence, pathogen levels on postintervention carcasses also are significant to public health issues. The data presented here suggest that only low levels of pathogens survive the application of antimicrobial interventions. Small numbers of *E. coli* O157:H7, *Salmonella,* and non-O157 STEC were recovered from the few contaminated, dressed carcasses (postintervention). Furthermore, data for *E. coli* O157:H7–positive preevisceration carcasses suggest that, like prevalence, the level of contamination drops between the preevisceration and postintervention processing sites. Different areas were sampled at each site, relatively few samples were analyzed, and some of the MPN scores (e.g., 0, 2, 0; 0, 0, 1; and 0, 1, 0) appear more often than would be expected by chance *(17),* so the data must be interpreted cautiously. However, the drop in *E. coli* O157:H7 levels from the preevisceration stage to the postintervention stage appears to have been substantial in some cases; for example, there was a drop from ca. 240 and ca. 1,100 cells per 320 cm^2 on two preevisceration carcasses to levels undetectable on ca. 2,500 cm^2 of the same carcasses at the postintervention stage (see Table 3). In conjunction with the prevalence rates, these data suggest that in general, few pathogen cells are introduced into beef products produced from fed-cattle carcasses.

The data presented here suggest that hides are the major source of contamination of beef carcasses with *E. coli* O157:H7, *Salmonella,* and non-O157 STEC. Significant seasonal differences in the prevalence levels of *E. coli* O157:H7 and *Salmonella* were found both preharvest (in feces and on hides) and postharvest (on carcasses). In addition, throughout the year the prevalence of cells carrying *stx* genes was higher than that of *E. coli* O157:H7 or *Salmonella.* These observations are consistent with seasonal rates for human clinical cases reported for *E. coli* O157:H7 and *Salmonella* in previous years but not with seasonal rates reported for non-O157 STEC *(21, 22)*. The discrepancy could reflect underreporting of non-O157 STEC disease cases, an inability of all STEC to cause disease, or a myriad of other factors *(7)*. However, these data cannot be used to establish a direct cause-and-effect relationship be-

tween pathogen prevalence during beef processing and disease cases caused by *E. coli* O157:H7, *Salmonella,* and non-O157 STEC. Further studies are needed to determine the relevance of these implications.

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REFERENCES

1. Acheson, D. W. K. 2000. How does *Escherichia coli* O157:H7 testing in meat compare with what we are seeing clinically? *J. Food Prot.* 63:819–821.
2. Animal and Plant Health Inspection Service. October 2001. *Escherichia coli* O157 in United States feedlots. NAHMS Feedlot '99. Available at: http://www.aphis.usda.gov:80/vs/ceah/cahm/Beef_Feedlot/bffeed.htm.
3. Animal and Plant Health Inspection Service. October 2001. *Salmonella* in United States feedlots. NAHMS Feedlot '99. Available at: http://www.aphis.usda.gov:80/vs/ceah/cahm/Beef_Feedlot/bffeed.htm.
4. Anonymous. 25 July 1996. Pathogen reduction; hazard analysis and critical control point (HACCP) systems; final rule. U.S. Department of Agriculture, Food Safety and Inspection Service. Code of Federal Regulations. *Fed. Regist.* 61:38805–38989.
5. Anonymous. 19 January 1999. Beef products contaminated with *Escherichia coli* O157:H7. U.S. Department of Agriculture, Food Safety and Inspection Service. Code of Federal Regulations. *Fed. Regist.* 64:2803–2805.
6. Arthur, T. M., G. A. Barkocy-Gallagher, M. Rivera-Betancourt, and M. Koohmaraie. 2002. Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. *Appl. Environ. Microbiol.* 68:4847–4852.
7. Arthur, T. M., G. A. Barkocy-Gallagher, M. Rivera-Betancourt, and M. Koohmaraie. 2002. Prevalence of non-O157 STEC in live animals and at various steps during harvest. *Proc. Recip. Meat Conf.* 55:21–27.
8. Avery, S. M., A. Small, C.-A. Reid, and S. Buncic. 2002. Pulsed-field gel electrophoresis characterization of Shiga toxin-producing *Escherichia coli* O157 from hides of cattle at slaughter. *J. Food Prot.* 65:1172–1176.
9. Bacon, R. T., J. N. Sofos, K. E. Belk, D. R. Hyatt, and G. C. Smith. 2002. Prevalence and antibiotic susceptibility of *Salmonella* isolated from beef animal hides and carcasses. *J. Food Prot.* 65:284–290.
10. Barham, A. R., B. L. Barham, A. K. Johnson, D. M. Allen, J. R. Blanton, Jr., and M. F. Miller. 2002. Effects of the transportation of beef cattle from the feedyard to the packing plant on prevalence levels of *Escherichia coli* O157 and *Salmonella* spp. *J. Food Prot.* 65:280–283.
11. Barkocy-Gallagher, G. A., T. M. Arthur, G. R. Siragusa, et al. 2001. Genotypic analyses of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the Midwestern states of the United States. *Appl. Environ. Microbiol.* 67:3810–3818.
12. Barkocy-Gallagher, G. A., E. D. Berry, M. Rivera-Betancourt, T. M. Arthur, X. Nou, and M. Koohmaraie. 2002. Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *J. Food Prot.* 65:1527–1534.
13. Barkocy-Gallagher, G. A., and M. Koohmaraie. 2002. Prevalence of *Escherichia coli* O157:H7 in cattle and during processing. *Proc. Recip. Meat Conf.* 55:15–20.
14. Beach, J. C., E. A. Murano, and G. R. Acuff. 2002. Prevalence of *Salmonella* and *Campylobacter* in beef cattle from transport to slaughter. *J. Food Prot.* 65:1687–1693.
15. Bell, R. G. 1997. Distribution and sources of microbial contamination on beef carcasses. *J. Appl. Microbiol.* 82:292–300.
16. Bettelheim, K. A. 2000. Role of non-O157 VTEC. *Soc. Appl. Microbiol. Symp.* 88:38S–50S.
17. Blodgett, R. Personal communication.
18. Bonardi, S., E. Maggi, G. Pizzin, S. Morabito, and A. Capriolo. 2001. Faecal carriage of verocytotoxin-producing *Escherichia coli* O157 and carcass contamination in cattle at slaughter in northern Italy. *Int. J. Food Microbiol.* 66:47–53.
19. Byrne, C. M., D. J. Bolton, J. J. Sheridan, D. A. McDowell, and I. S. Blair. 2000. The effects of preslaughter washing on the reduction of *Escherichia coli* O157:H7 transfer from cattle hides to carcasses during slaughter. *Lett. Appl. Microbiol.* 30:142–145.
20. Centers for Disease Control. 1993. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers—western United States. *Morb. Mortal. Wkly. Rep.* 42:258–263.
21. Centers for Disease Control. 2000. Surveillance for foodborne-disease outbreaks—United States, 1993–1997. *Morb. Mortal. Wkly. Rep.* 49:1–51.
22. Centers for Disease Control. 2002. Foodnet 2000 annual report. Foodnet Active Surveillance Project. Available at: http://www.cdc.gov/foodnet/annual/2000/2000_summary.htm.
23. Chapman, P. A. 2000. Methods available for the detection of *Escherichia coli* O157 in clinical, food and environmental samples. *World J. Microbiol. Biotechnol.* 16:733–740.
24. Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, J. Fox, and E. Crick. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect.* 111:439–447.
25. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
26. Fedorka-Cray, P. J., D. A. Dargatz, L. A. Thomas, and J. T. Gray. 1998. Survey of *Salmonella* serotypes in feedlot cattle. *J. Food Prot.* 61:525–530.
27. Fleiss, J. L. (ed.). 1981. Statistical methods for rates and proportions, 2nd ed. John Wiley and Sons, New York.
28. Food and Drug Administration. 1998. Bacteriological analytical manual, 8th ed., revision A. AOAC International, Gaithersburg, Md.
29. Food Safety and Inspection Service. 1994. Nationwide beef microbiological baseline data collection program: steers and heifers, October 1992–September 1993. U.S. Department of Agriculture, Food Safety and Inspection Service, Science and Technology, Microbiology Division, Office of Public Health and Science, Washington, D.C. Available at: http://www.fsis.usda.gov/OPHS/baseline/contents.htm.
30. Gill, C. O., J. C. McGinnis, and M. Badoni. 1996. Use of total or *Escherichia coli* counts to assess the hygienic characteristics of a beef carcass dressing process. *Int. J. Food Microbiol.* 31:181–196.
31. Hommel, G. 1988. A stagewise rejective multiple test procedure based on a modified Bonferroni test. *Biometrika* 75:383–386.
32. Hu, Y., Q. Zhang, and J. C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by a multiplex PCR. *J. Appl. Microbiol.* 87:867–876.
33. Keen, J. E., and R. O. Elder. 2002. Isolation of Shiga-toxigenic *Escherichia coli* O157 from hide surfaces and the oral cavity of finished beef feedlot cattle. *J. Am. Vet. Med. Assoc.* 220:756–763.
34. McEvoy, J. M., A. M. Doherty, M. Finnerty et al. 2000. The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Lett. Appl. Microbiol.* 30:390–395.
35. Meyer-Broseta, S., S. N. Bastian, P. D. Arné, O. Cerf, and M. Sanaa. 2001. Review of epidemiological surveys on the prevalence of contamination of healthy cattle with *Escherichia coli* serogroup O157:H7. *Int. J. Hyg. Environ. Health* 203:347–361.
36. Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* 11:142–201.
37. Paton, A. W., and J. C. Paton. 1998. Detection and characterization

of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eaeA*, enterohemorrhagic *E. coli hlyA*, rfb_{O111}, and rfb_{O157}. *J. Clin. Microbiol.* 36:598–602.

38. Phebus, R. K., A. L. Nutsch, D. E. Schafer, et al. 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. *J. Food Prot.* 60:476–484.
39. Pradel, N., V. Livrelli, C. de Champs, et al. 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *J. Clin. Microbiol.* 38:1023–1031.
40. Ransom, J. R., K. E. Belk, R. T. Bacon, J. N. Sofos, J. A. Scanga, and G. C. Smith. 2002. Comparison of sampling methods for microbiological testing of beef animal rectal/colonal feces, hides, and carcasses. *J. Food Prot.* 65:621–626.
41. Reissbrodt, R., H. Heier, H. Tschäpe, R. A. Kingsley, and P. H. Williams. 2000. Resuscitation by ferrioxamine E of stressed *Salmonella enterica* serovar Typhimurium from soil and water microcosms. *Appl. Environ. Microbiol.* 66:4128–4130.
42. Ridell, J., and H. Korkeala. 1993. Special treatment during slaughtering in Finland of cattle carrying an excessive load of dung; meat hygienic aspects. *Meat Sci.* 35:223–228.
43. Riley, L. W., R. S. Remis, S. D. Helgerson, et al. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308:681–685.
44. Rose, B. E., W. E. Hill, R. Umholtz, G. R. Ransom, and W. O. James. 2002. Testing for *Salmonella* in raw meat and poultry products collected at federally inspected establishments in the United States, 1998 through 2000. *J. Food Prot.* 65:937–947.
45. Silhavy, T. J., M. L. Berman, and L. W. Enquist (ed.). 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
46. Small, A., C.-A. Reid, S. M. Avery, N. Karabasil, C. Crowley, and S. Buncic. 2002. Potential for the spread of *Escherichia coli* O157, *Salmonella,* and *Campylobacter,* in the lairage environment at abattoirs. *J. Food Prot.* 65:931–936.
47. Sofos, J. N., S. L. Kochevar, G. R. Bellinger, et al. 1999. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. *J. Food Prot.* 62:140–145.
48. Thran, B. H., H. S. Hussein, M. R. Hall, and S. F. Khaiboullina. 2001. Shiga toxin–producing *Escherichia coli* in beef heifers grazing an irrigated pasture. *J. Food Prot.* 64:1613–1616.
49. Van Donkersgoed, J., T. Graham, and V. Gannon. 1999. The prevalence of verotoxins, *Escherichia coli* O157:H7, and *Salmonella* in the feces and rumen of cattle at processing. *Can. Vet. J.* 332–338.

Predictors of Hemolytic Uremic Syndrome in Children During a Large Outbreak of Escherichia coli O157:H7 Infections

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Predictors of Hemolytic Uremic Syndrome in Children During a Large Outbreak of *Escherichia coli* O157:H7 Infections

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ABSTRACT. *Objective.* **To evaluate risk factors for progression of *Escherichia coli* O157:H7 infection to the hemolytic uremic syndrome (HUS).**

Study Design. **We conducted a retrospective cohort study among 278 Washington State children <16 years old who developed symptomatic culture-confirmed *E coli* O157:H7 infection during a large 1993 outbreak. The purpose of the study was to determine the relative risk (RR) of developing HUS according to demographic characteristics, symptoms, laboratory test results, and medication use in the first 3 days of illness.**

Results. **Thirty-seven (14%) children developed HUS. In univariate analysis, no associations were observed between HUS risk and any demographic characteristic, the presence of bloody diarrhea or of fever, or medication use. In multivariate analysis, HUS risk was associated with, in the first 3 days of illness, use of antimotility agents (odds ratio [OR] = 2.9; 95% confidence interval [CI] 1.2–7.5) and, among children <5.5 years old, vomiting (OR = 4.2; 95% CI 1.4–12.7). Among the 128 children tested, those whose white blood cell (WBC) count was 13 000/μL in the first 3 days of illness had a 7-fold increased risk of developing HUS (RR 7.2; 95% CI 2.8–18.5). Thirteen (38%) of the 34 patients with a WBC count 13 000/μL developed HUS, but only 5 (5%) of the 94 children whose initial WBC count was <13 000/μL progressed to HUS. Among children who did not develop HUS, use of antimotility agents in the first 3 days of illness was associated with longer duration of bloody diarrhea.**

Conclusions. **Prospective studies are needed to further evaluate measures to prevent the progression of *E coli* O157:H7 infection to HUS and to assess further clinical and laboratory risk factors. These data argue against the use of antimotility agents in acute childhood diarrhea. Our finding that no intervention decreased HUS risk underscores the importance of preventing *E coli* O157:H7 infections. *Pediatrics* 1997;100(1). URL: http://www.pediatrics.org/cgi/content/full/100/1/e12; *antibiotics, antimotility agents, Escherichia coli O157:H7, kidney failure, leukocytosis.***

ABBREVIATIONS. HUS, hemolytic uremic syndrome; BUN, blood urea nitrogen; RR, relative risk; CI, confidence interval; TMP/SMZ, trimethoprim/sulfamethoxazole; OR, odds ratio; WBC, white blood cell; Stx, Shiga toxin.

Escherichia coli O157:H7 causes bloody and nonbloody diarrhea that progresses to hemolytic uremic syndrome (HUS) in a subset of patients.[1,2] Though the gastrointestinal manifestations of infection with *E coli* O157:H7 can be severe, HUS accounts for the major acute and chronic morbidity and mortality caused by this organism.

Associations between certain host-specific factors and the risk of progression of enteric infection with *E coli* O157:H7 to HUS have been reported. In population-based studies of HUS, age-specific incidence was highest among children <5 years of age, but these studies did not evaluate risk factors for progression of diarrhea or hemorrhagic colitis to HUS.[3–6] In some studies of children with *E coli* O157:H7 infection, girls were more likely than boys to develop HUS.[7,8] Patients who are infected with *E coli* O157:H7 and at the time of presentation to medical care have elevated white blood cell counts, fever, or bloody stools also have been noted to have a higher risk of progression to HUS than patients without these findings.[9–12]

Considerable interest has been focused on the effect of antimicrobial agents on the risk of patients with *E coli* O157:H7 infections developing HUS. In one prospective, randomized controlled trial, antimicrobial agents administered to 47 children late in the course of infection failed to demonstrate any effect.[13]

In 1993, a large outbreak of *E coli* O157:H7 infections resulted from the consumption of inadequately cooked hamburgers in restaurants of Chain A in Washington State, resulting in more than 500 cases.[14] Using interviews and medical-record review, we conducted a study among the cohort of children who sought medical attention during the outbreak to evaluate possible risk factors for progression of *E coli* O157:H7 infection to HUS.

METHODS

Inclusion Criteria

Patients were eligible for inclusion in the study if they had either symptomatic, culture-confirmed *E coli* O157:H7 infection or

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HUS beginning in January or February 1993, were <16 years of age, and resided in Washington State. They were considered to have primary cases of infection if illness began within 10 days of eating at a Chain A restaurant. Patients who became ill within 10 days of close contact with a case-patient and who had not eaten at Chain A during that time were considered to have secondary cases.

Data Collection

Data were collected from three sources. First, county health department staff, using a standard questionnaire, interviewed by telephone a parent of each patient within 2 weeks of illness onset and collected demographic data, the dates of illness onset and signs and symptoms of the illness, and the date of eating and food consumed at Chain A. Second, interviewers contacted patients' parents again by telephone 2 to 4 months later. Using a different standard questionnaire, they verified information obtained by the county health department and recorded more detailed information on patients' signs and symptoms, medication use, physician visits and hospitalization. Third, a study coordinator or one of the authors (B.P.B. or P.L.), using a standard data collection form, abstracted recorded signs and symptoms, medications, and test results from medical records.

Case Definition and Variables Examined

We used the onset dates of illness and signs and symptoms first reported by the parent. Diarrhea and vomiting were determined to be present if the family or patient reported these signs. Bloody diarrhea was defined as visible blood in the stool, also by parental report. Fever was defined as any temperature ≥38.5°C at any site. When the parent offered different dates at the time of the second interview, we compared the dates collected from the three sources and used those on which two sources agreed. When considering prescription medication use, we included only those patients where parental report and the medical record agreed on whether the medication was taken. We used the date the medication was started and the number of days taken as reported by the parent for both prescription and nonprescription medication.

Treatment was defined as at least 2 doses of therapy begun within 3 days of first symptoms. Antimotility agents included dicyclomine, diphenoxylate, Donnatal (phenobarbital, hyoscyamine sulfate, atropine sulfate, scopolamine [A.H. Robins, Richmond, VA]), hyoscyamine sulfate, loperamide, and opioid narcotics. Adsorptive agents were considered to be attapulgite or kaolinpectin. Continuous variables were also considered as categorical variables by dividing at the median or quartile values.

Complete HUS was defined as a platelet count <150 000/μL, hematocrit <30% with evidence of intravascular hemolysis on peripheral blood smear, and blood urea nitrogen (BUN) >20 mg/dL. Incomplete HUS was defined as two of these criteria. Complete and incomplete HUS were combined to form the outcome measure, termed HUS in this study. For children who subsequently developed HUS, only signs and symptoms occurring, and laboratory tests obtained, before HUS developed were included.

Microbiology

Stool samples were submitted to local laboratories, and tested for the presence of *E coli* O157:H7 by inoculation onto sorbitol-MacConkey agar. Serotyping, confirmation, and toxin typing of *E coli* O157:H7 recovered were performed as previously reported.[14]

Statistics

The χ^2 test, Fisher's exact test, Student's t test, and Pearson correlation coefficients were used to examine relationships among independent variables and HUS. Relative risks (RRs) and 95% confidence intervals (CIs) were calculated using standard methods or the exact method of Martin and Austin where appropriate.[15,16] Normality was assessed using the Shapiro-Wilk statistic, and for data that were not normally distributed, the Wilcoxon 2-sample and Kruskal-Wallis tests were used. In stratified analyses, possible differences in associations among strata were examined using Cochran-Mantel-Haenzel statistics and the Breslow-Day test. Logistic regression modelling was conducted using SAS (SAS Institute, Inc, Cary, NC) software.[17] Variables associated with the outcome in the univariate analysis at $P \leq .1$ and possible confounding variables were included in the initial model. The most parsimonious model was developed by evaluating the effects of covariates using the −2 Log Likelihood criteria.

RESULTS

Study Population

The study population consisted of 278 (86%) of the 324 children eligible for participation. Of the 46 children who did not participate, the parents of 10 (22%) refused to be interviewed, 25 (54%) refused medical record review, 8 (17%) could not be located, and 3 (7%) had either no medical record or had not visited a physician. The median age of study subjects was 6 years (range 0 to 15); 145 (52%) were female; 245 (88%) were white. Two hundred eighteen (78%) acquired their infection by eating a hamburger at Chain A and 30 (11%) had cases of secondary infection. The source of infection could not be determined in 30 (11%). Ninety-two (33%) patients were hospitalized.

Outcomes Identified

There were 33 children (12%) with complete HUS and 4 (2%) with incomplete HUS. These 4 patients had anemia (median hematocrit 27%; range 23–29) and thrombocytopenia (median 42 000/μL; range 29 000 to 122 000), but no azotemia; 3 had proteinuria or hematuria. The median interval between onset of the first symptom to hospitalization for HUS was 6 days (range 1 to 12).

Demographic Risk Factors Analyzed

We found no association between the risk of HUS and age, sex, or annual household income (Table 1). Although 2 of the 3 fatalities (all from complications of HUS) occurred in children who had secondary cases of infection, patients with secondary cases were no more likely to develop HUS (3/30; 10%) than were patients with primary cases (30/218; 14%). A similar proportion of patients with mental retardation or developmental delay developed HUS (1/8; 13%) as patients without these conditions (36/169; 21%).

TABLE 1. Risk of HUS by Selected Demographic Characteristics

Factor	Proportion of Patients With Factor Who Developed HUS	Rate Ratio (95% CI)	*P* Value
	Number/Total (%)		
Age (y)			
<3	12/76 (16)	2.1 (0.8–5.7)	.13
3–4	9/63 (14)	1.9 (0.7–5.4)	.21
5–8	11/72 (15)	2.1 (0.8–5.6)	.15
>8	5/67 (7)	Referent	
Sex			
Female	23/145 (16)	1.5 (0.8–2.8)	.19
Male	14/133 (11)	Referent	
Annual family income			
<$30 000	12/93 (13)	Referent	
$30 000–$50 000	11/81 (14)	1.0 (0.5–2.3)	.91
>$50 000	10/81 (12)	1.0 (0.4–2.1)	.90

Clinical Risk Factors Analyzed

We initially examined clinical characteristics present at any time before HUS developed. Children whose parents reported vomiting had 3 times the risk of developing HUS (29/153 [19%]) compared with children who did not (8/125 [6%]; RR = 3.0; 95% CI 1.4–6.2). HUS developed in a larger proportion of children with bloody diarrhea compared with children who had diarrhea without blood, and in a larger proportion of children with fever, but the differences were not statistically significant: 34/243 (14%) with bloody diarrhea developed HUS vs 2/28 (7%) without bloody diarrhea (RR = 2.0; 95% CI .5–7.7); 11/56 (20%) with fever developed HUS vs. 20/169 (12%) without fever (RR = 1.8; 95% CI .8–4.1).

To investigate early predictors of HUS, we evaluated the risk of HUS by clinical characteristics measured within the first 3 days of illness (Table 2). Vomiting during this interval was again significantly associated with HUS, conferring nearly twice the risk. This association was modified by age. Among children <5.5 years old, vomiting in the first 3 days of illness was strongly associated with HUS risk (RR = 3.5; 95% CI 1.4–9.4). In contrast, no association was observed between vomiting in the first 3 days of illness and HUS risk among children ≥5.5 years old (RR = 1.0; 95% CI .4–2.4).

Medication Risk Factors Analyzed

Fifty (18.0%) children received an antimicrobial agent, and 34 (12.5%) received an antimotility agent during the first 3 days of illness. No association was observed between treatment with any of these agents and age, sex, or hospitalization. Children treated with antimicrobial agents were more likely to live in households that had annual household incomes of more than $29 000 per year (RR = 1.7; 95% CI 1.0–2.8).

Thirty-one (62%) of the 50 children who received antimicrobial agents were treated with trimethoprim/sulfamethoxazole (TMP/SMZ), 13 (26%) received ampicillin or amoxicillin, 6 (12%) received a cephalosporin, and 4 (8%) received metronidazole. Tetracycline, erythromycin, ciprofloxacin, and gentamicin each were given to 1 child. Eleven children received more than 1 antimicrobial agent. There was no association between treatment with any antimicrobial agent or with TMP/SMZ, and HUS risk (Table 3).

In univariate analysis, children treated with antimotility agents had twice the risk of developing HUS, but this finding failed to reach statistical significance (Table 3). There was no association between receiving antimotility agents and the risk of developing HUS when these analyses were restricted to children <5.5 years old. No patients treated with adsorbents alone developed HUS.

Mulitivariate Modeling

In multivariate modeling, children who were treated with antimotility agents were more likely to develop HUS than were children who did not receive them (odds ratio [OR] = 2.9; 95% CI 1.2–7.5). The relationship between vomiting during the first 3 days of illness and HUS risk identified in the univariate analysis was also observed in the multivariate model, but the adjusted OR for the risk of HUS among children younger than 5.5 years with vomiting was larger (OR = 4.2; 95% CI 1.4–12.7). There was no association between vomiting and HUS risk among children at least 5.5 years old (OR = 1.0; 95% CI .4–3.0).

Laboratory Risk Factors Analyzed

Among the 128 children who had a laboratory test in the first 3 days of illness, patients with a total white blood (WBC) cell count >10 500/μL were at a 5-fold increased risk of HUS (Table 2); this association was not modified by age. The risk increased to 7-fold among children with a WBC count ≥13 000/μL, the upper quartile in this population. No other laboratory test result on specimens obtained during the first 3 days of illness was associated with the subsequent development of HUS.

TABLE 2. Risk of HUS by Selected Clinical Characteristics Beginning ≤3 Days After Diarrheal Illness Onset and by Laboratory Values Among Children Tested Within 3 Days of Diarrheal Illness Onset

Factor	Proportion of Patients With Factor Who Developed HUS	Proportion of Patients Without Factor Who Developed HUS	Rate Ratio (95% CI)	*P* Value
	Number/Total (%)	Number/Total (%)		
Vomiting	22/127 (17)	13/140 (9)	1.9 (1.0–3.5)	.05
Bloody diarrhea	29/213 (14)	7/59 (12)	1.1 (0.5–2.5)	.73
Fever	11/56 (20)	20/169 (12)	1.8 (0.8–4.1)	.14
Hematocrit				
>39%	10/70 (14)	8/58 (14)	1.0 (0.4–2.5)	.94
Platelets				
>310 000/μL	10/59 (17)	8/67 (12)	1.4 (0.6–3.6)	.42
BUN				
>9 mg/dL	7/64 (11)	8/49 (16)	0.7 (0.3–1.7)	.40
WBC count				
>10 500/μL	15/63 (24)	3/65 (5)	5.2 (1.6–17.0)	<.01
≥13 000/μL	13/34 (38)	5/94 (5)	7.2 (2.8–18.5)	<.01
Segmented neutrophils				
>69%	9/56 (16)	7/53 (13)	1.2 (0.5–3.0)	.67
Any band forms	11/57 (19)	5/52 (10)	2.0 (0.7–5.4)	.15

TABLE 3. Risk of HUS by Medications Begun Within 3 Days of Diarrheal Illness Onset

Factor	Proportion of Patients With Factor Who Developed HUS	Proportion of Patients Without Factor Who Developed HUS	Rate Ratio (95% CI)	*P* Value
	Number/Total (%)	Number/Total (%)		
Any antimicrobial agent	8/50 (16)	28/218 (13)	1.3 (0.6–2.6)	.56
TMP/SMZ*	6/31 (19)	30/234 (13)	1.5 (0.7–3.3)	.32
Antimotility agent†	8/34 (24)	28/238 (12)	2.0 (1.0–4.0)	.10
Adsorbent and/or antimotility agent‡	8/43 (19)	28/229 (12)	1.5 (0.7–3.1)	.26

* Trimethoprim/sulfamethoxazole.
† Includes dicyclomine, diphenoxylate, Donnatal, hyoscyamine sulfate, loperamide, or narcotic.
‡ Attapulgite, kaolin-pectin.

Less Severe Outcomes

Among children who did not develop HUS, we examined possible relationships between more common, but less severe, outcomes and treatment with antimicrobial or antimotility agents in the first 3 days of illness. We found no statistically significant associations between the number of days of diarrhea or of bloody diarrhea and antimicrobial agent use (Table 4). There was no difference in the median duration of diarrhea among children treated with antimotility agents compared with those who did not receive them, but the median duration of bloody diarrhea was longer (4 vs 3 days, respectively, $P < .05$) (Table 4).

DISCUSSION

We demonstrate that an elevated total WBC count early in the course of *E coli* O157:H7 infection is strongly associated with the development of HUS. Vomiting, the only sign associated with HUS risk, was not previously noted to be a factor for progression of *E coli* O157:H7 infection to HUS[8,9,11,18,19]. Vomiting may indicate severe gastrointestinal injury, higher intestinal concentrations of Shiga toxin (Stx), host susceptibility, or systemic toxemia with central nervous system effects.[20] We were unable to demonstrate an association between developmental delay and risk of progression to HUS, as has been previously noted.[21]

Reduced power may have contributed to our failure to associate bloody diarrhea or fever with HUS risk, because almost all children studied had bloody diarrhea, and parental recall might have caused imprecise reporting of fever. Also, we found no relationship between age, female sex, or higher socioeconomic status and HUS risk among infected patients. However, these risk factors for HUS might reflect differential exposure to *E coli* O157:H7 or ascertainment of HUS patients rather than increased risk of HUS among clinically infected patients. Because there were few nonwhite children in this study population, the relationship of race/ethnicity and HUS risk could not be assessed.

Our study had limited power to associate antimotility agents and HUS risk because few patients received these agents. Nonetheless, on multivariate analysis, there was a statistically significantly increased risk of progression of *E coli* O157:H7 infection to HUS among children who took agents that slowed peristalsis. Among children who did not develop HUS, antimotility agent use was associated with longer duration of bloody diarrhea. On the basis of these data, and in consideration of similar data from British Columbia,[8,19] we advise against administering agents that slow peristalsis to children with bloody diarrhea or *E coli* O157:H7 infection. In addition, because nonbloody diarrhea precedes bloody diarrhea in most *E coli* O157:H7 infections,[22] the absence of visible blood in stools does not indicate that a patient is not infected with *E coli* O157:H7 or another Stx-producing organism. In view of the lack of any demonstrated therapeutic benefit from these agents, we advise against administering agents that slow peristalsis to any child with acute diarrhea.

Antibiotics might injure or lyse intracolonic *E coli* O157:H7, liberating more Stx for systemic absorption,[23,24] or eliminate competitive colonic flora. Conversely, antibiotic-induced clearance of *E coli* O157:H7 might decrease the risk of progression to HUS. However, we demonstrate no benefit of antibiotics in *E coli* O157:H7 infection. In two *E coli* O157:H7 outbreaks, antimicrobials, particularly TMP/SMZ, increased the risk of HUS or of death,[9,11] a trend, albeit not statistically significant, also noted in Washington State in 1987.[22] Prolonged antimicrobial therapy was associated with reduced risk of HUS among patients in a case series from British Columbia, Canada, but the timing of administration with respect to illness onset was not reported,[8] and this outcome may reflect a bias toward continuation of antibiotics in children who were not destined to

TABLE 4. Duration of Diarrhea and Bloody Diarrhea by Medications Begun Within the First 3 Days of Illness Among Patients Who Did Not Develop HUS

Factor	Diarrhea		Bloody Diarrhea	
	No. of Days of Symptom, Median (Mean)	*P* Value	No. of Days of Symptom, Median (Mean)	*P* Value
Antimicrobial Agent				
Yes	6.0 (6.9)		4.5 (4.5)	
No	6.5 (7.7)	.2	4.0 (4.6)	.6
Antimotility Agent*				
Yes	7.0 (8.3)		5.0 (5.2)	
No	6.0 (7.5)	.3	4.0 (4.5)	.04
Adsorbent† and/or Antimotility Agent				
Yes	7.0 (8.3)		5.0 (4.8)	
No	6.0 (7.5)	.2	4.0 (4.6)	.3

* Dicyclomine, diphenoxylate, Donnatal, hyoscyamine sulfate, loperamide, or narcotic.
† Attapulgite, kaolin-pectin.

develop HUS. In an expanded case series, antimicrobial use and HUS risk were not associated.[20] In two prospective randomized, controlled studies, antimicrobial agents had no effect on the risk of HUS, but the sample sizes were small,[9] and therapy was commenced late.[13]

Our study has several strengths. By interviewing parents and reviewing medical records, we verified that prescribed antimicrobial agents were actually given, and, like Akashi et al,[12] we focused on the early stage of illness. By assessing the effect of early antimicrobial therapy, we may have reduced the possible confounding inherent in retrospective studies: antimicrobial agents may appear to have a deleterious effect because physicians prescribed them for sicker children (ie, those "destined" to develop HUS) as their illnesses became more severe. Furthermore, medication administered or continued[8] late in the course, once the pathophysiologic processes resulting in HUS have either begun or been avoided, might not affect illness outcome.

This is the largest study of its kind to date, and has additional strengths that improve the ability to generalize our findings. Because of the publicity surrounding this community-wide outbreak, a diverse group of children with a broad spectrum of disease severity may have been brought to medical attention. Only culture-confirmed cases of *E coli* O157:H7 infection were included and in almost all cases infection was caused by the same strain,[25] reducing bias from the inclusion of infections caused by *E coli* O157:H7 strains with varying virulence traits.[26]

This study has several limitations. Parental recall of the child's symptoms and of medications used, and completeness and accuracy of medical records, may have varied with the severity of the child's illness. Such differential reporting would bias toward an association between the factor and the risk of HUS. Second, the data in this retrospective study may have been imprecise. The information contained in the medical record was not collected in a standardized manner, and recall was requested for an event several weeks or months before the interview. Such nondifferential inaccuracies would reduce power and decrease the likelihood of finding an association. Third, power to detect an effect of antimicrobial agents on HUS risk would have been greater if a larger proportion of children had been treated; however, the study had sufficient power to detect a difference in risk of approximately 2.5-fold or greater. Finally, the results may not apply to disease caused by *E coli* O157:H7 that produces Stx 2 but not Stx 1. However, most *E coli* O157:H7 strains, like the outbreak strain, produce both Stx 1 and Stx 2.[26]

Our results may help clinicians. Only 5 of the 94 children with *E coli* O157:H7 infection tested in the first 3 days of illness who had a WBC count $<13\,000/\mu$L subsequently developed HUS (negative predictive value of 95%). This finding should be confirmed in other populations, but a low WBC count early in the course of *E coli* O157:H7 infection in children may indicate low HUS risk. Obtaining a WBC count early in the course of illness is possible; 49 (86%) of the 57 children who sought medical attention before the outbreak was publicized presented within 3 days of illness onset. A high WBC count was a moderately sensitive predictor of the likelihood to progression to HUS in our study; 13 (38%) of 34 children with a WBC count $\geq 13\,000/\mu$L in the first 3 days of illness subsequently developed HUS compared with 18 (14%) of the total of 128 children tested. However, because a normal WBC count does not absolutely indicate a benign course, all patients should be followed closely until diarrhea is resolved for several days to confirm that urine output remains adequate and mental status is normal.

These data may help plan trials to evaluate interventions to prevent HUS following *E coli* O157:H7 infection. Most infected patients recover spontaneously within approximately 1 week. If patients with a higher likelihood of developing HUS could be identified at presentation, this group could be studied in prospective, randomized, controlled trials to assess the role of antimicrobial agents, toxin binders, antitoxins, or other modalities to prevent HUS. Using the criteria of a WBC count $\geq 13\,000/\mu$L in the first 3 days of illness, we would have identified 13 (72%) of the 18 patients who developed HUS among the 128 patients in whom the laboratory test was obtained. Twenty-one (16%) patients meeting this criterion failed to develop HUS.

No intervention decreased the risk of developing HUS. Factors that reflect the host's early response to infection (vomiting and leukocytosis in the first three days of illness) predicted the subsequent development of HUS. These findings suggest that the pathophysiologic processes resulting in HUS often are initiated by the time medical attention is initially sought. These findings underscore the importance of preventing *E coli* O157:H7 infection as the most effective way to prevent HUS.

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REFERENCES

1. Cohen MB. *Escherichia coli* O157:H7 infections: a frequent cause of bloody diarrhea and the hemolytic-uremic syndrome. *Adv Pediatr*. 1996; 43:171–207
2. Boyce TG, Swerdlow DL, Griffin PM. *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. *N Engl J Med*. 1995;333:364–368
3. Tarr PI, Hickman RO. Hemolytic uremic syndrome epidemiology: a population-based study in King County, Washington, 1971–1980. *Pediatrics*. 1987;80:41–45
4. Rogers MJ, Rutherford GW, Alexander SR, et al. A population-based study of hemolytic uremic syndrome in Oregon, 1979–1982. *Am J Epidemiol*. 1986;123:137–142
5. Martin DL, MacDonald KL, White KE, Soler JT, Osterholm MT. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. *N Engl J Med*. 1990;323:1161–1167
6. Rowe PC, Orrbine E, Wells GA, et al. Epidemiology of hemolytic uremic syndrome in Canadian children from 1986–1988. *J Pediatr*. 1991;119: 218–224

7. Rowe PC, Walop W, Lior H, et al. Hemolytic anemia after childhood *Escherichia coli* O157:H7 infection: are females at increased risk? *Epidemiol Infect.* 1991;106:523–530
8. Cimolai N, Carter JE, Morrison BJ, Anderson JD. Risk factors for the progression of *Escherichia coli* O157:H7 enteritis to hemolytic-uremic syndrome. *J Pediatr.* 1990;116:589–592
9. Pavia AT, Nichols CR, Green DP, et al. Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. *J Pediatr.* 1990;116:544–551
10. Gransden WR, Damm MAS, Anderson JD, et al. Further evidence associating hemolytic uremic syndrome with infection by verotoxin-producing *Escherichia coli* O157:H7. *J Infect Dis.* 1986;154:522–524
11. Carter AO, Borczyk AA, Carlson JAK, et al. A severe outbreak of *Escherichia coli* O157:H7-associated hemorrhagic colitis in a nursing home. *N Engl J Med.* 1987;317:1496–1500
12. Akashi S, Joh K, Tsuji A, et al. A severe outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7 in Japan. *Eur J Pediatr.* 1994;153:65–655
13. Proulx F, Turgeon JP, Delage G, Lafleur L, Chicoine L. Randomized, controlled trial of antibiotic therapy for *Escherichia coli* O157:H7 enteritis. *J Pediatr.* 1992;121:299–303
14. Bell BP, Goldoft M, Griffin PM, et al. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: the Washington experience. *JAMA.* 1994;272:1349–1353
15. Kleinbaum DG, Kupper LL, Morgenstern H. *Epidemiologic Research: Principles and Quantitative Methods.* Belmont, CA: Wadsworth, Inc; 1982:299
16. Martin D, Austin H. An efficient program for computing conditional maximum likelihood estimates and exact confidence limits for a common odds ratio. *Epidemiology.* 1991;2:359–362
17. SAS Institute, Inc. *SAS/STAT User's Guide.* Version 6.08. Cary, NC: SAS Institute, Inc; 1989
18. Gianviti A, Rosmini F, Caprioli A, et al. Haemolytic-uraemic syndrome in childhood: surveillance and case-control studies in Italy. Italian HUS study group. *Pediatr Nephrol.* 1994;8:705–709
19. Cimolai N, Basalyga S, Mah DG, Morrison BJ, Carter JE. A continuing assessment of risk factors for the development of *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Clin Nephrol.* 1994;42:85–89
20. Fujii J, Kita T, Yoshida S, et al. Direct evidence of neuron impairment by oral infection with verotoxin-producing *Escherichia coli* O157:H7 in mitomycin-treated mice. *Infect Immunol.* 1994;62:3447–3453
21. Abu-Arafeh IA, Auchterlonie IA, Smail PJ. Risk of hemolytic-uremic syndrome in children with neurologic disorders. *J Pediatr.* 1992;120:834–835. Letter
22. Ostroff SM, Kobayashi JM, Lewis JH. Infections with *Escherichia coli* O157:H7 in Washington State: the first year of statewide disease surveillance. *JAMA.* 1989;262:355–359
23. Karch H, Stockbine N, O'Brien A. Growth of *Escherichia coli* in the presence of trimethoprim-sulfamethoxazole facilitates detection of Shiga-like toxin producing strains by colony blot assay. *FEMS Microbiol Lett.* 1986;35:141–145
24. Walterspiel JN, Ashkenazi S, Morrow AL, Cleary TG. Effect of subinhibitory concentrations of antibiotics on extracellular Shiga-like toxin I. *Infection.* 1992;20:25–29
25. Grimm LM, Goldoft M, Kobayashi J, et al. Molecular epidemiology of a fast-food restaurant-associated outbreak of *Escherichia coli* O157:H7 in Washington State. *J Clin Microbiol.* 1995;33:2155–2158
26. Ostroff SM, Tarr PI, Neill MA, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. *J Infect Dis.* 1989;160:994–998

Predictors of Hemolytic Uremic Syndrome in Children During a Large Outbreak of Escherichia coli O157:H7 Infections

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The Non-O157 Shiga-Toxigenic (Verocytotoxigenic) *Escherichia coli*; Under-Rated Pathogens

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Following a brief review of the ecology of *Escherichia coli* in general, the role of Shiga-Toxigenic (Verocytotoxigenic) *E. coli* (STEC) as pathogens is addressed. While STEC belonging to the serogroup O157 have been extensively studied and shown to be involved in many cases and outbreaks of human disease, the importance of STEC belonging to other serogroups has not been recognized as much. This review addresses the problems associated with these pathogens, demonstrating that increasing the awareness of them is a major part of the problem. This review then demonstrates how widespread isolations especially from food animals and human disease have been, discussing in particular STEC belonging to serogroups O8, O26, O103, O111, O113 and O128. The animal host-specificity of these STEC is also reviewed. In conclusion some methods of improving isolation of these pathogens is addressed.

Keywords *Escherichia coli*; Shiga-Toxigenic; verocytotoxigenic; O157; non-O157

INTRODUCTION

Escherichia coli are probably the most studied organisms in microbiology. Since their first description (Escherich 1885, 1988, 1989), they have become the model organism for much microbiological research, such that it is often forgotten that their main ecological niche is the alimentary tract of humans and most warm-blooded animals, where they appear ubiquitous. They are shed in the feces of warm-blooded animals and humans, however, they only comprise a very small percentage of the total fecal flora. According to one study (Mitsuoka and Hayakawa 1972) the enterobacterial flora ranges from values of 10^8 to 10^9, while the total flora ranged around 10^{10} CFU/g of large intestinal content. Only in neonates were there comparatively higher levels of *Enterobacteriaceae*. The normal *E. coli* flora of human infants is generally acquired from their mothers, associated adults and/or caregivers in their first week of life (Bettelheim et al. 1974; Bettelheim and Lennox-King 1976; O'Farrell et al. 1976). It is clear that both infants and adults continuously acquire new *E. coli* strains from food and the environment (Bettelheim et al. 1977; Stark and Lee 1982) and that food, especially meat, can be the source of much of the intestinal microbial flora harbored by humans (Bettelheim and Lennox-King 1976; Bettelheim et al. 1977; Majed et al. 1978; Smith 1965).

Over the last half-century it has become increasingly obvious that there are a number of different enteropathogenic groups of *E. coli*. At least six known pathotypes associated with gastrointestinal infections have been recognized, apart from those causing urinary tract infections, septicemia, and meningitis in humans and a number of similar diseases in animals.

The pathotypes associated with gastrointestinal infections currently recognized are:

- Enteropathogenic *E. coli* (EPEC)
- Enterotoxigenic *E. coli* (ETEC)
- Verocytotoxigenic *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC) of which Enterohemorrhagic *E. coli* (EHEC) are a subgroup.
- Enteroinvasive *E. coli* (EIEC)
- Enteroaggregative *E. coli* (EAggEC)
- Diffuse-adherent *E. coli* (DAEC).

Being typical *E. coli* in all respects, apart from possessing their respective virulence factors, these pathogenic *E. coli* behave in all respects including biochemically and ecologically like other non-pathogenic *E. coli*. Thus, if no distinct markers are available, their detection among commensal *E. coli* becomes very difficult and this is the main problem associated with the non-O157 Shiga-toxigenic (Verocytotoxigenic) *E. coli* (non-O157 STEC). Soon after the first description of the outbreaks due to the O157 STEC (Riley et al. 1983), it was realized that these types were unusual in not being able to ferment the carbohydrate sorbitol and a medium, which had originally been developed to select for certain EPEC (Rappaport and Heng

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TABLE 1
A selection of non-O157 STEC human outbreaks, reported in the world literature

Serotype	Country	Reference
O26:H-	Japan	Kudoh et al. 1994
O26:H11	Japan	Kudoh et al. 1994
O26:H11	Japan	Hiruta et al. 2001
O26:H11	Ireland	McMaster et al. 2001
O26:H11	Germany	Werber et al. 2002
O103:H2	Japan	Kudoh et al. 1994
O111:H-	Italy	Caprioli et al. 1994
O111:H-	Japan	Kudoh et al. 1994
O111:H-	Australia	Paton et al. 1996
O111:H2	Germany	Morabito et al. 1998
O111:H8	U.S.A.	Bergmire-Sweat et al. 2000; Brooks et al. 2004
O128:H2	Japan	Kudoh et al. 1994
O128:H2	Australia	Bettelheim et al. 1999
O145:H-	Japan	Kudoh et al. 1994

1952) was shown to be very effective in selecting O157 STEC from among the other commensal *E. coli* normally found on primary isolation of fecal specimens (March and Ratnam 1986). As ever-increasing numbers of cases and outbreaks due to O157 STEC were being reported in the world literature and even more effective media for their isolation were available (Bettelheim 1998a, 1998b, 2005), it appeared that the O157 STEC were the only types of STEC of any significance.

Although non-O157 STEC had been reported before 1982 (Konowalchuk et al. 1977; Wade et al. 1979; Wilson and Bettelheim 1980) and have regularly continued to be reported from human cases and outbreaks as well as from animals and environmental sources, very few laboratories look for them. The level of awareness of these pathogens is very low. It is the intention of this review to raise the level of awareness of these pathogens and of their role in human morbidity and mortality as well discuss their environmental importance, both in themselves as well as in connection with the O157 STEC.

THE RANGE OF CASES, OUTBREAKS, AND DISTRIBUTION OF NON-O157 STEC

Over the years there have been a number of outbreaks reported in the world literature associated with non-O157 STEC. A selection of these is listed in Table 1. It is noteworthy that certain serogroups like O26; O111, and O128 predominate among these outbreak reports.

When one considers single or at least not established outbreak strains then a much larger range of serogroups and serotypes of non-O157 STEC have been reported around the world. In a survey of the world literature (Bettelheim http://www.microbionet.com.au/vtectable.htm) there are noted 3760 isolations of STEC comprising 162 'O' groups. As many of these can be subtyped using flagellar antigen typing into a number of different serotypes it should be realized that there is a vast range of STEC serotypes, of which the serotypes O157:H7 and O157:H- are only two examples. The range of these serotypes is given in Tables 2 and 3. Table 2 lists the more common 'O' types with the sources from which these STEC have been isolated, while Table 3 lists the remaining 134 'O' types and the sources limited to just a few. As it is impossible in such a review to deal at all exhaustively with all these STEC serotypes, only some will be selected for further discussion. The ones to be discussed in this detail are O8, O26, O91, O103, O111, O113, and O128 as well as the serotype O5:H-.

The prevalence of STEC in cattle, food, and children was examined during a one-year prospective study in France, and it revealed that the majority of the STEC strains (66%) belonged to serotypes not previously associated with severe disease in humans (Pradel et al. 2000). However, the potential of these STEC as human pathogens should not be underestimated, because as long as non-O157 STEC are not regularly sought in clinical laboratories, they will neither be found nor reported. While no standard reliable method exists to isolate, characterize, and identify these pathogens, they will only be found and reported by the most enthusiastic laboratories.

STEC have been shown to be present in the intestinal tract of many domestic and wild animals, with the carriage rates being especially high in ruminants (Fagan et al. 1999). Transmission generally by a fecal route of these organisms can lead to contamination of foods derived from these animals. It has been shown many years ago that *E. coli* isolated from the feces of animals at slaughter can be isolated throughout the food chain (Shooter et al. 1970, 1974). STEC derived in this manner are likely to be a major source of human infection (Chapman et al. 1993). Farm animals, particularly cattle and sheep, are known reservoirs for STEC, especially non-O157 serotypes have been more commonly isolated from food, particularly meat of bovine or ovine origin (Read et al. 1990; Willshaw et al. 1993). Though many of the STEC serotypes found in cattle, sheep and other animals have rarely or never been isolated from humans, this may only be a matter of time or vigilance. Although some of these environmental non-O157 STEC appear to lack some of the virulence factors commonly found in strains isolated from patients and animals suffering from infection, they may well have other virulence factors. It was recently reported, that strains of STEC O113:H21, commonly found in cattle and associated with hemolytic uremic syndrome (HUS) lack Intimin (Paton et al. 1999), however, they produce another attachment factor (Paton and Paton 2002) as well as another toxin (Paton and Paton 2005). It may well be that when other non-O157 STEC are subjected to similar investigations further virulence factors will be identified.

TABLE 2

Sources of VTEC 'O' serogroups reported at least 30 times

	Cattle Healthy	Cattle Diseased	Sheep Healthy	Sheep Diseased	Poultry Healthy	Pultry Diseased	Pigs Healthy	Pigs Diseased	Beef	Sheep meat	Pork	Poultry Meat	Food	Other Human	Healthy Human	Human Diseased	Total
O1	6					4			2				1			18	31
O2	44	2	1			6	3		2		2		4	3		14	81
O5	16	5	13				1		1	2			2	5		16	61
O6	18		16				1		3	1	1		4	1		11	56
O8	28	4	7			1	12	1	15	2	3		6	1	5	18	103
O15	14	4	2			1	1		5				1	2	1	7	38
O22	37	2							11				9		2	10	71
O26	40	10	6				1						5	2	8	132	204
O75	1	1	14	1					2	3			1	1	2	6	32
O76	14		5										4	2	4	10	39
O84	22	4	1													7	34
O88	12	1	2						6				1	4		5	31
O91	32		19				7	1	10	3	2		9	1	6	50	140
O103	30	3	3			1			5				4	1	11	70	128
O111	22	14				1							1	1	2	102	143
O113	48	11	1						20	1			4	2	11	35	133
O116	27	3							7				1	1	2	3	44
O118	14	6	1												3	49	73
O119	22	3	3						1	1					1	8	39
O121	8		2				3	1					1		4	12	31
O128	6	1	28						4	1			1	9	9	39	98
O145	17												2	1	2	45	67
O146	9	3	16											8	7	22	65
O153	23	4	6						5				1	3		8	50
O156	18	3	8							1			2	1	2	6	41
O163	13	3	3				4		3	1	1		1			6	35
O174	26	6	7						6				2	2	7	25	81
Ont	175	27	45	1			14	1	34	7	2		31	16	23	65	441
OR	37	4	11			1			12	3		2	4	4	20	59	157
Total	779	124	220	2		15	47	4	154	26	11	2	102	71	132	858	2547
%	30.6	4.9	8.6	0.1	0.0	0.6	1.8	0.2	6.0	1.0	0.4	0.1	4.0	2.8	5.2	33.7	100.0

TABLE 3
Serogroups reported less than 30 times

	Cattle Healthy	Sheep Healthy	Human Diseased	Other	Total
O3	4	0	4	2	10
O4	5	3	9	3	20
O7	5	0	3	10	18
O9	0	1	7	7	15
O10	1	0	0	0	1
O11	3	0	3	1	7
O12	0	0	2	0	2
O14	0	0	2	0	2
O16	2	1	3	1	7
O17	2	0	1	4	7
O18	2	0	7	5	14
O20	10	3	5	8	26
O21	1	3	1	11	16
O23	1	0	7	1	9
O25	2	0	7	5	14
O27	0	0	1	4	5
O28	9	0	6	4	19
O29	1	0	0	1	2
O30	0	1	1	1	3
O31	0	0	1	1	2
O32	0	0	1	0	1
O35	1	1	0	3	5
O36	1	0	1	1	3
O38	7	0	2	1	10
O39	9	0	1	4	14
O40	2	0	3	5	10
O41	0	0	0	5	5
O42	1	1	0	0	2
O43	4	0	3	1	8
O44	1	0	0	1	2
O45	4	0	7	6	17
O46	10	0	4	4	18
O48	0	0	3	0	3
O49	5	1	0	2	8
O50	0	0	4	0	4
O51	1	0	0	1	2
O52	0	3	4	0	7
O53	2	0	1	0	3
O54	0	0	2	4	6
O55	3	3	18	2	26
O56	0	0	0	3	3
O57	0	0	0	2	2
O58	0	0	0	1	1
O60	0	0	5	4	9
O62	0	0	2	2	4
O64	0	0	0	4	4
O65	0	0	2	7	9
O66	0	0	1	0	1
O68	3	0	7	1	11
O69	4	1	4	3	12
O70	2	0	4	2	8
O71	0	2	3	4	9
O73	1	0	5	0	6
O74	12	0	6	4	22
O77	5	3	11	2	21
O78	0	0	2	9	11
O79	4	2	2	2	10
O80	5	0	2	4	11
O81	4	0	1	7	12
O82	14	0	1	9	24
O83	0	0	2	1	3
O85	1	0	1	0	2
O86	1	0	4	4	9
O87	6	5	2	4	17
O89	0	0	1	1	2
O90	1	3	0	4	8
O92	1	0	4	1	6
O93	2	0	4	1	7
O95	0	0	2	0	2
O96	3	0	2	4	9
O98	8	1	6	3	18
O100	0	0	4	9	13
O101	1	0	8	6	15
O102	1	0	2	4	7
O104	3	3	8	5	19
O105	6	0	4	5	15
O106	1	4	1	0	6
O107	0	1	2	1	4
O108	3	0	0	0	3
O109	4	0	1	1	6
O110	6	5	1	6	18
O112	8	6	7	5	26
O114	2	0	2	2	6
O115	5	0	8	5	18
O117	8	7	8	6	29
O120	4	1	1	7	13
O122	0	0	0	1	1
O123	0	10	3	8	21
O124	2	0	0	0	2
O125	2	3	5	0	10
O126	5	1	3	3	12
O127	0	0	1	3	4
O129	0	0	2	0	2
O130	9	0	0	4	13
O131	1	0	0	0	1
O132	9	0	2	3	14

TABLE 3
Serogroups reported less than 30 times *(Continued)*

	Cattle Healthy	Sheep Healthy	Human Diseased	Other	Total
O133	0	0	2	0	2
O134	0	0	1	1	2
O136	18	4	1	5	28
O137	0	0	1	0	1
O138	0	0	1	10	11
O139	1	0	0	4	5
O140	1	0	0	1	2
O141	6	1	0	8	15
O142	0	1	1	2	4
O143	0	0	1	0	1
O147	2	0	0	1	3
O148	1	0	2	1	4
O149	3	1	0	6	10
O150	2	0	4	3	9
O151	0	0	0	2	2
O152	3	0	2	3	8
O154	1	1	5	0	7
O159	1	0	0	8	9
O160	4	0	0	1	5
O161	5	0	0	0	5
O162	1	0	0	10	11
O165	7	1	16	1	25
O166	0	5	6	5	16
O167	0	0	0	1	1
O168	6	0	2	2	10
O169	1	0	1	0	2
O170	1	0	0	0	1
O171	14	0	0	7	21
O172	8	1	3	1	13
O173	0	0	1	1	2
O175	4	0	4	7	15
O176	1	4	0	0	5
O177	2	1	7	6	16
O178	5	0	3	9	17
O179	1	0	4	1	6
O180	0	0	0	1	1
O181	4	0	7	2	13
O182	1	0	0	0	1
Total	360	99	358	396	1213

Thus to summarize this introductory section, non-O157 STEC are widespread in domestic food animals, especially ruminants. From these they can be, and often are, transmitted along the food chain and can contaminate food for human consumption. Individual cases as well as outbreaks of human disease have been reported as being ascribed to them. While some types may lack some of the virulence factors typically associated with STEC, they may carry others, some of which have been identified. However, while identifying them is still considered a major problem by many laboratories, a full description of the ecology of non-O157 STEC cannot be given.

STEC BELONGING TO SEROGROUP O8

As shown in Table 2, there have been reported 103 isolations of strains of STEC O8. This shows that three serotypes predominate namely O8:H-, O8:H9, and O8:H19, which between them comprise 59 of the reported 103 isolations (57.3%; Table 4). A further examination of these three as well as the other O8 serotypes shows that though widespread in the environment including human food, which yielded 26 isolations (25.2%), there are only 18 (17.5%), which have been isolated from human disease. It thus has to be concluded that the O8 STEC serotypes, though capable of causing human disease, may only do so under certain conditions. Until non-O157 STEC are more thoroughly investigated no final conclusions of the role of these potential human pathogens can be drawn.

STEC BELONGING TO SEROGROUP O26

Here the situation is quite different from that observed with STEC serogroup O8 strains. The majority (189) of the 204 isolates (92.6%) belong to the two serotypes: O26:H- and O26:H11 (Table 5). It is quite possible that a number of the O26:H- strains when analyzed for their *fliC* type by PCR would show the presence of the H11 genotype, as was demonstrated by Beutin et al. (2004). In that study a number of non-motile strains belonging to other serogroups including O157 were similarly shown to have the H genotype, expected to be carried by them. Thus it should be considered that both O26:H- as well as O26:H11 STEC probably belong to one clone. When the sources of these serotypes are examined, it is immediately clear that the majority (125) of these 189 isolates are from human infections (66.1%) and that 31 (16.4%) are derived from healthy cattle and 13 (6.9%) from diseased cattle. Thus 89.4% are derived either from human infections or of bovine origin. It should therefore be concluded that this clone appears to have a particular propensity to survive in the bovine intestinal tract, rather than that of other food animals and to cause human infections, unlike strains of STEC O8, which were isolated from cattle, sheep and pigs. It is also noteworthy, that a number of outbreaks have been reported associated with the STEC O26:H11/H-clone (Table 1).

STEC BELONGING TO SEROGROUP O91

The situation is more akin with that observed with the STEC serogroup O8 strains. There are four serotypes (Table 6), which predominate: O91:H- (31.4%); O91:H10 (7.8%); O91:H14 (12.3%); and O91:H21 (39.2%). These together comprise 90.7% of all STEC O91 reports. With over a third (35.7%) of all STEC O91 reports being from cases of human disease, these organisms

TABLE 4
Distribution of O8 STEC serotypes according to the world literature

	Cattle Healthy	Cattle Diseased	Pigs Healthy	Sheep Healthy	Human Healthy	Human Diseased	Food	Other	Total
O8:H-	2	0	2	0	2	6	2	1	15
O8:H2	2	1	0	0	0	3	1	0	7
O8:H3	0	0	1	0	0	0	0	0	1
O8:H4	0	1	0	0	0	0	0	0	1
O8:H5	1	0	0	0	0	0	0	0	1
O8:H8	2	1	0	1	0	0	0	0	4
O8:H9	0	1	2	0	0	2	7	2	14
O8:H10	0	0	0	0	0	1	0	0	1
O8:H11	0	0	0	0	0	0	0	0	1
O8:H14	0	0	0	0	0	1	0	0	1
O8:H16	2	0	0	0	0	0	2	0	4
O8:H17	0	0	1	0	0	0	0	0	1
O8:H19	10	2	3	2	0	3	10	0	30
O8:H20	1	0	2	0	0	0	0	0	3
O8:H21	0	0	0	1	0	1	1	0	3
O8:H25	1	1	0	0	3	0	0	0	5
O8:H30	0	0	0	1	0	0	1	0	2
O8:H31	1	0	0	0	0	0	0	0	1
O8:H35	1	0	0	0	0	0	0	0	1
O8:H51	1	0	0	0	0	0	0	0	1
O8:Hnt	1	0	1	1	0	1	2	1	6
Total	25	7	12	6	5	18	26	4	103

should be seriously considered as pathogens. In the studies of Beutin et al. (2004), they found that a number of O91:H- strains, when analyzed for their *fliC* type by PCR would show the presence of the H14 genotype. This is interesting in this case, as O91:H21 is the most common serotype within this serogroup rather than O91:H14.

STEC BELONGING TO SEROGROUP O103

Among the serotypes of O103 serogroup, there are two serotypes, which predominate: O103:H- (15.6%) and O103:H2 (66.4%). These two comprise 82% of all reports of these STEC serotypes. While the studies of Beutin et al. (2004) did not reveal strains of O103:H- for their *fliC* type by PCR, it can be speculated that that they may well possess the H2 genotype. However, until such studies are performed on a number of O103:H- STEC strains the question has to remain open. Nevertheless, the results presented in Table 7, force one to the conclusion that STEC serotypes O103:H2/H- are important human pathogens and that their main reservoir is bovine, rarely being isolated from any other animals.

STEC BELONGING TO SEROGROUP O111

Among the reports of this serogroup again two serotypes predominate (Table 8). Again they are the non-motile O111:H- and the motile O111:H8. The studies of Beutin et al. (2004) found that strains of O111:H- when analyzed for their *fliC* type by PCR would show the presence of the H8 genotype. Again this suggests that one may well be dealing with the same type. In Table 1 are listed three outbreaks due to O111:H- and one due to O111:H8 in addition to one due to O111:H2. However, extensive studies to be discussed later on the Australian outbreak (Paton et al. 1996) clearly show a much more complex picture and as this may well relate to other STEC outbreaks it will be discussed separately. Nevertheless, strains of STEC O111:H8/H- should be considered as significant human pathogens. It is also noteworthy, that, apart from two isolations of O111:H45, one from diseased chickens in Canada (Parreira and Gyles 2002) and one from wild deer in Japan (Asakura et al. 1998), they were only isolated from healthy or diseased cattle. Although cattle appear to be the reservoir for these pathogens, they are only isolated very rarely and when they are isolated they occur as a minority in the specimens. As an example of this, the first reported isolation of STEC O111 from Australian cattle (Hornitzky et al. 2000) revealed only four colonies of the strain in a fecal culture on Vancomycin-Cefixime-Cefsulodin Blood agar surrounded by many other coliform colonies. The specimen came from a cow with profuse watery diarrhea. This was despite the fact that the most major STEC outbreak, so far

TABLE 5
Distribution of O26 STEC serotypes according to the world literature

	Cattle Healthy	Cattle Diseased	Sheep Healthy	Human Healthy	Human Diseased	Food	Other	Total
O26:H-	6	3	1	2	45	0	2	59
O26:H1	0	0	0	0	1	0	0	1
O26:H2	1	0	0	0	1	0	0	2
O26:H11	25	10	4	5	80	5	1	130
O26:H12	0	0	0	0	1	0	0	1
O26:H18	0	0	0	0	1	0	0	1
O26:H21	2	0	1	1	1	0	0	5
O26:H32	1	0	0	0	0	0	0	1
O26:Hnt	2	0	0	0	2	0	0	4
Total	37	13	6	8	132	5	3	204

References: Anon. 2002; Beutin et al. 1998; Bielaszewska et al. 1994; Bielaszewska et al. 1996; Blanco et al. 1997; 2003a, 2003b, 2004; Bockemühl et al. 1992, 1998; Bokete et al. 1997; Brooks et al. 1997; Caprioli et al. 1995; Carroll et al. 2003; Clarke et al. 1994; Cobbold and Desmarchelier 2000, 2001; Czirok et al. 1995; DesRosiers et al. 2001; Djordjevic et al. 2004; Dorn et al. 1993; Dreesman and Pulz 2004; Eklund et al. 2001, 2002; Enami et al. 1999; Evans et al. 2002; Farina et al. 1996; Fey et al. 2000; Friedrich et al. 2002; Fukushima and Seki 2004; Gallien et al. 2000; García-Aljaro et al. 2005; Gerber et al. 2002; Geue et al. 2002; Giammanco et al. 1996; Gioffré et al. 2002; Goldwater and Bettelheim 1994; Gunning et al. 2001; Guth et al. 2002, 2003; Hiruta et al. 2001; Hornitzky et al. 2001, 2002, 2005; Huppertz et al. 1996, 1999; Ishii et al. 2005; Jelacic et al. 2003; Jenkins et al. 2002; Karch et al. 1997b; Keskimäki et al. 1998; Kijima-Tanaka et al. 2005; Klein et al. 2002; Kudoh et al. 1994; Kugler et al. 1998; Leelaporn et al. 2003; Lehmacher et al. 1998; Ludwig et al. 2002; Mackenzie et al. 1998; McMaster et al. 2001; Mellman et al. 2005; Meng et al. 1998; Mercado et al. 2004; Misawa et al. 2000; Misselwitz et al. 2003; Miyamoto 2000; Ohara et al. 2002; Okitsu et al. 2001; Orden et al. 2003; Oteiza et al. 2005; Parma et al. 2000; Pichner et al. 2001; Pierard et al. 1994, 1997; Pradel et al. 2000; Prager et al. 2005; Pulz et al. 2003; Rahn et al. 1998; Read et al. 1992; Rivero et al. 2004; Sandhu et al. 1996, 1998; Scheutz 1995; Schmid et al. 2002, 1998, 1999; Schurman et al. 2000; Scotland et al. 1988; Smith et al. 1988; Takeda 1999; Tamura et al. 1996; Tatarczak et al. 2005; Thomas et al. 1993, 1996; Vaz et al. 2004; Verweyen et al. 1999; Wells et al. 1991; Werber et al. 2002; Wieler et al. 1996; Willshaw et al. 2001; Wilson and Bettelheim 1980; Wilson et al. 1996; Zhang et al. 2000; Zweifel et al. 2004.

described in Australia was predominantly due to STEC O111:H- (Paton et al. 1996) and this serotype is also the main STEC, that has been isolated from human cases of STEC infection, especially the more serious cases (Bettelheim 2001a). In a study of bovine feces from animals with gastrointestinal infections (Hornitzky et al. 2005), non-STEC strains of O111:H-, which otherwise had all the characteristics of typical STEC, including producing enterohemolysin and intimin were isolated as the most prevalent type. It can thus be speculated that these may be the reservoir and only acquire the capability to become typical STEC under certain conditions. Under normal circumstances studies involving the ecology of STEC would completely miss or ignore such strains.

STEC BELONGING TO SEROGROUP O113

Three serotypes predominate among strains of this serogroup: O113:H-, O113:H4, and O113:H21 (Table 9), of which the latter is by far the most prominent, comprising nearly half all reports of this serogroup. The studies of Beutin et al. (2004) found that strains of O113:H- when analyzed for their *fliC* type by PCR would show the presence of the H4 genotype and not the H21 genotype. Apart from one isolation each of O113:H4 from venison in Germany (Gallien et al. 2002) and one from sheep in Switzerland (Zweifel et al. 2004) and one of O113:H21 from a horse in Germany (Pichner et al. 2005), these STEC serotypes have to-date been only found in cattle, which are obviously the main reservoir. The recent finding by Paton and Paton (2005) that STEC O113:H21 strains produce a novel subtilase cytotoxin is significant, because they speculate it may well enhance the virulence of these organisms, which have in many cases been isolated from severe human infections.

STEC BELONGING TO SEROGROUP O128

In the case of this STEC serogroup there are again two serotypes, which predominate O128:H- and O128:H2 (Table 10). While the studies of Beutin et al. (2004) have not indicated that O128:H- STEC may carry the H2 genotype, there seems to be a strong similarity between the ecology of these two types, which suggests a strong similarity between them. The unusual feature of these serotypes is that apart from a few isolates from cattle, they predominate in sheep and this strongly indicates an ovine reservoir.

TABLE 6
Distribution of O91 STEC serotypes according to the world literature

	Cattle Healthy	Cattle Diseased	Pigs Healthy	Sheep Healthy	Human Healthy	Human Diseased	Food	Other	Total
O91:H-	4	0	2	15	3	12	7	1	45
O91:H7	1	0	0	0	0	0	0	0	1
O91:H8	1	0	0	0	0	0	0	0	1
O91:H10	2	0	0	0	0	9	0	0	11
O91:H12	0	0	1	0	0	0	0	0	1
O91:H14	2	0	1	1	3	7	1	1	16
O91:H16	0	0	0	1	0	0	0	0	1
O91:H19	0	1	0	0	0	0	0	0	1
O91:H21	17	1	0	1	0	17	15	0	51
O91:H29	0	0	0	1	0	0	0	0	1
O91:H38	0	0	1	0	0	0	0	0	1
O91:H40	0	0	0	0	0	3	0	0	3
O91:H44	0	0	1	0	0	0	0	0	1
O91:H49	1	0	0	0	0	0	1	0	2
O91:Hnt	1	0	2	0	0	2	0	0	5
Total	29	2	8	19	6	50	24	2	140

References: Anon. 2002; Atalla et al. 2000; Baker et al. 1999; Bennett and Bettelheim 2002; Bettelheim et al. 2000; Beutin et al. 1993, 1997, 1998, 2004; Blanco et al. 1997, 2003a, 2003b, 2004; Bockemühl et al. 1992; Bonardi et al. 2004; Bonnet et al. 1998; Brett et al. 2003a; Cantarelli et al. 2000; Clarke et al. 1994; Cortés et al. 2005; DesRosiers et al. 2001; Djordjevic et al. 2004; Dreesman and Pulz 2004; Eklund et al. 2001, 2002; Evans et al. 2002; Fratamico et al. 2004; Friedrich et al. 2002; Fukushima and Seki 2004; Gallien et al. 1997; Gallien et al. 2000; García-Aljaro et al. 2005; Goldwater and Bettelheim 1994; Hornitzky et al. 2001; Hornitzky et al. 2002; Hussein et al. 2003; Irino et al. 2005; Kaddu-Mulindwa et al. 2001; Keskimäki et al. 1998; Kijima-Tanaka et al. 2005; Kudva et al. 1997; Leung et al. 2001; McCluskey et al. 1999; Meng et al. 1998; Moreira et al. 2003; Orden et al. 2003; Pichner et al. 2001; Pierard et al. 1994, 1997; Pradel et al. 2000, 2001; Prager et al. 2005; Pulz et al. 2003; Rey et al. 2003; Richter et al. 1997; Rivero et al. 2004; Sandhu et al. 1996; Schmid et al. 2002; Schurman et al. 2000; Sidjabat-Tambunan et al. 1998; Stephan et al. 2000; Teufel et al. 1998; Timm et al. 1999; Todd et al. 1999; Urdahl et al. 2002; Urdahl et al. 2003; Vettorato et al. 2003; Willshaw et al. 1992, 1993; Wilson et al. 1994; Zweifel et al. 2004; Zweifel et al. 2005.

STEC O5:H-: ONE SEROTYPE—TWO PATHOTYPES?

The serotypes above have largely been conceived as belonging each to a separate clone and it is additionally suggested that the non-motile serotypes may well belong to the same clone as one of the motile serotype such as O26:H11/H-, O111:H8/H-, and O113:H4/H-. However, a number of observations suggest that strains belonging to apparently the same serotype O5:H- may belong to two separate pathotypes, one ovine-derived: Pathotype O and one bovine-derived Pathotype B (McLean et al. 2005). Pathotype O typically possesses the Shiga-toxin subtypes $stx_{1c}/stx_1 + stx_{2c}$ and produces enterohemolysin but does not posses Intimin (Brett et al. 2003b; Ramachandran et al. 2001) is prevalent in healthy sheep (Djordjevic et al. 2001, 2004) as well as being the causative agent of a case of HUS (Starr et al. 1998). Pathotype B has been typically isolated from calves with diarrhea as well as healthy calves and possesses the Shiga-toxin subtype stx_1, and produces enterohemolysin and does posses Intimin (Int-β subtype) (Wieler et al. 1996; Djordjevic et al. 2004). Pathotype B rarely produces Shiga-toxin 2 and typically produces urease, which pathotype O and most other *E. coli* do not (Brett et al. 2003a; Hornitzky et al. 2005; Mercado et al. 2004; Wieler et al. 1996) and a strain of this pathotype B was isolated from an adult case of bloody diarrhea (McLean et al. 2005). Such different pathotypes, may well be reflected among other STEC serotypes. It is only when a full understanding of these pathogens is achieved and their real epidemiological significance ascertained that further conclusions can be drawn.

ARE NON-O157 STEC IMPORTANT PATHOGENS?

In many senses this question has already been answered. However, it is considered important to extend this discussion further as it will lead to the next section, which suggests, why they are not reported more often. The outbreaks listed in Table 1 should be evidence enough that non-O157 STEC are pathogens. The Shiga toxins these organisms produce are very similar to those produced by the O157 STEC as are the other virulence factors. In some cases non-O157 STEC do not produce Intimin and/or enterohemolysin, but in some cases they have been shown to produce other or similar virulence factors.

TABLE 7
Distribution of O103 STEC serotypes according to the world literature

	Cattle Healthy	Cattle Diseased	Sheep Healthy	Human Healthy	Human Diseased	Food	Total
O103:H-	6	2	1	2	9	0	20
O103:H2	17	2	1	8	51	6	85
O103:H6	0	0	0	0	1	0	1
O103:H7	0	0	0	0	0	1	1
O103:H11	1	0	0	0	0	0	1
O103:H12	0	1	0	0	0	0	1
O103:H14	0	0	0	0	0	2	2
O103:H18	0	0	0	1	3	0	4
O103:H21	0	1	0	0	1	1	3
O103:H25	1	0	0	3	0	0	4
O103:H38	0	0	1	0	0	0	1
O103:H42	1	0	0	0	0	0	1
O103:H43	0	0	0	0	0	1	2
O103:Hnt	1	0	0	0	2	0	3
Total	27	6	3	14	67	11	128

References: Allerberger et al. 1996; Anon. 2002; Asakura et al. 1998; Banatvala et al. 1996; Bergmire-Sweat et al. 2000; Beutin et al. 1998, 2004; Bielaszewska et al. 1994, 1996; Blanco et al. 2003a, 2004; Bockemühl et al. 1992, 1998; Bokete et al. 1997; Brett et al. 2003a; Brooks et al. 2004; Caprioli et al. 1994; Carroll et al. 2003; Clarke et al. 1994; Dorn et al. 1989, 1993; Dreesman and Pulz 2004; Eklund et al. 2001, 2002; Farina et al. 1996; Fey et al. 2000; Friedrich et al. 2002; Gallien et al. 2000; Gerber et al. 2002; Giammanco et al. 1996; Giraldi et al. 1990; Goldwater and Bettelheim 1994; Gunzburg et al. 1988; Hornitzky et al. 2000; Hornitzky et al. 2001; Huppertz et al. 1996; Irino et al. 2005; Karch et al. 1997b; Klein et al. 2002; Kudoh et al. 1994; Leelaporn et al. 2003; Lehmacher et al. 1998; Leomil et al. 2003; Leung et al. 2001; Ludwig et al. 2002; Mackenzie et al. 1998; Mellman et al. 2005; Meng et al. 1998; Mercado et al. 2004; Misawa et al. 2000; Miyamoto 2000; Morabito et al. 1998; Ohara et al. 2002; Okitsu et al. 2001; Park et al. 1996; Parreira and Gyles 2002; Paton et al. 1996; Pierard et al. 1990; Pierard et al. 1994; Pierard et al. 1997; Prager et al. 2005; Pryor et al. 1990; Pulz et al. 2003; Sandhu et al. 1996; Sandhu et al. 1998; Sandhu et al. 1999; Scheutz 1995; Schmidt et al. 1998, 1999; Sperandio et al. 1998; Strockbine et al. 1997; Tamura et al. 1996; Tatarczak et al. 2005; Tzipori et al. 1988; Vaz et al.2004; Verweyen et al. 1999; Wells et al. 1991; Wieler et al. 1996; Willshaw et al. 1992; Wilson et al. 1996; Zweifel et al. 2005.

Strains of STEC O113:H21 have been shown to produce a subtilase cytotoxin in addition to the Shiga toxin 2, which they typically produce (Paton and Paton 2005). Although strains of this STEC serotypes do not produce Intimin, they produce another adhesin designated the STEC autoagglutinating adhesin (saa) (Paton and Paton 2002). Thus if some non-O157 STEC lack one of the generally accepted virulence factors, it should not be assumed that it is therefore not as pathogenic as the typical O157 STEC.

Extensive studies by many laboratories have shown that there are a number of subtypes of both the Shiga toxins as well as of the Intimin types. One typical such study on bovine non-O157 STEC (Brett et al. 2003) showed that STEC of bovine origin commonly possessed certain stx_2 subtypes, while ovine non-O157 STEC possessed a characteristic stx_1 subtype (Brett et al. 2003b). Also among Intimin types many varieties exist, which have been designated by letters of the Greek alphabet as α_1, α_2, β, γ, κ, ε, η, ι, λ, θ, and ζ and with newer types described μ, ν, and ξ (Ramachandran et al. 2003). This study showed that there was a strong relationship between STEC or EPEC serotype and Intimin subtype. Serotypes O157:H- and O157:H7 were found to carry Intimin subtype γ, O111:H- carried Intimin subtype θ, O103:H2 carried Intimin subtype ε, and O26:H11 carried Intimin subtype β. It does not appear that there are major differences in virulence between the different subtypes of Intimin or Shiga toxins. However, these subtypes taken together with the serotype appear to have a major effect on the ecology of the STEC.

In a recent review Beutin (2006) describes the motile/nonmotile strains of O26:H11/H-, O103:H2, O111:H8/H-, and O145:H28/H- as well as O157:H7/H- as the well-known pathogenic "gang of five." Apart from those he draws attention to further emerging highly virulent STEC types including O118:H16/H- and O121:H19/H- (Maidhof et al. 2002; Tarr et al. 2002). It is noteworthy that at the time of writing this review, Utah (U.S.A.) health officials reported that Salinas Valley lettuce may have been responsible for an STEC O121:H19 outbreak in their state in June 2006 that sickened 73 people, including 3 who developed kidney failure (A ProMED-mail post (http://www.promedmail.org); 30.8.2006).

TABLE 8
Distribution of O111 STEC serotypes according to the world literature

	Cattle Healthy	Cattle Diseased	Human Healthy	Human Diseased	Food	Other	Total
O111:H-	12	8	2	68	1	0	91
O111:H2	1			4			5
O111:H7				1			1
O111:H8	5	3		17			25
O111:H11	1	2		1			4
O111:H16	1						1
O111:H21	1			2			3
O111:H30				1			1
O111:H45					0	2	2
O111:Hnt	1	1		7			9
Total	22	14	2	101	1	2	142

References: Allerberger et al. 1996; Atalla et al. 2000; Baker et al. 1999; Bettelheim et al. 1990; Beutin and Müller 1998; Beutin et al. 1993, 1998, 2004; Blanco et al. 2003a, 2004; Bockemühl et al. 1992, 1998; Bokete et al. 1997; Brett et al. 2003a; Brooks et al. 2004; Caprioli et al. 1994; Carroll et al. 2003; Clarke et al. 1994; Dorn et al. 1989, 1993; Dreesman and Pulz 2004; Eklund et al. 2001; Eklund et al. 2002; Farina et al. 1996; Fey et al. 2000; Friedrich et al. 2002; Gallien et al. 2000; Gerber et al. 2002; Giammanco et al. 1996; Giraldi et al. 1990; Goldwater and Bettelheim 1994; Gunzburg et al. 1988; Hornitzky et al. 2000; Hornitzky et al. 2001; Huppertz et al. 1996; Irino et al. 2005; Karch et al. 1997b; Klein et al. 2002; Kudoh et al. 1994; Leelaporn et al. 2003; Lehmacher et al. 1998; Leomil et al. 2003; Leung et al. 2001; Ludwig et al. 2002; Mackenzie et al. 1998; Mellman et al. 2005; Meng et al. 1998; Mercado et al. 2004; Misawa et al. 2000; Miyamoto 2000; Morabito et al. 1998; Ohara et al. 2002; Okitsu et al. 2001; Park et al. 1996; Parreira and Gyles 2002; Paton et al. 1996; Pierard et al. 1990, 1994, 1997; Prager et al. 2005; Pryor et al. 1990; Pulz et al. 2003; Sandhu et al. 1996; Sandhu et al. 1998; Sandhu et al. 1999; Scheutz 1995; Schmidt et al. 1998; Schmidt et al. 1999; Sperandio et al. 1998; Strockbine et al. 1997; Tamura et al. 1996; Tatarczak et al. 2005; Tzipori et al. 1988; Vaz et al.2004; Verweyen et al. 1999; Wells et al. 1991; Wieler et al. 1996; Willshaw et al. 1992; Wilson et al. 1996; Zweifel et al. 2005.

It should also be noted that the serotygroups O121 and O145 did not make 'the cut' as the most prominently reported serogroups (Table 2), and it is very possible that others might well similarly emerge. As Beutin (2006) comments that as STEC undergo frequent genetic rearrangements in their chromosome, their virulence plasmids, *stx*phages and in their LEE pathogenicity islands, which code for the attaching and effacing phenotype (Intimin production) (Beutin 2005; Brunder et al. 1999; Garrido et al. 2006), there is an extensive range of future pathogenic non-O157 STEC, that will cause human morbidity and mortality.

ISOLATION, IDENTIFICATION, AND CHARACTERIZATION OF NON-O157 STEC

The problems associated with the isolation of the non-O157 STEC are the major problem in the realization of their importance as human pathogens. However, before even the problems of isolation, comes the lack of awareness of these potential pathogens. There are now a number of regulations in many countries banning meat and associated products from human consumption if they carry strains of O157, some authorities even just specify strains of O157:H7. It is well known that a meat specimen may have present in it a non-toxigenic strain of O157 and a toxigenic non-O157 STEC. This would be banned. However, if it only had the latter it would be passed for human consumption and if it only had the former it would be banned.

This point is especially noted in a study of an outbreak of gastroenteritis, which was finally ascribed to Noroviruses (Marshall et al. 2001). In the examination of fecal specimens from the patients by PCR, Shiga toxin genes were noted, but STEC could not be isolated by the methods available at the time. In addition four of the patients yielded strains of non-toxigenic *E. coli* O157:H39. If one had relied exclusively on fecal PCR tests for the presence of Shiga toxin genes and the O157 antigen and not extended the study to virological tests, this outbreak would quite falsely have been ascribed to STEC O157. How many other outbreaks have been falsely ascribed in this way?

Having established the importance of awareness, it is realized that the lack of awareness, is mainly due to the difficulty of isolating non-O157 STEC. As they are not often isolated (mainly because they are not sought) there is a lack of awareness. It is part of the aim of this critical review to break into this vicious cycle and suggest that it is not that difficult to isolate non-O157 STEC and hopefully in future many outbreaks will be correctly identified.

TABLE 9
Distribution of O113 STEC serotypes according to the world literature

	Cattle Healthy	Cattle Diseased	Human Healthy	Human Diseased	Food	Other	Total
O113:H-	9	2	3	2	2		18
O113:H2	1						1
O113:H4	10	1	5	6	10	2	32
O113:H5				1			1
O113:H6				1			1
O113:H7		1			1		2
O113:H11	2						2
O113:H17				1			1
O113:H18				1			1
O113:H19	2						2
O113:H21	24	6		19	12	1	61
O113:H27	1						1
O113:H32				1			1
O113:H53				2			2
O113:Hnt				1			1
O113:HR			3				3
Total	49	10	8	35	25	3	130

References: Allerberger 1996; Atalla et al. 2000; Baker et al. 1999; Bettelheim et al. 1990; Beutin and Müller 1998; Beutin et al. 1993, 1998, 2004; Blanco et al. 2003a, 2004; Brett et al. 2003a; Cerqueira et al. 1997; Clarke et al. 1994; Friedrich et al. 2002; Gallien et al. 1999; Gallien et al. 2002; García-Aljaro et al. 2005; Goldwater et al. 1998; Guth et al. 2003; Heuvelink et al. 1996; Hornitzky et al. 2002; Hornitzky et al. 2002; Hornitzky et al. 2005; Irino et al. 2005; Jenkins et al. 2002; Kaddu-Mulindwa et al. 2001; Kijima-Tanaka et al. 2005; Klein and Bülte 2003; Kugler et al. 1998; Lehmacher et al. 1998; Leomil et al. 2003; Meng et al. 1998; Miyao et al. 1998; Paton et al. 1999; Pichner et al. 2005; Piérard et al. 1994, 1997; Pradel et al. 2000; Prager et al. 2005; Pulz et al. 2003; Read et al. 1992; Richter et al. 1997; Rivero et al. 2004; Sandhu et al. 1996; Sandhu et al. 1998; Schmid et al. 2002; Schurman et al. 2000; Sidjabat-Tambunan et al. 1998; Stephan and Hoelzle 2000; Stephan and Untermann 1999; Stephan et al. 2000; Suthienkul et al. 1990; Tamura et al. 1996; Tatarczak et al. 2005; Teufel et al. 1998; Thran et al. 2001; Timm et al. 1999; Urdahl et al. 2003; Willshaw et al. 1993; Willshaw et al. 1996; Wilson et al. 1996; Zweifel et al. 2004; Zweifel et al. 2005.

When attempting to isolate and identify possible non-O157 STEC from clinical specimens, the O157 STEC also have to be considered. They may well be the prime STEC present or may be present as well as the non-O157 STEC. It has to be realized that patient specimens or more importantly food or environmental specimens involved in cases of intestinal infection may not necessarily carry just one pathogen but any number. It should be recognized, that any competent microbiologist should be able to detect and isolate any STEC from such specimens. For primary isolations, methods specifically designed to isolate the O157 STEC, can also be used to detect the non-O157 STEC.

Based on the realization that most O157 STEC cannot ferment sorbitol, the sorbitol MacConkey agar (SMAC) was developed (March and Ratnam 1986). This was followed by a number of more specific media including the Rainbow Agar (Bettelheim 1998a), CHROMagar® (Bettelheim 1998b), and O157:H7 ID agar (O157 H7 ID-F) (Bettelheim 2005). When examining any of these media for the presence of O157 STEC, if other typical *E. coli*-like colonies are selected and subjected to further investigation, it is possible that some may well be non-O157 STEC. It is also always possible that sorbitol-fermenting or other atypical O157 STEC may also be identified in this way (Bettelheim et al. 2002).

Unfortunately, at present, there are no specific media for non-O157 SMAC, although recently Hiramatsu et al. (2002) developed a selecting medium using rhamnose to isolate STEC O26. No doubt such media will be developed in future, based on earlier observations that various STEC clones do have characteristic fermentation patterns when tested with different substrates (Bettelheim 1997). Immunomagentic beads specifically designed for the capture of strains of *E. coli* O157 have been in use for a number of years. More recently similar beads for the to capture of important non-O157 STEC, including O26, O103, O111, and O145 have also become available (Dynal Biotech Ltd).

Many, but not all STEC, produce enterohemolysin and some enterohemolysin producing *E. coli* are not STEC. However, enterohemolysin-production is a very useful indicator for STEC and a medium was developed which will specifically detect enterohemolysin-producing organisms (Beutin et al. 1989) and

TABLE 10
Distribution of O128 STEC serotypes according to the world literature

	Cattle Healthy	Cattle Diseased	Sheep Healthy	Human Healthy	Human Diseased	Food	Other	Total
O128:H-	1	1	8	1	7		4	22
O128:H2	1		11	7	24	5	4	52
O128:H7					1			1
O128:H8	1		2		2			5
O128:H10			1	1				2
O128:H12					1			1
O128:H16			1					1
O128:H19							1	1
O128:H21	1		2					3
O128:H35	1					1		2
O128:H45					3			3
O128:Hnt	1		2		1			4
O128:HR			1					1
Total	6	1	28	9	39	6	9	98

References: Anon. 2002; Bennett and Bettelheim 2002; Bettelheim et al. 1999; Beutin et al. 1993, 1997, 1998, 2004; Blanco et al. 2003a, 2003b, 2004; Bockemühl et al. 1992; Brooks et al. 1997; Clarke et al. 1994; Cortés et al. 2005; Djordjevic et al. 2004; Domingue et al. 2003; Dreesman and Pulz 2004; Evans et al. 2002; Friedrich et al. 2002; Gallien et al. 2002; Geue et al. 2002; Giammanco et al. 1996; Hussein et al. 2003; Jenkins et al. 2002; Kudoh et al. 1994; Kudva et al. 1997; Leelaporn et al. 2003; McCluskey et al. 1999; Ohara et al. 2002; Orden et al. 2003; Piérard et al. 1994; Piérard et al. 1997; Pradel et al. 2000; Prager et al. 2005; Pulz et al. 2003; Read et al. 1992; Rey et al. 2003; Schmid et al. 2002; Stephan and Hoelzle 2000; Stephan et al. 2000; Strockbine et al. 1997; Tamura et al. 1996; Thomas et al. 1993; Urdahl et al. 2002; Urdahl et al. 2003; Wieler et al. 1996; Willshaw et al. 1992, 1993; Zweifel et al. 2004.

this medium was shown to be most effective in selecting for most of the STEC (Bettelheim 1995). This medium was further developed by the addition of antibiotics, which makes it more selective (Lehmacher et al. 1998). In order to characterize whether putative strains of *E. coli* selected are STEC, the only method is to determine whether they produce Shiga toxin(s) and there are a number of methods with which to achieve this.

The use of the commercially available VTEC-Screen "SEIKEN" (Denka Seiken Co., Ltd. Tokyo, Japan) is based on the principle of the agglutination by free Shiga toxin(s) of latex particles sensitized with specific anti-Shigatoxin antibodies. Chart et al. (2001) compared this assay with the Verocell (American Type Culture Collection, Washington DC, U.S.A.) assay (Konowalchuk et al. 1977) for the detection of STEC. Both bacterial supernatants as well as patients' fecal extracts were found to give comparable results in this reversed-passive latex agglutination (RPLA) test, although it was slightly less sensitive than the Verocell assay. Their conclusion was that the "RPLA test proved to be a simple, rapid and convenient method of detecting VT in bacterial culture supernatant fluids and in the feces of patients infected with VTEC."

If the recently developed adaptation of the method (Bettelheim 2001b) is used the suspect colonies can be identified as to whether they are STEC in under three hours. Thus careful examination of the primary isolation media, selecting as great a variety of colonial types, with no preconceived ideas, will very likely pick an STEC within three hours of the examination of the plates. As this adaptation uses very little of the reagents, many colonies can be examined.

The Shiga Toxin colony immunoblot is a novel recently described technique, which has now been made commercially available (Roche Diagnostics GmbH, Mannheim, Germany) as a kit for the detection and isolation of single STEC colonies from a primary isolation plate (Rüger et al. 2001). The basis of these techniques is that colony blots are made from primary or secondary isolation plates and the presence of VTEC on these plates is then detected by either immunological or nucleic acid probes.

The use of the Premier-EHEC (Meridian Bioscience, Inc., Cincinnati, OH, USA) ELISA can detect Shiga toxin in specimens immunologically and has been successfully used to screen specimens for the presence of STEC (Kirchgatterer et al. 2002; Klein et al. 2002).

In a recent review (Bettelheim and Beutin 2003) a number of different methods to detect STEC were evaluated and it was shown that there are many methods available to detect STEC regardless of serotype. However, it was also shown in this review, that there were many commercially available test kits, which can be used for these purposes, but a note of caution was introduced, because a number of these kits have not been subjected to a full

evaluation, which includes testing for all the known Shiga toxin subtypes.

Standard established methods of confirming that the STEC isolate is an *E. coli* and serogrouping, using commercially available antisera, should enable any competent laboratory to isolate, identify, and characterize non-O157 STEC.

THE IMPORTANCE OF NON-O157 STEC

The intensive investigations, which were performed following the outbreak in Australia, which was ascribed to STEC O111:H- will demonstrate the pitfalls of ignoring these pathogens. As a result of a number of previous observations of the importance of STEC O111:H- as a pathogen in Australia (Goldwater and Bettelheim 1994; Gunzburg et al. 1988; Pryor et al. 1990), a rapid PCR technique had been developed in Adelaide, where the outbreak was centered (Paton et al. 1996). This enabled the rapid identification of the presence of STEC O111:H- in a number of patients with HUS, as well as in the mettwurst sausage, which had also been epidemiologically linked to the outbreak. However, one of the patients associated with the outbreak also yielded a strain of STEC O157:H- and further investigation of some of the specimens from both the patients with HUS as well as the mettwurst sausage and some other patients who had diarrhea indicated that as well as O157, a number of other STEC were also involved including strains of serogroups O23, O26, and O91. Of the 23 children with HUS STEC O111:H- was isolated from 19 and from the mettwurst, while STEC O157:H- from three children with HUS and from the mettwurst. This led to the speculation that if the rapid O111 PCR had not been available, the outbreak might well have been falsely ascribed to STEC O157:H- (Goldwater and Bettelheim 1996). This speculation suggested that a number of outbreaks ascribed to STEC O157 may well have been due to other STEC, which were not sought and that STEC O157 may only have been present in small numbers as well as the main STEC serotype. One of the examples quoted in that speculation was a reported HUS outbreak from Argentina where only specimens from one patient (2%) out of 51 children with HUS yielded STEC O157:H7 (Lopez et al. 1989). In their review (Su and Brandt 1995) put an overall figure of 46% to 58% as the incidence range of STEC O157 infections in cases of HUS. The question to be asked, therefore, what about the role of the non-O157 STEC, which then were not sought and in many incidents nowadays are still not sought.

This outbreak in Adelaide was further examined serologically (Kulkarni et al. 2002). This study was the first and so far only comprehensive serological investigation of an HUS outbreak, in which a wide range of *E. coli* 'O' group antigens were employed, to examine the antibody responses. The 51 'O' antigens used in this study comprised four groups, which were those isolated from patients involved in the outbreak, standard strains of the same 'O' groups, other 'O' groups reported in the literature as associated with HUS and 'O' groups belonging to strains commonly isolated from humans, but rarely if ever associated with HUS. In all 49 serum samples from 21 children with HUS from the outbreak were examined as well as 14 single samples from contemporaneous age-matched controls. The serological studies were performed completely independently of the clinical assessments of the patients. While there was minimal seroreactivity from the contemporaneous age-matched controls sera and minimal seroreactivity by the clinical sera with the non-HUS linked 'O' group antigens, there were extensive serological reactions with the sera from the patients. The conclusions, reached from these studies, clearly showed that the more serious the clinical condition of the patient, the greater was the number of different HUS-associated 'O' group antigens against which that patient's serum gave a positive reaction. This suggests that severity of HUS may well be reflected by multiplicity of infecting STEC. Thus, again stressing that looking for only STEC O157 may well give a totally erroneous picture of an outbreak, even if it is successfully cultivated.

E. coli outbreak

In September 2006, reports emerged in the popular press, as well as ProMED, of an outbreak of STEC O157, in eight of states of the United States, in which at least 49 persons had got sick, eight with HUS and one person had died. On the basis of molecular biological studies on the isolates from patients as well as fresh bagged spinach, which had been epidemiologically linked to the outbreak, it appeared that the same STEC O157:H7 strain was involved in all cases. A warning was therefore issued that consumers should avoid eating such fresh bagged spinach. By 14.09.06 ten states including 58 patients were affected and Californian spinach was suspected of being involved. Based on a number of earlier studies, the FDA warned people about eating such spinach and also stated that washing it is unlikely to remove the pathogens completely. The number of reported cases rose to 94 with 14 of HUS by the 15th and the number of affected states doubled from 10 to 20. It was also reported that the potentially contaminated spinach may have been exported to Canada and Mexico. The company supplying the spinach from San Juan Bautista, California had started recalling all its products that contain spinach in all the brands they pack with "Best if Used by Dates" of "17 Aug 2006 through 1 Oct 2006." By the 16th, the number cases had risen to 102 with 16 of HUS. The investigations are continuing. The most likely source of contamination of the spinach is water, contaminated with fecal matter of bovine origin. Cattle commonly shed STEC, including O157 and this can contaminate ground water and thus sources for irrigation of crops like spinach and lettuce. It is noteworthy that on the 14th September there was also a report from Sweden of what was described as the largest outbreak of STEC O157 ever recorded in humans in any Nordic country. This was ascribed to contaminated lettuce. The report listed 135 cases, which included 11 with HUS. Studies had revealed the presence of an identical strain isolated from cattle of a nearby farm, upstream the irrigation point of the lettuce. However, the investigators were unable to isolate the strain from the lettuce. These two reports confirm that STEC, especially O157, remain important pathogens, however, in the reports of neither outbreak was there a mention as to whether non-O157 STEC were sought. Water used to irrigate growing vegetables, contaminated by fecal matter of bovine origin is likely to contain a variety of *E. coli* most probably including non-O157 STEC as well as the O157 STEC. It could be speculated that the HUS cases and

other more serious cases of infection may well have been due to ingestion of more than one STEC type.

CONCLUSIONS

Non-O157 STEC have been shown to be important pathogens, despite being severely under-reported, because in many laboratories the facility to isolate, identify and characterize them do not exist. Where they do exist non-O157 STEC are found. They may cause many outbreaks either alone or in conjunction with other pathogens including O157 STEC (Goldwater and Bettelheim 1996), and viruses (Bettelheim et al. 1999; Marshall et al. 2001). Unless outbreaks are subjected to full microbiological assessments, no adequate conclusions about the role of the pathogen or pathogens, which have been identified, can be made. Similarly, there have been numerous studies on the ecology, distribution and related microbiological factors on STEC O157, without taking into consideration the presence of other STEC or even of the *E. coli* flora as a whole, thus it seems that many false conclusions can be made. Such studies should take into consideration that there is a great diversity of STEC and *E. coli* in general in the feces of humans (Bettelheim et al. 1972) and animals such as cattle (Bettelheim et al. 2005). As microbiologists, we ignore the non-O157 STEC at our peril.

REFERENCES

Allerberger, F. 1996. Enterohemorrhagic *E. coli* (EHEC) infections in tourists (Austria). Notiziaro dell'Istituto Superiore di Sanita 9,N.11(Suppl.3):3.

Allerberger, F., Rossbpth, D., Direrich, M.P., Aleksic, S., Schmidt, H., and Karch, H. 1996. Prevalence and clinical manifestations of Shiga Toxin-producing *Escherichia coli* infections in Austrian children. *Europ. J. Clin. Microbiol. Infect. Dis.* 15, 545–550.

Anon, 2002. Verotoxigenic *Escherichia coli*. Annual Report on Zoonoses in Denmark 2002.

Asakura, H., Makino, S., Shirahata, T., Tsukamoto, T., Kurazono, H., Ikeda, T., and Takeshi, K. 1998. Detection and genetical characterization of shiga toxin-producing *Escherichia coli* from wild deer. *Microbiol. Immunol.* 42, 815–822.

Atalla, H.N., Johnson, R., McEwen, S., Usborne, R.W., and Gyles, C.L. 2000. Use of a Shiga toxin (Stx)-enzyme-linked immunosorbent assay and immunoblot for detection and isolation of Stx-producing *Escherichia coli* from naturally contaminated beef. *J. Food Protec.* 63, 1167–1172.

Baker, M., Bennett, J., and Nicol, C. 1999. Emergence of verotoxigenic *Escherichia coli* (VTEC) in New Zealand. *New Zealand Publ. Hlth. Rpt.* 6, 9–12.

Banatvala, N., Debeukelaer, M.M., Griffin, P.M., Barrett, T.J., Greene, K.D., Green, J.H., and Wells, J.G. 1996. Shiga-like toxin producing *Escherichia coli* O111 and associated hemolytic-uremic syndrome: a family outbreak. *Ped. Infect. Dis. J.* 15, 1008–1011.

Bennett, J., and Bettelheim, K.A. 2002. Serotypes of non-O157 verocytotoxigenic *Escherichia coli* isolated from meat in New Zealand. *Comp. Immunol. Microbiol. Infect. Dis.* 25(2), 77–84.

Bergmire-Sweat, D., Marengo, L., Pendergrass, P., Hendricks, K., Garcia, M., Drumgoole, R., Baldwin, T., Kingsley, K., Walsh, B., Lang, S., Prine, L., Busby, T., Trujillo, L., Perrotta, D., Hathaway, A., Jones, B., and Jaiyeola, A. 2000. *Escherichia coli* O111:H8 outbreak among teenage campers—Texas, 1999. *JAMA* 283, 2517.

Bettelheim, K.A. 1995. Identification of enterohaemorrhagic *Escherichia coli* by means of their production of enterohaemolysin. *J. Appl. Bacteriol.* 79, 178–180.

Bettelheim, K.A. 1997. Biochemical Studies on Enterohaemorrhagic *Escherichia coli* (EHEC). In: D. Kay and C. Fricker, eds. *Coliforms and E. coli Problem or Solution*, , pp. 243–248, Cambridge, United Kingdom: The Royal Society of Chemistry Information Services.

Bettelheim, K.A. 1998a. Studies of *Escherichia coli* cultured on Rainbow™ agar O157 with particular reference to Enterohaemorrhagic *Escherichia coli* (EHEC). *Microbiol. Immunol.* 42, 265–269.

Bettelheim, K.A. 1998b. Reliability of CHROMagar® for the detection of Enterohaemorrhagic *Escherichia coli* (EHEC) O157 but not EHEC belonging to other serogroups. *J. Appl. Bacteriol* 85, 425–428.

Bettelheim, K.A. 2001a. Enterohaemorrhagic *Escherichia coli* O157:H7: a red herring? *J. Med. Microbiol.* 50, 201–202.

Bettelheim, K.A. 2001b. Development of a rapid method for the detection of verocytotoxin-producing *Escherichia coli* (VTEC). *Lett. Appl. Microbiol.* 33, 31–35.

Bettelheim, K.A. 2005. Reliability of O157:H7 ID agar (O157H7 ID-F) for the detection and isolation of verocytotoxigenic strains of *Escherichia coli* Microbione belonging to serogroup O157. *J. Appl. Microbiol.* 99, 408–410.

Bettelheim, K.A. Microbionet. http://www.microbionet.com.au/vtectable.htm. Accessed October 2006.

Bettelheim, K.A., Bensink, J.C., and Tambunan, H.S. 2000. Serotypes of verotoxin-producing (Shiga toxin-producing) *Escherichia coli* isolated from healthy sheep. *Comp. Immunol. Microbiol. Infect. Dis.* 23, 1–7.

Bettelheim, K.A., Bowden, D.S., Doultree, J., Catton, M.G., Chibo, D., Ryan, N.J.P., Wright, P.J., Gunesekere, I.C., Griffith, J.M., Lightfoot, D., Hogg, G.G., Bennett-Wood, V., and Marshall, J.A. 1999. Combined infection of Norwalk-like virus and Verotoxin-producing bacteria associated with a gastroenteritis outbreak. *J. Diarrhoeal Dis. Res.* 17, 34–36.

Bettelheim, K.A., Breadon, A., Faiers, S.M., O'Farrell, S.M., and Shooter, R.A. 1974. The origin of "O" serotypes of *Escherichia coli* in babies after normal delivery. *J. Hyg. Camb.* 73, 67–70.

Bettelheim, K.A., Brown, J.E., Lolokha, S., and Echeverria, P. 1990. Serotypes of *Escherichia coli* that hybridized with DNA probes for genes encoding Shiga-like Toxin I, Shiga-like Toxin II and serogroup O157 enterohemorrhagic *E. coli* fimbriae isolated from adults with diarrhea in Thailand. *J. Clin. Microbiol.* 28, 293–295.

Bettelheim, K.A., Cooke, E.M., O'Farrell, S.M., and Shooter, R.A. 1977. The effect of diet on intestinal *Escherichia coli*. *J. Hyg., Camb.* 79, 43–45.

Bettelheim, K.A., Faiers, M., and Shooter, R.A. 1972. Serotypes of *Escherichia coli* in normal stools. *Lancet*. ii, 1224–1226.

Bettelheim, K.A., Kuzevski, A., Gilbert, R.A., Krause, D.O., and McSweeney, C.S. 2005. The diversity of *Escherichia coli* serotypes and biotypes in cattle faeces. *J. Appl. Microbiol.* 98, 699–709.

Bettelheim, K.A., and Lennox-King, S.M. 1976. The acquisition of *Escherichia coli* by newborn babies. *Infection* 4, 174–179.

Bettelheim, K.A., Whipp, M., Djordjevic, S.P., and Ramachandran, V. 2002. First isolation outside Europe of sorbitol-fermenting verocytotoxigenic *Escherichia coli* (VTEC) belonging to O group O157. *J. Med. Microbiol.* 51, 713–714.

Beutin, L. 2006. Emerging enterohaemorrhagic *Escherichia coli*, causes and effects of the rise of a human pathogen. *J. Vet. Med. B* 53, 299–305.

Beutin, L., Montenegro, M.A., Ørskov, I., Ørskov, F., Prada, J., Zimmermann, S., and Stephan, R. 1989. Close association of verocytotoxin (Shiga-like Toxin) production with enterohemolysin production in strains of *Escherichia coli*. *J. Clin. Microbiol.* 27, 2559–2564.

Beutin, L., Geier, D., Steinrück, H., Zimmermann, S., and Scheutz, F. 1993. Prevalence and some properies of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J. Clin. Microbiol.* 31, 2483–2488.

Beutin, L., Geier, D., Zimmermann, S., Aleksic, S., Gillespie, H.A., and Whittam, T.S. 1997. Epidemiological relatedness and clonal types of natural populations of *Escherichia coli* strains producing shiga toxins in separate populations of cattle and sheep. *Appl. Environm. Microbiol.* 63, 2175–2180.

Beutin, L., Kaulfuss, S., Herold, S., Oswald, E., and Schmidt, H. 2005. Genetic analysis of enteropathogenic and enterohemorrhagic *Escherichia coli* O103 strains by molecular typing of virulence and housekeeping genes and pulsed-field gel electrophoresis. *J. Clin. Microbiol.* 43, 1552–1563.

Beutin, L., Krause, G., Zimmermann, S., Kaulfuss, S., and Gleier, K. 2004. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. *J. Clin. Microbiol.* 42, 1099–1108.

Beutin, L., and Müller, W. 1998. Cattle and verotoxigenic *Escherichia coli* (VTEC), an old relationship. *Vet. Rec* 142, 283–284.

Beutin, L., Zimmermann, S., and Gleier, K. 1998. Human infections with Shiga toxin-producing *Escherichia coli* other than serogroup O157 in Germany. *Emerg. Infect. Dis.* 4, 635–639.

Bielaszewska, M., Janda, J., Bláhova, K., Srámkova, L., Havlík, J., and Potuzník, V. 1994. Verocytotoxin-producing *Escherichia coli* in children with hemolytic uremic syndrome and diarrhea in the Czech Republic. In: Ed. Karmali and A.G. Goglio, eds. *Recent Advances in Verocytotoxin-producing Escherichia Coli Infections*, , pp. 37–40: Excerpta Medica International Congress Series 1072, Elsevier Science, Amsterdam, The Netherlands.

Bielaszewska, M., Janda, J., Bláhova, K., Feber, J., Potuzník, V., and Souckova, A. 1996. Verocytotoxin-producing *Escherichia coli* in children with hemolytic uremic syndrome in the Czech Republic. *Clin. Nephrol.* 46, 42–44.

Blanco, M., Blanco, J.E., Gonzalez, E.A., Mora, A., Jansen, W., Gomes, T.A.T., Zerbini, L.F., Yano, T., Decastro, A.F.P., and Blanco, J. 1997. Genes coding for enterotoxins and verotoxins in porcine *Escherichia coli* strains belonging to different O:K:H serotypes: Relationship with toxic phenotypes. *J. Clin. Microbiol.* 35, 2958–2963.

Blanco, J., Blanco, M., Blanco, J.E., Mora, A., González, E.A., Bernárdez, M.I., Alonso, M.P., Coira, A., Rodriguez, A., Rey, J., Alonso, J.M., and Usera, M.A. 2003a. Verotoxin-producing *Escherichia coli* (VTEC) in Spain: prevalence, serotypes, and virulence genes of O157:H7 and non-O157:H7 VTEC in ruminants, raw beef products, and humans. *Exp. Biol. Med.* 228, 345–351.

Blanco, M., Blanco, J.E., Blanco, J., Mora, A., Prado, C., Alonso, M.P., Mourino, M., Madrid, C., Balsalobre, C., and Veter, J.A. 1997. Distribution and characterization of faecal verotoxin-producing *Escherichia coli* (VTEC) isolated from healthy cattle. *Vet. Microbiol.* 54, 309–319.

Blanco, M., Blanco, J.E., Mora, A., Rey, J., Alonso, J.M., Hermoso, M., Hermoso, J., Alonso, M.P., Dahbi, G., González, E.A., Bernárdez, M.I., and Blanco, J. 2003b. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from healthy sheep in Spain. *J. Clin. Microbiol.* 41, 1351–1356.

Blanco, A., Blanco, J.E., Mora, A., Dahbi, G., Alonso, A.P., Gonzalez, E.A., Bernardez, M.I., and Blanco, J. 2004. Serotypes, virulence genes, and intimin types of shiga toxin (verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae-ξ). *J. Clin. Microbiol.* 42, 645–651.

Bockemühl, J., Aleksic, S., and Karch, H. 1992. Serological and biochemical properties of Shiga-like toxin (Verotoxin)-producing strains of *Escherichia coli*, other than O-group 157, from patients in Germany. *Zblt. Bakt.* 276, 189–195.

Bockemühl, J., Karch, H., and Tschäpe H. 1998. Zur Situation der Infektionen des Menschen durch enterohämorrhagische *Escherichia coli* (EHEC) in Deutschland 1997. *Bundesgesundheitsblatt Sonderheft Oktober* 1998. 2–5.

Bokete, T.N., Whittam, T.S., Wilson, R.A., Clausen, C.R., Ocallahan, C.M., Moseley, S.L., Fritsche, T.R., and Tarr, P.I. 1997. Genetic and phenotypic analysis of *Escherichia coli* with enteropathogenic characteristics isolated from Seattle children. *J. Infect. Dis.* 175, 1382–1389.

Bonardi, S., Foni, E., Brindani, F., Bacci, C., Chiapponi, C., and Cavallini, P. 2004. Detection and characterization of verocytotoxin-producing *Escherichia coli* (VTEC) O157 and non-O157 in cattle at slaughter. *Microbiologica* 27, 255–261.

Bonnet, R., Souweine, B., Gauthier, G., Rich, C., Livrelli, V., Sirot, J., Joly, B., and Forrestier, C. 1998. Non-O157:H7 Stx2-producing *Escherichia coli* strains associated with hemolytic uremic syndrome in adults. *J. Clin. Microbiol.* 36, 1777–1780.

Brett, K.N., Hornitzky, M.A., Bettelheim, K.A., Walker, K., and Djordjevic, S.P. 2003a. Bovine non-O157 Shiga toxin 2-containing *Escherichia coli* isolates commonly possess stx2-EDL933 and/or stx2vhb subtypes. *J. Clin. Microbiol.* 41, 2716–2722.

Brett, K.N., Ramachandran, V., Hornitzky, M.A., Bettelheim, K.A., Walker, K., and Djordjevic, S.P. 2003b. *stx*1c Is the most common Shiga toxin 1 subtype among Shiga toxin-producing *Escherichia coli* isolates from sheep but Not among isolates from cattle. *J. Clin. Microbiol.* 41, 926–936.

Brooks, H.J., Bettelheim, K.A., Todd, B., and Holdaway, M.D. 1997. Non-O157 Vero cytotoxin producing *Escherichia coli*: Aetiological agents of diarrhoea in children in Dunedin, New Zealand. *Comp. Immunol. Microbiol. Infect. Dis.* 20, 163–170.

Brooks, J.T., Bergmire-Sweat, D., Kennedy, M., Hendricks, K., Garcia, M., Marengo, L., Wells, J., Ying, M., Bibb, W., Griffin, P.M., Hoekstra, R.M., and Friedman, C.R. 2004. Outbreak of Shiga toxin—Producing Escherichia coli O111:H8 infections among attendees of a high school cheerleading camp. *Clin. Infect. Dis.* 38, 190–198.

Brunder, W., Schmidt, H., Frosch, M., and Karch, H. 1999. The large plasmids of Shiga toxin-producing *Escherichia coli* (STEC) are highly variable genetic elements. *Microbiology UK* 145, 1005–1014.

Cantarelli, V., Nagayama, K., Takahashi, A., Honda, T., Caudoro, P.F., Dias, C.A., Mezzari, A., and Brodt, T. 2000. Isolation of shiga toxin-producing *Escherichia coli* (STEC) serotype O91:H21 from a child with diarrhea in Porto Alegre City, RS, Brazil. *Brazilian J. Microbiol.* 31, 266–270.

Caprioli, A., and Luzzi, I. 1995. VT2-producing *E. coli* O26:H11. *Notiziaro dell'Istituto Superiore di Sanita* 8, 4.

Caprioli, A., Luzzi, I., Rosmini, F., Resti, C., Edelfonti, A., Perfumo, F., Farina, C., Goglio, A., Gianviti, A., and Rizzoni, G. 1994. Community wide outbreak of hemolytic uremic syndrome associated with non-O157 verocytotoxin-producing *Escherichia coli*. *J. Infect. Dis.* 169, 208–211.

Carroll, K.C., Adamson, K., Korgenski, K., Croft, A., Hankemeier, R., Daly, J., and Park, C.H. 2003. Comparison of a commercial reversed passive latex agglutination assay to an enzyme immunoassay for the detection of shiga toxin-producing *Escherichia coli*. *Europ. J. Clin. Microbiol. Infect. Dis.* 22(11), 689–692.

Cerqueira, A.M., Tibana, A., and Guth, B.E.C. 1997. High occurrence of Shiga-like toxin-producing *Escherichia coli* isolated from raw beef products in Rio de Janeiro City, Brazil. *J. Food Protect.* 60, 177–180.

Chapman, P.A., Siddons, C.A., Wright, D.J., Norman, P., Fox, J., and Crick, E. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect.* 111, 439–447.

Chart, H., Willshaw, G.A., and Cheasty, T. 2001. Evaluation of a reversed passive latex agglutination test for the detection of Verocytotoxin (VT) expressed by strains of VT-producing *Escherichia coli*. *Lett. Appl. Microbiol.* 32, 370–374.

Clarke, R.C., Wilson, J.B., Read, S.C., Renwick, S., Rahn, K., Johnson, R.P., Alves, D., Karmali, M.A., Lior, H., McEwen, S.A., Spika, J., and Gyles, C.L. 1994. Verocytotoxin-producing *Escherichia coli* (VTEC) in the food chain: preharvest and processing perspectives. Recent Advances in Verocytotoxin-producing Escherichia Coli Infections.Verocytotoxin-producing *Escherichia coli* in children with hemolytic uremic syndrome and diarrhea in the Czech Republic. In: Ed. Karmali and A.G. Goglio, eds. *Recent Advances in Verocytotoxin-producing Escherichia Coli Infections*, , pp. 17–24: Excerpta Medica International Congress Series 1072, Elsevier Science, Amsterdam, The Netherlands.

Cobbold, R., and Desmarchelier, P. 2000. A longitudinal study of Shiga-toxigenic *Escherichia coli* (STEC) prevalence in three Australian dairy herds. *Vet. Microbiol.* 71, 125–137.

Cobbold, R., and Desmarchelier, P. 2001. Characterisation and clonal relationships of Shiga-toxigenic Escherichia coli (STEC) isolated from Australian dairy cattle. *Vet. Microbiol.* 79, 323–335.

Cortés, C., De la Fuente, R., Blanco, J., Blanco, M., Blanco, J.E., Dhabi, G., Mora, A., Justel, P., Contreras, A., Sánchez, A., Corrales, J.C., and Orden, J.A. 2005. Serotypes, virulence genes and intimin types of verotoxin-producing *Escherichia coli* and enteropathogenic *E. coli* isolated from healthy dairy goats in Spain. *Vet. Microbiol.* 110, 67–76.

Czirok, E., and Herpay, M. 1995. National surveillance of VTEC infection (Hungary). *Notiziaro dell'Istituto Superiore di Sanita.* 8, 2.

DesRosiers, A., Fairbrother, J.M., Johnson, R.P., Desautels, C., Letellier, A., and Quessy, S. 2001. Phenotypic and genotypic characterization of *Escherichia coli* verotoxin-producing isolates from humans and pigs. *J. Food Protect.* 64, 1904–1911.

Djordjevic, S.P., Hornitzky, M.A., Bailey, P.G., Vanselow, B., Walker, K., and Bettelheim, K.A. 2001. Virulence properties and serotypes of Shiga toxin-producing *Escherichia coli* from healthy Australian slaughter-age sheep. *J. Clin. Microbiol.* 39, 2017–2021.

Djordjevic, S.P., Ramachandran, V., Bettelheim, K.A., Vanselow, B., Holst, P., Bailey, P.G., and Hornitzky, M.A. 2004. Serotypes and virulence gene profiles of Shiga toxin-producing *Escherichia coli* strains isolated from feces of pasture-fed and lot-fed sheep. *Appl. Environ. Microbiol.* 70, 3910–3917.

Domingue, G., Willshaw, G.A., Smith, H.R., Perry, N., Radford, D., and Cheasty, T. 2003. DNA-based subtyping of verocytotoxin-producing *Escherichia coli* (VTEC) O128ab:H2 strains from human and raw meat sources. *Lett. Appl. Microbiol.* 37, 433–437.

Dorn, C.R., Scotland, S.M., Smith, H.R., Willshaw, G.A., and Rowe, B. 1989. Properties of Verocytotoxin-producing *Escherichia coli* of human and animal origin belonging to serogroups other than O157:H7. *Epidemiol. Infect.* 103, 83–95.

Dorn, C.R., Francis, D.H., Angrick, E.J., Wilgohs, J.A., Wilson, R.A., Collins, J.E., Jenke, B.H., and Shawd, S.J. 1993. Characteristics of verocytotoxin-producing *Escherichia coli* associated with intestinal colonization and diarrhea in calves. *Vet. Microbiol.* 36, 149–159.

Dreesman, J., and Pulz, M. 2004. On the epidemiology of human enterohemorrhagic *Escherichia coli*-infections in Lower Saxony. *Dtsche Tierärzt. Wchnschrift* 111, 317–320.

Eklund, M., Scheutz, F., and Siitonen, A. 2001. Clinical isolates of non-0157 shiga toxin-producing *Escherichia coli*: Serotypes, virulence characteristics, and molecular profiles of strains of the same serotype. *J. Clin. Microbiol.* 39, 2829–2834.

Eklund, M., Leino, K., and Siitonen, A. 2002. Clinical *Escherichia coli* strains carrying stx genes: stx variants and stx-positive virulence profiles. *J. Clin. Microbiol.* 40, 4585–4593.

Enami, M., Nakasone, N., Honma, Y., Kakinohana, S., Kudaka, J., and Iwanaga, M. 1999. Expression of type I pili is abolished in verotoxin-producing *Escherichia coli* O157. *FEMS Microbiol. Lett.* 179, 467–472.

Escherich, Th. 1885. Die Darmbakterien des Neugeborenen un Säuglings. *Fortschritte der Medizin* 3, 547–554.

Escherich, Th. Translated by Bettelheim, K.A. 1988. The Intestinal Bacteria of the Neonate and Breast-Fed Infant. Pt.1. *Rev. Infect. Dis.* 10:1220–1225

Escherich, Th. Translated by Bettelheim, K.A. 1989. The Intestinal Bacteria of the Neonate and Breast-Fed Infant. Pt.2. *Rev. Infect. Dis.* 11:352–356

Evans, J., Wilson, A., Willshaw, G.A., Cheasty, T., Tompkins, D.S., Wheeler, J.G., and Smith, H.R. 2002. Vero cytotoxin-producing *Escherichia coli* in a study of infectious intestinal disease in England. *Clin. Microbiol. Infect.* 8, 183–186.

Fagan, P.K., Hornitzky, M.A., Bettelheim, K.A., and Djordjevic, S.P. 1999. Detection of Shiga-like toxin (*stx1* and *stx2*), Intimin (*eaeA*), and Enterohemorhagic *Escherichia coli* (EHEC) Hemolysin EHEC *hlyA*) genes in animal feces by multiplex PCR. *Appl. Environ. Microbiol.* 65, 868–872.

Farina, C., Goglio, A., Conedera, G., Minelli, F., and Caprioli, A. 1996. Antimicrobial Susceptibility of *Escherichia coli* O157 and other enterohaemorrhagic *Escherichia coli* isolated in Italy. *Europ. J. Clin. Microbiol. Infect. Dis.* 15, 351–353.

Fey, P.D., Wickert, R.S., Rupp, M.E., Safranek, T.J., and Hinrichs, S.H. 2000. Prevalence of non-O157:H7 shiga toxin-producing *Escherichia coli* in diarrheal stool samples from Nebraska. *Emerg. Infect. Dis.* 6, 530–533.

Fratamico, P.M., Bagi, L.K., Bush, E.J., and Solow, B.T. 2004. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* in swine feces recovered in the National Animal Health Monitoring System's Swine 2000 Study. *Appl. Environ. Microbiol.* 70, 7173–7178.

Friedrich, A.W., Bielaszewska, M., Zhang, W.L., Pulz, M., Kuczius, T., Ammon, A., and Karch, H. 2002. *Escherichia coli* harboring shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J. Infect. Dis.* 185, 74–84.

Fukushima, H., and Seki, R. 2004. High numbers of Shiga toxin-producing *Escherichia coli* found in bovine faeces collected at slaughter in Japan. *FEMS Microbiol. Lett.* 238, 189–197.

Gallien, P., Richter, H., Klie, H., Timm, M., Karch, H., Perlberg, K.-W., Steinrück, H., Riemer, S., Djuren, M., and Protz, D. 1997. Detection of STEC and epidemiological investigationsin surrounding of a HUS patient. *Berlin. Münch. Tierärzt. Wochensch.* 110, 342–346.

Gallien, P., Much, C., Perlberg, K.W., and Protz, D. 1999. Subtyping of stx-genes in Shigatoxin producing *Escherichia coli* (STEC). Presence in raw foods and correlation to other factors. *Fleischwirtschaft.* 79, 99–103.

Gallien, P., Karch, H., Much, C., Steinruck, H., Lehmann, S., Timm, M., Richter, H., Perlberg, K.W., and Protz, D. 2000. Subtyped eae-genes in Shigatoxin-producing *Escherichia coli* (STEC)—Occurence in raw or undercooked food samples and comparison of isolates from faecal samples and stool samples. *Fleischwirtschaft* 80, 84–89.

Gallien, P., Timm, M., and Lehmann, S. 1999. Existence of the pathogenic island LPA in shigatoxin-producing *Escherichia coli*. *Fleischwirtschaft.* 82, 119–122.

García-Aljaro, C., Muniesa, M., Blanco, J.E., Blanco, M., Blanco, J., Jofre, J., and Blanch, A.R. 2005. Characterization of Shiga toxin-producing *Escherichia coli* isolated from aquatic environments. *FEMS Microbiol. Lett.* 246, 55–65.

Garrido, P., Blanco, M., Moreno-Paz, M., Briones, C., Dahbi, G., Blanco, J., Blanco, J., and Parro, V. 2006. STEC-EPEC oligonucleotide microarray: A new tool for typing genetic variants of the LEE pathogenicity island of human and animal shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) strains. *Clin. Chem.* 52, 192–201.

Gerber, A., Karch, H., Allerberger, F., Verweyen, H.M., and Zimmerhackl, L.B. 2002. Clinical course and the role of Shiga toxin-producing *Escherichia coli* infection in the hemolytic-uremic syndrome in pediatric patients, 1997–2000, in Germany and Austria: A prospective study. *J. Infect. Dis.* 186, 493–500.

Geue, L., Segura-Alvarez, M., Conraths, F.J., Kuczius, T., Bockemühl, J., Karch, H., and Gallien, P. 2002. A long-term study on the prevalence of shiga toxin-producing *Escherichia coli* (STEC) on four German cattle farms. *Epidemiol. Infect.* 129, 173–185.

Giammanco, A., Maggio, M., Giammanco, G., Morelli, R., Minelli, F., Scheutz, F., and Caprioli, A. 1996. Characteristics of *Escherichia coli* strains belonging to enteropathogenic *E. coli* serogroups isolated in Italy from children with diarrhea. *J. Clin. Microbiol.* 34, 689–694.

Gioffré, A., Meichtri, L., Miliwebsky, E., Baschkier, A., Chillemi, G., Romano, M.I., Estani, S.S., Cataldi, A., Rodríguez, R., and Rivas, A. 2002. Detection of Shiga toxin-producing *Escherichia coli* by PCR in cattle in Argentina. Evaluation of two procedures. *Vet. Microbiol.* 87, 301–313.

Giraldi, R., Guth, B.E.C., and Trabulsi, L.R. 199 Production of Shiga-like toxin among Escherichia coli strains and other bacteria isolated from diarrhea in São Paulo, Brazil. *J. Clin. Microbiol.* 28, 1460–1462.

Gobius, K.S., Higgs, G.M., and Desmarchelier, P.M. 2003. Presence of activatable Shiga toxin genotype (stx2 d) in Shiga toxigenic *Escherichia coli* from livestock sources. *J. Clin. Microbiol.* 41, 3777–3783.

Goldwater, P.N., and Bettelheim, K.A. 1994. The role of enterohaemorrhagic *E. coli* serotypes other than O157:H7 as causes of disease. Recent Advances in Verocytotoxin-producing Escherichia Coli Infections. In Verocytotoxin-producing *Escherichia coli* in children with hemolytic uremic syndrome and diarrhea in the Czech Republic. In: Ed. Karmali and A.G. Goglio, eds. *Recent Advances in Verocytotoxin-producing Escherichia Coli Infections*, , pp. 57–60, Amsterdam, The Netherlands: Excerpta Medica International Congress Series 1072, Elsevier Science.

Goldwater, P.N., and Bettelheim, K.A. 1996. An outbreak of Hemolytic Uremic Syndrome due to *Escherichia coli* O157:H7: Or was it? *Emerg. Infect. Dis.* 2, 153–154.

Goldwater, P.N., Giles, N., and Bettelheim, K.A. 1998. An unusual case of microangiopathic haemolytic anaemia associated with enterohaemorrhagic *Escherichia coli* O113:H21 infection, a verocytotoxin-2/shiga toxin-2 producing serotype. *J. Infect.* 37, 302–304.

Gunning, R.F., Wales, A.D., Pearson, G.R., Done, E., Cookson, A.L., and Woodward, M.J. 2001. Attaching and effacing lesions in the intestines of two calves associated with natural infection with Escherichia coli O26:H11. *Vet. Rec.* 148, 780–782.

Gunzburg, S., Gracey, M., Forbes, D., Hewitt, I., and Bettelheim, K.A. 1988. Haemolytric-uraemic syndrome and verotoxigenic *Esch. coli*. *Med. J. Australia* 149, 54–55.

Guth, B.E.C., Chinen, I., Miliwebsky, E., Cerqueira, A.M.F., Chillemi, G., Andrade, J.R.C., Baschkier, A., and Rivas, M. 2003. Serotypes and Shiga toxin genotypes among *Escherichia coli* isolated from animals and food in Argentina and Brazil. *Vet. Microbiol.* 92(4), 335–349.

Guth, B.E.C., de Souza, R.L., Vaz, T.M.I., and Irino, K. 2002. First Shiga toxin-producing *Escherichia coli* isolate from a patient with hemolytic uremic syndrome, Brazil. *Emerg. Infect. Dis.* 8(5), 535–536.

Guth, B.E.C., Vaz, T.M.I., Gomes, T.A.T., Chinarelli, S.H., Rocha, M.M.M., de Castro, A.F.P., and Irino, K. 2005. Re-emergence of O103:H2 Shiga toxin-producing *Escherichia coli* infections in Sao Paulo, Brazil. *J. Med. Microbiol.* 54, 805–806.

Heuvelink, A.E., Wernars, K., and de Boer, E. 1996. Occurrence of *Escherichia coli* O157 and other verocytotoxin-producing *E. coli* in retail raw meats in the Netherlands. *J. Food Protect.* 59, 1267–1272.

Hiramatsu, R., Matsumoto, M., Miwa, Y., Suzuki, Y., Saito, M., and Miyazaki, Y. 2002. Characterization of Shiga toxin-producing *Escherichia coli* O26 strains and establishment of selective isolation media for these strains. *J. Clin. Microbiol.* 40, 922–925.

Hiruta, N., Murase, T., and Okamura, N. 2001. An outbreak of diarrhoea due to multiple antinicrobial-resistant Shiga toxin-producing *Escherichia coli* O26:H11 in a nursery. *Epidemiol. Infect.* 127, 221–227.

Hornitzky, M.A., Bettelheim, K.A., and Djordjevic, S.P. 2000. The isolation of enterohaemorrhagic *Escherichia coli* O111:H- from Australian cattle. *Aust. Vet. J.* 78, 636–637.

Hornitzky, M.A., Bettelheim, K.A., and Djordjevic, S.P. 2001. The detection of Shiga toxin-producing *Escherichia coli* in diagnostic bovine faecal samples using vancomycin-cefixime-cefsulodin blood agar and PCR. *FEMS Microbiol. Lett.* 198, 17–22.

Hornitzky, M.A., Vanselow, B.A., Walker, K., Bettelheim, K.A., Corney, B., Gill, P., Bailey, G., and Djordjevic, S.P. 2002. Virulence properties and serotypes of Shiga toxin-producing *Escherichia coli* from healthy Australian cattle. *Appl. Environ. Microbiol.* 68, 6439–6445.

Hornitzky, M.A., Mercieca, K., Bettelheim, K.A., and Djordjevic, S.P. 2005. Bovine feces from animals with gastrointestinal infections are a source of serologically diverse atypical enteropathogenic *Escherichia coli* and Shiga toxin-producing *E. coli* strains that commonly possess intimin. *Appl. Environ. Microbiol.* 71, 3405–3412.

Huppertz, H.-I., Busch, D., Schmidt, H., Aleksic, S., and Karch, H. 1996. Diarrhea in young children associated with *Escherichia coli* non-O157 organisms that produce Shiga-like toxin. *J. Ped* 128, 341–346.

Huppertz, H.I., Rutkowski, S., Busch, D.H., Eisebit, R., Lissner, R., and Karch, H. 1999. Bovine colostrum ameliorates diarrhea in infection with diarrheagenic *Escherichia coli*, Shiga toxin-producing *E. coli*, and *E. coli* expressing intimin and hemolysin. *J. Ped. Gastroenterol. Nutrit* 29, 452–456.

Hussein, H.S., Thran, B.H., Hall, M.R., Kvasnicka, W.G., and Torell, R.C. 2003. Verotoxin-producing *Escherichia coli* in culled beef cows grazing rangeland forages. *Exp. Biol. Med.* 228, 352–357.

Hussein, H.S., Thran, B.H., and Glimp, H.A. 2003. Verotoxin-producing *Escherichia coli* in sheep grazing an irrigated pasture or arid rangeland forages. *Exp. Biol. Med.* 228, 358–364.

Irino, K., Kato, M.A., Vaz, T.M.I., Ramos, I.I., Souza, M.A., Cruz, A.S., Gomes, T.A.T., Vieira, M.A.M., and Guth, B.E.C. 2005. Serotypes and virulence markers of Shiga toxin-producing *Escherichia coli* (STEC) isolated from dairy cattle in Sao Paulo State, Brazil. *Vet. Microbiol.* 105, 29–36.

Ishii, Y., Kimura, S., Alba, J., Shiroto, K., Otsuka, M., Hashizume, N., Tamura, K., and Yamaguchi, K. 2005. Extended-spectrum β-lactamase-producing Shiga toxin gene (stx1)–positive *Escherichia coli* O26:H11: a new concern. *J. Clin. Microbiol.* 43, 1072–1075.

Jelacic, J.K., Damrow, T., Chen, G.S., Jelacic, S., Bielaszewska, M., Ciol, M., Carvalho, H.M., Melton-Celsa, A.R., O'Brien, A.D., and Tarr, P.I. 2003. Shiga toxin-producing *Escherichia coli* in Montana: Bacterial genotypes and clinical profiles. *J. Infect. Dis.* 188, 719–729.

Jenkins, C., Pearce, M.C., Chart, H., Cheasty, T., Willshaw, G.A., Gunn, G.J., Dougan, G., Smith, H.R., Synge, B.A., and Frankel, G. 2002. An eight-month study of a population of verocytotoxigenic *Escherichia coli* (VTEC) in a Scottish cattle herd. *J. Appl. Microbiol.* 93, 994–953.

Jensen, C., Schiellerup, P., Olsen, K.E., Scheutz, F., Petersen, E., Gerner-Smidt, P., and Mølbak, K. 2005. Antimicrobial treatment of asymptomatic carriers of verocytotoxin-producing *Escherichia coli*: An empiric study. *Scand. J. Infect. Dis.* 37, 61–63.

Kaddu-Mulindwa, D.H., Aisu, T., Gleier, K., Zimmermann, S., and Beutin, L. 2001. Occurrence of Shiga toxin-producing *Escherichia coli* in fecal samples from children with diarrhea and from healthy zebu cattle in Uganda. *Int. J. Food Microbiol.* 66, 95–101.

Karch, H., Geitz, C., and Schmidt, H. 1997a. Increased Incidence of Infections with EHEC O103:H2. *Notiziaro dell'Istituto Superiore di Sanita* 10,N.3(Suppl.1), 2.

Karch, H., Huppertz, H.I., Bockemühl, J., Schmidt, H., Schwarzkopf, A., and Lissner, R. 1997b. Shiga toxin-producing *Escherichia coli* infections in Germany. *J. Food Protect.* 60, 1454–1457.

Keskimäki, M., Saari, M., Heiskanen, T., and Siitonen, A. 1998. Shiga toxin-producing *Escherichia coli* in Finland from 1990 through 1997: Prevalence and characteristics of isolates. *J. Clin. Microbiol.* 36, 3641–3646.

Kijima-Tanaka, M., Ishihara, K., Kojima, A., Morioka, A., Nagata, R., Kawanishi, M., Nakazawa, M., Tamura, Y., and Takahashi, T. 2005. A national surveillance of Shiga toxin-producing *Escherichia coli* in food-producing animals in Japan. *J. Vet. Med. Series B-Infect. Dis. Vet. Publ. Hlth.* 52, 230–237.

Kirchgatterer, A., Weber, T., Hinterreiter, M., Knoflach, P., and Allerberger, F. 2002. Haemorrhagic colitis due to *Escherichia coli* O103:H2 associated with infliximab therapy in a patient with rheumatoid arthritis. *Rheumatology* 41, 355–356.

Klein, G., and Bülte, M. 2003. Antibiotic susceptibility pattern of *Escherichia coli* strains with verocytotoxic *E. coli*-associated virulence factors from food and animal faeces. *Food Microbiol.* 20, 27–33.

Klein, E.J., Stapp, J.R., Clausen, C.R., Boster, D.R., Wells, J.G., Qin, X., Swerdlow, D.L., and Tarr, P.I. 2002. Shiga toxin-producing *Escherichia coli* in children with diarrhea: A prospective point-of-care study. *J. Ped.* 141, 172–177.

Konowalchuk, J., Speirs, J.L., and Stavric, S. 1977. Vero response to a cytotoxin of *Escherichia coli*. *Infect. Immun.* 18, 775–779.

Kudoh, Y., Kai, A., Obata, H., Kusunoki, J., Monma, C., Shingaki, M., Yanagawa, Y., Yamada, S., Mtsushita, S., Itoh, T., and Ohta, K. 1994. Epidemiological surveys on verocytotoxin-producing Escherichia coli infections in Japan. Recent Advances in Verocytotoxin-producing Escherichia Coli Infections. Verocytotoxin-producing *Escherichia coli* in children with hemolytic uremic syndrome and diarrhea in the Czech Republic. In: Ed. Karmali and A.G. Goglio, eds. *Recent Advances in Verocytotoxin-producing Escherichia Coli Infections*, , pp. 53–56: Excerpta Medica International Congress Series 1072, Elsevier Science, Amsterdam, The Netherlands.

Kudva, I.T., Hatfield, P.G., and Hovde, C.J. 1997. Characterization of *Escherichia coli* O157:H7 and other Shiga Toxin-producing *E. coli* serotypes isolated from sheep. *J. Clin. Microbiol.* 35, 892–899.

Kugler, R., Busch, U., Bayer, M., Gerber, L., Hellein, G., Barankay, V., and Huber H.Ch. 1998. Vergleich von ELISA, Verozelltest, PCR und immunomagnetischer Separation (IMS) in der Diagnostik von enterohämorrhagischen *Escherichia coli* (EHEC) in Stuhlproben. *Bundesgesundheitsblatt Sonderheft Oktober* 1998. 13–19.

Kulkarni, H., Goldwater, P.N., Martin, A., and Bettelheim, K.A. 2002. *Escherichia coli* 'O' group serological responses and clinical correlations in epidemic HUS patients. *Comp. Immunol. Microbiol. Infect. Dis.* 25(4), 249–268.

Leelaporn, A., Phengmak, M., Eampoklap, B., Manatsathit, S., Tritilanunt, S., Siritantikorn, S., Nagayama, K., Iida, T., Niyasom, C., and Komolpit, P. 2003. Shiga toxin- and enterotoxin-producing *Escherichia coli* isolated from subjects with bloody and nonbloody diarrhea in Bangkok, Thailand. *Diag. Microbiol. Infect. Dis.* 46, 173–180.

Lehmacher, A., Meier, H., Aleksic, S., and Bockemühl, J. 1998. Detection of hemolysin variants of shiga toxin-producing *Escherichia coli* by PCR and culture on vancomycin-cefixime-cefsulodin blood agar. *Appl. Environ. Microbiol.* 64, 2449–2453.

Leomil, L., Aidar-Ugrinovich, L., Guth, B.E.C., Irino, K., Vettorato, M.P., Onuma, D.L., and de Castro, A.F.P. 2003. Frequency of Shiga toxin-producing *Escherichia coli* (STEC) isolates among diarrheic and non-diarrheic calves in Brazil. *Vet. Microbiol.* 97, 103–109.

Leung, P.H.M., Yam, W.C., Ng, W.W.S., and Peiris, J.S.M. 2001. The prevalence and characterization of verotoxin-producing *Escherichia coli* isolated from cattle and pigs in an abattoir in Hong Kong. *Epidem. Infect.* 126, 173–179.

Lopez, E.L., Diaz, M., Grinstein, S., Devoto, S., Mendilaharzu, F., Murray, B.E., Ashkenazi, S., Rubeglio, E., Woloj, M., Vasquez, M., Turco, M., Pickering, L.K., and Cleary, T.J. 1989. Hemolytic Uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. *J. Infect. Dis.* 160, 469–475.

Ludwig, K., Sarkim, V., Bitzan, M., Karmali, M.A., Bobrowski, C., Ruder, H., Laufs, R., Sobottka, I., Petric, M., Karch, H., and Müller-Wiefel, D.E. 2002. Shiga toxin-producing *Escherichia coli* infection and antibodies against Stx2 and Stx1 in household contacts of children with enteropathic hemolytic-uremic syndrome. *J. Clin. Microbiol.* 40, 1773–1782.

Mackenzie, A.M., Lebel, P., Orrbine, E., Rowe, P.C., Hyde, L., Chan, F., Johnson, W., McLaine, P.N., and the Synsorb PK Study Investigators. 1998. Sensitivities and specificities of premier *E. coli* O157 and premier EHEC enzyme immunoassays for diagnosis of infection with verotoxin (shiga-like toxin)-producing *Escherichia coli*. *J. Clin. Microbiol.* 36, 1608–1611.

Maidhof, H., Guerra, B., Abbas, S., Elsheikha, H.M., Whittam, T.S., and Beutin, L. 2002. A multiresistant clone of Shiga toxin-producing *Escherichia coli* O118:[H16] is spread in cattle and humans over different European countries. *Appl. Environ. Microbiol.* 68, 5834–5842.

Majed, N.I., Bettelheim, K.A., Shooter, R.A., and Moorhouse, E. 1978. The effect of travel on faecal *Escherichia coli* serotypes. *J. Hyg. Camb.* 81, 481–487.

March, S.B., and Ratnam, S. 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157.H7 associated with haemorrhagic colitis. *J. Clin. Microbiol.* 23, 869–872.

Mariani-Kurkdjian, P., Denamur, E., Milon, A., Picard, B., Cave, H., Lambert-Zechovsky, N., Loirat, C., Goullet, P., Sansonetti, P., and Elion, J. 1993. Identification of a clone of *Escherichia coli* O103:H2 as a potential agent of hemolytic uremic syndrome in France. *J. Clin. Microbiol.* 31, 296–301.

Marshall, J.A., Yuen, L.K.W., Catton, M.G., Gunesekere, I.C., Wright, P.J., Bettelheim, K.A., Griffith, J.M., Lightfoot, D., Hogg, G.G., Gregory, J., Wilby, R., and Gaston, J. 2001. Multiple outbreaks of Norwalk-like virus gastroenteritis associated with a Mediterranean-style restaurant. *J. Med. Microbiol.* 50, 143–151.

McCluskey, B.J., Rice, D.H., Hancock, D.D., Hovde, C.J., Besser, T.E., and Gray, S. 1999. Prevalence of *Escherichia coli* O157 and other Shiga-toxin-producing *E. coli* in lambs at slaughter. *J. Vet. Diagn. Invest.* 11, 563–565.

McLean, C., Bettelheim, K.A., Kuzevski, A., Falconer, L., and Djordjevic, S.P. 2005. Isolation of *Escherichia coli* O5:H-, possessing genes for Shiga toxin 1, intimin-β and enterohaemolysin, from an intestinal biopsy from an adult case of bloody diarrhoea: evidence for two distinct O5:H- pathotypes. *J. Med. Microbiol.* 54, 605–607.

McMaster, C., Roch, E.A., Willshaw, G.A., Doherty, A., Kinnear, W., and Cheasty, T. 2001. Verocytotoxin-producing Escherichia coli serotype 026:H11 outbreak in an Irish crèche. *Europ. J. Clin. Microbiol. Infect. Dis.* 20, 430–432.

Meng, J.H., Zhao, S.H., and Doyle, M.P. 1998. Virulence genes of Shiga toxin-producing *Escherichia coli* isolated from food, animals and humans. *Int. J. Food Microbiol.* 45, 229–235.

Mellman, A., Bielaszewska, L., Zimmerhackl, L.B., Prager, R., Harmsen, D., Tschäpe, H., and Karch, H. 2005. Enterohemorrhagic *Escherichia coli* in human infection: In vivo evolution of a bacterial pathogen. *Clin. Infect. Dis.* 41, 785–792.

Mercado, E.C., Gioffré, A., Rodríguez, S.M., Cataldi, A., Irino, K., Elizondo, A.M., Cipolla, A.L., Romano, M.I., Malena, R., and Méndez, M.A. 2004. Non-O157 Shiga toxin-producing *Escherichia coli* isolated from diarrhoeic calves in Argentina. *J. Vet. Med. Series B-Infect. Dis. Vet. Publ. Hlth* 51, 82–88.

Misawa, N., Sueyoshi, N., Uemura, R., Kakemizu, Y., Kawashima, K., Nagatomo, H., Kondo, F., Murakami, T., and Takahashi, Y. 2000. Effect of bicozamycin on the eradication of Shiga toxin-producing *Escherichia coli* in calves. *Microbiol. Immun* 44, 891–896.

Misselwitz, J., Karch, H., Bielazewska, M., John, U., Ringelmann, F., Ronnefarth, G., and Patzer, L. 2003. Cluster of hemolytic-uremic syndrome caused by Shiga toxin-producing *Escherichia coli* O26:H11. *Ped. Infect. Dis. J* 22, 349–354.

Mitsuoka, T., and Hayakawa, K. 1972. Die Faecalflora bei Menschen. I. Mitteilung: Die Zusammensetzung der Faecalflora der verschiedenen Altersgruppen. *Zbl. Bakt. Mikrobiol. Hyg., I. Abt. Orig. A* 223, 333–342.

Miyamoto, H. 2000. Statistical and epidemiological Analyses on food poisonings and infections and applications by microbiological analyses. National Reports on "Emerging and Reemerging Infectious Diseases" 1997–1999; Ministry of Health and Welfare, Japan.

Miyao, Y., Kataoka, T., Nomoto, T., Kai, A., Itoh, T., and Itoh, K. 1998. Prevalence of verotoxin-producing *Escherichia coli* harbored in the intestine of cattle in Japan. *Vet. Microbiol.* 61, 137–143.

Morabito, S., Karch, H., Mariani-Kurkdjian, P., Schmidt, H., Minelli, F., Bingen, E., and Caprioli, A. 1998. Enteroaggregative, shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of hemolytic-uremic syndrome. *J. Clin. Microbiol.* 36(3), 840–842.

Moreira, C.N., Pereira, M.A., Brod, C.S., Rodrigues, D.P., Carvalhal, J.B., and Aleixo, J.A. 2003. Shiga toxin-producing *Escherichia coli* (STEC) isolated from healthy dairy cattle in southern Brazil. *Vet. Microbiol.* 93(3), 179–183.

O'Farrell, S.M., Lennox-King, S.M.J., Bettelheim, K.A., Shaw, E.J., and Shooter, R.A. 1976. *Escherichia coli* in a maternity ward. *Infection* 4, 146–152.

Ohara, T., Kojio, S., Taneike, I., Nakagawa, S., Gondaira, F., Tamura, Y., Gejyo, F., Zhang, H.M., and Yamamoto, T. 2002. Effects of azithromycin on Shiga toxin production by *Escherichia coli* and subsequent host inflammatory response. *Antimicrob. Ag. Chemother.* 46, 3478–3483.

Okitsu, T., Kusumoto, M., Suzuki, R., Sata, S., Nishiya, Y., Kawamura, Y., and Yamai, S. 2001. Identification of Shiga toxin-producing *Escherichia coli* possessing insertionally inactivated Shiga toxin gene. *Microb. Immunol.* 45, 319–322.

Orden, J.A., Ruiz-Santa-Quiteria, J.A., Blanco, M., Blanco, J.E., Mora, A., Cid, C., González, E.A., Blanco, J., and de la Fuente, R. 2003. Prevalence and characterization of Vero cytotoxin-producing Escherichia coli isolated from diarrhoeic and healthy sheep and goats. *Epidemiol. Infect.* 130, 313–321.

Oteiza, J.M., Chinen, I., Miliwebsky, E., and Rivas, M. 2005. Isolation and characterization of Shiga toxin-producing *Escherichia coli* from precooked sausages (morcillas). *Food Microbiol.* 23(3), 283–288.

Padola, N.L., Sanz, M.E., Blanco, J.E., Blanco, M., Blanco, J., Etcheverria, A.I., Arroyo, G.H., Usera, M.A., and Parma, A.E. 2004. Serotypes and virulence genes of bovine Shigatoxigenic *Escherichia coli* (STEC) isolated from a feedlot in Argentina. *Vet. Microbiol.* 100, 3–9.

Park, C.H., Gates, K.M., Vandel, N.M., and Hixon, D.L. 1996. Isolation of Shiga-like Toxin producing *Escherichia coli* (O157 and non-O157) in a community hospital. *Diag. Microbiol. Infect. Dis.* 26, 69–72.

Parma, A.E., Sanz, M.E., Blanco, J.E., Blanco, J., Viñas, M.R., Blanco, M., Padola, N.L., and Etcheverria, A.I. 2000. Virulence genotypes and serotypes of verotoxigenic *Escherichia coli* isolated from cattle and foods in Argentina—Importance in public health. *Europ. J. Epidemiol.* 16, 757–762.

Parreira, V.R., and Gyles, C.L. 2002. Shiga toxin genes in avian *Escherichia coli*. *Vet. Microbiol.* 87(4), 341–352.

Paton, A.W., and Paton, J.C. 2002. Direct detection and characterization of Shiga toxigenic *Escherichia coli* by multiplex PCR for *stx*1, *stx*2, *eae*, *ehxA*, and *saa*. *J. Clin. Microbiol.* 40, 271–274.

Paton, A.W., and Paton, J.C. 2005. Multiplex PCR for direct detection of Shiga toxigenic *Escherichia coli* strains producing the novel subtilase cytotoxin. *J. Clin. Microbiol.* 43, 2944–2947.

Paton, A.W., Ratcliff, R.M., Doyle, R.M., Seymour-Murray, J., Davos, D., Lancer, J.A., and Paton, J.C. 1996. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. *J. Clin. Microbiol.* 34, 1622–1627.

Paton, A.W., Woodrow, M.C., Doyle, R.M., Lanser, J.A., and Paton, J.C. 1999. Molecular characterization of a shiga toxigenic *Escherichia coli* O113:H21 strain lacking *eae* responsible for a cluster of cases of hemolytic-uremic syndrome. *J. Clin. Microbiol.* 37, 3357–3361.

Pichner, R., Hechelmann, H.G., Steinrück, H., and Gareis, M. 2001. Verotoxin-producing *E. coli* (VTEC) and *E. coli* counts in fresh raw sausages. *Fleischwirtschaft.* 81, 191–192.

Pichner, R., Sander, A., Steinrück, H., and Gareis, M. 2005. Occurrence of *Salmonella* spp. and shigatoxin-producing *Escherichia coli* (STEC) in horse faeces and horse meat products. *Berlin. Münch. Tierärztl. Wchnschrft.* 118, 321–325.

Piérard, D., Etterijck, R., Breynaert, J., Moriau, L., and Lauwers, S. 1990. Results of screening for Verocytotoxin-producing *Escherichia coli* in faeces in Belgium. *Europ. J. Clin. Microbiol. Infect. Dis.* 9, 198–201.

Piérard, D., Stevens, D., Moriau, L., Lior, H., and Lauwers, S. 1994. Three years PCR screening for VTEC in human stools in Brussels. Recent Advances in Verocytotoxin-producing Escherichia Coli Infections. In: Ed. Karmali and A.G. Goglio, eds. *Recent Advances in Verocytotoxin-producing Escherichia Coli Infections*, , pp. 33–36: Excerpta Medica International Congress Series 1072, Elsevier Science, Amsterdam, The Netherlands.

Piérard, D., Stevens, D., Moriau, L., Lior, H., and Lauwers, S. 1997. Isolation and virulence factors of verocytotoxin-producing *Escherichia coli* in human stools. *Clin. Microbiol. Infect.* 3, 531–540.

Pradel, N., Livrelli, V., de Champs, C., Palcoux, J.B., Reynaud, A., Scheutz, F., Sirot, J., Joly, B., and Forestier, C. 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *J. Clin. Microbiol.* 38, 1023–1031.

Pradel, N., Boukhors, K., Bertin, Y., Forestier, C., Martin, C., and Livrelli, V. 2001. Heterogeneity of shiga toxin-producing *Escherichia coli* strains isolated from hemolytic-uremic syndrome patients, cattle, and food samples in central France. *Appl. Environ. Microbiol.* 67(6), 2460–2468.

Pradel, N., Leroy-Setrin, S., Joly, B., and Livrelli, V. 2002. Genomic subtraction to identify and characterize sequences of Shiga toxin-producing *Escherichia coli* O91:H21. *Appl. Environm. Microbiol.* 68, 2316–2325.

Pradel, N., Livrelli, V., de Champs, C., Palcoux, J.B., Reynaud, A., Scheutz, F., Sirot, J., Joly, B., and Forestier, C. 2000. Prevalence and characterization of shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *J. Clin. Microbiol.* 38, 1023–1031.

Prager, R., Annemüller, S., and Tschäpe, H. 2005. Diversity of virulence patterns among shiga toxin-producing *Escherichia coli* from human clinical cases—need for more detailed diagnostics. *International J. Med. Microbiol.* 295(1), 29–38.

Pryor, W., Hodson, H., McIntyre, P., and Bettelheim, K.A. 1990. Toxigenic *Escherichia coli* in uraemic haemolytic syndrome. *Med. J. Australia* 152, 221–222.

Pulz, M., Matussek, A., Monazahian, M., Tittel, A., Nikolic, E., Hartmann, M., Bellin, T., Buer, J., and Gunzer, F. 2003. Comparison of a shiga toxin enzyme-linked immunosorbent assay and two types of PCR for detection of shiga toxin-producing *Escherichia coli* in human stool specimens. *J. Clin. Microbiol.* 41, 4671–4675.

Rahn, K., Renwick, S.A., Johnson, R.P., Wilson, J.B., Clarke, R.C., Alves, D., McEwen, S.A., Lior, H., and Spika, J. 1998. Follow-up study of verocytotoxigenic *Escherichia coli* infection in dairy farm families. *J. Infect. Dis.* 177, 1139–1140.

Ramachandran, V., Brett, K.N., Hornitzky, M.A., Dowton, M., Bettelheim, K.A., Walker, K., and Djordjevic, S.P. 2003. Distribution of intimin subtypes among *Escherichia coli* isolates from ruminant and human sources. *J. Clin. Microbiol.* 41, 5022–5032.

Ramachandran, V., Hornitzky, M.A., Bettelheim, K.A., Walker, M.J., and Djordjevic, S.P. 2001. The common ovine Shiga toxin 2-containing *Escherichia coli* serotypes and human isolates of the same serotypes possess a Stx2 d toxin type. *J. Clin. Microbiol.* 39, 1932–1937.

Rappaport, F., and Heng, E. 1952. Media for the isolation and differentiation of pathogenic *Esch. coli* (serotypes O111 and O55). *J. Clin. Pathol.* 5, 361–362.

Read, S.C., Clarke, R.C., Martin, A., de Grandis, S.A., Hii, J., and Gyles, C.L. 1992. Polymerase chain reaction for detection of verocytotoxigenic *Escherichia coli* isolated from animal and food sources. *Mol. Cell. Probes.* 6, 153–161.

Read, S.C., Gyles, C.L., Clarke, R.C., Lior, H., and McEwen, S. 1990. Prevalence of verocytotoxigenic *Escherichia coli* in ground beef, pork and chicken in Ontario. *Epidemiol. Infect.* 105, 11–20.

Rey, J., Blanco, J.J., Blanco, M., Mora, A., Dahbi, G., Alonson, J.M., Hermosa, M., Hermosa, J., Alonso, M.P., Usera, M.A., González, E.A., Bernárdez, M.I., and Blanco, J. 2003. Serotypes, phage types and virulence genes of Shiga-producing *Escherichia coli* isolated from sheep in Spain. *Vet. Microbiol.* 94, 47–56.

Richter, H., Klie, H., Timm, M., Gallien, P., Steinruck, H., Perlberg, K.W., and Protz, D. 1997. Verotoxin-producing *E. coli* (VTEC) in faeces from cattle slaughtered in Germany. *Berlin. Münch. Tierärztl. Wchnschrft.* 110, 121–127.

Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, G.J., Herbert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., Blake, P.A., and Cohen, M.L. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New Engl. J. Med.* 308, 681–685.

Rivero, M.A., Padola, N.L., Etcheverria, A.I., and Parma, A.E. 2004. Enterohemorrhagic *Escherichia coil* and hemolytic-uremic syndrome in Argentina. *Medicina-Buenos Aires.* 64, 352–356.

Rüger, H.J., Karch, H., Timm, M., Richter, H., Musielski, H., Rüger, K., Pollex, G., and Mohr, J. 2001. Development and evaluation of a colony

immunoblot assay for isolation of Shiga-toxin producing *Escherichia coli*. DGHM Congress, Aachen, 2001; abstract P8. *Int. J. Med. Microbiol.* 291, P5–P8.

Sandhu, K.S., Clarke, R.C., McFadden, K., Brouwer, A., Loouie, M., Wilson, J., Lior, H., and Gyles, C.L. 1996. Prevalence of the eaeA gene in verotoxigenic *Escherichia coli* strains from dairy cattle in Southwest Ontario. *Epidemiol. Infect.* 116, 1–7.

Sandhu, K.S., Clarke, R.C., and Gyles, C.L. 1999. Virulence markers in shiga toxin-producing *Escherichia coli* isolated from cattle. *Can. J. Vet. Res.* 63, 177–184.

Scheutz, F. 1995. VTEC isolated from Danish Patients, 1986–1994. *Notiziaro dell'Istituto Superiore di Sanita.* 8, 3.

Schmid, H., Burnens, A.P., Baumgartner, A., Boerlin, P., Bille, J., Liassine, N., Friderich, P., and Breer, C. 2002. Verocytotoxin-producing *Escherichia coli* in patients with diarrhoea in Switzerland. *Europ. J. Clin. Microbiol. Infect. Dis.* 21, 810–813.

Schmidt, H., Geitz, C., Tarr, P.I., Frosch, M., and Karch, H. 1999. Non-O157:H7 pathogenic shiga toxin-producing *Escherichia coli*: Phenotypic and genetic profiling of virulence traits and evidence for clonality. *J. Infect. Dis.* 179, 115–123.

Schmidt, H., von Maldeghem, J., Frosch, M., and Karch, H. 1998. Antibiotic susceptibilities of verocytotoxin-producing *Escherichia coli* O157 and non-O157 strains isolated from patients and healthy subjects in Germany during 1996. *J. Antimicrob. Chemother.* 42, 548–550.

Schurman, R.D., Hariharan, H., Heaney, S.B., and Rahn, K. 2000. Prevalence and characteristics of shiga toxin-producing *Escherichia coli* in beef cattle slaughtered on Prince Edward Island. *J. Food Protect.* 63, 1583–1586.

Scotland, S.M., Rowe, B., Smith, H.R., Willshaw, G.A., and Gross, R.J. 1988. Verocytotoxin-producing strains of *Escherichia coli* from children with haemolytic uraemic syndrome and their detection by specific DNA probes. *J. Med. Microbiol.* 25, 237–243.

Shooter, R.A., Cooke, M.E., O'Farrell, S.M., Bettelheim, K.A., Chandler, M.E., and Bushrod, F.M. 1974. The isolation of *Escherichia coli* serotypes from a poultry packing station and an abattoir. *J. Hyg., Camb.* 73, 245–247.

Shooter, R.A., Cooke, E.M., Rousseau, S.A., and Breaden, A.L. 1970. Animal sources of common serotypes of *E. coli* in the food of hospital patients. Possible significance in urinary tract infection. *Lancet.* ii, 226–228.

Sidjabat-Tambunan, H., Bensink, J.C., and Bettelheim, K.A. 1998. Isolation of verocytotoxin-producing *Escherichia coli* from mutton carcases. *Aust. Vet. J.* 76, 364–365.

Smith, H.W. 1965. The development of the flora of the alimentary tract in young animals. *J. Path. Bact.* 90, 495–513.

Smith, H.R., Scotland, S.M., Willshaw, G.A., Wray, C., McLaren, I.M., Cheasty, T., and Rowe, B. 1988. Verocytotoxin production and the presence of VT genes in *Escherichia coli* strains of animal origin. *J. Gen. Microbiol.* 134, 829–834.

Sperandio, V., Kaper, J.B., Bortolini, M.R., Neves, B.C., Keller, R., and Trabulsi, L.R. 1998. Characterization of the locus of enterocyte effacement (LEE) in different enteropathogenic *Escherichia coli* (EPEC) and shiga-toxin producing *Escherichia coli* (STEC) serotypes. *FEMS Microbiol. Lett.* 164, 133–139.

Stark, P.L., and Lee, A. 1982. The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. *J. Med. Microbiol.* 15, 189–203.

Starr, M., Bennettwood, V., Bigham, A.K., Dekoningward, T.P., Bordun, A.M., Lightfoot, D., Bettelheim, K.A., Jones, C.L., and Robinsbrowne, R.M. 1998. Hemolytic-uremic syndrome following urinary tract infection with enterohemorrhagic *Escherichia coli*: case report and review. *Clin. Infect. Dis.* 27, 310–315.

Stephan, R., and Hoelzle, L.E. 2000. Characterization of shiga toxin type 2 variant B-subunit in *Escherichia coli* strains from asymptomatic human carriers by PCR-RFLP. *Lett. Appl. Microbiol.* 31, 139–142.

Stephan, R., and Kuhn, K. 1999. Prevalence of Verotoxin-producing *Escherichia coli* (VTEC) in bovine coli mastitis and their antibiotic resistance patterns. *J. Vet. Med. B* 46, 423–427.

Stephan, R., Ragettli, S., and Untermann, F. 2000. Prevalence and characteristics of verotoxin-producing *Escherichia coli* (VTEC) in stool samples from asymptomatic human carriers working in the meat processing industry in Switzerland. *J. Appl. Microbiol.* 88, 335–341.

Stephan, R., and Untermann, F. 1999. Virulence factors and phenotypical traits of verotoxin-producing Escherichia coli strains isolated from asymptomatic human carriers. *J. Clin. Microbiol.* 37, 1570–1572.

Strockbine, N., Sowers, E., Greene, K., Hayes, P., Griffin, P., and Wells, J. 1997. Characterization of Shiga Toxin-producing non-O157 Escherichia coli from the United States 1983–1997. *VTEC '97 Abstract V235/III*, p. 67.

Su, C., and Brandt, L.J. 1995. *Escherichia coli* O157:H7 infections in humans. *Ann. Intern. Med* 123, 698–714.

Suthienkul, O., Brown, J.E., Seriwatana, J., Tienthongdee, S., Sastravaha, S., and Echeverria, P. 1990. Shiga-like-toxin producing *Escherichia coli* in retail meats and cattle in Thailand. *Appl. Environ. Microbiol.* 56, 1135–1139.

Takeda, Y. 1999. Enterohemorrhagic *Escherichia coli* infection in Japan. *Pediat. Internat* 41, 198–201.

Tamura, K., Sakazaki, R., Murase, M., and Kosako, Y. 1996. Serotyping and categorisation of *Escherichia coli* strains isolated between 1958 and 1992 from diarrhoeal diseases in Asia. *J. Med. Microbiol.* 45, 353–358.

Tarr, C.L., Large, T.M., Moeller, C.L., Lacher, D.W., Tarr, P.I., Acheson, D.W., and Whittam, T.S. 2002. Molecular characterization of a serotype O121:H19 clone, a distinct Shiga toxin-producing clone of pathogenic *Echerichia coli*. *Infect. Immun.* 70, 6853–6859.

Tatarczak, M., Wieczorek, K., Posse, B., and Osek, J. 2005. Identification of putative adhesin genes in shigatoxigenic *Escherichia coli* isolated from different sources. *Vet. Microbiol.* 110, 77–85.

Teufel, P., Bartelt, E., Bräunig, J., Ellerbroek, L., Telo, A., Wichmann-Schauer, H., Gallien, P., Richter, H., Klie, H., Timm, M., and Perlberg, K.-W. 1998. Nachweis von Verotoxin-produzierenden *E. coli* (VTEC) in Rinderhackfleisch und Weichkäse. *Bundesgesundheitsblatt Sonderheft Oktober 1998*, 31–36.

Thomas, A., Chart, H., Cheasty, T., Smith, H.R., Frost, J.A., and Rowe, B. 1993. Verocytotoxin-producing *Escherichia coli* particularly serogroup O157 associated with human infections in the United Kingdom. *Epidemiol. Infect.* 110, 591–600.

Thomas, A., Cheasty, T., Frost, J.A., Chart, H., Smith, H.R., and Rowe, B. 1996. Vero cytotoxin-producing *Escherichia coli*, particularly serogroup O157, associated with human infections in England and Wales: 1992–4. *Epidemiol. Infect.* 117, 1–10.

Thran, B.H., Hussein, H.S., Hall, M.R., and Khaiboullina, S.F. 2001. Shiga toxin-producing *Escherichia coli* in beef heifers grazing an irrigated pasture. *J. Food Protect* 64, 1613–1616.

Timm, M., Klie, H., Richter, H., Gallien, P., Perlberg, K.W., Lehmann, S., and Protz, D. 1999. Detection and prevalence of Verotoxin-producing *Escherichia coli* (VTEC) in raw sausages. *Berlin. Münch. Tierärztl. Wchnschrft.* 112, 385–389.

Todd, E.C.D., Szabo, R.A., MacKenzie, J.M., Martin, A., Rahn, K., Gyles, C., Gao, A., Alves, D., and Yee, A.J. 1999. Application of a DNA hybridization-hydrophobic-grid membrane filter method for detection and isolation of verotoxigenic *Escherichia coli*. *Appl. Environm. Microbiol.* 65, 4775–4780.

Tzipori, S., Wachsmuth, K.I., Smithers, J., and Jackson, C. 1988. Studies in gnotobioltic piglets on non-O157:H7 *Escherichia coli* serotypes isolated from patients with hemorrhagic colitis. *Gastroenterology.* 94, 590–597.

Urdahl, A.M., Beutin, L., Skjerve, E., and Wasteson, Y. 2002. Serotypes and virulence factors of Shiga toxin-producing *Escherichia coli* isolated from healthy Norwegian sheep. *J. Appl. Microbiol.* 93, 1026–1033.

Urdahl, A.M., Beutin, L., Skerve, E., Zimmermann, S., and Wasteson, Y. 2003. Animal host associated differences in Shiga toxin-producing *Escherichia coli* isolated from sheep and cattle on the same farm. *J. Appl. Microbiol.* 95, 92–101.

Vaz, T.M.I., Irino, K., Kato, M.A.M.F., Dias, A.M.G., Gomes, T.A.T., Medeiros, M.I.C., Rocha, M.M.A., and Guth, B.E.C. 2004. Virulence properties and characteristics of Shiga toxin-producing *Escherichia coli* in São Paulo, Brazil, from 1976 through 1999. *J. Clin. Microbiol.* 42, 903–905.

Verweyen, H.M., Karch, H., Allerberger, F., and Zimmerhackl, L.B. 1999. Enterohemorrhagic *Escherichia coli* (EHEC) in pediatric hemolytic-uremic syndrome: A prospective study in Germany and Austria. *Infection* 27, 341–347.

Vettorato, M.P., Leomil, L., Guth, B.E.C., Irino, K., and Castro, A.F.P. 2003. Properties of Shiga toxin-producing *Escherichia coli* (STEC) isolates from sheep in the State of São Paulo, Brazil. *Vet. Microbiol.* 95, 103–109.

Wells, J.G., Shipman, L.D., Greene, K.D., Sowers, E.G., Green, J.H., Cameron, D.N., Downes, F.P., Martin, M.L., Griffin, P.M., Ostroff, S.M., Potter, M.E., Tauxe, R.V., and Wachsmuth, I.K. 1991. Isolation of *Escherichia coli* O157:H7 and other Shiga-like toxin-producing *E. coli* from dairy cattle. *J. Clin. Microbiol.* 29, 985–989.

Wade, W.G., Thom, B.T., and Evans, B.T. 1979. Cytotoxic Enteropathogenic *Escherichia coli*. *The Lancet*. ii, 1235–1236.

Werber, D., Fruth, A., Liesegang, A., Littmann, M., Buchholz, U., Prager, R., Karch, H., Breuer, T., Tschäpe, H., and Ammon, A. 2002. A multistate outbreak of Shiga toxin-producing *Escherichia coli* O26:H11 infections in Germany, detected by molecular subtyping surveillance. *J. Infect. Dis.* 186, 419–422.

Wieler, L.H., Vieler, E., Erpenstein, C., Schlapp, T., Steinrück, H., Bauerfeind, R., Byomi, A., and Baljer, G. 1996. Shiga toxin-producing *Escherichia coli* strains from bovines: Assiation of adhesion with carriage of *eae* and other genes. *J. Clin. Microbiol.* 34, 2980–2984.

Willshaw, G.A., Scotland, S.M., Smith, H.R., and Rowe, B. 1992. Properties of Verocytotoxin-producing *Escherichia coli* of human origin of O groups other than O157. *J. Infect. Dis.* 166, 797–802.

Willshaw, G.A., Smith, H.R., Roberts, D., Thirlwell, J., Cheasty, T., and Rowe, B. 1993. Examination of raw beef products for the presence of Verocytotoxin producing *Escherichia coli*, particularly those of serogroup O157. *J. Appl. Bact.* 75, 420–426.

Willshaw, G.A., Cheasty, T., Smith, H.R., O'Brien, S.J., and Adak, G.K. 2001. Verocytotoxin-producing *Escherichia coli* (VTEC) O157 and other VTEC from human infections in England and Wales: 1995–1998. *J. Med. Microbiol.* 50, 135–142.

Wilson, J.B., Clarke, R.C., Renwick, S., Rahn, K., Johnson, R.P., Alves, D., Karmali, M.A., Lior, H., McEwen, S.A., and Spika, J. 1994. Verocytotoxigenic *Escherichia coli* infection on dairy farms in Southern Ontario. Recent Advances in Verocytotoxin-producing Escherichia Coli Infections. In: Ed. Karmali and A.G. Goglio, eds. *Recent Advances in Verocytotoxin-producing Escherichia Coli Infections*, , pp. 61–64: Excerpta Medica International Congress Series 1072, Elsevier Science, Amsterdam, The Netherlands.

Wilson, J.B., Clarke, R.C., Renwick, S., Rahn, K., Johnson, R.P., Karmali, M.A., Lior, H., Alves, D., Gyles, C.L., Sandhu, K.S., McEwen, S.A., and Spika, J.S. 1996. Vero cytotoxigenic *Escherichia coli* infection in dairy farm families. *J. Infect. Dis.* 174, 1021–1027.

Wilson, M.W., and Bettelheim, K.A. 1980. Cytotoxic *Escherichia coli* serotypes. *Lancet*. i, 201.

Zhang, W.L., Bielaszewska, M., Liesegang, A., Tschäpe, H., Schmidt, H., Bitzan, M., and Karch, H. 2000. Molecular characteristics and epidemiological significance of Shiga toxin-producing *Escherichia coli* O26 strains. *J. Clin. Microbiol.* 38, 2134–2140.

Zweifel, C., Blanco, J.E., Blanco, M., Blanco, J., and Stephan, R. 2004. Serotypes and virulence genes of ovine non-O157 shiga toxin-producing *Escherichia coli* in Switzerland. *Int. J. Food Microbiol.* 15, 19–27.

Zweifel, C., Schumacher, S., Blanco, M., Blanco, J.E., Tasara, T., Blanco, J., and Stephan, R. 2005. Phenotypic and genotypic characteristics of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) from Swiss cattle. *Vet. Microbiol.* 105, 37–45.

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Microbiological Characterization of Imported and Domestic Boneless Beef Trim Used for Ground Beef†

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ABSTRACT

The United States imports lean boneless beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) to meet demand for ground beef production. The reported incidence of and etiological agents responsible for foodborne diseases differ between these countries and the United States. Our objective was to determine whether current U.S. microbiological profiling adequately addresses the potential differences between foreign and domestic beef trim. We compared the hygienic status of imported and domestic (USA) beef trim by enumeration of aerobic bacteria, *Enterobacteriaceae,* coliforms, *Escherichia coli,* and *Staphylococcus aureus.* We also compared the prevalence of pathogens between imported and domestic samples by screening for the presence of *Salmonella, Campylobacter* spp., *Listeria* spp., and non-O157 Shiga toxin–producing *E. coli* (STEC). A total of 1,186 samples (487 USA, 220 AUS, 223 NZL, and 256 URY) of boneless beef trim were analyzed. Results of enumeration revealed significant differences between samples from all countries, with the lowest pathogen numbers in samples from AUS and the highest in samples from URY. Six *Salmonella* isolates (1 NZL, 1 URY, and 4 USA), 79 *L. monocytogenes* isolates (4 AUS, 5 NZL, 53 URY, and 17 USA), and 7 *Campylobacter* isolates (1 NZL, 1 URY, 5 USA) were found among the trim samples tested. Non-O157 STEC prevalence was 10% in NZL samples and about 30% in all of the other samples; 99 STEC strains were isolated. Serotyping of these isolates revealed that serotypes associated with hemolytic uremic syndrome were not different in prevalence between imported and domestic beef trim. Although it may be tempting to do so, these data cannot be used to compare the microbiological quality of beef trim between the countries examined. However, these results indicate that the current pathogen monitoring procedures in the United States are adequate for evaluation of imported beef trim.

The fed beef industry of the United States produces an excess of 50% (50-50) lean-fat beef trim but not enough 90-10 lean-fat beef trim to mix with it to meet the domestic demand for lean ground beef. According to imported beef and veal statistics recorded by the U.S. Department of Agriculture (USDA) Economic Research Service, 3.6 billion pounds (1.6 billion kilograms) of beef were imported into the United States in 2004 *(51).* Much of this beef was lean beef destined to be mixed with domestic 50-50 trim.

More than half of the beef imported annually originates in Australia (AUS), New Zealand (NZL), and Uruguay (URY) *(52),* countries in which the incidence of foodborne disease and etiological agents responsible for it differ substantially from those found in the United States and Canada *(18, 25).* One example of these differences is the variation in serotypes of Shiga toxin–producing *Escherichia coli* (STEC) that have been implicated in several foodborne diseases *(32, 37).* STEC cause symptoms that range from mild diarrhea to a very severe and life-threatening condition, hemolytic uremic syndrome (HUS). The STEC serotype most frequently associated with clinical disease in the United States and other countries of the Northern Hemisphere is O157:H7 *(29, 31, 32).* However, other non-O157 STEC serotypes have been associated with illness and outbreaks of severe disease in countries of the Southern Hemisphere *(1, 32).*

During the production of ground beef, imported beef trim is treated in the same manner as is domestic beef trim. This approach could be problematic because even though foreign sources are required to supply beef that has been tested for *E. coli* O157:H7 and *Salmonella,* once the foreign beef trim is blended and ground with domestic trim it is difficult to trace the origins of any subsequently identified pathogens. Molecular identification techniques are being employed more often in these tracing efforts. Current data regarding the microbial status of imported beef once it is received in the United States are limited. The objective of this project was to determine if and how the hygienic status and pathogen prevalence of imported beef trim from AUS, NZL, and URY differ from those of domestic beef trim. Hygienic status was determined by enumeration of aerobic bacteria, *Enterobacteriaceae,* coliforms, *E. coli,* and *Staphylococcus aureus.* The difference in pathogen prevalence between imported and domestic samples was determined by screening for the presence of *Salmonella, Campylobacter* spp., *Listeria* spp., and non-O157 STEC. Any of these organisms present were isolated and identified to species and/or serotype.

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† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

MATERIALS AND METHODS

Sample processing. Between 1 January and 31 March 2005, 1,186 samples of domestic and imported boneless beef trim were collected and sent to the U.S. Meat Animal Research Center (USMARC) for analysis. The domestic samples were collected from mostly chilled beef from fed and cull beef production facilities. The imported trim originated in AUS, NZL, or URY, was frozen, and may have contained calf or veal trim. The beef trim came from multiple processing plants or establishments within each source country. AUS trim was packed an average of 104 days (53 to 176 days), NZL trim was packed an average of 98 days (46 to 201 days), and URY trim was packed an average of 86 days (39 to 272 days) before samples were collected in the United States. Most U.S. trim (97.5%) was fresh chilled beef that was packed 7 or fewer days (average of 3 days) before samples were collected. The remaining U.S. trim samples (2.5%) were collected from beef trim that had been frozen for 14 to 68 days.

Quality assurance personnel collected samples at each participating supplier by randomly selecting pieces of trim from 27-kg cartons of imported trim or 2,000-lb (907-kg) combos of domestic trim before blending and grinding of ground beef. The pieces of trim were placed in Whirl-Pak bags (Nasco, Fort Atkinson, Wis.) and held frozen (−20°C) until shipment on ice to the USMARC. Samples were received frozen and held at −20°C until processed. Most imported trim was 3- to 4-in. (7.6- to 10.2-cm) long slices, 0.5- to 1-in. (1.2- to 2.5-cm) cubes, or shredded beef. Domestic trim samples were similar but also included coarse ground beef and reduced fat beef. To test the maximal surface area per unit of weight of large or cubed pieces of beef trim, the outside edges of numerous pieces were trimmed away and combined for analysis. Samples received as ground beef, small shredded pieces, or reduced fat beef were used directly in analysis. Once prepared, samples were subdivided for pathogen detection and enumeration assays. A 10-g sample was used to enumerate total aerobic bacteria, *Enterobacteriaceae,* coliform bacteria, *E. coli,* and *S. aureus.* Another 10-g sample was used to determine the prevalence of *Campylobacter.* One 25-g sample was used to determine the prevalence of *Listeria,* and a second 25-g sample was used to determine the prevalence of non-O157 STEC and *Salmonella.*

Enumeration of bacteria. Aerobic plate counts (APCs) were performed, and *Enterobacteriaceae* (EB), coliform bacteria (CF), *E. coli* (EC), and *S. aureus* (SA) were enumerated after preparing a 1:10 ratio of beef trim samples to peptone water (Difco, Becton Dickinson, Sparks, Md.). Each dilution was prepared in a Whirl-Pak filter bag and then stomached in a laboratory blender (BagMixer 400VW, Interscience Laboratories Inc., Weymouth, Mass.) at high speed (nine strokes per second) for 30 s. One milliliter of each sample was taken from the filter side of the bag and plated onto Petrifilm (3M Microbiology, St. Paul, Minn.) aerobic count plates for APC, Petrifilm *Enterobacteriaceae* count plates for EB, Petrifilm *E. coli*/coliform count plates for EC and CF, and Petrifilm Staph Express count plates with Staph Express indicator disks for SA. Petrifilm plates were incubated according to the manufacturer's recommendations, and colonies were counted manually. Results were log transformed for analysis using SAS software (SAS Institute, Inc., Cary, N.C.). EB, CF, EC, and SA plates with no colonies (i.e., less than 10 CFU) were not used for determination of mean log CFU per gram, but a value of 1 CFU/g was assigned in the analysis of APC data when no colonies were present.

***Salmonella* isolation and characterization.** *Salmonella* was isolated by enriching a 25-g portion of each sample in 225 ml of tryptic soy broth (TSB; Difco, Becton Dickinson) according to established USMARC protocols *(18)* with the following modifications. Immunomagnetic separation was performed without the addition of protamine. The captured anti-*Salmonella* separation beads were selectively enriched in Oxoid Rappaport-Vassiliadis Soya peptone broth (Remel, Lenexa, Kans.) instead of Rappaport-Vassiliadis broth before plating to Hektoen enteric agar (HE) and brilliant green agar with sulfadiazol (BGS). Suspect colonies on either of these agar plates were confirmed to be *Salmonella* using PCR for the *invA* gene *(39).*

All confirmed *Salmonella* isolates were serogrouped using Wellcolex Colour *Salmonella* tests (Remel) according to the manufacturer's recommendations and were serotyped *(3)* using antisera for the identification of somatic and flagellar antigens (Remel). The antibiotic susceptibility of all *Salmonella* isolates was determined by performing MIC tests with the defined National Antimicrobial Resistance Monitoring System *Salmonella* antibiotic panels (CMV1AGNF, Trek Diagnostic Systems, Inc., Cleveland, Ohio) and a Sensititre AutoInoculator and AutoReader (Trek). The antibiotics in this panel were amikacin, amoxicillin–clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole.

***Campylobacter* isolation and identification.** The *Campylobacter* isolation procedure was performed using procedures found in the *Bacteriological Analytical Manual (21).* One hundred milliliters of 30°C prewarmed Bolton broth base (Neogen Corp., Lansing, Mich.) containing 5% sheep blood was added to 10 g of trim in a Whirl-Pak bag. This sample was then gently suspended by stomaching at the lowest speed (six strokes per second) for 1 min in a laboratory blender (Interscience Laboratories). Because trim samples had been frozen for storage and transit, a preenrichment step was used, which consisted of shaking (50 rpm) for 4 h at 37°C in a microaerobic atmosphere of 10% CO_2, 5% O_2, and 85% N. A selective enrichment followed, which consisted of the addition of antibiotics (20 mg/liter cefoperazone, 20 mg/liter trimethoprim, 20 mg/liter vancomycin, and 50 mg/liter cycloheximide) and incubation at 42°C for 48 h under microaerobic conditions. The enriched samples were streaked for isolation on modified CCDA plates (*Campylobacter* Blood-Free Selective Agar, Neogen) that were prepared as recommended by the manufacturer. These plates were incubated at 42°C for up to 48 h under microaerobic conditions. Suspect *Campylobacter* on CCDA plates were confirmed by a genus-specific PCR assay *(22)* and a species-specific multiplex PCR assay *(28)* that identified the thermotolerant *Campylobacter* species (*C. jejuni, C. coli, C. lari,* and *C. upsaliensis*).

***Listeria* isolation and characterization.** The prevalence of *Listeria* spp. in trim samples was determined using standard enrichment procedures *(19)* and PCR assay confirmation *(13).* Portions of trim (25 g) were placed in Whirl-Pak bags with 225 ml of University of Vermont medium (Neogen) and stomached (nine strokes per second) for 30 s in a laboratory blender (Interscience Laboratories). Each sample was then incubated at 35°C for 20 h. An aliquot (0.1 ml) of this enrichment was subcultured in 10 ml of Fraser broth (Neogen) that was incubated at 35°C for 40 h; this subculture was then streaked for isolation onto Oxford agar (Neogen) to isolate presumptive *Listeria* colonies. A multiplex PCR assay *(13)* was used to identify *Listeria* spp. (*L. monocytogenes, L. grayii, L. innocua, L. seeligeri, L. welshimeri,* and *L. ivanovii*). Isolates of *L. monocytogenes* (LM) were further characterized by serogrouping, using a combination of PCR assay *(14)* and specific antisera (Difco, Becton Dickinson) according to established protocols *(9).*

Non-O157 STEC isolation and characterization. The TSB enrichments for *Salmonella* were also used for the isolation of STEC as described previously *(6)* with minor modifications. Two 1-ml aliquots of each enrichment were mixed with 0.5 ml of 50% glycerol and stored at −70°C, and a 5-μl aliquot was used in a multiplex PCR assay to detect the *stx* genes *(35).* Enrichments of samples with positive signals for stx_1 and/or stx_2 were used for isolation of STEC. The corresponding −70°C glycerol stock of each stx_1- and/or stx_2-positive sample was diluted and spread plated to yield approximately 1,000 colonies per plate on 150-mm petri dishes of modified EC broth (Difco, Becton Dickinson) containing 1.5% Difco agar (Becton Dickinson) and 1% glucose. Colonies were lifted to Hybond-N+ membranes (GE Healthcare, Piscataway, N.J.) and used in hybridizations with combined stx_1 and stx_2 DNA probes *(6)* labeled using the ECL Direct Nucleic Acid Labeling and Detection System (GE Healthcare) according to the manufacturer's recommendations. Positive colonies were subcultured and confirmed to be STEC by PCR assay *(35).* Biochemical assays using Fluorocult LMX Broth (Merck KGaA, Darmstadt, Germany) and Sensititre Gram-negative ID panels (Trek) confirmed the organism to be *E. coli.* The serotype of each STEC isolate was determined by serologic identification of the O serogroup and molecular identification of the H group performed by the Gastroenteric Diseases Laboratory (College of Animal Sciences, Pennsylvania State University, University Park, Pa.).

Statistical analyses. Results from the enumeration of bacterial groups were analyzed by analysis of variance using the GLM procedures of SAS. The model included the main effect of country of origin. For significant main effects ($P < 0.05$), least squares means separation was carried out with the PDIFF option (a pairwise *t* test). Data for enumerations were log transformed before the analysis of variance. Pairwise comparisons of frequencies of EB, CF, EC, SA, and other pathogens were made using the PROC FREQ and Mantel-Haenszel chi-square analysis of SAS.

RESULTS

Enumerations. Populations of aerobic bacteria, EB, EC, CF, and SA (Table 1) revealed that URY samples had the highest concentrations of each indicator organism and AUS and NZL samples had the lowest. The APCs were different ($P < 0.05$) for each country. URY beef trim had the highest mean APC (2.8 log CFU/g), and U.S., NZL, and AUS trim had mean APCs of 2.5, 2.2, and 1.6 log CFU/g, respectively. Mean EB concentrations were the same ($P > 0.05$) for AUS, NZL, and U.S. trim (1.4, 1.5, and 1.5 log CFU/g, respectively) but were significantly higher ($P < 0.05$) for URY trim (2.0 log CFU/g). The frequency of samples with EB concentrations higher than 10 CFU/g was lowest and not different ($P > 0.05$) in AUS and NZL samples (8.2 and 9.0%, respectively). However, this frequency was significantly higher in both URY and U.S. samples (31.3 and 37.8%, respectively). The CF and EC results were similar in pattern to those for EB.

URY trim had the highest frequency of SA-positive samples (29.5%). The frequency of SA-positive samples was second highest in NZL samples (8.2%) and slightly different ($P < 0.05$) from U.S. trim (4.2%), but the same ($P > 0.05$) as AUS trim (4.0%). The level of SA contamination was statistically similar for URY (1.61 log CFU/g) and U.S. (1.35 CFU/g) samples but was significantly higher

TABLE 1. *Frequency and concentration of bacterial indicators of contamination in imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	Aerobic bacteria		EB			EC			CF			SA		
	No. of samples	Mean log CFU/g	No. of samples	No. (%) of samples above LOD[c]	Mean log CFU/g	No. of samples	No. (%) of samples above LOD	Mean log CFU/g	No. of samples	No. (%) of samples above LOD	Mean log CFU/g	No. of samples	No. (%) of samples above LOD	Mean log CFU/g
AUS	219	1.6 D	219	18 (8.2) B	1.4 B	198	2 (1.0) B	1.2[d]	198	9 (4.5) B	1.4 AB	198	8 (4.0) BC	1.1 B
NZL	223	2.2 C	223	20 (9.0) B	1.5 B	219	1 (0.5) B	1.0[d]	219	10 (4.6) B	1.5 AB	219	18 (8.2) B	1.3 B
URY	256	2.8 A	256	80 (31.3) A	2.0 A	241	23 (9.5) A	1.8 A	241	63 (26.1) A	2.0 A	241	71 (29.5) A	1.6 A
USA	486	2.5 B	487	184 (37.8) A	1.5 B	377	27 (7.2) A	1.2 B	377	96 (25.5) A	1.6 B	377	16 (4.2) C	1.4 AB

[a] Manual counts were done on appropriate Petrifilm count plates (3M Microbiology). EB, *Enterobacteriaceae*; EC, *E. coli*; CF, coliforms; SA, *Staphylococcus aureus*. Bacterial concentrations and frequencies within a column followed by the same letter are not significantly different ($P > 0.05$).

[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).

[c] Number of samples for which plate counts were above the limit of detection (LOD) of the 10 CFU/g.

[d] No statistical analysis was conducted when only one or two samples were above the LOD.

TABLE 2. *Frequency and characterization of* Salmonella *in imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	No. of samples	No. (%) of positive samples[c]	No. of samples resistant to antibiotics[d]
AUS	220	0	NA[e]
NZL	223	1 (0.4)[f]	1[g]
URY	256	1 (0.4)[h]	0
USA	487	4 (0.8)[i]	0

[a] *Salmonella* strains were isolated by immunomagnetic separation and selective enrichment. Suspect colonies on HE and BGS were confirmed by PCR assay, serogrouped, and serotyped.
[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).
[c] Prevalences for each country are not significantly different ($P > 0.05$).
[d] Antibiotic resistance was determined using NARMS MIC panels and the Trek Diagnostics Sensititre.
[e] NA, not applicable.
[f] New Zealand *Salmonella* isolate was serotype Typhimurium O5 null (Copenhagen).
[g] Isolate was resistant to ampicillin, chloramphenicol, streptomycin, sulphazoxazole, and tetracycline.
[h] Uruguay *Salmonella* isolate was serotype Newport.
[i] Domestic *Salmonella* isolates were serotypes Anatum, Enteritidis, and Montevideo. One isolate was untypeable.

TABLE 3. *Frequency and characterization of* Campylobacter *isolates from imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	No. of samples	No. (%) of positive samples[c]	No. of samples infected with: Undetermined species	*C. jejuni*	*C. coli*
AUS	151	0			
NZL	216	1 (0.5)	1	0	0
URY	250	1 (0.4)	0	1	0
USA	393	5 (1.3)	0	4	1

[a] *Campylobacter* strains were isolated by selective enrichment in Bolton broth followed by selective growth on modified CCDA. Suspect colonies were confirmed by latex agglutination and species-specific PCR. Thermotolerant *Campylobacter* species were identified by PCR assay of the *lpxA* gene.
[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).
[c] Prevalences are not significantly different ($P > 0.05$).

in URY samples than in both AUS and NZL samples ($P < 0.05$).

Salmonella. The prevalence of *Salmonella* was less than 1% in all samples and the same ($P > 0.05$) in the samples from all countries (Table 2). One *Salmonella* strain was isolated from NZL samples and one was isolated from URY samples. Four *Salmonella* strains were isolated from U.S. samples. No *Salmonella* was found in the 220 samples from Australia. Each *Salmonella* isolate was of a different serotype. The NZL *Salmonella* was serotype Typhimurium O5 null (Copenhagen), and the URY *Salmonella* isolate was serotype Newport. One of the *Salmonella* isolates in the U.S. samples was untypeable. The others were identified as *Salmonella* Anatum, *Salmonella* Enteritidis, and *Salmonella* Montevideo. All of the *Salmonella* isolates were characterized for antibiotic resistance; one isolate possessed resistance to multiple antibiotics. The *Salmonella* Typhimurium from New Zealand was resistant to ampicillin (MIC > 32 μg/ml), chloramphenicol (MIC > 32 μg/ml), streptomycin (MIC $= 64$ μg/ml), sulfamethoxazole (MIC > 256 μg/ml), and tetracycline (MIC $= 32$ μg/ml). This isolate was also intermediately resistant to amoxicillin–clavulanic acid (MIC $= 16$ μg/ml).

Campylobacter. The prevalence of *Campylobacter* was low and was not different ($P > 0.05$) among the samples tested. Some difficulties in maintaining microaerophilic conditions existed during these experiments, leading to the loss of some samples; seven *Campylobacter* were isolated (Table 3). Four *C. jejuni* and one *C. coli* strains were isolated from 393 U.S. samples. One *C. jejuni* strain was isolated from 250 URY samples. One *Campylobacter* strain that was neither *C. jejuni* nor *C. coli* also was isolated from 216 NZL samples. No *Campylobacter* strains were isolated from 151 AUS samples.

Listeria. The prevalence of *Listeria* spp. and LM was significantly higher ($P < 0.05$) in URY samples than in samples from other countries (Table 4). Twenty-nine percent (66 of 226) of URY trim samples contained at least one species of *Listeria.* Of the 66 *Listeria*-positive samples, 53 (almost 25% of all the URY samples tested) contained LM. Fifteen of the 66 *Listeria*-positive samples contained multiple species of *Listeria* and/or multiple serovars of LM. Other *Listeria* species identified in URY samples were *L. innocua and L. seeligeri,* and four isolates were untypeable. Twenty-two (6.5%) of the U.S. samples contained *Listeria.* Seventeen of these samples contained LM, and the other five contained other *Listeria* species (*L. ivanovii, L. welshimeri,* and *L. innocua*). One U.S. sample contained *L. innocua* and two serovars of LM. Six NZL and six AUS samples contained *Listeria*; five and four of these samples, respectively, were positive for LM. The only other *Listeria* species isolated from NZL and AUS samples was *L. innocua.*

Different serovars of LM were predominant in U.S., NZL, and AUS samples. All NZL LM isolates were serovar 1/2c, and these five isolates originated from two different establishments on five different pack dates. Three of four AUS LM strains were of the 4b serovar, and these three originated from one establishment but pack dates spanned a 3-month period. The predominant LM serovar in U.S. samples was 1/2a; 10 of these isolates originated from six different establishments. No LM serovar predominated in the URY samples, where serovars 1/2b, 1/2c, and 4b were equally prevalent ($P > 0.05$). Serovar 4b was found in 22 of the 58 LM-positive URY samples. These 22 serovar 4b LM stains originated from 7 of the 17 URY establishments

TABLE 4. *Frequency and characterization of* Listeria *and* L. monocytogenes *(LM) isolates from imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	No. of samples	No. (%) of samples positive for[c]:			No. of LM isolates of each serovar[d]:				
		LM	Other *Listeria*	Total	1/2a	1/2b	1/2c	4b	3a or 3b
AUS	198	4 (2.0) B	2 (1.0) B	6 (3.0) BC	0 Y	0 Y	1 XY	3 X	0 Y
NZL	219	5 (2.3) B	1 (0.5) B	6 (2.7) C	0 Y	0 Y	5 X	0 Y	0 Y
URY	226	53 (24) A	13 (5.8) A	66 (29) A	3 Y	16 X	17 X	22 X	0 Y
USA	341	17 (5.0) B	5 (1.5) B	22 (6.5) B	10 X	4 Y	0 Z	0 Z	4 Y

[a] *Listeria* detection was performed using standard culture techniques, and LM was identified by PCR assay of the *iap* and *prs* genes.
[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).
[c] Some samples contained multiple species of *Listeria* and/or serovars of LM. Within the same column values followed by the same letter are not significantly different ($P > 0.05$).
[d] LM serovars were determined using a combination of serology and PCR assay for specific sequences within Imo0737, Imo1118, ORF 2819, and ORF 2110. Within the same row in this section, values followed by the same letter are not significantly different ($P > 0.05$).

that provided beef trim. Serovar 1/2b originated from multiple establishments, whereas over 75% of serovar 1/2c strains originated from the same establishment.

Non-O157 STEC. The prevalence of Shiga toxin genes (*stx*) in each sample was determined by PCR assay (Table 5). Two different *stx* genes were evaluated, and trim samples were positive for stx_1, stx_2, or both stx_1 and stx_2. About 30% of the U.S., AUS, and URY samples were PCR positive for the *stx* genes. NZL samples were least often positive (10% of samples; $P < 0.05$). The distribution of stx_1 and stx_2 genes among the *stx*-positive samples varied by country of origin. The frequency of stx_1 alone was lower in *stx*-positive URY samples ($P < 0.05$) than in *stx*-positive AUS, NZL, and U.S. samples, whereas the frequency of stx_2 alone was not different ($P > 0.05$) among all source countries. The frequency of both genes in a single sample was highest in *stx*-positive URY samples ($P < 0.05$) and lowest in *stx*-positive AUS and NZL samples ($P < 0.05$).

The samples that were PCR positive in the *stx* gene screen were then further processed in an effort to isolate the source of the *stx* gene(s) (Table 6). At least one STEC was isolated from 81 of the 309 samples that were positive for *stx* genes. A total of 99 STEC were isolated. In 10 samples, two different STEC were isolated, and in four samples three different STEC were isolated. We were able to confirm the source of the *stx* gene(s) in 28 U.S., 9 AUS, 4 NZL, and 40 URY samples by isolation of an STEC. The STEC isolates were serotyped, and 63 different serotypes were identified. At least one serotype associated with human illness was isolated in samples from each country. Thirteen serotypes associated with HUS were isolated from U.S., URY, and NZL samples. No HUS-related strains were isolated from AUS samples. The prevalence of HUS-related serotypes among the isolated STEC strains from U.S. samples was not different ($P > 0.05$) from the prevalence in NZL, AUS, and URY samples.

TABLE 5. *Frequency of Shiga toxin 1 and Shiga toxin 2 genes in imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	No. of samples	No. (%) of samples[c] positive for:			
		stx_1	stx_2	stx_1 and stx_2	Total
AUS	220	44 (66) A	15 (22) A	8 (12) BC	67 (30) A
NZL	223	16 (70) A	5 (22) A	2 (8) BC	23 (9.7) B
URY	256	14 (20) B	24 (33) A	34 (47) A	72 (28) A
USA	487	77 (52) A	38 (26) A	32 (22) B	147 (30) A

[a] Shiga toxin genes were detected by PCR assays from TSB enrichments of each sample.
[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).
[c] Within each column, values followed by the same letter are not significantly different ($P > 0.05$).

DISCUSSION

Approximately 40 countries are approved to export to the United States (listed in the U.S. Code of Federal Regulations 9 CFR 327.2[b] and 381.196[b]). Countries that export the most beef to the United States are Australia, Canada, and New Zealand followed by Uruguay, Denmark, Argentina, Brazil, Nicaragua, and Costa Rica *(52)* In 2004, the United States imported 3.6 billion pounds (1.6 billion kilograms) of beef and veal, about 1 billion pounds (0.5 billion kilograms) of which originated in Canada *(51).* Although Canada is a significant source of imported beef, it does not primarily provide lean beef trim destined for ground beef. The North American cattle herds are similar with regard to relevant foodborne pathogens *(18).*

The number of tests performed in these studies consumed the entire sample, and in some cases insufficient sample was available. In situations where results were inconclusive, no results were recorded. For this reason, only 341 of the 487 U.S. samples produced results for *Listeria* (Table 4). This situation also was encountered with other enumerations (Table 1). While processing samples for *Campylobacter* (Table 3), the inability to maintain the microaerophilic atmosphere for a number of samples precluded valid results. Ap-

TABLE 6. *Serotypes of STEC isolated from imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	No. of positive samples[c]	No. of STEC isolated	Serotypes[d]					No. of HUS-related serotypes[e]
AUS	9	10[f]	O33:H11	O73:H35	O113:H36 (3)	O113:H51	O147:H7	0 B
			O171:H$^+$	ONT:H$^+$[g]	ONT:H2			
NZL	4	4	O26:H8	O26:H11[g,h]	O64:H9	O163:H19[g,h]		2 A
URY	40	52[i]	O2:H25	O6:H30	O6:H34	O8:H3	O8:H19 (2)[g,h]	6 B
			O15:H27 (3)[g]	O20:H19 (4)[g,h]	O39:H14	O55/83:H15	O74:H28 (2)	
			O82:H8	O82:H15	O83:H8 (2)	O83:H11	O88:H38 (2)	
			O113:H21[g,h]	O113:H36	O116:H36	O130:H11	O163:H19 (3)[g,h]	
			O163:H26	0168:H$^+$[g,h]	O174:H11	O174:H28 (2)	O174:H36 (2)[g]	
			ONT:H$^+$ (2)[g]	ONT:H11 (2)[g,h]	ONT:H18[g]	ONT:H19 (2)[g]	ONT:H32	
			ONT:H34	ONT:H46 (4)[g]	ONT:H51	ONT:H52		
USA	28	32[j]	O5:H36	O8:H19 (3)[g,h]	O20:H19[g,h]	O55/83:H15	O73:H$^+$	5 AB
			O73:H18	O79:H7[g,h]	O83:H$^+$	O83:H38	O83/132:H2	
			O88:H38	O113:H4[g]	O113:H51	O116:H21	O117:H$^+$	
			O132:H$^+$	O132:H38	O142:H34	O150:H2 or 35	O165:Hneg[g,h]	
			O171:H2	O172:H10	O174:H36	OX25:H11	ONT:H2	
			ONT:H7	ONT:H32	ONT:H51	Orough:Hneg[g,h]		

[a] Serotyping of STEC isolates was performed by the Gastroenteric Diseases Laboratory (Pennsylvania State University).
[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).
[c] Number of Shiga toxin 1– or Shiga toxin 2–positive samples from which an STEC isolate was recovered.
[d] Numbers in parentheses are the number of samples in which this serotype was found when more than 1.
[e] Values followed by the same letter are not different ($P > 0.05$).
[f] One sample contained two STEC serotypes.
[g] Serotype associated with human illness.
[h] Serotype associated with HUS *(11)*.
[i] Five samples contained two STEC serotypes, and one sample contained three STEC serotypes.
[j] Seven samples contained two STEC serotypes, and three samples contained three STEC serotypes.

proximately 200 or more samples from each country were available in each assay for statistical analyses, except for AUS samples tested for *Campylobacter*.

In this study, the foreign samples destined for export to the United States were packed from September through December, which are spring months in the Southern Hemisphere. The samples arrived and were compared with U.S. samples that were collected from December through February, which are winter months in the Northern Hemisphere. The seasonality of pathogens such as *Salmonella (7), E. coli (7),* and *Campylobacter (44)* is well documented. Pathogens increase in the spring, become highly prevalent through the summer and into the autumn, and then decrease during the winter. Therefore, the U.S. samples in this study were obtained during the low-prevalence season whereas the imported samples were from a high-prevalence season. These seasonal effects may be responsible for some of the differences observed between URY and U.S. samples, but when the results from the AUS and NZL samples are considered, the differences between the URY and U.S. samples seem more likely to be associated with the processing environment and processes in use rather than seasonal variations in bacterial prevalences.

The concentrations of these target indicator bacteria in boneless beef trim appear to have changed little since the publication of earlier studies. In previous studies, U.S. trim samples had a mean APC of 2.7 log CFU/g (range, 1.9 to 3.6 log CFU/g) and a mean CF count of 1.7 log CFU/g (range, 0.8 to 2.5 log CFU/g) *(26, 27)*. The differences in these populations between countries of origin are likely due to the number of specific instances of high counts. APCs for U.S. trim ranged as high as 4.85 log CFU/g as did APCs of URY trim, but the number of instances in which this concentration was observed in the U.S. trim was half that for the URY trim (4 and 9%, respectively). Both NZL and AUS trim had individual APCs that were higher than 4 log CFU/g, but the occurrence of these high concentrations was less frequent (1%) than it was in U.S. or URY trim samples. This frequency of putative contamination events also was reflected by the number of EB, CF, and EC colonies that were countable in all the samples.

All AUS processing establishments are required to operate under a single standard for the production, transportation, and export control of meat products for human consumption *(43)*. In a recent report on the microbiological quality of beef carcasses and frozen beef in Australia *(38),* beef produced at 24 establishments was examined. The results of that report correlate well with the results of our study for mean counts of total viable cells (APCs), coliforms, *E. coli,* and *Enterobacteriaceae*. The exception was the SA frequency. Coagulase-positive staphylococci were detected in 20.3% of the samples at a mean concentration of 0.80 log CFU/g and a maximum concentration 2.32 log CFU/g. We detected SA at a frequency of only 4% in AUS

samples. This discrepancy may be due to differences in the methodologies used to detect and identify SA *(38).*

C. jejuni is the most commonly reported bacterial cause of foodborne infection in the United States *(2).* The major risk factors for human campylobacteriosis are mishandling of raw poultry and consumption of undercooked poultry. The possible presence of *Campylobacter* previously has not been considered a significant food safety issue associated with beef. However, in beef cattle *Campylobacter* has been reported to be chronically shed by 85% of animals, and *C. jejuni* prevalence as high as 38% was reported *(23, 24).* Because of these high prevalences before processing, *Campylobacter* could be a significant carcass and subsequent trim contaminant. Despite the potential for contamination, a very low prevalence was found in the U.S. beef trim samples. Retail beef *Campylobacter* prevalence of <1% has been reported *(2, 54).*

We expected to find a higher prevalence of *Campylobacter* in AUS and NZL samples, as indicated by a 2001 Australian (OzFoodNet) survey performed in conjunction with the U.S. counterpart (FoodNet) in which the incidence of culture-confirmed *Campylobacter* infections was more than six times higher in Australia than in the United States *(53).* In New Zealand, *Campylobacter* infections were reported to have quadrupled in the last 15 years, and the incidence of *Campylobacter* infections was reported to be about five times that in Australia *(5).* We found a very low incidence of *Campylobacter* in the AUS and NZL samples, as has been previously reported for retail red meat in these two countries *(33).* Little is known about *Campylobacter* prevalence in URY, but this pathogen is recognized as a significant cause of diarrhea in that country *(46).* Our results indicate that *Campylobacter* does not appear to be a significant contaminant of beef trim and ground beef.

Salmonella is a major cause of foodborne illness in the United States, resulting in an estimated 1.3 million human cases, 15,600 hospitalizations, and 550 deaths each year *(31).* The most common *Salmonella* serotypes associated with human illness in the United States are Typhimurium, Enteritidis, Newport, Heidelberg, and Muenchen *(31).* We isolated *Salmonella* serotypes Anatum, Montevideo, and Enteritidis from the U.S. trim samples in this study. According to the USDA National Animal Health Monitoring System, *Salmonella* Montevideo was one of the five serotypes most frequently shed by feedlot cattle *(49),* and *Salmonella* Anatum was one of the five serotypes most frequently shed by dairy cattle *(48, 50). Salmonella* Enteritidis is usually associated with eggs and poultry and is uncommon in cattle *(42).*

Isolates of *Salmonella* serotypes Newport and Typhimurium have exhibited various antimicrobial susceptibility patterns, and many have shown resistance to multiple antimicrobials *(10, 55).* A multidrug-resistant *Salmonella* Newport of particular concern is resistant to eight antimicrobials: ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, amoxicillin–clavulanic acid, ceftiofur, and cephalothin. Multidrug-resistant *Salmonella* Typhimurium can have either the same antibiotic resistance as *Salmonella* Newport or can be resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). The *Salmonella* Newport isolated from a URY trim sample was not resistant to these antimicrobials. We isolated *Salmonella* Typhimurium, a commonly identified serotype from bovine sources in New Zealand *(4),* from one NZL trim sample, and it was ACSSuT resistant.

LM is the causative agent of epidemic and sporadic listeriosis, and the consequences from contracting listeriosis can be particularly severe in pregnant women, newborns (<1 year of age), the elderly (>65 years of age), and immunocompromised individuals *(16, 30, 41).* LM is present throughout the environment and is routinely isolated from numerous animal sources, including cattle, but comprehensive information on the prevalence of LM in U.S. and foreign beef trim is minimal *(40).* LM is considered a public health threat in ready-to-eat foods (e.g., cold cuts, cheese, and ice cream), whereas its presence in raw meat, which will presumably be cooked, is not considered as great a threat. In our study, the prevalence of LM in beef trim was generally below 5%, except for samples from Uruguay, where almost one in four samples contained LM.

Thirteen serovars of LM have been described, but four serovars (1/2a, 1/2b, 1/2c, and 4b) make up 95% of the isolates recovered from foods and infected humans *(14, 45).* Serovar 4b has been described as the cause of major outbreaks of invasive listeriosis *(14, 30).* We isolated LM 4b only from AUS and URY trim samples. Three of the four LM isolates from AUS samples were serovar 4b and were traceable to a single establishment, which provided only four samples for this study. Thirty-eight percent of the LM isolated from URY samples were serovar 4b, and two thirds of these isolates could be traced back to four establishments. Such high prevalence of a single serotype suggest that its source is the processing environment, but additional tracking data are needed for confirmation. The five LM isolates found in the NZL samples came from 2 of the 44 establishments that provided samples. The 17 LM isolates from U.S. samples could be traced back to nine establishments. These data suggest that URY processors need to examine their production environments for sources of LM contamination so that contamination with this organism can be reduced.

STEC strains have been implicated as the causative agents in human diseases ranging from mild diarrhea to life-threatening HUS *(32, 37).* STEC strains can contain either or both of the *stx* genes; however, severe disease is more commonly associated with strains that possess the stx_2 gene *(37).* A large difference in the distribution of the stx_2 gene was observed in the trim samples. Only 10% of NZL samples contained an *stx* gene, and in 70% of the cases it was the less pathogenic stx_1 gene. URY, AUS, and U.S. samples had similar frequencies of *stx* gene carriage, but 80% of the *stx*-positive URY samples carried the more pathogenic stx_2 gene, whereas only 34% of AUS and 48% of U.S. samples carried stx_2.

Statistical analysis of recovery rates for STEC isolates from samples that were PCR positive for the *stx* genes revealed that URY trim was different from U.S., AUS, and NZL trim. The rate of recovery of STEC isolates from U.S.,

AUS, or NZL samples was less than 20%, whereas STEC isolates were recovered from 56% of URY samples that were PCR positive for *stx*. The PCR assay used may have detected *stx* genes carried by other organisms such as *Citrobacter freundii* or *Enterobacter cloacae (34, 47)*; however, it is more likely that the *E. coli* contamination in the URY samples, based on the results of *E. coli* enumeration, is the explanation for the higher rate of recovery of STEC in the URY samples.

Many STEC serotypes have been isolated, characterized, and catalogued. Some of the non-O157 STEC serotypes associated with clinical disease are O8, O26, 045, O103, O111, O113, O121, and 0145 *(1, 12, 15, 32)*. However, many STEC strains have been neither isolated from humans nor associated with human disease *(11)*. Statistical analysis revealed that the serotype distribution of the HUS-associated strains from imported and U.S. beef trim was not different.

The STEC serotype most frequently isolated from the AUS trim in our study was O113:H36. Although STEC O113 is described as a common disease-related serotype, the H36 antigen associated with this isolate in the AUS samples has not been associated with HUS *(11, 36)*. In our study, only 4 of the 223 NZL samples examined contained an isolatable STEC, and two of the four isolates had HUS-related serotypes. A much larger sample set would be needed to determine whether NZL beef trim posed any higher risk of infection than does U.S. trim. In our study, the incidence of HUS-associated serotypes in NZL samples (two of the four isolates) was not different ($P > 0.05$) from that in U.S. samples (7 of 32 isolates). In studies performed for the New Zealand Food Safety Authority (NZFSA), the majority of STEC isolates from NZL red meats were of serotypes that were infrequently a cause of disease *(29)*. In that study, STEC contamination was low in New Zealand by international standards, and infections were rare. The majority of STEC infections in New Zealand were attributed to serotype O157:H7, and only one death was attributed to a non-O157 STEC infection *(29)*. We isolated STEC serotypes O163:H19 and O26:H11 from NZL samples. Serotype O163:H19 has been isolated by NZFSA from minced beef *(20)*, and serotype O26:H11 was previously isolated from NZL bobby veal *(17)*. The presence of veal or calf trim in the imported beef trim from NZL could not be determined. According to previous reports and ongoing surveillance, the other serotypes of STEC we isolated from NZL samples, O26:H8 and O64:H9, were unique.

To the best of our knowledge, this is the first report of STEC serotypes O5:H36, O8:H3, O33:H11, O39:H14, O73:H35, O82:H15, O83:H8, O83:H38, O113:H36, O113:H51, and O163:H26. Three of these serotypes (O88:H38, O113:H36, and O113:H51) were isolated from beef trim originating in different countries. These results indicate that STEC is common in multiple countries, it is likely that there are many STEC serotypes yet to be identified.

The results of this study indicate that microbiological differences exist between imported and U.S. boneless beef trim destined for ground beef, but these differences do not necessitate changes to the current monitoring system used in the United States. Although it may be tempting to use these data to compare the microbiological quality of beef trim from the countries examined, such comparisons are best made using data collected before export or data from agencies such as OzFoodNet or the New Zealand National Microbiological Database. Generally, AUS and NZL lean beef trim had lower levels of contamination than did U.S. trim, whereas in URY samples levels of some contaminants were higher after arrival in the United States. Compared with U.S. trim, URY trim also had a higher frequency of illness-related serovars of LM and HUS-associated STEC. During the collection of these data, preliminary results were shared with the participants. The importers who supplied the beef trim for this work communicated with their URY suppliers in an effort to reduce contamination and improve slaughter practices. It has been reported to us that URY processors have adopted improved methods and that current imports of URY beef trim into the United States have improved in microbial quality.

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REFERENCES

1. Acheson, D. W. 2000. How does *Escherichia coli* O157:H7 testing in meat compare with what we are seeing clinically? *J. Food Prot.* 63:819–821.
2. Altekruse, S. F., N. J. Stern, P. I. Fields, and D. L. Swerdlow. 1999. *Campylobacter jejuni*—an emerging foodborne pathogen. *Emerg. Infect. Dis.* 5:28–35.
3. Andrews, W. H., and T. S. Hammack. 1998. *Salmonella,* chap. 5. *In* Bacteriological analytical manual, 8th ed., rev. A. Available at: http://www.cfsan.fda.gov/~ebam/bam-toc.html. Accessed 6 January 2006.
4. Anonymous. 2005. Non-human *Salmonella* isolates, 2004. Public Health Surveillance Report. Available at: http://www.surv.esr.cri.nz/enteric_reference/nonhuman_salmonella.php?we_objected=579. Accessed 6 January 2006.
5. Anonymous. 2005. Notifiable and other diseases in New Zealand. Annual report 2004. Available at: http://www.surv.esr.cri.nz/PDF_surveillance/AnnSurvRpt/2004AnnualSurvRpt.pdf. Accessed 6 January 2006.
6. Arthur, T. M., G. A. Barkocy-Gallagher, M. Rivera-Betancourt, and M. Koohmaraie. 2002. Prevalence and characterization of non-O157 Shiga toxin–producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. *Appl. Environ. Microbiol.* 68:4847–4852.
7. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli,* including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
8. Barkocy-Gallagher, G. A., E. D. Berry, M. Rivera-Betancourt, T. M. Arthur, X. Nou, and M. Koohmaraie. 2002. Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *J. Food Prot.* 65:1527–1534.
9. Bennett, R. W., and R. E. Weaver. 1998. Serodiagnosis of *Listeria*

monocytogenes, chap. 11. *In* Bacteriological analytical manual, 8th ed., rev. A. Available at: http://www.cfsan.fda.gov/~ebam/bam-toc.html. Accessed 6 January 2006.
10. Berge, A. C., J. M. Adaska, and W. M. Sischo. 2004. Use of antibiotic susceptibility patterns and pulsed-field gel electrophoresis to compare historic and contemporary isolates of multi-drug-resistant *Salmonella enterica* subsp. *enterica* serovar Newport. *Appl. Environ. Microbiol.* 70:318–323.
11. Bettelheim, K. A. 2006. Serotypes of VTEC. The VTEC table. Available at: http://www.microbionet.com.au/vtectable.htm. Accessed 20 January 2006.
12. Brooks, H. J., K. A. Bettelheim, B. Todd, and M. D. Holdaway. 1997. Non-O157 vero cytotoxin producing *Escherichia coli*: aetiological agents of diarrhoea in children in Dunedin, New Zealand. *Comp. Immunol. Microbiol. Infect. Dis.* 20:163–170.
13. Bubert, A., I. Hein, M. Rauch, A. Lehner, B. Yoon, W. Goebel, and M. Wagner. 1999. Detection and differentiation of *Listeria* spp. by a single reaction based on multiplex PCR. *Appl. Environ. Microbiol.* 65:4688–4692.
14. Doumith, M., C. Buchrieser, P. Glaser, C. Jacquet, and P. Martin. 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* 42:3819–3822.
15. Friedrich, A. W., M. Bielaszewska, W. L. Zhang, M. Pulz, T. Kuczius, A. Ammon, and H. Karch. 2002. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J. Infect. Dis.* 185:74–84.
16. Gasanov, U., D. Hughes, and P. M. Hansbro. 2005. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. *FEMS Microbiol. Rev.* 29:851–875.
17. Hathaway, S. (Programme Development Group, New Zealand Food Safety Authority). 2005. Personal communication.
18. Health Canada. 2003. Canadian integrated surveillance report: *Salmonella, Campylobacter,* pathogenic *E. coli,* and *Shigella* from 1996 to 1999. CCDR 2003;29S1. Available at: http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/03vol29/29s1/index.html. Accessed 6 January 2006.
19. Hitchins, A. D. 2002. Detection and enumeration of *Listeria monocytogenes* in foods, chap. 10. *In* Bacteriological analytical manual, 8th ed., rev. A. Draft rev., September 2002. Available at: http://www.cfsan.fda.gov/~ebam/bam-toc.html. Accessed 6 January 2006.
20. Hudson, J. A., C. Nicol., J. Capill, and J. Bennett. 2000. Isolation of Shiga toxin–producing *Escherichia coli* (STEC) from foods using EHEC agar. *Lett. Appl. Microbiol.* 30:109–113.
21. Hunt, J. M., C. Abeyta, and T. Tran. 1998. *Campylobacter,* chap. 7. *In* Bacteriological analytical manual, 8th ed., rev. A. Available at: http://www.cfsan.fda.gov/~ebam/bam-toc.html. Accessed 6 January 2006.
22. Inglis, G. D., and L. D. Kalischhuk. 2003. Use of PCR for direct detection of *Campylobacter* species in bovine feces. *Appl. Environ. Microbiol.* 69:3435–3447.
23. Inglis, G. D., L. D. Kaliscuk, and H. W. Busz. 2003. A survey of *Campylobacter* species shed in faeces of beef cattle using polymerase chain reaction. *Can. J. Microbiol.* 49:655–661.
24. Inglis, G. D., L. D. Kaliscuk, and H. W. Busz. 2004. Chronic shedding of *Campylobacter* species in beef cattle. *J. Appl. Microbiol.* 97:410–420.
25. Kaferstein, F. K., Y. Motarjemi, and D. W. Bettcher. 1997. Foodborne disease control: a transnational challenge. *Emerg. Infect. Dis.* 3:503–510.
26. Kang, D. H., M. Koohmaraie, W. J. Dorsa, and G. R. Siragusa. 2001. Development of a multiple-step process for the microbial decontamination of beef trim. *J. Food Prot.* 64:63–71.
27. Kang, D. H., M. Koohmaraie, and G. R. Siragusa. 2001. Application of multiple antimicrobial interventions for microbial decontamination of commercial beef trim. *J. Food Prot.* 64:168–171.
28. Klena, J. D., C. T. Parker, K. Knabb, J. C. Ibbitt, P. M. L. Devane, S. T. Horn, W. G. Miller, and M. E. Konkel. 2004. Differentiation of *Campylobacter coli, Campylobacter jejuni, Campylobacter lari,* and *Campylobacter upsaliensis* by a multiplex PCR developed from the nucleotide sequence of the lipid A gene *lpxA. J. Clin. Microbiol.* 42:5549–5557.
29. Lake, R., A. Hudson, and P. Cressey. 2002. Risk profile: Shiga toxin–producing *Escherichia coli* in red meat and meat products. Institute of Environmental Science and Research, Ltd., Porirua, New Zealand.
30. Mead, P. S., E. F. Dunne, L. Graves, M. Wiedmann, M. Patrick, S. Hunter, E. Salehi, F. Mostashari, A. Craig, P. Mshar, T. Bannerman, B. D. Sauders, P. Hayes, W. Dewitt, P. Sparling, P. Griffin, D. Morse, L. Slutsker, and B. Swaminathan. 2006. Nationwide outbreak of listeriosis due to contaminated meat. *Epidemiol. Infect.,* in press.
31. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.
32. Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli. Clin. Microbiol. Rev.* 11:142–201.
33. OzFoodNet Working Group. 2005. Reported foodborne illness and gastroenteritis in Australia: annual report of the OzFoodNet network, 2004. *Commun. Dis. Intell.* 29:164–190.
34. Paton, A. W., and J. C. Paton. 1996. *Enterobacter cloacae* producing a Shiga-like toxin II–related cytotoxin associated with a case of hemolytic-uremic syndrome. *J. Clin. Microbiol.* 34:463–465.
35. Paton, A. W., and J. C. Paton. 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1, stx2, eaeA,* enterohemorrhagic *E. coli hlyA, rfb*O111, and *rfb*O157. *J. Clin. Microbiol.* 36:598–602.
36. Paton, A. W., R. M. Ratcliff, R. M. Doyle, J. A. Lanser, and J. C. Paton. 1996. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin–producing *Escherichia coli. J. Clin. Microbiol.* 34:1622–1627.
37. Paton, J. C., and A. W. Paton. 1998. Pathogenesis and diagnosis of Shiga toxin–producing *Escherichia coli* infections. *Clin. Microbiol. Rev.* 11:450–479.
38. Phillips, D., D. Jordan, S. Morris, I. Jenson, and J. Sumner. 2006. A national survey of the microbiological quality of beef carcasses and frozen boneless beef in Australia. *J. Food Prot.* 69:1113–1117.
39. Rahn, K., S. A. De Grandis, R. C. Clarke, S. A. McEwen, J. E. Galan, G. Ginocchio, R. Curtiss, and C. L. Gyles. 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella. Mol. Cell. Probes* 6:271–279.
40. Rivera-Betancourt, M., S. D. Shackelford, T. M. Arthur, K. E. Westmoreland, G. Bellinger, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* in two geographically distant commercial beef processing plants in the United States. *J. Food Prot.* 67:295–302.
41. Schlech, W. F. 2000. Foodborne listeriosis. *Clin. Infect. Dis.* 31:770–775.
42. Sorensen, O., J. V. Donkersgoed, M. McFall, K. Manninen, G. Gensler, and G. Ollis. 2002. *Salmonella* spp. shedding by Alberta beef cattle and the detection of *Salmonella* spp. in ground beef. *J. Food Prot.* 65:484–491.
43. Standards Australia. 2002. Australian standard for production and transportation of meat and meat products for human consumption, AS 4696. SAI Global, Sydney.
44. Stanley, K. N., J. S. Wallace, J. E. Currie, P. J. Diggle, and K. Jones. 1998. The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves. *J. Appl. Microbiol.* 85:472–480.
45. Tappero, J., A. Schuchat, K. A. Deaver, L. Mascola, and J. D. Wenger. 1995. Reduction in listeriosis: effectiveness of prevention efforts? *JAMA* 273:1118–1122.
46. Torres, M. E., M. C. Pírez, F. Schelotto, G. Varela, V. Parodi, F. Allende, E. Falconi, L. Dell'Acqua, P. Gaione, M. V. Méndez, A. M. Ferrari, A. Montano, E. Zanetta, A. M. Acuña, H. Chiparelli, and E. Ingold. 2001. Etiology of children's diarrhea in Montevideo, Uruguay: associated pathogens and unusual isolates. *J. Clin. Microbiol.* 39:2134–2139.
47. Tschape, H., R. Prager, W. Streckel, A. Fruth, E. Tietze, and G. Bohme. 1995. Verocytotoxigenic *Citrobacter freundii* associated

with severe gastroenteritis and cases of haemolytic uraemic syndrome in a nursery school: green butter as the infection source. *Epidemiol. Infect.* 114:441–450.

48. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 1994. *Salmonella* in dairy calves. Centers for Epidemiology and Animal Health, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Fort Collins, Colo.
49. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 1995. *Salmonella* shedding by feedlot cattle. Centers for Epidemiology and Animal Health, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Fort Collins, Colo.
50. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 1998. *E. coli* O157:H7 and *Salmonella* status on U.S. dairy operations. Centers for Epidemiology and Animal Health, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Fort Collins, Colo.
51. U.S. Department of Agriculture, Economic Research Service. 2005. Background statistics: U.S. beef and cattle industry. Available at: http://www.ers.usda.gov/news/BSECoverage.htm. Accessed 6 January 2006.
52. U.S. Department of Agriculture, Foreign Agricultural Service. 2005. BICO import commodity aggregations. Available at: http://www.fas.usda.gov/USTRADE/USTImBICO.asp?QI=. Accessed 6 January 2006.
53. Vally, H., M. D. Kirk, E. Scallan, F. J. Angulo, and G. V. Hall. 2004. Higher incidence of *Campylobacter* infection in Australia compared with the United States in 2001. FoodNet publication. Available at: www.cdc.gov/foodnet/pub/publications/2004/vally_2004.pdf. Accessed 6 January 2006.
54. Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli,* and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington, D.C. area. *Appl. Environ. Microbiol.* 67:5431–5436.
55. Zhao, S., P. J. Fedorka-Cray, S. Friedman, P. F. McDermott, R. D. Walker, S. Qaiyumi, S. L. Foley, S. K. Hubert, S. Ayers, L. English, D. A. Dargatz, B. Salamone, and D. G. White. 2005. Characterization of *Salmonella* Typhimurium of animal origin obtained from the National Antimicrobial Resistance Monitoring System. *Foodborne Pathol. Dis.* 2:169–181.

MAJOR ARTICLE

Non-O157 Shiga Toxin–Producing *Escherichia coli* Infections in the United States, 1983–2002

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Background. Shiga toxin–producing *Escherichia coli* (STEC) O157:H7 is a well-recognized cause of bloody diarrhea and hemolytic-uremic syndrome (HUS). Non-O157 STEC contribute to this burden of illness but have been underrecognized as a result of diagnostic limitations and inadequate surveillance.

Methods. Between 1983 and 2002, 43 state public health laboratories submitted 940 human non-O157 STEC isolates from persons with sporadic illnesses to the Centers for Diseases Control and Prevention reference laboratory for confirmation and serotyping.

Results. The most common serogroups were O26 (22%), O111 (16%), O103 (12%), O121 (8%), O45 (7%), and O145 (5%). Non-O157 STEC infections were most frequent during the summer and among young persons (median age, 12 years; interquartile range, 3–37 years). Virulence gene profiles were as follows: 61% stx_1 but not stx_2; 22% stx_2 but not stx_1; 17% both stx_1 and stx_2; 84% intimin *(eae);* and 86% enterohemolysin (E-*hly*). stx_2 was strongly associated with an increased risk of HUS, and *eae* was strongly associated with an increased risk of bloody diarrhea. STEC O111 accounted for most cases of HUS and was also the cause of 3 of 7 non-O157 STEC outbreaks reported in the United States.

Conclusions. Non-O157 STEC can cause severe illness that is comparable to the illness caused by STEC O157. Strains that produce Shiga toxin 2 are much more likely to cause HUS than are those that produce Shiga toxin 1 alone. Improving surveillance will more fully elucidate the incidence and pathological spectrum of these emerging agents. These efforts require increased clinical suspicion, improved clinical laboratory isolation, and continued serotyping of isolates in public health laboratories.

More than 100 serotypes of Shiga toxin–producing *Escherichia coli* (STEC) have been associated with human disease [1–3], causing illnesses that range from mild diarrhea to bloody diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) [4, 5]. *E. coli* O157:H7 is the STEC most frequently isolated in North America and is the serotype most often associated with bloody diarrhea and HUS. Non-O157 STEC have also caused sporadic illness and outbreaks of bloody diarrhea and HUS, both in the United States [6–10] and overseas [11–21]. It has been estimated that STEC O157:H7 causes 73,000 illnesses annually in the United States and that non-O157 STEC serotypes cause at least 37,000 illnesses [22]. Surveys from North America demonstrate that STEC causes diarrhea at frequencies similar to those of other important enteric bacterial pathogens (e.g., *Salmonella* and *Shigella* species), depending on the population studied and its geographic location, with STEC O157 isolated in one-half of the illnesses and a variety of non-O157 STEC serotypes isolated in the other half [23–34]. In continental Europe, infections with non-O157 STEC serotypes are more common than infections with O157:H7 STEC

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[35, 36]. Not all non-O157 STEC cause human illness, and some non-O157 STEC isolates recovered from diarrheal stool are likely not pathogens. The full spectrum of pathogenic non-O157 serogroups and the illnesses they cause remain poorly defined.

When infection with a non-O157 STEC is suspected, clinical laboratories may refer isolates to public health laboratories for identification and further characterization. The Foodborne and Diarrheal Diseases Laboratory of the Centers for Diseases Control and Prevention (CDC) serves as the national reference laboratory for the confirmation of suspect non-O157 STEC isolates submitted by clinical laboratories to state and local public health departments and is one of the few laboratories in North America with the capacity to serotype confirmed isolates. Until 2000, non-O157 STEC infections were not a nationally notifiable disease. Since 2000, the Council of State and Territorial Epidemiologists has requested that public health departments report STEC infections to the National Notifiable Diseases Surveillance System. In the present article, we summarize the data from a large convenience sample of sporadic human non-O157 STEC isolates from the United States that have been confirmed by the CDC, describing the frequencies of serotypes, their seasonality, age distribution, the presence of various virulence factors, and associations between virulence factors and clinical syndromes.

SUBJECTS, MATERIALS, AND METHODS

Data collection. We reviewed the records for non-O157 STEC isolates forwarded by state public health laboratories to the CDC's reference laboratory between 1983 and 2002 for confirmation and serotyping. The documentation required when submitting specimens to the CDC included the name of the submitting state, the date of illness and/or specimen collection, the source (e.g., stool or blood), and the age and sex of the person from whom the specimen was collected. A presumptive diagnosis (e.g., diarrhea or HUS) and illness symptoms were requested but not required. Some of the isolates included in this national data set (<10% total) may have been included in previous analyses from contributing jurisdictions [31, 32, 34, 37]. The present investigation followed the guidelines of the US Department of Health and Human Services with regard to the protection of human subjects.

Laboratory procedures. Submitted isolates were streaked onto tryptose blood plates with washed sheep blood (Smith River Biologicals) and were incubated at 35°C for 18–24 h [38] The plates were examined at 4 and 18 h for production of enterohemolysin. Individual colonies, both hemolytic and nonhemolytic, were then plated on trypticase soy agar with 5% sheep blood, incubated for 18–24 h, and tested by polymerase chain reaction for gene sequences encoding the following virulence factors: Shiga toxins 1 and 2 (stx_1 and stx_2) [39–41], intimin *(eae)* [42], and enterohemolysin (E-*hly*) [43]. Isolates that were positive for either or both Shiga toxins were serologically characterized for O and H antigens [44].

Statistical methods. We created a data set with a unique record for each isolate submitted, eliminating duplicate isolates that had been submitted from the same person for the same illness episode. We limited our descriptive analysis to sporadic disease by excluding any non-O157 STEC isolates that had been sent to the CDC from the few known US outbreaks. We analyzed dichotomous variables by the χ^2 test and Fisher's exact test and continuous variables by the Wilcoxon rank sum test (SAS; version 8.0e; SAS Institute). Multivariate analyses were performed by logistic regression with a backward-elimination process. We considered associations to be significant if the 95% confidence interval (CI) excluded 1.0 and $P \leq .05$ (2-tailed).

RESULTS

Between 1983 and 2002, the CDC confirmed 940 non-O157 STEC isolates that had been submitted by 42 state public health laboratories and the District of Columbia; 866 (92%) of the isolates were received after 1996 (figure 1). For 801 (85%) isolates, the clinical specimen source was provided: 794 were from stool, 4 were from urine, and 3 were from blood. The O-groups for 123 (13%) isolates could not be determined (i.e., they were nontypeable or formed rough colonies). The remainder included 209 (22%) O26:H11 or NM; 152 (16%) O111:H8 or NM; 117 (12%) O103:H2, H11, H25, or NM; 80 (8%) O121:H19 or H7; 63 (7%) O45:H2 or NM; 43 (5%) O145:NM; and 147 other isolates from 55 O-groups (each accounting for ≤1% of all isolates) (table 1). The 6 most common serogroups accounted for 664 (71%) of all isolates. No cases were identified in which >1 STEC strain was isolated during a single illness episode. Most isolates were collected during the North American summer, between June and September. Incidence peaked in August, when 18% of all isolates were collected (figure 2). Other than this seasonality, there was no other evident clustering of isolates from any serotype in time or space in a fashion that would have suggested an unrecognized outbreak. We found no statistically significant differences in the proportional distributions of the 6 most common serotype north and south of latitudes 36°N and 42°N (data not shown).

Of 676 isolates from persons whose sex was included on the specimen submission form, 372 (55%) were from women. Of 501 (53%) isolates collected from persons for whom age was provided, the median age was 12 years (interquartile range [IQR], 3–37 years); 285 (57%) isolates were from persons ≥10 years old. There were 357 persons of known age from whom isolates belonging to the 6 most common serogroups were isolated. The median age of these persons was significantly lower than that of the 61 persons with isolates belonging to other serogroups (10 vs. 21 years; $P = .04$), after exclusion of 83

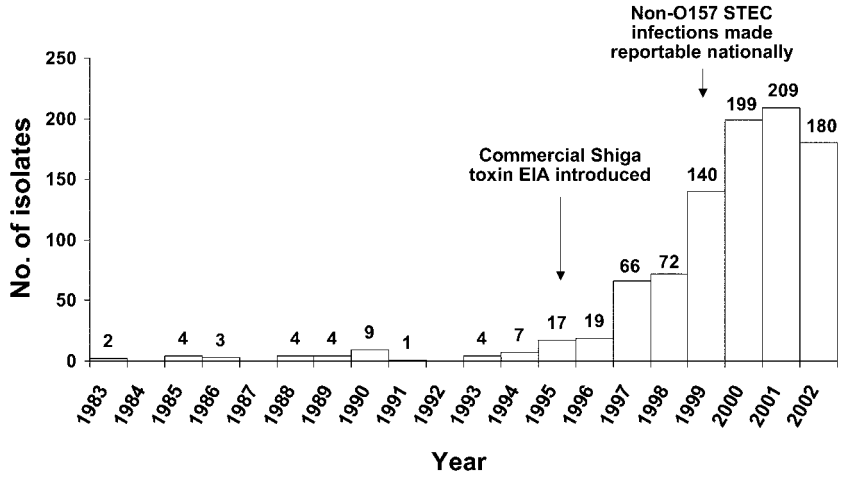

Figure 1. Human non-O157 Shiga toxin–producing *Escherichia coli* (STEC) isolates submitted to the Centers for Disease Control and Prevention for confirmation, by year, 1983–2002 ($n = 940$).

persons of known age from whom non-O157 STEC isolates of undetermined serogroup were isolated.

Of 292 (31%) isolates submitted with definitive clinical data, 21 (7%) were from persons with HUS and 75 (26%) were from persons with bloody diarrhea but not HUS (table 2). Data on age were available for 13 (62%) of the 21 persons from whom isolates associated with HUS were collected; among these 13, the median age was 6 years (IQR, 2–10 years). Data on age were available for 49 (65%) of the 75 persons from whom isolates associated with bloody diarrhea but not HUS were collected; among these 49, the median age was 17 years (IQR, 5–51 years).

STEC O111 was the only serogroup statistically associated with HUS; it was isolated from 10 (48%) of the 21 persons with HUS, compared with 42 (16%) of the 271 persons without HUS (relative risk [RR], 4.20 [95% CI, 1.18–9.36]; $P = .001$). STEC O121 was the only serogroup statistically associated with bloody diarrhea; it was isolated from 13 (17%) of the 75 persons with bloody diarrhea but not HUS, compared with 9 (4%) of the 217 persons without bloody diarrhea (RR, 2.57 [95% CI, 1.71–3.88]; $P = .001$).

Multivariate analyses examining the relationships between virulence gene profiles (singly and in all permutations, up to and including the combination of all 4 genes evaluated) and HUS demonstrated a statistically significant increased risk of HUS associated with stx_2: 20 (93%) of the 21 persons with HUS provided isolates with detectable stx_2, compared with 88 (33%) of the 271 persons without HUS (estimated odds ratio [OR], 32.0 [95% CI, 4.26–240]; $P = .008$). Similar analyses demonstrated a statistically significant increased risk of bloody diarrhea without HUS associated with *eae:* 70 (93%) of the 75 persons with bloody diarrhea but without HUS provided isolates with detectable *eae,* compared with 184 (86%) of the 214 persons without bloody diarrhea (OR 5.26 [95% CI, 1.60–17.3]; $P = .006$). All associations remained robust and of comparable magnitude when the univariate and multivariate analyses included all isolates, regardless of clinical data.

DISCUSSION

This comprehensive evaluation of non-O157 STEC isolates from persons in the United States found that 6 (O26, O111, O103, O121, O45, and O145) of the 61 serogroups identified accounted for 71% of the isolates recovered from 1983 to 2002. Three of these serogroups (O26, O111, and O103) accounted for 50% of the isolates. The non-O157 STEC demonstrated a summer seasonality, similar to that for the STEC O157:H7 [45, 46], and were isolated more frequently from children.

The virulence gene stx_2 was significantly associated with an increased risk of HUS in persons infected with non-O157 STEC in the United States, which is consistent with similar findings reported from the United Kingdom [47]. Surveys have shown that the vast majority of North American STEC O157:H7 isolates associated with HUS possess stx_2 alone or in combination with stx_1 and that only a small fraction possess stx_1 but not stx_2 [4, 46, 48]; we found a similar distribution of Shiga toxin genes among the non-O157 STEC isolates associated with HUS. Studies conducted elsewhere in the world examining the association between HUS and virulence factors in STEC collections, some of which included O157:H7, have similarly shown that stx_2 is the factor most strongly associated with the development of HUS [20, 47, 49–54]. Although the mechanism by which Shiga toxin 2 causes HUS is not fully understood, the active site of this molecule is considerably more accessible than the active site of Shiga toxin 1, which may explain the stronger association between this virulence factor and disease [55].

Table 1. Human non-O157 Shiga toxin–producing *Escherichia coli* isolates submitted to the Centers for Disease Control and Prevention (CDC) for confirmation and prevalence of associated virulence genes, by serotype, 1983–2002.

Serogroup	Age of donor, median (IQR), years[a]	Shiga toxin			*eae*	E-*hly*
		stx$_1$ alone	*stx*$_2$ alone	*stx*$_1$ and *stx*$_2$		
O26 (n = 209)	6 (2–18)	182/208 (88)	4/208 (2)	22/208 (11)	198/202 (98)	195/202 (96)
O111 (n = 152)	6 (2–21)	65/152 (43)	0/152 (0)	87/152 (57)	146/149 (98)	139/149 (93)
O103 (n = 117)	17 (3–31)	117/117 (100)	0/117 (0)	0/117 (0)	115/115 (100)	114/115 (99)
O121 (n = 80)	10 (4–34)	1/80 (1)	72/80 (90)	7/80 (9)	73/75 (97)	69/75 (92)
O45 (n = 63)	25 (13–48)	62/63 (98)	0/63 (0)	1/63 (2)	62/63 (98)	60/63 (95)
O145 (n = 43)	18 (6–42)	18/43 (42)	19/43 (44)	6/43 (14)	42/42 (100)	42/42 (100)
O165 (n = 14)	20 (8–66)	0/14 (0)	7/14 (50)	7/14 (50)	13/14 (93)	13/14 (93)
O118 (n = 9)	36 (2–52)	9/9 (100)	0/9 (0)	0/9 (0)	8/8 (100)	8/8 (100)
O91 (n = 8)	42 (30–46)	4/8 (50)	1/8 (12)	3/8 (38)	0/8 (0)	4/8 (50)
O113 (n = 8)	19 (10–41)	0/8 (0)	6/8 (75)	2/8 (25)	0/8 (0)	5/8 (62)
O153 (n = 7)	10 (9–11)	7/7 (100)	0/7 (0)	0/7 (0)	7/7 (100)	7/7 (100)
O146 (n = 6)	69 (64–74)	5/6 (83)	1/6 (17)	0/7 (0)	0/6 (0)	2/6 (33)
O174 (n = 6)	51 (21–75)	0/6 (0)	6/6 (100)	0/6 (0)	0/6 (0)	0/6 (0)
Other (n = 95)[b]	27 (7–62)	29/95 (30)	46/95 (48)	20/95 (20)	29/94 (31)	39/93 (42)
Undetermined (n = 123)	27 (7–62)	71/123 (58)	38/123 (31)	14/123 (11)	82/121 (68)	96/121 (79)
Total (n = 940)	15 (5–41)	570/939 (61)	200/939 (21)	169/939 (18)	775/918 (84)	793/917 (86)

NOTE. Data are proportion (%) of isolates, unless otherwise noted. IQR, interquartile range.

[a] For isolates for which the age of the person providing the specimen was included with submission to the CDC.

[b] For O22, O28, and O88, 5 isolates each (<1%); for 0119, O128, and O172, 4 isolates each (<1%); for O6, O8, O60, O63, O104, O117, and O126, 3 isolates each (<1%); for O2, O14, O49, O50, O55, O73, O75, O96, O109, O110, O137, and O163, 2 isolates each (<1%); and for O1, O4, O5, O21, O38, O46, O48, O51, O77, O79, O83, O84, O86, O116, O124, O125, O140, O142, O143, O159, O168, O171, and O53/O117, 1 isolate each (<1%).

We also identified an independent association between *eae* and bloody diarrhea; *eae* encodes the protein intimin, which facilitates attaching and effacing lesions on the gut epithelium and may explain, in part, the association between this virulence factor and bloody diarrhea. Bloody diarrhea has also been associated with *stx*$_2$, with or without other factors [52, 56]. The association with *stx*$_2$ remains controversial [34, 57], with some authors proposing that the bloody diarrhea observed during STEC infections may more likely be related to virulence factors other than Shiga toxins [34, 58].

Our data demonstrate that STEC O111 is the second most common bacterial cause of HUS in the United States, after STEC O157:H7. STEC O111 was identified as the etiological agent for 3 of 7 reported outbreaks of non-O157 STEC serotypes; 2 of the STEC O111 outbreaks included cases of HUS. Eleven (52%) of the 21 non-O157 STEC isolates associated with HUS were STEC O111; none were isolates from the 3 recognized outbreaks. An association between STEC O111 and HUS has also been observed in other countries [59].

The association we observed between HUS and *stx*$_2$ was not, however, a marker for infection with STEC O111, even though 9 (90%) of the 10 STEC O111 isolates associated with HUS were *stx*$_2$ positive. Our multivariate analysis included as variables only virulence genes, which (like other investigators) we believe to be the driving force in the etiology of STEC-associated illness. When serotype was included in the multivariate models, we were not surprised to find a statistically significant association between HUS and STEC O111 (OR 2.89 [95% CI 1.15–7.23]; P = .024); however, the association between HUS and *stx*$_2$ remained robust and was more significant (OR, 27.2 [95% CI, 3.60–205]; P = .001).

We did not receive submissions from cases of illness yielding 2 or more different STEC isolates. The lack of these cases in our collection is likely due, in large part, to common culturing practices that are not sensitive enough to recover >1 different STEC isolate from a stool specimen. Studies are needed to evaluate specimens for mixed STEC infections and to assess the prevalence of such infections, particularly their association with severe illness.

Our survey was limited to isolates sent to the CDC reference laboratory and does not represent the results of formal surveillance. Nonetheless, this large convenience sample provides the most informative data on non-O157 STEC infections in the United States. A convenience sample is subject to a number of biases. Most infections are not detected, because clinical laboratories do not test specimens for non-O157 STEC. Conversely, laboratories equipped to identify STEC may have isolated strains and not sent them to the CDC. However, the number of non-O157 STEC isolates not submitted to the CDC was likely small, because most US clinical and public health laboratories lacked the resources for serotyping *E. coli* during the survey period. It is likely that some clinical laboratories identified Shiga toxin–positive specimens but did not submit an isolate for serotyping. Although we cannot assess the extent

Table 2. Prevalence of virulence genes among 21 human non-O157 Shiga toxin–producing *Escherichia coli* (STEC) isolates associated with hemolytic-uremic syndrome (HUS) and 75 non-O157 STEC isolates associated with bloody diarrhea, 1983–2002.

Disease, serogroup	Shiga toxin			*eae*	E-*hly*
	stx_1 alone	stx_2 alone	stx_1 and stx_2		
HUS					
O111 (n = 10)	1/10 (10)	0/10 (0)	9/10 (90)	9/9 (100)	8/9 (89)
Other[a] (n = 11)	0/11 (0)	8/11 (73)	3/11 (27)	7/11 (64)	8/11 (73)
Total (n = 21)	1/21 (5)	8/21 (38)	12/21 (57)	16/20 (80)	16/20 (80)
Bloody diarrhea					
O26 (n = 12)	10/12 (83)	0/12 (0)	2/12 (17)	12/12 (100)	12/12 (89)
O111 (n = 8)	4/8 (50)	0/8 (0)	4/8 (50)	7/8 (88)	8/8 (100)
O103 (n = 12)	12/12 (100)	0/12 (0)	0/12 (0)	12/12 (100)	12/12 (100)
O121 (n = 13)	0/13 (0)	13/13 (100)	0/13 (0)	12/13 (92)	12/13 (92)
O45 (n = 10)	10/10 (100)	0/10 (0)	0/10 (0)	10/10 (100)	9/10 (90)
O145 (n = 4)	1/4 (25)	2/4 (50)	1/4 (25)	4/4 (100)	4/4 (100)
Other[b] (n = 9)	3/9 (33)	5/9 (56)	1/9 (11)	6/9 (67)	6/9 (67)
Undetermined (n = 7)	6/7 (86)	0/7 (0)	1/7 (14)	7/7 (100)	7/7 (100)
Total (n = 75)	46/75 (61)	20/75 (27)	9/75 (12)	70/75 (93)	70/75 (93)

NOTE. Data are proportion (%) of isolates.

[a] For O14, O22, O26, O50, O79, O113, O121, O137, O145, O165, and O172, 1 isolate each.

[b] For O8, O49, O79, O117, O118, O137, O140, O143, and O165, 1 isolate each.

of this activity, we have no reason to believe that there was a systematic exclusion bias that would have altered the distribution of serogroups we describe here. Formal surveillance could ameliorate these biases and provide more-informative data by better describing the spectrum of STEC serotypes that cause disease, their frequency and geographic distribution, and the clinical illness they cause.

We received clinical data for only one-third of specimens; we assumed that the isolates we received were forwarded to public health laboratories by clinical facilities for the diagnostic evaluation of illness. Although both diarrhea and HUS are well-characterized conditions, use of a standardized diagnosis on the specimen submission form was not required; the diagnoses included on the forms were assigned at the discretion of the submitter on the basis of available clinical information, which may have been limited and led to underreporting. Diagnosis of non-O157 STEC infection may have been pursued more aggressively in very ill persons, particularly those with bloody diarrhea or HUS. A high proportion of isolates that were included in small, previous series were from patients with more-severe illness [34], suggesting that some of the patients with missing clinical data also had severe illness. This classification error would alter the statistical associations of HUS and bloody diarrhea with non-O157 serogroups and virulence factors we observed; however, without better clinical data, we cannot speculate reliably to what degree. The associations we found are consistent with the findings of previous reports and are biologically plausible. The present study was unable to analyze important questions regarding the clinical care of persons with STEC infection, such as the benefit or harm resulting from

Table 3. Reported outbreaks of human non-O157 Shiga toxin–producing *Escherichia coli* infections in the United States, 1983–2002.

Year	Serogroup	State	No. of ill persons	Serologically confirmed	Suspected exposure/ vehicle	HUS reported	Exposure/ vehicle confirmed	Reference
1990	O111	Ohio	5	Yes	Undetermined	Yes	No	[7]
1994	O104	Montana	18	Yes	Milk	No	No	[6]
1999	O121	Connecticut	11	Yes	Lake water	Yes	No	[60]
1999	O111	Texas	56	Yes	Salad bar	Yes	No	[10]
2000	O103	Washington	18	Yes	Punch	Yes	No	Unpublished[a]
2001	O111	South Dakota	3	No	Day care	No	No	[61]
2001	O26	Minnesota	4	No	Lake water	No	No	Unpublished[a]

NOTE. HUS, hemolytic-uremic syndrome.

[a] Unpublished data reported to the Centers for Disease Control and Prevention (presented with permission from the Washington State Department of Health and the Minnesota Department of Public Health).

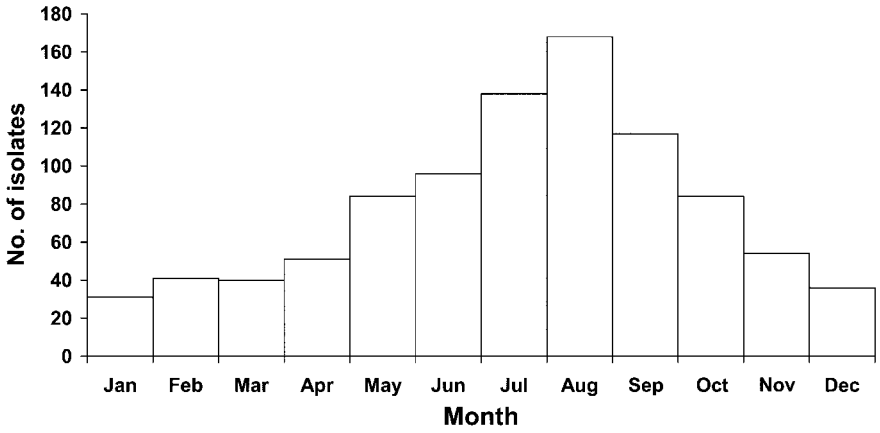

Figure 2. Human non-O157 Shiga toxin–producing *Escherichia coli* isolates submitted to the Centers for Disease Control and Prevention for confirmation, by month of illness onset or specimen collection, 1983–2002 ($n = 940$).

the use of antibiotics in the treatment of non-O157 STEC infection; clinical complications of these infections other than HUS (e.g., stroke); length of illness; and period of bacterial shedding. At a minimum, future surveillance-based case reporting of non-O157 STEC infections should indicate definitively whether bloody diarrhea and HUS were present or absent by use of standardized definitions.

The present study included only sporadic infections with non-O157 STEC. From 1992 to 2002, 7 outbreaks of non-O157 STEC infections were reported in the United States (table 3); 5 were serologically confirmed. Three were caused by STEC O111, and 1 each was caused by STEC O26, O103, O104, and O121. Epidemiologic investigation associated 6 outbreaks with specific exposures (3 foodborne and 3 environmental); however, a vehicle was microbiologically confirmed in none. Improved surveillance and awareness of non-O157 STEC as pathogens will improve the identification and reporting of outbreaks, which may be currently underrecognized.

Most non-O157 STEC cannot be visually distinguished from nonpathogenic *E. coli* on differential plating media (e.g., sorbitol MacConkey's agar [SMAC]), which until recently was the principal method used to screen bloody diarrhea and specimens from persons with HUS for STEC O157:H7. O157:H7 colonies appear pale, whereas other *E. coli,* including most non-O157 STEC, appear pink. Screening for non-O157 STEC has been facilitated by new assays that detect Shiga toxin; submission of non-O157 STEC isolates to the CDC for confirmation increased substantially after the commercial introduction of Shiga toxin EIAs (figure 1). Notably, simultaneous culture of stools on SMAC and screening for Shiga toxin by EIA has identified more STEC than has either procedure alone [33, 62].

The advent of Shiga toxin EIAs has substantially increased the capacity of community-based laboratories to diagnose non-O157 STEC infections. Before the mid-1990s, diagnosis was limited to a few academic and public health laboratories with specialized capacity. Although the 74 isolates received before the mid-1990s may be less representative of community-based illness, we have presented data from both before and after the development of Shiga toxin EIAs to illustrate how these assays can enhance routine surveillance for non-O157 STEC. The proportional distribution of serotypes among the 866 isolates received during the period 1997–2002 was not significantly different from that among all 940 isolates. Inclusion of isolates from both periods would not affect our analysis of the associations between clinical syndromes and virulence factors.

Although direct testing of stool by use of these EIAs to detect the presence of Shiga toxin–producing bacteria might seem to obviate the clinical need to culture stool, the importance of obtaining STEC isolates for public health surveillance cannot be overemphasized. Non-O157 STEC may be as common a cause of diarrhea as other, better recognized bacterial agents. Notably, most of the isolates we received from persons described as having diarrhea were not described as having either bloody diarrhea or HUS, and these persons may have presented with clinical symptoms similar to those of infections with other enteric pathogens. The low infectious dose of some non-O157 STEC genotypes and their potential to cause severe life-threatening illness, particularly among children, make these agents an important public health concern. For this reason, it is essential that Shiga toxin–positive specimens or isolates be forwarded to public health laboratories for serological and molecular characterization of the Shiga toxin–producing organisms; identification of the toxin alone is inadequate and should not be used to replace culture and serotyping.

Improved national surveillance will advance our understanding of the epidemiology of non-O157 STEC infections and allow us to monitor changes in the frequency with which major serogroups cause illness over time. We strongly encourage clinicians to consider STEC infection when diagnosing illnesses and clinical laboratories to consider screening all specimens

from persons with diarrhea for STEC, both O157 and non-O157. If screening all specimens for non-O157 STEC is impractical, then we advise, at a minimum, evaluating specimens from persons with bloody diarrhea or HUS; this practice could be reserved for specimens that do not yield STEC O157. Molecular methods, such as Shiga toxin EIAs, should be used in tandem with culture and serotyping and never alone. At this time, isolating and serotyping *E. coli* isolates that are positive for Shiga toxin is the only way to monitor trends, to detect emerging STEC serotypes, and to define their epidemiology. Providing public health laboratories with the expertise and antisera needed to identify STEC that belong to the 6 major non-O157 serogroups is a first step that could speed the identification of outbreaks and improve our overall understanding of STEC.

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References

1. Johnson RP, Clarke RC, Wilson JB, et al. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *Escherichia coli.* J Food Prot **1996**; 59:1112–22.
2. Strockbine NA, Wells JG, Bopp CA, Barrett TJ. Overview of detection and subtyping methods. In: Kaper JB, O'Brien AD, eds. *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. Washington, DC: American Society for Microbiology Press, **1998**:331–56.
3. Bettelheim KA. Role of non-O157 VTEC. Symp Ser Soc Appl Microbiol **2000**; 88(Suppl):S38–50.
4. Mead PS, Griffin PM. *Escherichia coli* O157:H7. Lancet **1998**; 352: 1207–12.
5. Besser RE, Griffin PM, Slutsker L. *Escherichia coli* O157:H7 gastroenteritis and the hemolytic uremic syndrome: an emerging infectious disease. Annu Rev Med **1999**; 50:355–67.
6. Anonymous. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21—Helena, Montana, 1994. MMWR Morb Mortal Wkly Rep **1995**; 44:501–3.
7. Banatvala N, Debeukelaer MM, Griffin PM, et al. Shiga-like toxin-producing *Escherichia coli* O111 and associated hemolytic-uremic syndrome: a family outbreak. Pediatr Infect Dis J **1996**; 15:1008–11.
8. Tarr PI, Fouser LS, Stapleton AE, et al. Hemolytic-uremic syndrome in a six-year-old girl after a urinary tract infection with Shiga-toxin-producing *Escherichia coli* O103:H2. N Engl J Med **1996**; 335:635–8.
9. Fey PD, Wickert RS, Rupp ME, Safranek TJ, Hinrichs SH. Prevalence of non-O157:H7 Shiga toxin-producing *Escherichia coli* in diarrheal stool from Nebraska. Emerg Infect Dis **2000**; 6:530–3.
10. Brooks JT, Bergmire-Sweat D, Kennedy M, et al. Outbreak of Shiga toxin–producing *Escherichia coli* O111:H8 infections among attendees of a high school cheerleading camp. Clin Infect Dis **2004**; 38:190–8.
11. Tanaka H, Ohseto M, Yamashita Y, et al. Bacteriological investigation on an outbreak of acute enteritis associated with verotoxin-producing *Escherichia coli* O111:H- [in Japanese]. Kansenshogaku Zasshi **1989**; 63:1187–94.
12. Caprioli A, Luzzi I, Rosmini F, et al. Community-wide outbreak of hemolytic-uremic syndrome associated with non-O157 verocytotoxin-producing *Escherichia coli.* J Infect Dis **1994**; 169:208–11.
13. Anonymous. Community outbreak of hemolytic uremic syndrome attributable to *Escherichia coli* O111:NM—South Australia, 1995. MMWR Morb Mortal Wkly Rep **1995**; 44:550–1, 7–8.
14. Blanco JE, Blanco M, Molinero ME, Peiro E, Mora A, Blanco J. Brote de gastroenteritis asociado con un *Escherichia coli* vertoxigenico O111: H- VT1+ eae+. Alimentaria **1996**; 275:109–13.
15. Huppertz HI, Busch D, Schmidt H, Aleksic S, Karch H. Diarrhea in young children associated with *Escherichia coli* non-O157 organisms that produce Shiga-like toxin. J Pediatr **1996**; 128:341–6.
16. Pierard D, Moriau L, Stevens D, Lauwers S. Ten years of screening for VTEC in stool culture samples [abstract 217]. In: Abstracts from the 4th International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections (Kyoto). Kyoto: VTEC 2000 Organizing Committee, **2000**.
17. Caprioli A, Tozzi AE, Rizzoni G, Karch H. Non-O157 Shiga toxin-producing *Escherichia coli* infections in Europe [letter]. Emerg Infect Dis **1997**; 3:578–9.
18. Beers M, Lanser J. Screening for Shiga toxin-producing *Escherichia coli* in South Australia. Microbiol Aust **1997**; 18:16–7.
19. Bonnet R, Souweine B, Gauthier G, et al. Non-O157:H7 stx2-producing *Escherichia coli* strains associated with sporadic cases of hemolytic-uremic syndrome in adults. J Clin Microbiol **1998**; 36:1777–80.
20. Beutin L, Zimmerman S, Gleier K. Human infections with Shiga-toxin producing *Escherichia coli* other than serogroup O157 in Germany. Emerg Infect Dis **1998**; 4:635–9.
21. Werber D, Fruth A, Liesegang A, et al. A multistate outbreak of Shiga toxin–producing *Escherichia coli* O26:H11 infections in Germany, detected by molecular subtyping surveillance. J Infect Dis **2002**; 186:419–22.
22. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. Emerg Infect Dis **1999**; 5:607–25.
23. Pai CH, Ahmed N, Lior H, Johnson WM, Sims HV, Woods DE. Epidemiology of sporadic diarrhea due to verocytotoxin-producing *Escherichia coli:* a two-year prospective study. J Infect Dis **1988**; 157:1054–7.
24. Ritchie M, Partington S, Jessop J, Kelly MT. Comparison of a direct fecal Shiga-like toxin assay and sorbitol-MacConkey agar culture for laboratory diagnosis of enterohemorrhagic *Escherichia coli* infection. J Clin Microbiol **1992**; 30:461–4.
25. Bokete TN, O'Callahan CM, Clausen CR, et al. Shiga-like toxin-producing *Escherichia coli* in Seattle children: a prospective study. Gastroenterology **1993**; 105:1724–31.
26. Begue RE, Neill MA, Papa EF, Dennehy PH. A prospective study of Shiga-like toxin-associated diarrhea in a pediatric population. J Pediatr Gastroenterol Nutr **1994**; 19:164–9.
27. Ramotar K, Henderon E, Szumski R, Louie TJ. Impact of free verotoxin testing on epidemiology of diarrhea caused by verotoxin-producing *Escherichia coli.* J Clin Microbiol **1995**; 33:1114–20.
28. Park CH, Gates KM, Vandel NM, Hixon DL. Isolation of Shiga-like toxin producing *Escherichia coli* (O157 and non-O157) in a community hospital. Diagn Microbiol Infect Dis **1996**; 26:69–72.
29. Kehl K, Havens SP, Behnke CE, Acheson DWK. Evaluation of the premier EHEC assay for detection of Shiga toxin-producing *Escherichia coli.* J Clin Microbiol **1997**; 35:2051–4.
30. Acheson D, Ngo T, Chitrakar R. Prevalence of O157 and non-O157 STEC in the United States [abstract 225]. In: Abstracts from the 4th International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections (Kyoto). Kyoto: VTEC 2000 Organizing Committee, **2000**.
31. Fiorentino T, Hurd S, Howard R, Welles C, Phan Q, Hadler JL. Emergence of nonculture methods for detecting Shiga toxin-producing *E. coli* (STEC) in Connecticut (CT) laboratories, 2000 [abstract C-166]. In: Abstracts and program book for the 101st General Meeting of the American Society for Microbiology (Orlando). Orlando: American Society for Microbiology, **2001**. Available at: http://www.asm.org/memonly/abstracts/AbstractView.asp?AbstractID=47955. Last accessed 2 September 2005.

32. Nims L, Horensky DS, Buck LL, et al. Isolation of Shiga toxin-producing *Escherichia coli* in New Mexico [abstract C-165]. In: Abstracts and program book for the 101st General Meeting of the American Society for Microbiology (Orlando). Orlando: American Society for Microbiology, **2001**. Available at: http://www.asm.org/memonly/abstracts/AbstractView.asp?AbstractID=47865. Last accessed 2 September 2005.
33. Klein EJ, Stapp JR, Clausen CR, et al. Shiga toxin-producing *Escherichia coli* in children with diarrhea: a prospective point-of-care study. J Pediatr **2002**; 141:172–7.
34. Jelacic JK, Damrow T, Chen GS, et al. Shiga toxin-producing *Escherichia coli* in Montana: bacterial genotypes and clinical profiles. J Infect Dis **2003**; 188:719–29.
35. Caprioli A, Tozzi AE. Epidemiology of Shiga toxin-producing *Escherichia coli* infections in continental Europe. In: Kaper JB, O'Brien AD, eds. *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. Washington, DC: American Society for Microbiology Press, **1998**:38–48.
36. Blanco JE, Blanco M, Alonso MP, et al. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from human patients: prevalence in Lugo, Spain, from 1992 through 1999. J Clin Microbiol **2004**; 42:311–9.
37. Penney TM, Doing KM. Prevalence and detection of Shiga-toxin producing *E. coli* in Maine [abstract C-516]. In: Abstracts and program book for the 101st General Meeting of the American Society for Microbiology (Orlando). Orlando: American Society for Microbiology, **2001**. Available at: http://www.asm.org/memonly/abstracts/AbstractView.asp?AbstractID=47909. Last accessed 2 September 2005.
38. Beutin L, Montenegro MA, Orskov I, et al. Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. J Clin Microbiol **1989**; 27:2559–64.
39. Olsvik O, Rimstad E, Hornes E, et al. A nested PCR followed by magnetic separation of amplified fragments for detection of *Escherichia coli* Shiga-like toxin genes. Mol and Cell Probes **1991**; 5:429–35.
40. Louie M, de Azavedo J, Clarke R, et al. Sequence heterogeneity of the *eae* gene and detection of verotoxin-producing *Escherichia coli* using serotype-specific primers. Epidemiol Infect **1994**; 112:449–61.
41. Reischl U, Youssef MT, Kilwinski J, et al. Real-time fluorescence PCR assays for detection and characterization of Shiga toxin, intimin, and enterohemolysin genes from Shiga toxin-producing *Escherichia coli*. J Clin Microbiol **2002**; 40:2555–65.
42. Karch H, Bohm H, Schmidt H, Gunzer F, Aleksic S, Heesemann J. Clonal structure and pathogenicity of Shiga-like toxin-producing, sorbitol-fermenting *Escherichia coli* O157:H-. J Clin Microbiol **1993**; 31: 1200–5.
43. Fratamico J, Franke S, Schmidt A, et al. Detection of *Escherichia coli* O157:H7 by multiplex PCR. J Clin Microbiol **1995**; 33:2188–91.
44. Ewing WH. Indentification of *Enterobacteriaciae*. 4th ed. New York: Elsevier Science Publishing, **1986**.
45. Parry SM, Palmer SR. The public health significance of VTEC O157. Symp Ser Soc Appl Microbiol **2000**; 88(Suppl):S1-9.
46. Slutsker L, Ries AA, Greene KD, Wells JG, Hutwagner L, Griffin PM. *Escherichia coli* O157:H7 diarrhea in the United States: clinical and epidemiologic features. Ann Intern Med **1997**; 126:505–13.
47. Jenkins C, Willshaw GA, Evans J, et al. Subtyping of virulence genes in verocytotoxin-producing *Escherichia coli* (VTEC) other than serogroup O157 associated with disease in the United Kingdom. J Med Microbiol **2003**; 52:941–7.
48. Cimolai N, Basalyga S, Mah DG, Morrison BJ, Carter JE. A continuing assessment of risk factors for the development of *Escherichia coli* 0157:H7-associated hemolytic uremic syndrome. Clin Nephrol **1994**; 42:85–9.
49. Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J Infect Dis **1989**; 160: 994–8.
50. Eklund M, Leino K, Siitonen A. Clinical *Escherichia coli* strains carrying *stx* genes: *stx* variants and *stx*-positive virulence profiles. J Clin Microbiol **2002**; 40:4585–93.
51. Friedrich AW, Bielaszewska M, Zhang WL, et al. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J Infect Dis **2002**; 185:74–84.
52. Ethelberg S, Olsen KEP, Scheutz F, et al. Virulence factors for hemolytic uremic syndrome, Denmark. Emerg Infect Dis **2004**; 10:842–7.
53. Boerlin P, McEwen SA, Bioerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. J Clin Microbiol **1999**;37: 497–503.
54. Werber D, Fruth A, Buchholz U, et al. Strong association between Shiga toxin-producing *Escherichia coli* O157 and virulence genes *stx*2 and *eae* as possible explanation for predominance of serogroup O157 in patients with haemolytic uraemic syndrome. Eur J Clin Microbiol Infect Dis **2003**; 22:726–30.
55. Fraser ME, Fujinaga M, Cherney MM, et al. Structure of Shiga toxin type 2 (Stx2) from *Escherichia coli* O157:H7. J Biol Chem **2004**; 279: 27511–7.
56. Werber D, Fruth A, Heissenhuber A, et al. Shiga toxin–producing *Escherichia coli* O157 more frequently cause bloody diarrhea than do non-O157 strains [letter]. J Infect Dis **2004**; 189:1335–6.
57. Tarr PI, Damrow T. Reply to Werber et al. [letter]. J Infect Dis **2004**;189: 1336–7.
58. Welinder-Olsson C, Badenfors M, Cheasty T, Kjellni E, Kaijser B. Genetic profiling of enterohemorrhagic *Escherichia coli* strains in relation to clonality and clinical signs of infection. J Clin Microbiol **2002**; 40: 959–64.
59. Scheutz F, Beutin L, Smith HR. Characterization of non-O157 verocytotoxigenic *E. coli* (VTEC) isolated from patients with haemolytic uremic syndrome (HUS) world-wide from 1982–2000 [abstract 203]. In: Abstracts from the 4th International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections (Kyoto). Kyoto: VTEC 2000 Organizing Committee, **2000**.
60. McCarthy TA, Barrett NL, Hadler JL, et al. Hemolytic-uremic syndrome and *Escherichia coli* O121 at a lake in Connecticut, 1999. Pediatrics **2001**; 108:E59.
61. Carlson C. Investigation of an *Escherichia coli* O111:NM outbreak in a daycare in South Dakota [abstract: session 28, board 80]. In: International Conference on Emerging Infectious Diseases 2002 (Atlanta). Atlanta: Centers for Disease Control and Prevention, **2002**.
62. Cohen M. Shiga toxin-producing *E. coli:* Two tests are better than one [commentary]. J Pediatr **2002**; 141:155–6.

Bacterial Foodborne and Diarrheal Disease National Case Surveillance

Annual Report, 2005

Enteric Diseases Epidemiology Branch
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Contents

Executive Summary

The Enteric Diseases Epidemiology Branch (EDEB), Division of Foodborne, Bacterial, and Mycotic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases is responsible for surveillance of bacterial enteric pathogens. National case surveillance encompasses two systems administered outside EDEB: the National Notifiable Diseases Surveillance System (NNDSS), which is clinical case-based, and the Public Health Laboratory Information System (PHLIS), which is a laboratory isolation-based reporting system. The laboratory-based system alone includes data on important pathogen characteristics data such as serotype for *Salmonella*, *Shigella*, and Shiga toxin-producing *Escherichia coli* isolates. Serotype information for these pathogens is crucial for surveillance, outbreak detection, and investigation. PHLIS also includes some pathogens that are not formally nationally notifiable, but may be notifiable at the state level. In addition EDEB primarily collects information for botulism, typhoid fever, cholera and other *Vibrio* illnesses, as well as for Shiga toxin-producing *E. coli*, non-O157. Information in this report includes case and isolate counts in 2005, as of March 2007; the numbers may have changed compared with previous publications of 2005 surveillance data.

The number of reported cases of diseases under surveillance is a vast underestimate of the true burden, because most episodes of disease never reach the reporting systems. Many ill persons do not seek medical care, medical practitioners may not order the tests to make a specific diagnosis, and laboratories may not conduct the appropriate tests to isolate the causative pathogens. Some pathogens are not included on the list of nationally notifiable diseases (e.g., *Campylobacter* and *Yersinia*) and are not included in this report, though individual states may require reporting and collect surveillance data. The completeness of surveillance data is variable. The Foodborne Diseases Active Surveillance Network (FoodNet) conducted more intensive surveillance in ten sites in 2005; more information is available at http://www.cdc.gov/foodnet/.

Many illnesses are not included in any surveillance of individual cases, in part because there are no standard clinical tests to detect them. Examples include illnesses due to enterotoxigenic *E. coli* and due to enterotoxins produced by *Bacillus cereus, Clostridium perfringens,* and *Staphylococcus aureus*. For such conditions, reports of foodborne outbreak investigations provide the best available surveillance information. Foodborne outbreak reports are available at http://www.cdc.gov/foodborneoutbreaks/. It should be noted that all surveillance reports from state and territorial departments of public health to the Centers for Disease Control and Prevention (CDC) are voluntary.

Each year, EDEB summarizes surveillance results in multiple formats, including letters to state and territorial epidemiologists and public health laboratory directors, reports in the CDC publication *Morbidity and Mortality Weekly Report (MMWR)*, and publications in peer-reviewed scientific journals. More information about these documents is available at the end of this report in the following sections: Sources and Contacts for Bacterial Foodborne and Diarrheal Diseases, Publications by the Enteric Diseases Epidemiology Branch, 2005, and CDC Internet sites for Foodborne and Diarrheal Diseases.

This report is the third in an annual series summarizing results from nationally notifiable bacterial foodborne and diarrheal diseases case surveillance systems. A description of the surveillance systems is included to explain the differences between these systems and why they sometimes have different case counts for the same disease entity (see the Data Sources and Background section of this report for more information). The specialized sentinel site surveillance system, FoodNet, provides complementary information for a range of foodborne infections of public health concern from 10 sites. FoodNet annual summaries are available at http://www.cdc.gov/foodnet/reports.htm.

Looking forward, EDEB is actively involved in advancing the nation's surveillance for foodborne and diarrheal diseases. CDC-wide integrated surveillance systems are under construction, which may make national surveillance for many types of diseases more efficient. We are working to make more surveillance tools available to state and local public health personnel and more surveillance information available to public health workers, policy makers, and the general public through combined reports and information available on the Internet.

The case and isolate counts for eight diseases and pathogens for 2005 are presented in Table 1-1 and described on the following pages.

Table 1-1. Case and isolate counts for foodborne and diarrheal diseases and pathogens, 2005

Pathogen/Disease	Comments	Nationally Notifiable	Data Source		
			NNDSS* No. cases	PHLIS† No. isolates	EDEB‡ No. cases or isolates
Botulism	Includes foodborne, wound, infant and other types	Yes	135	NA	145
E. coli O157:H7		Yes	2,621	2,368	NA
E. coli, Shiga toxin-producing, non-O157		Yes	501	224	348
Hemolytic uremic syndrome		Yes	221	NA	NA
Listeriosis		Yes	896	NA	NA
Salmonella Typhi (typhoid fever)		Yes	324	348	143§
Salmonella, non-Typhi (salmonellosis)	Includes >2,400 Serotypes	Yes	45,322	35,836	NA
Shigella (shigellosis)	Includes 4 subgroups	Yes	16,168	8,520	NA
Vibrio cholerae, toxigenic	Includes O1 and O139 serotypes (that causes cholera)	Yes	8	NA	12
Other *Vibrios* (vibriosis)	Some species may not be pathogenic	No	NA	NA	546

*NNDSS (National Notifiable Diseases Surveillance System)
†PHLIS (Public Health Laboratory Information System)
‡EDEB (Enteric Diseases Epidemiology Branch)
§ Preliminary data

Botulism

A total of 145 cases of foodborne (18), wound (28), infant (96), and other types (3) of botulism were reported to the EDEB botulism surveillance system, including three deaths (attributed to foodborne botulism [2] and unknown [1]) and four outbreaks (defined as two or more cases as a result of persons ingesting the same food).

Escherichia coli O157:H7 and Other Shiga Toxin-Producing *E. coli*

Escherichia coli O157:H7 has been nationally notifiable since 1994. In 2000, the Council for State and Territorial Epidemiologists (CSTE) passed a resolution in which all Shiga toxin-producing *E. coli* were made nationally notifiable under the name Enterohemorrhagic *Escherichia coli* or EHEC; national surveillance for EHEC began in 2001. Reported infections with the most well-known pathogen in this group, *E. coli* O157:H7, has increased annually since becoming nationally notifiable to a peak number of 4,744 in 1999. The steady increase in the number of cases was due in part to an increasing ability of laboratories to identify this

pathogen. Coordinated efforts by regulators and industry have been effective in reducing contamination and illness related to ground beef. During 2004, 2,544 cases were reported through NNDSS.

The National *E. coli* Reference Laboratory at CDC provides serotyping and molecular characterization of virulence factors as a service to state public health laboratories. In 2005, CDC received 348 isolates of Shiga toxin-producing *E. coli*, non-O157. Isolates originated from 39 states and included 29 different O groups. The three most common O groups were O26 (24%), O103 (17%), and O111 (13%). A total of 501 cases of Shiga toxin-producing *E. coli* non-O157 were reported to NNDSS.

Hemolytic Uremic Syndrome (HUS), Post-diarrheal
HUS is defined by the triad of hemolytic anemia, thrombocytopenia, and renal insufficiency. The patients reported in national notifiable diseases surveillance include only those with antecedent diarrheal illness. The most common etiology in the United States is infection with a Shiga toxin-producing *E. coli*, principally *E. coli* O157:H7. About 8% of persons infected with *E. coli* O157:H7 develop HUS. Of the 221 cases of HUS reported in 2005, 75% occurred in children younger than age 10 years.

Listeriosis
Listeriosis became nationally notifiable in 2000. Surveillance is conducted through NNDSS. Forty-five states and one territory reported at least one case, for a total of 896 cases.

***Salmonella* Typhi (Typhoid Fever)**
Infection with *Salmonella* serotype Typhi leads to typhoid fever. The number of cases of typhoid fever (324 in NNDSS) has been relatively small and constant, mostly associated with travel outside the United States. *S.* Typhi isolates are reported through the National Salmonellosis Surveillance System; 348 isolates were reported in 2005.

***Salmonella*, Non-Typhi (Salmonellosis)**
A total of 35,836 non-Typhi *Salmonella* isolates were reported in 2005. The national rate was 12.2 per 100,000 population. Similar to other years, children younger than age 5 years accounted for 20% of *Salmonella* isolates. About 10% of isolates came from persons in each of the second through fifth decades of life, with lower proportions from persons in later decades of life.

The thirty most common serotypes of *Salmonella* in 2005 represent 82% of all *Salmonella* isolates. The four most common serotypes in 2005 (Typhimurium, Enteritidis, Newport, and Heidelberg; 52% of all isolates) have been the most common serotypes since 1995, except for 2004 when serotype Javiana replaced Heidelberg as the fourth most common serotype. Serotype Typhimurium has been the most commonly isolated serotype since 1997, though Enteritidis was a very close second in 2005. Serotypes Typhimurium and Enteritidis have both declined substantially (28% and 34%, respectively) since 1995; the total number of *Salmonella* isolates has also declined during this period, though not as substantially as serotypes Typhimurium and Enteritidis.

***Shigella* (Shigellosis)**

Shigella transmission occurs via the fecal-oral route. Most *Shigella sonnei* infections occur in young children and are associated with crowding and poor personal hygiene. Daycare centers have been implicated in many *S. sonnei* outbreaks.

A total of 10,484 *Shigella* isolates were reported to PHLIS in 2005. This represents a stabilization of *Shigella* rates from the sharp decreases that occurred in 2004. The national rate was 3.5 per 100,000 population, based on 2005 census population estimates for the United States. Similar to previous years, children younger than age 5 years accounted for 28.2% of all *Shigella* isolates. About 34.2% came from persons aged 5–19 years, and 26.6% from persons aged 20–59, with lower proportions from persons in later decades of life.

Of the 10,484 isolates, 9,420 (89.7%) were subgrouped. The relative proportions of the different subgroups remained similar to previous years, with subgroup D (*S. sonnei*) accounting for the largest percentage of isolates (74.4%), followed by subgroup B (*S. flexneri*, 13.6%), subgroup C (*S. boydii*, 1.2%) and subgroup A (*S. dysenteriae*, 0.5%).

Cholera and Non-Cholera *Vibrio*

In 2005, 12 patients with toxigenic *V. cholerae* were reported. Five patients were hospitalized and no deaths were reported. No isolates of toxigenic *V. cholerae* O139 were identified. All 12 patients were infected with toxigenic *V. cholerae* serogroup O1. Infection was acquired during international travel for five isolated cases. Exposure to domestic seafood was the source of infection for four patients. Source of infection was unknown for three cases.

Other *Vibrio* isolates (excluding *V. cholerae* serogroup O1 and O139) were not nationally notifiable in 2005, and not all states report cases. States bordering the Gulf of Mexico have a reporting agreement with CDC; others do not, but are encouraged to report cases. In 2005, 578 *Vibrio* isolates from 546 patients were reported to the Cholera and Other *Vibrio* Illness Surveillance System. Among patients for whom information was available, 232 (46%) of 506 were hospitalized and 40 (8%) of 485 died. *V. parahaemolyticus* was isolated from 218 (40%) patients, and was the most frequently reported *Vibrio* species. Of the 546 patients infected with *V. parahaemolyticus*, 23% were hospitalized and 1% died. *V. vulnificus* was isolated from 121 (22%) patients; 90% were hospitalized and 26% died.

Expanded Surveillance Summaries for Selected Pathogens and Diseases, 2005

The following bacterial foodborne and diarrheal diseases case surveillance summaries for 2005 are derived from individual reports sent to state and territorial epidemiologists and public health laboratory directors. They are compiled here to provide more detailed text, tables, and figures. An expanded summary of *E. coli* O157 infections, listeriosis, typhoid fever, and hemolytic uremic syndrome surveillance (HUS) data is not included in this report; more comprehensive surveillance data concerning these are available in FoodNet reports at http://www.cdc.gov/foodnet/. Only a few select tables and figures are included here from the *Salmonella Annual Summary, 2005* and the *Shigella Annual Summary, 2005*. These complete reports are available at http://www.cdc.gov/ncidod/dbmd/phlisdata.

Botulism

The botulism surveillance case definition is available at http://www.cdc.gov/EPO/DPHSI/casedef/botulism_current.htm. Botulism is a rare but serious paralytic illness caused by a neurotoxin produced by the bacterium *Clostridium botulinum.* There are three main forms of botulism. Foodborne botulism is caused by eating foods that contain the botulism toxin. Wound botulism is caused by toxin produced from a wound infected with *Clostridium botulinum*. Infant botulism is caused by consumption of spores of the *Clostridium botulinum* organism, which then grow in the intestine of infants and release toxin. All forms of botulism can be fatal. Because many people can eat a food contaminated with the botulism toxin, every case of botulism suspected to be foodborne is considered a public health emergency.

EDEB staff members are available to consult with health department and physicians 24 hours a day. CDC also maintains the only source of antitoxin used to treat botulism in the United States. The request for consultation and release of antitoxin by health departments and physicians is the basis of surveillance for most cases of foodborne and wound botulism. States report cases of infant botulism to EDEB on a yearly basis; therapeutic human antitoxin licensed for treatment of infant botulism is available from the California Department of Health Services. Suspected botulism cases should be reported immediately to local or state public health officials, who then should call the CDC Emergency Operations Center at (770) 488-7100; CDC will immediately connect callers with an on-call botulism consultant. For consultation on suspected infant botulism occurring in any state, the Infant Botulism Treatment and Prevention Program of the California Department of Health Services should be contacted at (510) 231-7600.

A total of 145 cases of botulinum intoxication were reported to CDC in 2005 (Tables 2-1 and 2-2). Among the 18 cases of foodborne intoxication, toxin type A accounted for 7 (39%) cases, toxin type B 1 (5%) case, and toxin type E for 10 (56%) cases (Table 2-3). The median age of patients was 35 years. Two deaths were reported. There were 4 multi-case outbreaks. They were caused by fish (type unspecified), stinkfish, stinkhead, and suspected leftovers containing several ingredients including salmon, respectively.

There were 96 reported cases of infant botulism in 2005 (Table 2-4). Toxin type B accounted for 52 (54%) cases and toxin type A for 44 (46%) cases. The median age of patients was 16.5 weeks; no deaths were reported.

There were 28 reported cases of wound botulism in 2005 (Table 2-5). Toxin type A accounted for 25 (89%) cases, toxin type B 2 (7%) cases, and unknown toxin type 1 (4%) cases. All cases occurred in injecting drug users. The median age of patients was 44.5 years; no deaths were reported.

There were 3 reported cases of botulism of other or unknown source in 2005 (Table 2-6). Toxin type F accounted for 2 (67%) cases, and type A accounted for 1 (33%) case. One toxin type F case was associated with adult intestinal colonization and exposure to gardening soil. The sources of the other type F case and the type A case were unknown. The case patients were 56, 84, and 74 years of age respectively; one death was reported.

Table 2-1. Summary of cases of botulism reported to the Botulism Surveillance System, 2005

Type	Cases	Median age	Sex	Toxin type	Comments
Foodborne	18 cases (2 reported deaths)	35 years (range: 1–82 years)	10 (56%) male	7 (39%) type A 1 (5%) type B 10 (56%) type E	4 multicase outbreaks
Infant	96 cases (No reported deaths)	16.5 weeks (range: 2–60 weeks)	53 (55%) male	44 (46%) type A 52 (54%) type B	.
Wound	28 cases (No reported deaths; 2 without information)	44.5 years (range: 28–57 years)	23 (82%) male	25 (89%) type A 2 (7%) type B 1 (4%) toxin type undetermined	
Other, unknown	3 cases (1 reported death)	74 years (range: 56–84 years)	3 (100%) male	1 (33%) type A 2 (67%) type F *(Clostridium baratii)*	

Table 2-2. Cases of botulism reported to the Botulism Surveillance System, by state and type, 2005

State/District	Foodborne	Wound	Infant	Other	Total
Alaska	8				8
Alabama			1		1
Arizona			1		1
California	3	24	42		69
Colorado			1		1
Delaware			2		2
Florida			1		1
Idaho			1		1
Illinois			1	1*	2
Kentucky			1		1
Louisiana			1		1
Massachusetts	1				1
Maryland			5		5
Michigan	1				1
Missouri			1		1
Montana				1†	1
North Carolina	1		1		2
New Hampshire			1		1
New Jersey	2		9		11
New Mexico			1		1
Nevada			1		1
New York City			4		4
Oklahoma	1		1		2
Oregon			2		2
Pennsylvania	1		11		12
Texas			1	1*	2
Utah			3		3
Virginia			1		1
Washington		4	2		6
Total	**18**	**28**	**96**	**3**	**145**

*Unknown source
†Adult intestinal; gardening soil

Table 2-3. Cases of foodborne botulism reported to the Botulism Surveillance System, by month, 2005 (N = 18)

Month	State	Age (years)	Sex	Toxin Type	Vehicle	Death
January	Massachusetts	75	Male	A	Chili with rice and lotus root	No
	Michigan	82	Male	B	Unknown†	Yes
May	California	30	Male	A	"Pruno"‡	No
July	New Jersey*	45	Male	E	Fish	No
	New Jersey*	16	Female	E	Fish	No
	Oklahoma	14	Female	A	Home-canned venison stew	No
August	Alaska*[1]	27	Female	E§	Stinkfish	No
	Alaska*[1]	33	Male	E	Stinkfish	No
	Alaska*[1]	37	Female	E§	Stinkfish	No
	Alaska*[1]	69	Male	E	Stinkfish	No
	Alaska*[2]	19	Female	E	Stinkhead	No
	Alaska*[2]	23	Female	E	Stinkhead	No
	Alaska*[2]	47	Female	E	Stinkhead	No
	Alaska*[2]	1	Male	E	Stinkhead	No
September	North Carolina	64	Female	A	Homemade nutritional juice	No
November	California*	82	Male	A	Leftovers containing salmon	Yes
	California*	17	Male	A	Leftovers containing salmon	No
	Pennsylvania	63	Male	A	History of homecanning	No

*Cases involved in multicase outbreaks
[1] Group 1 multicase outbreak in Alaska
[2] Group 2 multicase outbreak in Alaska
†Multiple suspected sources due to history of improper food storage
‡Homemade alcoholic beverage
§Toxin type derived from epidemiologically-linked case

Table 2-4. Cases of infant botulism reported to the Infant Botulism Treatment and Prevention Program, by month, 2005 (N = 96)

Month	State	Onset Age (weeks)	Sex	Toxin Type	Death
January	California	7	Male	B	No
	California	29	Male	A	No
	California	12	Female	A	No
	New Jersey	50	Female	B	No
	New York City	11	Male	B	No
	Pennsylvania	13	Female	B	No
	Pennsylvania	16	Male	B	No
	Utah	20	Female	A	No
February	California	18	Male	B	No
	California	34	Male	A	No
	California	43	Female	A	No
	New Jersey	12	Female	A	No
	Pennsylvania	28	Male	B	No
	Pennsylvania	20	Male	B	No
	Utah	16	Male	A	No
March	California	14	Male	A	No
	California	8	Male	A	No
	Delaware	39	Female	B	No
	Florida	34	Female	A	No
	Maryland	25	Female	B	No
	Oklahoma	10	Female	A	No
	Pennsylvania	20	Male	B	No
April	California	12	Female	B	No
	Pennsylvania	16	Female	B	No
May	California	24	Male	B	No
	California	6	Female	A	No
	California	3	Male	A	No
	Illinois	12	Male	A	No
	Kentucky	12	Male	B	No
	Maryland	28	Male	B	No
	Maryland	13	Male	B	No
	New Jersey	15	Female	B	No
	New Jersey	12	Male	B	No
	New York City	22	Female	B	No
	Pennsylvania	24	Male	B	No
	Pennsylvania	21	Female	B	No
	Utah	10	Male	A	No
	Virginia	21	Male	B	No
June	California	26	Male	A	No
	California	19	Male	B	No
	California	23	Female	A	No
	California	28	Female	B	No
	California	19	Male	A	No
	California	20	Male	A	No

Month	State	Onset Age (weeks)	Sex	Toxin Type	Death
	Delaware	40	Male	B	No
	Louisiana	3	Male	B	No
	Nevada	60	Female	A	No
	Pennsylvania	22	Male	B	No
July	Alabama	5	Male	B	No
	California	16	Male	A	No
	California	19	Female	B	No
	California	30	Male	A	No
	California	6	Female	B	No
	New Hampshire	17	Female	B	No
	New Jersey	21	Male	B	No
	New Jersey	17	Male	B	No
August	Arizona	13	Male	A	No
	California	4	Female	B	No
	California	20	Female	B	No
	California	16	Male	B	No
	California	10	Female	B	No
	California	20	Male	A	No
	Maryland	3	Male	B	No
	New Jersey	4	Male	B	No
	Washington	31	Male	A	No
September	California	24	Female	A	No
	California	23	Female	B	No
	California	14	Male	A	No
	California	20	Female	A	No
	California	4	Female	A	No
	Oregon	16	Female	A	No
	Oregon	19	Female	A	No
	Texas	10	Male	A	No
October	California	17	Female	A	No
	California	46	Female	A	No
	California	9	Female	A	No
	California	6	Male	A	No
	California	30	Male	A	No
	Idaho	16	Male	A	No
	New Jersey	4	Female	B	No
	New York City	19	Male	B	No
	Pennsylvania	2	Female	B	No
	Pennsylvania	17	Female	B	No
November	California	8	Female	A	No
	California	9	Female	A	No
	Colorado	8	Female	A	No
	New York City	6	Female	B	No
December	California	5	Male	B	No
	California	19	Female	B	No
	California	24	Male	A	No
	Maryland	15	Male	B	No
	Missouri	9	Female	A	No
	North Carolina	12	Male	B	No
	New Jersey	10	Male	B	No
	New Mexico	52	Male	B	No
	Washington	17	Male	A	No

Table 2-5. Cases of wound botulism reported to the Botulism Surveillance System, by month, 2005 (N = 28)

Month	State	Age (years)	Sex	Toxin Type	Exposure*	Death
January	California	38	Female	A	IDU	No
	Washington	37	Male	A	IDU	Unknown
	Washington	50	Male	A	IDU	Unknown
February	California	28	Male	A	IDU	No
March	California	47	Male	A	IDU	No
	California	43	Male	B	IDU	No
	California	57	Male	A	IDU	No
	California	43	Male	A	IDU	No
	Washington	34	Female	A	IDU	No
May	California	51	Female	A	IDU	No
June	California	40	Male	A	IDU	No
July	California	48	Male	A	IDU	No
	California	38	Male	B	IDU	No
	California	53	Male	Unknown†	IDU	No
	California	35	Male	A	IDU	No
August	California	43	Male	A	IDU	No
	California	49	Male	A	IDU	No
	California	51	Male	A	IDU	No
	California	34	Male	A	IDU	No
	California	50	Female	A	IDU	No
September	California	30	Male	A	IDU	No
	California	51	Male	A	IDU	No
	California	53	Female	A	IDU	No
	California	36	Male	A	IDU	No
October	California	56	Male	A	IDU	No
	California	36	Male	A	IDU	No
November	California	46	Male	A	IDU	No
	Washington	51	Male	A	IDU	No

*IDU = injecting drug user
† Serum quantity not sufficient for toxin typing

Table 2-6. Cases of botulism, other, reported to the Botulism Surveillance System, 2005 (N= 3)

Month	State	Age (years)	Gender	Toxin Type	Exposure	Death
March	Montana	56	Female	F (*C. baratii*)*	Adult intestinal; gardening soil	No
June	Illinois	84	Female	F(*C. baratii*)*	Unknown	Yes
September	Texas	74	Female	A	Unknown	No

*Botulinum toxin Type F produced by *Clostridium baratii*

Escherichia coli, Shiga Toxin-Producing non-O157

The surveillance case definition for Shiga toxin-producing *Escherichia coli* (STEC) is available at http://www.cdc.gov/EPO/DPHSI/casedef/escherichia_coli_current.htm. Shiga toxin-producing *Escherichia coli* (STEC) strains cause diarrhea and hemolytic uremic syndrome (HUS). The most common STEC that causes illness in the United States is *E. coli* O157:H7. Non-O157 STEC strains are also important pathogens; they have caused several U.S. outbreaks and, in some U.S. studies, they have been isolated from diarrheal stools as frequently as *E. coli* O157:H7. STEC is indicated as enterohemorrhagic *Escherichia coli* (EHEC) in the Nationally Notifiable Diseases Surveillance System (NNDSS) for 2005.

In June 2000, the Council of State and Territorial Epidemiologists (CSTE) passed a position statement recommending inclusion of *E. coli* O157 and non-O157 STEC that cause human illness as nationally notifiable. Reporting of non-O157 STEC has increased every year since implementation in 2001.

During 2005, 501 cases of non-O157 STEC were reported through NNDSS. To better understand the non-O157 STEC serogroups associated with human illness, CDC encourages state health laboratories to forward suspected non-O157 STEC isolates to the CDC's National *Escherichia coli* Reference Laboratory, where confirmatory testing for Shiga toxin genes and serotyping are offered. In 2005, 348 isolates were received by CDC from 39 states (Figure 3-1).

The non-O157 isolates received by CDC in 2005 included 29 different O groups. The predominant groups were O26 (24%) and O103 (17%), followed by O111 (13%), O45 (8%), and O121 (7 %). These five O groups made up 69 % of all isolates (Table 3-1). *E. coli* O26 was also the most commonly isolated non-O157 STEC in 2004 and 2003. In 2001, *E. coli* O111 was the most common.

Identification of an STEC requires demonstrating the ability of the *E. coli* isolate to produce Shiga toxin. Before 1995, Shiga toxin was detected by using highly technical assays available only at reference and research laboratories. Since 1995, the U.S. Food and Drug Administration (FDA) has licensed several rapid enzyme immunoassays (EIA) for the detection of Shiga toxin in human stool specimens and culture broth. Since these EIA kits have become commercially available and the use of polymerase chain reaction (PCR) to identity toxin genes has increased, the number of non-O157 STEC isolates sent to CDC for serotyping has increased each year.

Healthcare providers evaluating patients with diarrhea or HUS should consider infection with non-O157 STEC in addition to *E. coli* O157. A small number of persons have developed HUS after urinary tract infection with STEC strains; in these cases, urine culture has yielded the pathogen when stool culture was negative.

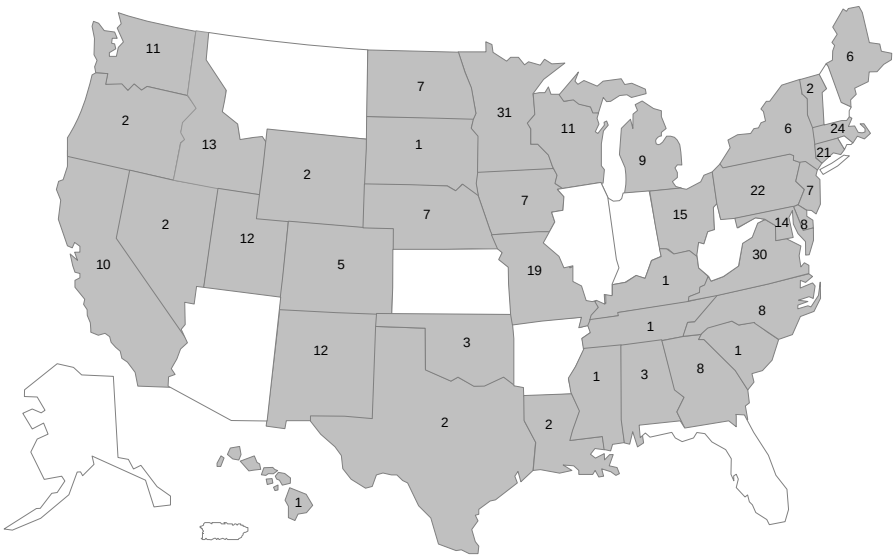
Healthcare providers should notify clinical diagnostic laboratories when STEC O157 infection is suspected so that appropriate testing methods can be applied. Clinical laboratories should strongly consider including STEC O157 in their routine bacterial enteric panel (with *Salmonella*, *Shigella*, and *Campylobacter*). The best way to identify all STEC infections is to screen all stool samples submitted for routine enteric bacterial testing for Shiga toxins using

EIA or PCR. Ideally, the clinical diagnostic laboratory should culture simultaneously for STEC O157 (e.g., on a sorbitol-containing medium such as sorbitol MacConkey agar). Clinical diagnostic laboratories that use a Shiga toxin EIA but do not perform simultaneous culture for STEC O157 should culture all Shiga toxin-positive broths for STEC O157 as soon as possible and forward these isolates to a state or local public health laboratory for confirmation and subtyping. When a Shiga toxin-positive broth does not yield STEC O157, then broth culture should be forwarded to the state of local public health laboratory for identification of non-O157 STEC. State and local public health laboratories should confirm the presence of Shiga toxin in broths and should attempt to obtain a STEC isolate. All non-O157 STEC isolates should be sent by public health laboratories to CDC for confirmation and further characterization.

Table 3-1. Serogroup of non-O157 STEC isolates from humans sent to National *Escherichia coli* Reference Laboratory and Epidemic Investigation and Surveillance Laboratory, 2005

Serogroup	Number	Percent
O26	83	23.9%
O103	58	16.7%
O111	44	12.6%
O45	27	7.8%
O121	26	7.5%
O145	12	3.4%
O91	8	2.3%
O76	5	1.4%
O177	4	1.1%
O28	4	1.1%
O118	3	0.9%
O165	3	0.9%
O112	2	0.6%
O123	2	0.6%
O153	2	0.6%
O174	2	0.6%
O63	2	0.6%
O84	2	0.6%
O22	1	0.3%
O51	1	0.3%
O69	1	0.3%
O8	1	0.3%
O87	1	0.3%
O88	1	0.3%
O116	1	0.3%
O117	1	0.3%
O126	1	0.3%
O143	1	0.3%
O181	1	0.3%
Rough	20	5.7%
Undetermined	26	7.5%
Unknown	2	0.6%
Total	348	100.0%

Figure 3-1. States that submitted non-O157 STEC isolates to CDC, 2005 (N = 39)*



* Data obtained from the National *Escherichia coli* reference Laboratory and the Epidemic Investigation and Surveillance Laboratory
Note: Numbers on map indicate the number of isolates submitted for that state.

Listeria

The listeriosis surveillance case definition is available at http://www.cdc.gov/EPO/DPHSI/casedef/listeriosis_current.htm. Infection with *Listeria monocytogenes* is characterized by fever and muscle aches, and sometimes nausea or diarrhea. The nervous system can be affected, resulting in meningitis and cerebritis, with symptoms such as headache, stiff neck, confusion or convulsions. Pregnant women, newborns, and adults with weakened immune systems are at greatest risk of developing listeriosis. Infection during pregnancy may be asymptomatic but can result in miscarriage, premature delivery, or infection of the newborn.

Listeriosis has been a nationally reportable disease since 2000. Reports of listeriosis are submitted to CDC through NNDSS. There were 896 cases of listeriosis reported to NNDSS during 2005 (0.3 cases per 100,000 population). The rate of listeriosis was highest among neonates (1.6 cases per 100,000 population), followed by adults older than age 70 years (1.5 cases per 100,000 population). More comprehensive surveillance data on listeriosis incidence rates are available in FoodNet reports at http://www.cdc.gov/foodnet/.

The Listeriosis Initiative is an effort to aid in investigations of future *Listeria* outbreaks and clusters. Timely isolation and subtyping of all isolates of *L. monocytogenes* and prompt interviews of patients are means to improving outbreak investigation. Data collected using a standard, detailed report form are maintained in a central database for rapid analysis in the event of an outbreak. These data can be used for case-control analysis of a cluster, where people with non-matching isolates serve as controls. Prompt data collection and analysis could allow earlier public health intervention during an outbreak. During 2004–2005, there were 131 forms submitted from eleven states.

All isolates of *Listeria* should be submitted for subtyping to state or national laboratories. Public health professionals and health care providers should consider interviewing all cases of listeriosis using the standard interview form, available at http://www.cdc.gov/foodborneoutbreaks/documents/ListeriaCaseReportFormOMB0920-0004.pdf.

Salmonella

The *Salmonella* surveillance case definition is available at http://www.cdc.gov/epo/dphsi/casedef/salmonellosis_current.htm. The National *Salmonella* Surveillance System collects reports of isolates of *Salmonella* from human sources from every state. *Salmonella* isolates are submitted to the state public health laboratory by clinical diagnostic laboratories. The state and territorial laboratories confirm the isolates as *Salmonella,* perform serotyping according to the Kauffmann-White scheme, and report the data electronically through the PHLIS to EDEB. Unusual or difficult isolates are forwarded to the National *Salmonella* Reference Laboratory at CDC for further characterization or confirmation. These results are reported back to the state laboratory, where they are reported to CDC through PHLIS. Duplicates are removed from the file at the end of the year. Every 20th isolate is forwarded to the National Antimicrobial Resistance Monitoring System (NARMS) at CDC for

susceptibility testing.

The capture of isolates in the National *Salmonella* Surveillance System is considered to be fairly complete. However, some *Salmonella* isolates may not be forwarded to public health laboratories, and therefore are not reported. In addition, irrespective of the surveillance system, many cases of *Salmonella* illness are not reported because the ill person does not seek medical care, the healthcare provider does not obtain a specimen for diagnosis, or the laboratory does not perform the necessary diagnostics tests. The results of surveillance reported here should be considered underestimates of the true number of infections.

The reporting state represents the state where laboratory confirmation and serotyping were performed. In some instances, the reporting state is not the state of residence of the person from whom the isolate was obtained. For *Salmonella* serotype Typhi, only the first isolation in one year for each person is counted.

A total of 36,184 *Salmonella* isolates were reported from participating public health laboratories in 2005. This represents a 12% decrease compared with 1995, and a slight increase compared with 2004 (1.4%). The national rate was 12.2 per 100,000 population.

Similar to other years, children younger than age 5 years accounted for 20% of all *Salmonella* isolates. Less than 10% of isolates came from persons in each of the second through fifth decades of life, with lower proportions from persons in later decades of life. The distribution of isolates between the sexes was different, with a greater proportion of isolates from male than female infants and children, and a smaller proportion of isolates from male than female adults.

The thirty most common serotypes of *Salmonella* in 2005 are listed in Table 4-1. These represent 82% of all *Salmonella* isolates. The four most common serotypes in 2005 (Typhimurium, Enteritidis, Newport, and Heidelberg; 52% of all isolates) have been the most common serotypes since 1995, except for 2004 when serotype Javiana replaced Heidelberg as the fourth most common serotype (Figure 4-1). Serotype Typhimurium has been the most commonly isolated serotype since 1997, though Enteritidis was a very close second in 2005. Serotypes Typhimurium and Enteritidis have both declined substantially (28% and 34%, respectively) since 1995; the total number of *Salmonella* isolates has also declined during this period, though not as substantially as serotypes Typhimurium and Enteritidis.

Among the thirty most common serotypes in 2005, *Salmonella* Hadar has had the largest percent decline during the past decade. Serotype Hadar was the fifth most common serotype in 1995 and has steadily declined to the 21st most common serotype in 2005, a 75% decline in number of isolates. Serotype Poona has declined 63% since 1995, although most of the decline was between 1995 and 1997. *Salmonella* Mississippi has had the most dramatic increase, 184% since 1995, most since 2002. *Salmonella* Newport had a large increase in numbers between 1997 and 2002, but has been declining since then. Similarly, serotype Javiana had substantial increases in 2003 and 2004, but declined 25% in 2005.

Salmonella serotype I 4,[5],12:i:- was introduced as the 18th most common serotype in 2002 and has increased in rank to sixth in 2005. The serotype has been tracked in the National *Salmonella*

Surveillance system since 1998, though many isolates were classified as only Subspecies I or Group B in the past. Since the 2003 *Salmonella* Surveillance Summary was published, we reexamined the surveillance data for 1995–2003 and were able to reclassify some isolates submitted in these years as I 4,[5],12:i:- based on additional data submitted. Recent efforts to correctly classify this serotype may be responsible for at least some of the increase in numbers. It is unknown how many of the 479 isolates reported as Subspecies I, Group B in 2005 could be this serotype. In 1998, this serotype was the fourth most commonly identified in Spain; genetic analysis of the Spanish isolates revealed a close relationship to serotype Typhimurium (1). Many U.S. isolates of this serotype were characterized by pulsed-field gel electrophoresis (PFGE) and the patterns submitted to PulseNet, the National Molecular Subtyping Network for Foodborne Disease Surveillance. The PFGE patterns for most serotype I 4,[5],12:i- isolates were closely related to serotype Typhimurium PFGE patterns, indicating that they are most likely variants of serotype Typhimurium.

A large proportion of serotype Typhimurium isolates were resistant to multiple antimicrobial drugs; in a 2004 national survey, 39% were resistant to one or more drugs and 23% had a five-drug resistance pattern characteristic of a single phage type, DT104. Similarly, serotype Newport has emerged as a major multidrug-resistant pathogen. In 2004, 28 (15%) of 190 serotype Newport isolates submitted to the National Antimicrobial Resistance Monitoring System were resistant to at least seven of 17 antimicrobial agents tested, including extended-spectrum cephalosporins. Similar to other years, there were marked regional differences in the frequency of *Salmonella* isolates among serotypes. The rate of isolations by region has been followed closely for serotype Enteritidis as a means of assessing the impact of egg safety regulations and industry improvements. As indicated in Figure 4-2, serotype Enteritidis rates of isolation had been relatively high in New England, Mid-Atlantic, and Pacific regions, but have shown significant decreases since 1995. However, since 2003 all regions have had small increases in serotype Enteritidis rates of isolation.

Table 4-1. The 30 *Salmonella* serotypes most frequently reported to PHLIS, 2005

Rank	Serotype	Number	Percent
1	Typhimurium*	6982	19.3%
2	Enteritidis	6730	18.6%
3	Newport	3295	9.1%
4	Heidelberg	1903	5.3%
5	Javiana	1324	3.7%
6	I 4,[5],12:i:-	822	2.3%
7	Montevideo	809	2.2%
8	Muenchen	733	2.0%
9	Saintpaul	683	1.9%
10	Braenderup	603	1.7%
11	Oranienburg	590	1.6%
12	Mississippi	565	1.6%
13	Infantis	505	1.4%
14	Paratyphi B var. L(+) tartrate+	460	1.3%
15	Thompson	428	1.2%
16	Agona	367	1%
17	Typhi	348	1%
18	Hartford	239	0.7%
19	Stanley	224	0.6%
20	Berta	209	0.6%
21	Hadar	205	0.6%
22	Bareilly	201	0.6%
23	Anatum	197	0.5%
24	Poona	196	0.5%
25	Mbandaka	190	0.5%
26	Panama	148	0.4%
27	Litchfield	141	0.4%
28	Sandiego	138	0.4%
29	Schwarzengrund	138	0.4%
30	Brandenburg	134	0.4%
Subtotal		**29,507**	**81.5%**
All other serotyped		3,841	10.6%
Unknown		1113	3.1%
Partially serotyped isolates		1684	4.7%
Rough or nonmotile isolates		39	0.1%
Subtotal		**6,677**	**18.5%**
Total		**36,184**	**100%**

* Typhimurium includes var. Copenhagen

Figure 4-1. Isolation rate per 100,000 population for the top four serotypes of *Salmonella* reported to PHLIS, 1970–2005

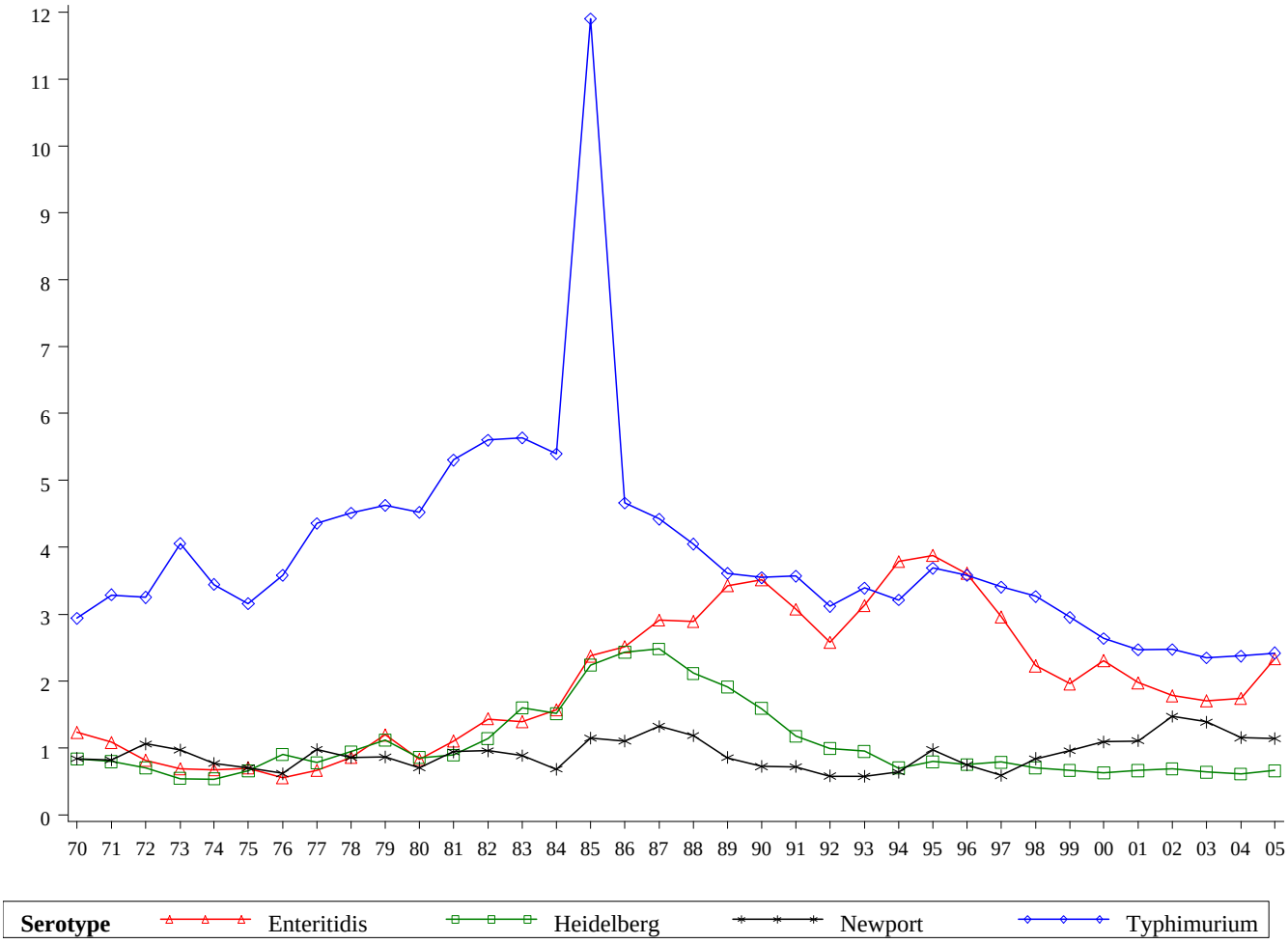
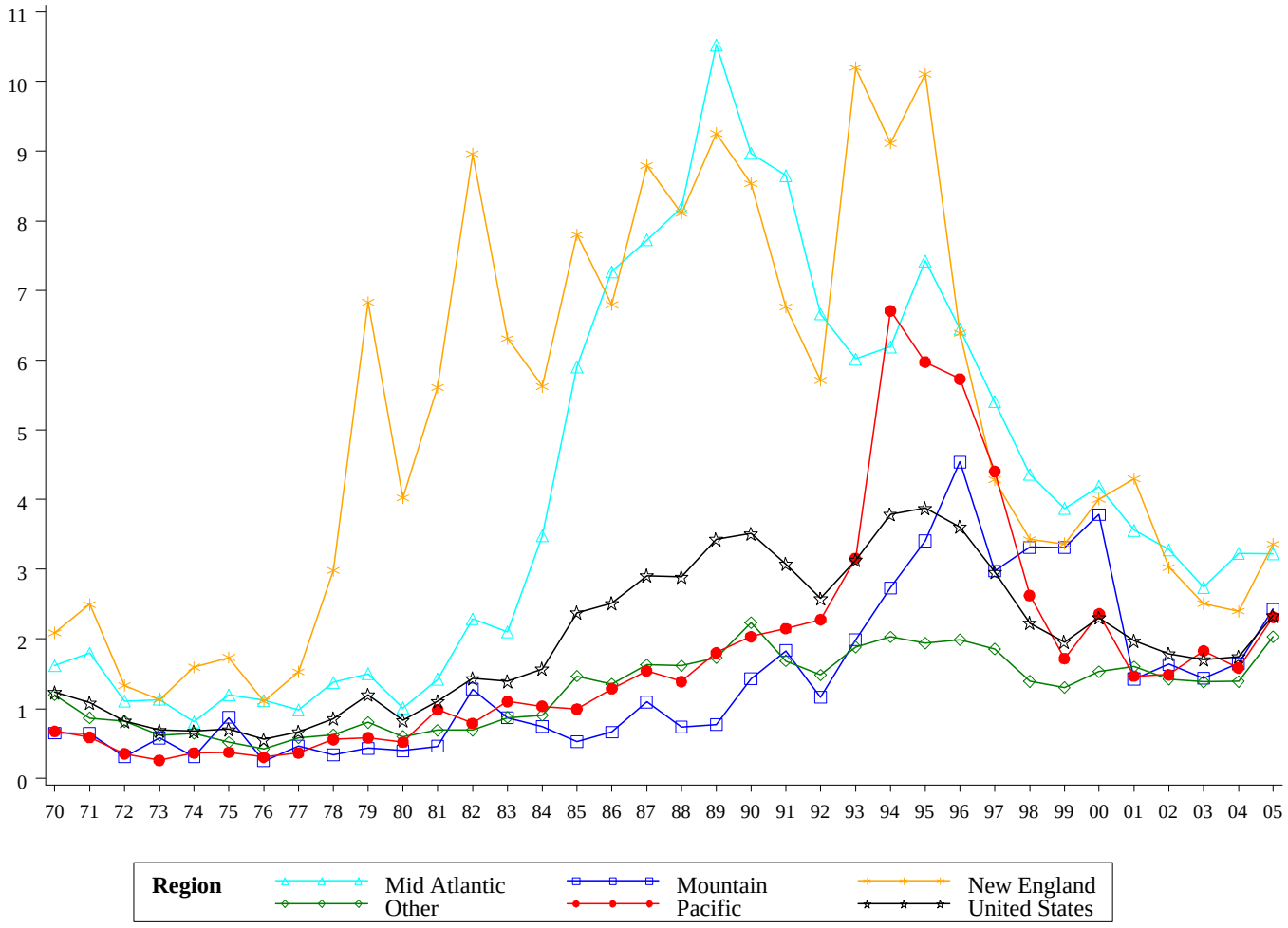


Figure 4-2. Isolation rate per 100,000 population for *Salmonella* Enteritidis reported to PHLIS, by region, 1970–2005



Shigella

The *Shigella* surveillance case definition is available at http://www.cdc.gov/epo/dphsi/casedef/shigellosis_current.htm. The National *Shigella* Surveillance System collects reports of isolates of *Shigella* from every state. *Shigella* isolates are submitted to the state public health laboratory by clinical diagnostic laboratories. The state and territorial laboratories confirm the isolates as *Shigella*, perform subtyping, and report the data electronically through PHLIS to EDEB. Unusual or untypable isolates are forwarded to the National *Shigella* Reference Laboratory at CDC for further characterization or confirmation. These results are reported back to the state laboratory, where they are reported to CDC through PHLIS. Duplicates are removed from the file at the end of the year.

The capture of isolates in the National *Shigella* Surveillance System is considered to be consistent. However, some *Shigella* isolates may not be forwarded or reported to state public health laboratories, and therefore are not captured. In addition, irrespective of the surveillance system, many cases of *Shigella* illness are not reported because the ill person does not seek medical care, the healthcare provider does not obtain a specimen for diagnosis, or the laboratory does not perform culture for *Shigella*. The results of surveillance reported here are therefore substantial underestimates of the true number of infections.

The reporting state represents the state where laboratory confirmation and subtyping were performed. In some instances, the reporting state is not the same as the state of residence of the person from whom the isolate was obtained.

There are four major subgroups and 44 recognized serotypes of *Shigella* that are differentiated from one another by their biochemical traits (such as ability to ferment mannitol) and antigenic properties (Table 5-1).

A total of 10,484 *Shigella* isolates were reported from public health laboratories in 50 states in 2005 (Table 5-2). The national rate was 3.5 per 100,000 population. Similar to previous years, children younger than age 5 years accounted for 28.2% of all *Shigella* isolates. About 34.2% came from persons aged 5–19 years, and 26.6% from persons aged 20–59, with lower proportions from persons in later decades of life. The overall distribution of *Shigella* isolates between the sexes was similar, with females accounting for 53.0% of persons from whom *Shigella* was isolated. Females accounted for more cases than males in all age groups except 40–49 years (47.5% female). The female predominance was particularly evident among persons aged 20–29 years (67.7%). Among reported isolates of *Shigella flexneri*, a male predominance is seen, particularly in the age groups 20–29 (57.3%), 30–39 (68.5%), 40–49 (73.6%), and 50–59 (61.2%). Gender information was not reported for 7.0% of all isolates and age information was not reported for 5.6% of isolates.

The frequency of reported subgroups, and the frequency of reported serotypes within these groups for all *Shigella* isolates are shown in Tables 5-2 and 5-3. Of the 10,484 isolates, 9,402 (89.7%) were subgrouped. The relative proportions of subgroups remained constant, with subgroup D (*S. sonnei*) accounting for the largest percentage of isolates (74.4%), followed by subgroup B (*S. flexneri*, 13.6%), subgroup C (*S. boydii*, 1.2%) and subgroup A (*S. dysenteriae*,

0.5%). Over the past decade, the numbers of reported *Shigella* isolates in subgroups A, B and C, and the proportions of all reported *Shigella* isolates caused by these three subgroups have declined. The number (1,082) and the proportion (10.3%) of all reported *Shigella* isolates that were not identified as belonging to a specific subgroup also decreased.

Table 5-1. Classification of *Shigella* subgroups

Subgroup	Subgroup	Serotypes	Fermentation of D-Mannitol	Subgroup B Group Antigens
A	*S. dysenteriae*	15	-	-
B	*S. flexneri*	8*	+	+
C	*S. boydii*	20	+	-
D	*S. sonnei*	1	+	-

* Serotypes 1–5 are subdivided into 11 subserotypes

Table 5-2. *Shigella* subgroups reported to PHLIS, 2005

Rank	Subgroup	Number	Percent
1	*S. sonnei*	7,795	74.4%
2	*S. flexneri*	1,430	13.6%
3	*S. boydii*	124	1.2%
4	*S. dysenteriae*	53	0.5%
Subtotal		**9,402**	**89.7%**
Unknown		**1,082**	**10.3%**
Total		**10,484**	**100.0%**

Table 5-3. Rank and number of isolates of *Shigella* serotypes reported to PHLIS, 2005

Rank	Serotype	Number	Percent
1	*S. sonnei*	7795	74.4%
2	*S. flexneri* unspecified	838	8.0%
3	*S. flexneri* 2 unspecified	108	1.0%
4	*S. boydii* unspecified	91	0.9%
5	*S. flex*neri 2a	88	0.8%
6	*S. flexneri* 1 unspecified	86	0.8%
7	*S. flexneri* 3 unspecified	51	0.5%
8	*S. flexneri* 4 unspecified	50	0.5%
9	*S. flexneri* 4a	47	0.5%
10	*S. dysenteriae* unspecified	34	0.3%
11	*S. flexneri* 1b	34	0.3%
12	*S. flexneri* 3a	31	0.3%
13	*S. flexneri* 6	28	0.3%
14	*S. flexneri* variant y	26	0.3%
15	*S. flexneri* 2b	17	0.2%
16	*S. flexneri* 3b	17	0.2%
17	*S. boydii* 2	11	0.1%
18	*S. boydii* 1	8	0.1%
19	*S. dysenteriae* 2	5	0.1%
20	*S. boydii* 4	4	0.0%
21	*S. dysenteriae* 3	4	0.0%
22	*S. dysenteriae* 4	4	0.0%
23	*S. boydii* 14	3	0.0%
24	*S. dysenteriae* 1	3	0.0%
25	*S. flexneri* 1a	3	0.0%
26	*S. boydii* 10	2	0.0%
27	*S. boydii* 15	2	0.0%
28	*S. boydii* 20	2	0.0%
29	*S. flexneri* 5 unspecified	2	0.0%
30	*S. boydii* 8	1	0.0%
31	*S. dysenteriae* 12	1	0.0%
32	*S. dysenteriae* 3162-96	1	0.0%
33	*S. dysenteriae* 6	1	0.0%
34	*S. flexneri* 4b	1	0.0%
35	*S. flexneri* 5a	1	0.0%
36	*S. flexneri* 88-893	1	0.0%
37	*S. flexneri* variant x	1	0.0%
Subtotal		**9,402**	**89.7%**
Unknown		**1,082**	**10.3%**
Total		**10,484**	**100.0%**

Vibrio

The cholera and vibriosis (non-cholera *Vibrio* species) surveillance case definitions are available at http://www.cdc.gov/epo/dphsi/casedef/cholera_current.htm and http://www.cdc.gov/epo/dphsi/casedef/vibriosis.htm. Infections with toxigenic *Vibrio cholerae* O1 and O139, the causative agents of cholera, have been reportable in the United States for many years. More recently, toxigenic *V. cholerae* O141 has emerged as a cause of illness, but it does not cause cholera and is not notifiable.

The Cholera and Other *Vibrio* Illness Surveillance System (COVIS) was initiated by CDC, FDA, and the Gulf Coast states (Alabama, Florida, Louisiana, Mississippi, and Texas) in 1988. CDC has maintained a database of reported *Vibrio* infections from humans in order to obtain reliable information on illnesses associated with *Vibrio* species. Participating health officials collect clinical data, information about underlying illness, history of seafood consumption, and exposure to seawater in the seven days before illness, and then conduct tracebacks of implicated oysters. This information has been used to educate consumers about the health risks of seafood, as well as to help determine host, food, and environmental risk factors. Since 1997, many other states have also reported *Vibrio* isolates. However, only toxigenic *V. cholerae* O1 and O139, the causative agents of cholera, were nationally notifiable during 2005; thus the true number of *Vibrio* isolates is greater than reported. CDC serotypes all *V. parahaemolyticus* isolates received from state health departments, and screens for cholera toxin production in all *V. cholerae* isolates.

Results are summarized using CDC form 52.79, Cholera and Other *Vibrio* Illnesses Surveillance Report and presented in two categories: *V. cholerae* isolates that produce cholera toxin (referred to as toxigenic *V. cholerae*), and all other *Vibrio* isolates, including those *V. cholerae* isolates that do not produce cholera toxin. Results are presented separately for Gulf Coast states versus other states, to be consistent with previous reports. Additionally, results are presented by anatomic site of isolation. It is important to note that isolation of some *Vibrio* species from a patient with illness does not necessarily indicate causation. While many *Vibrio* species are well-recognized pathogens, the status of *V. damsela, V. furnissii, V. metschnikovii*, and *V. cincinnatiensis* as enteric pathogens is less clear.

In June 2006, the Council of State and Territorial Epidemiologists (CSTE) adopted a resolution to add all *Vibrio* species infections (vibriosis) to the list of nationally notifiable diseases reported to NNDSS. Reporting for vibriosis is in addition to and distinct from reporting of *V. cholerae* currently conducted through NNDSS. The position statement, "National Reporting for non-cholera *Vibrio* Infections (Vibriosis)," can be found at http://www.cste.org/PS/2006pdfs/PSFINAL2006/06-ID-05FINAL.pdf. In addition to reporting through NNDSS, CDC requests that states collect information using the standard surveillance form for COVIS available at http://www.cdc.gov/foodborneoutbreaks/.

Isolates of toxigenic *Vibrio cholerae*

In 2005, 12 patients with toxigenic *V. cholerae* were reported (Table 6-1). Five patients were hospitalized, and no deaths were reported. All 12 patients were infected with toxigenic *V. cholerae* serogroup O1; no isolates of toxigenic *V. cholerae* O139 were identified. Infection was acquired during international travel in five isolated cases (three patients acquired

infection while traveling in Pakistan, and two patients traveled in the Philippines). Exposure to domestic seafood was the source of infection for four patients, two of whom were a husband and wife in Louisiana who ate crab and shrimp harvested from the Gulf Coast. The other two cases associated with domestic seafood were unrelated and occurred in patients who ate seafood acquired in Hawaii. Source of infection was unknown for three cases in Guam, of whom two patients were related, but had brief contact with each other and did not share any meals in the two weeks before illness onset. They, however, did receive drinking water from the same municipal aquifer supply and ate finfish in the week before illness. The third patient in Guam reported eating tuna fish and shrimp, in the week before illness.

Other *Vibrio* isolates (excluding toxigenic *V. cholerae*)

In 2005, 578 *Vibrio* isolates, excluding toxigenic *V. cholerae*, from 546 patients were reported to the COVIS. Among patients for whom information was available, 232 (46%) of 506 were hospitalized and 40 (8%) of 485 died. *V. parahaemolyticus* was isolated from 218 (40%) patients, and was the most frequently reported *Vibrio* species. Of the patients infected with *V. parahaemolyticus*, 23% were hospitalized, and 1% died. *V. vulnificus* was isolated from 121 (22%) patients; 90% were hospitalized, and 26% died. The following sections provide further information on these non-toxigenic *Vibrio* isolates:

Geographic location: In 2005, CDC received 219 (40%) reports of *Vibrio* illness from Gulf Coast states, 143 (26%) from Pacific Coast states, 151 (28%) from Atlantic Coast states (excluding Florida), and 33 (6%) from inland states (Figure 6-1). The most frequent *Vibrio* species reported from Gulf Coast states were *V. vulnificus* (39%), *V. parahaemolyticus* (23%), *V. alginolyticus* (11%), and non-toxigenic *V. cholerae* (11%) (Table 6-2). The most frequent *Vibrio* species reported from non-Gulf Coast states were *V. parahaemolyticus* (51%), *V. alginolyticus* (12%), *V. vulnificus* (11%), and non-toxigenic *V. cholerae* (10%) (Table 6-3).

Anatomic site of isolation: Among the 578 *Vibrio* isolates, 243 (42%) were from stool, 105 (18%) from blood, and 164 (28%) from wounds. In addition, 18 (3%) isolates were obtained from the ear, and 48 (8%) were from urine, sputum, or other sites. *V. parahaemolyticus* was the species most frequently isolated from stool (150 [62%] of 243 isolates from stool); *V. vulnificus* was the species most frequently isolated from blood (68 [65%] of 105 isolates from blood) and from wounds (51 [31%] of 164 isolates from wounds).

Seasonality: The number of patients from whom *Vibrio* species was isolated had a clear seasonal peak during the summer months (Figure 6-2). The greatest frequency of cases occurred in August for Gulf Coast states and non-Gulf Coast states.

Exposures: 153 (28%) patients reported having a wound either before or during exposure to *Vibrio*. Of those, 100 (65%) reported water activities such as swimming and boating, 34 (22%) reported handling seafood, and 40 (26%) reported contact with marine wildlife. Excluding patients from whom *Vibrio* was classified as a wound, and among the 255 for whom a food history was available, 223(87%) reported eating seafood in the seven days before illness onset. Among the 86 who reported eating a single seafood item, 49% ate oysters (91% of whom consumed them raw), 10% ate shrimp, and 15% ate finfish (Table 6-4). International travel in the seven days before illness onset was reported by 41 (9%) of 449

patients, for whom information was available.

Laboratory: For reports where laboratory confirmation was available, the state public health laboratory confirmed the identification of 234 (97%) of 242 human *Vibrio* isolates. CDC received 126 isolates of *V. parahaemolyticus* from 115 patients. Of these, 111 were viable, and four were not viable. Of the viable *V. parahaemolyticus* isolates, 28 (25%) from 11 health jurisdictions were serotype O4:K12 (Colorado, Hawaii, Louisiana, Maryland, Maine, Montana, North Carolina, New York State, New York City, Oregon, and Washington); 16 (14%) isolates from seven states were serotype O3:K6 (Arizona, Colorado, Georgia, Louisiana, Maryland, New Mexico, and Washington); 13 (12%) isolates from eight states were serotype O1:K56 (Hawaii, Louisiana, Maine, Montana, Oregon, Texas, Virginia, and Washington); and the remaining 54 isolates were one of 25 serotypes.

Outbreaks: Illnesses following Hurricane Katrina were reported to COVIS from eight states (Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, Oklahoma, and Texas). The species reported were 26 *V. vulnificus* (72%), 6 non-cholerigenic *V. cholerae* (17%), 3 *V. parahaemolyticus* (8%), and 1 (3%) unidentified *Vibrio* species. For patients with available information, 20 (91%) of 22 were considered wound infections because they reported having a wound either before or during exposure to *Vibrio*.

Table 6-1. Isolates of toxigenic *V. cholerae* reported to COVIS, 2005

State	Age	Sex	Onset	Exposure	Serogroup	Serotype
Hawaii	85	Female	4/8/2005	Domestic (seafood)	*V. cholerae* O1	Ogawa
Hawaii	34	Male	5/10/2005	Domestic (seafood)	*V. cholerae* O1	Ogawa
Montana	44	Female	8/2/2005	Travel in Pakistan	*V. cholerae* O1	Inaba
New York	44	Female	7/26/2005	Travel in Pakistan	*V. cholerae* O1	Inaba
Louisiana	43	Male	10/15/2005	Domestic (crab and shrimp) - Gulf Coast	*V. cholerae* O1	Inaba
Louisiana	46	Female	10/15/2005	Domestic (crab and shrimp) - Gulf Coast	*V. cholerae* O1	Inaba
Guam	32	Male	10/26/2005	Unknown	*V. cholerae* O1	Ogawa
Guam	29	Male	10/19/2005	Unknown	*V. cholerae* O1	Ogawa
Michigan	53	Male	11/3/2005	Travel in Pakistan	*V. cholerae* O1	Inaba
Michigan	34	Male	11/26/2005	Travel in the Philippines	*V. cholerae* O1	Ogawa
California	46	Male	12/22/2005	Travel in the Philippines	*V. cholerae* O1	Ogawa
Guam	26	Female	12/26/2005	Unknown	*V. cholerae* O1	Ogawa

Table 6-2. Number of *Vibrio* illnesses (excluding toxigenic *V. cholerae*) reported to COVIS, by species, complications and site of isolation in patients from Gulf Coast states, 2005

Vibrio Species	Patients		Complications* Hospitalized		Complications* Deaths		Isolates		Site of Isolation: Stool	Blood	Wound	Other†
	N	(%)	n/N	(%)	n/N	(%)	N	(%)				
V. alginolyticus	23	(11)	5/21	(24)	0/21	(0)	23	(10)	1	1	15	6
V. cholerae (non-toxigenic) ‡	23	(11)	12/20	(60)	3/19	(16)	23	(10)	12	5	1	4
V. damsela	3	(1)	0/2	(0)	0/2	(0)	3	(1)	0	0	3	0
V. fluvialis	11	(5)	7/11	(64)	2/11	(18)	12	(5)	5	2	4	1
V. hollisae	3	(1)	2/3	(67)	0/3	(0)	3	(1)	1	0	1	1
V. mimicus	4	(2)	2/4	(50)	1/4	(25)	4	(2)	3	0	0	1
V. parahaemolyticus	50	(23)	17/44	(39)	2/46	(4)	52	(22)	20	4	21	7
V. vulnificus	85	(39)	70/77	(91)	15/65	(23)	93	(39)	3	45	40	5
Species not identified	9	(4)	4/9	(44)	0/8	(0)	9	(4)	5	0	1	3
Multiple species§	8	(4)	4/8	(50)	1/6	(17)	18	(8)	6	4	8	0
Total	**219**	**(100)**	**123/199**	**(62)**	**24/185**	**(13)**	**240**	**(100)**	**56**	**61**	**94**	**29**

* Denominators indicate patients for whom information is known.
† Includes ear, endotracheal secretion, sputum, and urine.
‡ Non-toxigenic *V. cholerae*. Includes non-toxigenic *V. cholerae* O1 (1 isolates) and other non-toxigenic *V. cholerae* [non-O1 non-O139] (22 isolates).
§ *V. parahaemolyticus* and *V. alginolyticus* were isolated from two patients, *V. parahaemolyticus* and *V. mimicus* were isolated from one patient, *V. parahaemolyticus* and *V. fluvialis* were isolated from one patient, *V. parahaemolyticus* and an unidentified *Vibrio* species were isolated from one patient, *V. parahaemolyticus* and *V. vulnificus* were isolated from one patient, *V. vulnificus* and *V. alginolyticus* was isolated from one patient, and *V. vulnificus* and an unidentified *Vibrio* species were isolated from one patient.

Table 6-3. Number of *Vibrio* illnesses (excluding toxigenic *V. cholerae*) reported to COVIS, by species, complications, and site of isolation in patients from non-Gulf Coast states, 2005

Vibrio Species	Patients		Complications*				Isolates		Site of Isolation			
			Hospitalized		Deaths				Stool	Blood	Wound	Other†
	N	(%)	n/N	(%)	n/N	(%)	N	(%)				
V. alginolyticus	40	(12)	9/37	(24)	0/37	(0)	40	(12)	2	1	25	12
V. cholerae (non-toxigenic)[3]	33	(10)	14/31	(45)	2/28	(7)	35	(10)	19	10	4	2
V. damsela	4	(1)	3/4	(75)	0/4	(0)	4	(1)	0	0	3	1
V. fluvialis	17	(5)	8/17	(47)	1/17	(6)	17	(5)	12	2	2	1
V. furnissii	2	(0.6)	2/2	(100)	1/2	(50)	2	(0.6)	1	1	0	0
V. hollisae	4	(1)	3/4	(75)	0/3	(0)	4	(1)	4	0	0	0
V. mimicus	6	(2)	3/6	(50)	1/6	(17)	6	(2)	3	0	2	1
V. parahaemolyticus	168	(51)	29/155	(19)	0/155	(0)	168	(50)	130	3	22	13
V. vulnificus	36	(11)	31/35	(89)	10/32	(31)	37	(11)	1	23	11	2
Species not identified	10	(3)	3/9	(33)	0/9	(0)	10	(3)	6	1	1	2
Other	1	(0.3)	0/1	(0)	0/1	(0)	2	(1)	1	0	0	1
Multiple species§	6	(2)	4/6	(67)	1/6	(17)	13	(4)	8	3	0	2
Total	**327**	**(100)**	**109/307**	**(36)**	**16/300**	**(5)**	**338**	**(100)**	**187**	**44**	**70**	**37**

* Denominators indicate patients for whom information is known.
† Includes ear, peritoneal fluid, sinus, sputum, and urine.
‡ Non-toxigenic *V. cholerae*. Includes non-toxigenic *V. cholerae* O1 (3 isolates), non-toxigenic *V. cholerae* O139 (1 isolate), and other non-toxigenic *V. cholerae* (non-O1 non-O139) (29 isolates).
§ *V. parahaemolyticus* and *V. alginolyticus* were isolated from one patient; *V. parahaemolyticus* and *V. fluvialis* were isolated from two patients; *V. parahaemolyticus* and an unidentified *Vibrio* species were isolated from one patient; *V. alginolyticus* and *V. fluvialis* were isolated from one patient; and *V. cholerae* non-O1, non-O139, *V. fluvialis*, and *V. vulnificus* were isolated from one patient.

Table 6-4. Seafood exposure among patients with foodborne *Vibrio* infection (excluding toxigenic *V. cholerae*) who reported eating a single seafood item in the week before illness onset, 2005

	Mollusks			Crustaceans						
	Oysters	**Clams**	**Mussels**	**Shrimp**	**Lobster**	**Crab**	**Crayfish**	**Other Shellfish***	**Finfish†**	**Total**
Ate (%)	42 (49)	3 (3)	1 (1)	9 (10)	1 (1%)	13 (15%)	2 (2%)	2 (2%)	13 (15%)	86
Ate raw (%)	91	67	100	44	0	0	0	0	27	49

* Other shellfish reported: scallops

† Finfish reported: ahi poke, cat fish, flounder, perch, red snapper, rockfish filet, salmon, sunfish, sushi, tuna, yellow fin, and whiting.

Figure 6-1. Number of patients with *Vibrio* isolates (excluding toxigenic *V. cholerae*) reported to COVIS, by state, 2005 (N = 546 patients)

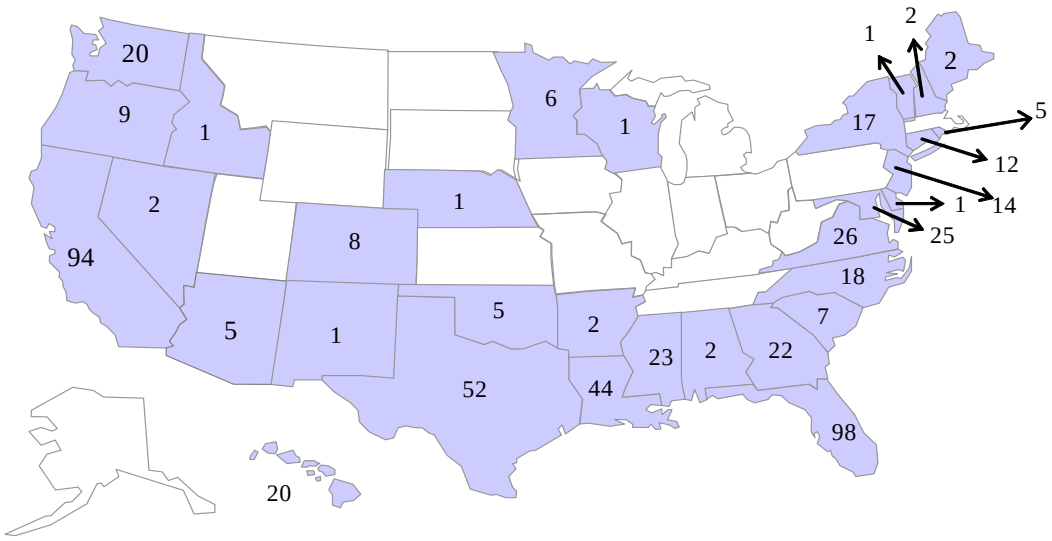
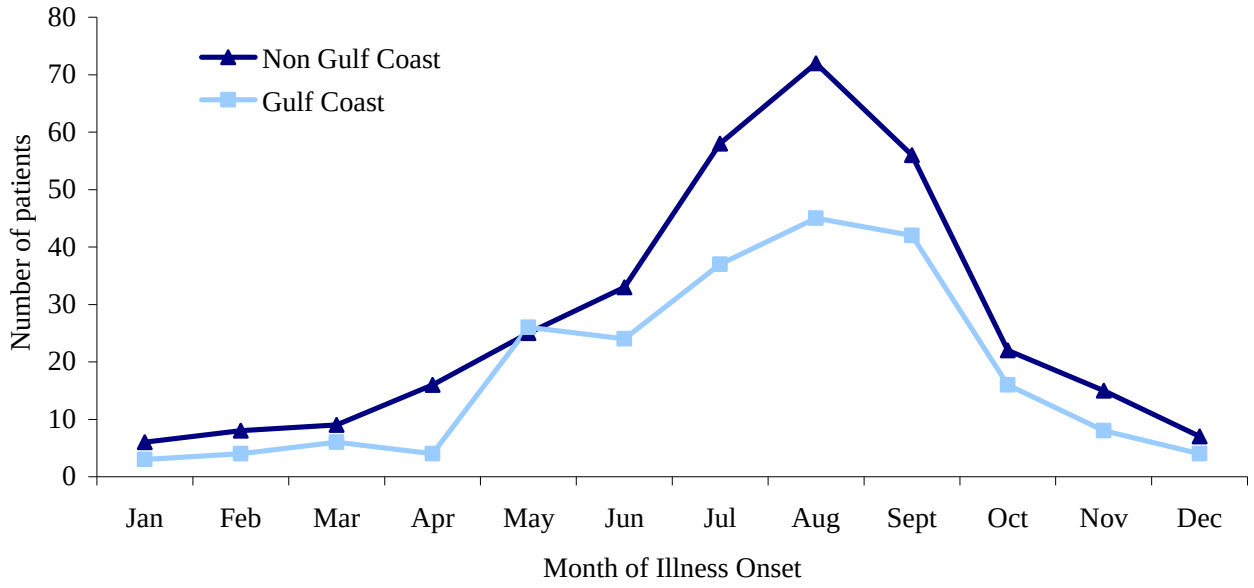


Figure 6-2. Number of patients with *Vibrio* isolates (excluding toxigenic *V. cholerae*) reported to COVIS, by month of illness onset or specimen isolation, Gulf Coast states vs. other states, 2005 (N = 546)



Data Sources and Background

CDC conducts national surveillance to define the magnitude and burden of diseases, to identify outbreaks or high risk groups so that preventive actions can be taken, and to track the effectiveness of control and prevention measures.

The surveillance systems for different foodborne pathogens have evolved over time. There are many distinct surveillance systems, some managed by individual program areas (e.g., botulism surveillance), and others administered and used more broadly.

National Notifiable Diseases Surveillance System (NNDSS) and the National Electronic Telecommunications System for Surveillance (NETSS)
The origins of NNDSS date back to 1878 when Congress authorized the U.S. Marine Hospital Service to collect morbidity reports regarding cholera, smallpox, plague, and yellow fever from U.S. consuls oversees. Today, the NNDSS is operated by CDC in collaboration with the Council of State and Territorial Epidemiologist (CSTE) and serves as a timely source of national disease data. NETSS is the software and electronic communication pathway by which NNDSS data reach the CDC; this whole system is often identified by the NETSS acronym. NETSS is administered by the CDC National Center for Public Health Informatics (NCPHI).

There are several sources of NETSS surveillance information for individual infections. For many diseases, public health authorities at state health departments request or require that physicians and other health care workers report cases to the local health department. For some diseases, authorities also request or require clinical laboratories to report the identification or isolation of certain pathogens. These reports are summarized and forwarded to the state department of health, which then sends the information to CDC, if the disease is nationally notifiable.

Public Health Laboratory Information System (PHLIS)
In addition to allowing public health authorities to track diagnosed cases of notifiable disease, sending pathogens isolated from patients to public health laboratories to confirm the identity of the organism and its subtype provides an additional public health benefit. This process can identify clusters of specific subtypes and link events from widely dispersed locations. An example is surveillance for serotype of *Salmonella*. In 1962, CDC, CSTE, and the Association of State and Territorial Public Health Laboratory Directors agreed to serotype *Salmonella* isolates and send the resulting information to CDC weekly. Eight states participated initially. Eventually, all 50 states began transmitting information through PHLIS, an electronic network tool developed in the 1980s. PHLIS collects laboratory surveillance information for a large number of pathogens (foodborne and non-foodborne). In 2004, it was administered by the Biostatistics and Information Management Branch of the Division of Bacterial and Mycotic Diseases, located in CDC's National Center for Infectious Diseases. PHLIS information has been used to identify, investigate, and control outbreaks of salmonellosis and other foodborne diseases at local, regional, national, and international levels.

Limitations Common to NETSS and PHLIS
Most surveillance systems for foodborne and diarrheal diseases tend to underestimate the

burden of disease. Diseases that cause severe clinical illness are most likely to be reported accurately, if they were diagnosed by a physician. However, persons who have diseases that are clinically mild, and infrequently associated with severe consequences, might not seek medical care from a healthcare provider, and these diseases are never diagnosed. Even if these less severe diseases are diagnosed, they are less likely to be reported in surveillance systems.

The information reported about each case is typically limited to age, sex, county of residence, date of diagnosis, and a small number of other variables. The degree of completeness of data reporting is also influenced by the diagnostic facilities available; the control measures in effect; the public awareness of a specific disease; and the interests, resources, and priorities of state and local officials responsible for disease control and public health surveillance. Factors such as changes in the case definitions for public health surveillance, the introduction of new diagnostic tests, or the discovery of new disease entities can cause changes in disease reporting that are independent of the true incidence of disease.

Some important infections that are difficult to diagnose are not included in general surveillance. For example, the diagnosis of enterotoxigenic *E. coli* (ETEC) remains restricted to a few research and large public health laboratories, and tests for this pathogen are not performed in standard clinical laboratories. Surveillance systems cannot track infections by this cause of foodborne diarrheal illness.

Limitations specific to NETSS and PHLIS

NETSS is a passive surveillance system that relies on a mix of clinicians and laboratories that vary by state and by pathogen to report cases or pathogen isolations. The system includes cases that are diagnosed only clinically (on the basis of symptoms, signs and the epidemiological setting) as well as cases that are diagnosed by a definitive laboratory test. The willingness of clinicians to report cases varies from disease to disease, and the completeness and timeliness of reporting is problematic for some diseases. The data do not include the specific findings of the public health laboratory, such as a subtype, and therefore are not useful for detecting clusters of a particular subtype. The lack of subtyping for common pathogens makes detection of outbreaks difficult, especially those that are multi-jurisdictional. This is particularly true for *Salmonella* and *Shigella* infections.

PHLIS, a public health laboratory-based surveillance system, is also limited as a passive system; it relies on clinical laboratories to send *Salmonella* and other isolates to the state public health laboratory for subtyping. For example, because there is no routine referral or subtyping of *Campylobacter* strains in the United States, state public health laboratories may report only those strains that they isolate themselves (e.g., from patients in public health clinics or from specimens collected in outbreak investigations). The number of *Campylobacter* isolates reported through PHLIS is typically a small fraction of the number that is diagnosed. The need to send an isolate from the original clinical laboratory to the state public health laboratory and the need for the state laboratory to do the serotyping means that reports may be delayed. Training and support are required to ensure that state laboratories have the specialized skills and reagents needed to perform serotyping or other subtyping methods. The PHLIS software, written first in the late 1980s, has not been fully integrated into other software used in the states, and its use requires training.

State-to-State Variations in Reported Cases
There is substantial variation in the number of reported cases from one state compared to another, even when taking into account the differences in population sizes among states. One major source of variation is that a given disease may be reportable in one state but not in another, even for nationally notifiable diseases. Reporting requirements are under state jurisdiction. There may also be substantial variation from one state to another, depending on local resources, interests, and priorities. When more than one route is available for reporting surveillance data within the public health system, states may choose to use one or the other or more than one. For example, some state public health laboratories report *E. coli* O157:H7 isolates that they receive for confirmation through PHLIS, and some state epidemiology offices report infections with this organism through NETSS.

Some states may chose to submit reports on diseases for which they have collected information, but which are not nationally notifiable. These data indicate the interest and concern with that disease within that specific state, but are not part of the nationally notifiable disease system.

In addition, there are substantial state-to-state and regional differences in the incidence of certain diseases. For example, PHLIS has demonstrated that some *Salmonella* serotypes are isolated with similar frequency in persons in all U.S. regions, while other serotypes are highly localized. The PHLIS *Salmonella* Surveillance System is a stable system that has been functioning well for several decades with full national participation, so these results are considered valid.

Program-Specific Surveillance Systems
Because both NETSS and PHLIS collect little information beyond very basic patient demographics (e.g., age, sex, race, place, and time) and pathogen characteristics (e.g. *Salmonella* serotype in PHLIS), EDEB collects more detailed information on individual cases for some diseases because this information is needed for accurate monitoring and effective intervention. The diseases included are botulism, typhoid fever, and cholera and *Vibrio* species infections. For botulism, typhoid fever, and cholera, reporting is nationwide. For the non-cholera *Vibrio* species reporting is mainly through a surveillance alliance with the Gulf Coast states of Alabama, Florida, Louisiana, and Texas. *Vibrio* surveillance also includes voluntary reporting from many other states. These systems and their resulting databases are distinct and separate from each other and from NETSS and PHLIS.

Botulism surveillance has unique attributes. Botulism is an extreme hazard that can be fatal if untreated, and it has caused rare but catastrophic foodborne outbreaks that are public health emergencies. CDC provides the antitoxin used to treat the illness, and releases it for treatment of suspected botulism from airport quarantine stations at the request of a state epidemiologist. Clinicians who suspect a patient has botulism can call their state health department or CDC to arrange emergency release through a 24-hour emergency response system. This drug release mechanism means that CDC gets immediate information about suspected cases of botulism, which functions as an early alert surveillance system.

Though not formally part of a surveillance system, EDEB tracks the number and type of non-

O157 Shiga toxin-producing *E. coli* received from public health laboratories around the country. Among public health and clinical laboratories in the United States, only CDC has the capacity to serotype and characterize a wide variety of these isolates. Thus, our collection of isolates is likely representative of those isolated and forwarded to public health laboratories.

Surveillance at Selected Sites

For nine foodborne infections, the most detailed and accurate surveillance information comes from Foodborne Diseases Active Surveillance Network (FoodNet). In 2005, FoodNet included 10 surveillance sites, each comprised of several counties within a state, or a whole state, and covering a population of approximately 44.5 million, or 15% of the U.S. population. FoodNet actively gathers information about nine infections or conditions, integrates it with available laboratory information, and also collects information about the severity and outcome of the illness. In addition, FoodNet also conducts population surveys to determine the burden of illness, and how many ill persons visited a physician and got tested, as well as surveys of clinical laboratories to determine which pathogens are sought. Because standard surveillance methods are used, FoodNet data can be used to compare rates of illness over time and from one site to another.

Enhancements to Surveillance Systems

Public health surveillance is an evolving effort. As new disease entities are identified and defined as public health problems, surveillance for them begins and improves. As better understanding leads to better prevention, cases may level off, decline, and ultimately disappear. On the list of nationally notifiable diseases, there are several that were once large public health problems, but are now rarely reported. The official list of nationally notifiable diseases changes in accordance with resolutions issued by CSTE.

The methods and information obtained for surveillance also continue to evolve. Active surveillance in sentinel populations (such as FoodNet) can provide reliable and detailed information about detected infections and eliminate the undercount caused by lack of resources or reporting effort. However, this effort is expensive and cannot be applied everywhere. The ongoing revolution in biotechnology is bringing new subtyping and fingerprinting technologies, such as pulsed-field gel electrophoresis (PFGE), into state and local public health laboratories. PulseNet is a national network of public health and food regulatory agency laboratories coordinated by CDC; PulseNet participants use PFGE to characterize isolates of foodborne disease pathogens. Isolate DNA patterns generated by PFGE are submitted electronically to the PulseNet database at CDC, where they are analyzed to identify clusters of illness caused by the same pathogen subtype. This approach is enhancing our capacity to detect outbreaks rapidly, to link widely separated cases, and to track more precisely the results of specific control measures. New electronic reporting media have accelerated reporting and have made possible practical automated cluster detection algorithms, such as the Statistical Outbreak Detection Algorithm (SODA), which has been in operation using PHLIS data for *Salmonella* since 1995. CDC's efforts to produce a new integrated surveillance system, which will bring information directly from the clinical laboratory into a public health database, should improve the timeliness and consistency of reporting for many diseases.

Sources and Contacts for Surveillance of Bacterial Foodborne and Diarrheal Diseases

Many staff members both within and outside EDEB are responsible for foodborne and diarrheal diseases national surveillance. For the purpose of this report, EDEB national case surveillance activity is considered separate from foodborne outbreak surveillance, FoodNet, and the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS-EB). Information concerning FoodNet and NARMS is cited in the reference section. Surveillance for foodborne disease outbreaks is contained in the report from the EDEB Outbreak Response and Surveillance Team. Note also that EDEB activities concern bacterial pathogens. Surveillance information concerning viral and parasitic diseases is reported by Division of Viral and Rickettsial Diseases and the Division of Parasitic Diseases, respectively, and surveillance information regarding chemical intoxications is reported by the National Center for Environmental Health.

Sources and Contacts for Surveillance of Bacterial Foodborne and Diarrheal Diseases

System	Cases Reported	Contact	Title	CDC Division
NNDSS/NETSS	Clinical-case reporting of Campylobacteriosis, Botulism, EHEC, Hemolytic Uremic Syndrome, Listeriosis, Typhoid Fever, Salmonellosis, Shigellosis, Cholera	Ruth Ann Jajosky	Epidemiologist	Integrated Surveillance Systems and Services
PHLIS	Laboratory-based reporting of STEC, *Salmonella*, *Shigella*	Richard Bishop	Analyst	Foodborne, Bacterial, and Mycotic Diseases
National Botulism Surveillance System	Detail case information for all U.S. botulism cases, including foodborne, infant, wound, and other forms	Jeremy Sobel	Epidemiologist, EDEB	Foodborne, Bacterial, and Mycotic Diseases
Typhoid Fever Surveillance System	Detailed case information for all U.S. typhoid fever cases	Liz Blanton	Epidemiologist, EDEB	Foodborne, Bacterial, and Mycotic Diseases
Vibrio Surveillance System	Detailed case information for all U. S. cholera and other *Vibrio* species infections	Martha Iwamoto (vibriosis) Liz Blanton (cholera)	Epidemiologist, EDEB Epidemiologist, EDEB	Foodborne, Bacterial, and Mycotic Diseases Foodborne, Bacterial, and Mycotic Diseases
National *Salmonella*, *Campylobacter*, and *Helicobacter* Reference Lab	Isolates received at CDC for serotyping and characterization	Patricia Fields	Chief, Enteric Diseases Laboratory Branch	Foodborne, Bacterial, and Mycotic Diseases
National *E. coli*, *Shigella*, *Yersinia*, and *Vibrio* Reference Lab	Isolates received at CDC for serotyping and characterization	Nancy Strockbine	Team Lead, National *E. coli*, *Shigella*, *Yersinia*, and *Vibrio* Reference Lab	Foodborne, Bacterial, and Mycotic Diseases

List of Acronyms

BSO……. . Biostatistics Office

CDC……... Centers for Disease Control and Prevention

CSTE……..Council of State and Territorial Epidemiologist

DFBMD…… Division of Foodborne, Bacterial, and Mycotic Diseases

EHEC…….Enterohemorrhagic *Escherichia coli*

EIA…….Enzyme Immunoassays

ETEC……..Enterotoxigenic *Escherichia coli*

EDEB…….Enteric Diseases Epidemiology Branch

FDA……Food and Drug Administration

FoodNet……..Foodborne Diseases Active Surveillance Network

HUS……Hemolytic Uremic Syndrome

MMWR……..Morbidity Mortality Weekly Report

NARMS-EB……...National Antimicrobial Resistance Monitoring System for Enteric Bacteria

NCID……..National Center for Infectious Diseases

NETSS……... National Electronic Telecommunications System for Surveillance

NNDSS…….. National Notifiable Diseases Surveillance System

PCR……Polymerase Chain Reaction

PFGE……..Pulsed-field Gel Electrophoresis

PHLIS…… Public Health Laboratory Information System

SODA…….Statistical Outbreak Detection Algorithm

STEC……..Shiga toxin-producing *Escherichia coli*

Publications by the Enteric Diseases Epidemiology Branch, 2005

Anderson A, Nelson M, Baker N, Rossiter S, Angulo F. Public health consequences of use of antimicrobial agents in agriculture. In: Smulders FJM, Collins JD, eds. Food safety assurance and veterinary public health: Wageningen Academic, 2005:173–184.

Batz M, Doyle M, Morris G, et al. Attributing illness to food. Emerging Infectious Diseases 2005;11:993–999.

Brooks J, Sowers E, Wells J, et al. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. Journal of Infectious Diseases 2005;192:1422–1429.

Burr R, Effler P, Kanenaka R, Nakata M, Holland B, Angulo F. Emergence of *Salmonella* serotype Enteritidis phage type 4 in Hawaii traced to locally-produced eggs. International Society for Infectious Diseases 2005;9:340–346.

CDC. *Escherichia coli* O157:H7 infections associated with ground beef from a U.S. military installation, Okinawa, Japan, February 2004. Morbidity and Mortality Weekly Report 2005;54:40–42.

CDC. Outbreak of multidrug-resistant *Salmonella* Typhimurium associated with rodents purchased at retail pet stores—United States, December 2003–October 2004. Morbidity and Mortality Weekly Report 2005;54:429.

CDC. Outbreaks of *Salmonella* infections associated with eating roma tomatoes—United States and Canada, 2004. Morbidity and Mortality Weekly Report 2005;54:326–328.

CDC. Rapid health response, assessment, and surveillance after a tsunami—Thailand, 2004–2005. Morbidity and Mortality Weekly Report 2005;54:61-64.

CDC. Salmonellosis associated with pet turtles—Wisconsin and Wyoming, 2004. Morbidity and Mortality Weekly Report 2005;54:223–226.

Crump J, Otieno P, Slutsker L, et al. Household based treatment of drinking water with flocculant-disinfectant for preventing diarrhoea in areas with turbid source water in rural western Kenya: cluster randomised controlled. BMJ 2005;10:1–6.

Devasia R, Varma J, Whichard J, et al. Antimicrobial use and outcomes in patients with multidrug-resistant and pansusceptible *Salmonella* Newport infection, 2002–2003. Microbial Drug Resistance 2005;11:371–377.

Doumith M, Jacquet C, Gerner-Smidt P, et al. Multicenter validation of a multiplex PCR assay for differentiating the major *Listeria monocytogenes* serovars 1/2a, 1/2b, 1/2c, and 4b: toward an international standard. Journal of Food Protection 2005;68:2648–2650.

Fischer T, Ashley D, Kerin T, et al. Rotavirus antigenemia in patients with acute gastroenteritis.

Journal of Infectious Diseases 2005;192:913–919.

Fisk T, Lundberg B, Guest J, et al. Invasive infection with multidrug-resistant *Salmonella* enterica Serotype Typhimurium Definitive Type 104 among HIV-Infected Adults. Clinical Infectious Disease 2005;40:1016–1021.

Flint J, Duynhoven YTV, Angulo F, et al. Estimating the burden of acute gastroenteritis, foodborne disease, and pathogens commonly transmitted by food: an international review. Clinical Infectious Diseases 2005;41:698–704.

Frenzen P, Drake A, Angulo F. Economic cost of illness due to *Escherichia coli* O157 infections in the United States. Journal of Food Protection 2005;68:2623–2630.

Fry A, Braden C, Griffin P, Hughes J. Foodborne disease. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. Philadelphia PA: Churchill Livingston; 2005. p. 1286–1301.

Gerner-Smidt P, Kincaid J, Kubota K, et al. Molecular surveillance of shiga toxigenic *Escherichia coli* O157 by PulseNet USA. Journal of Food Protection 2005;68:1926–1931.

Graves L, Hunter S, Rae Ong A, et al. Microbiological aspects of the investigation that traced the 1998 outbreak of listeriosis in the United States to contaminated hot dogs and establishment of molecular subtyping-based surveillance for *Listeria monocytogenes* in the PulseNet Network. Journal of Clinical Microbiology 2005;43:2350–2355.

Green L, Selman C, Banerjee A, et al. Food service worker's self-reported food preparation practices: an EHS-Net study. International Journal of Hygiene and Environmental Health 2005;208:27–35.

Green LR, Selman C, Scallan E, Jones TF, Marcus R. Beliefs about meals eaten outside the home as sources of gastrointestinal illness. Journal of Food Protection 2005;68:2184–2189.

Gupta A, Nelson J, Barrett L, et al. Antimicrobial resistance among *Campylobacter* strains, United States,1997–2001. Emerging Infectious Diseases 2005;10:1102–1109.

Gupta A, Sivapalasingam S, Olsen S, Mintz E, Bibb W, Hoekstra R. Evaluation of community-based serologic screening for identification of chronic *Salmonella* Typhi carriers in Vietnam. International Journal of Infectious Diseases 2005;1–6.

Gupta A, Summer C, Castor M, Maslanka S, Sobel J. Adult botulism type F in the United States, 1981-2002. Neurology 2005;65:1694–1700.

Hannah EL, Angulo FJ, Johnson JR, Haddadin B, Williamson J, Matthew S. Drug-resistant *Escherichia coli*, Rural Idaho. Emerging Infectious Diseases 2005;11:1614–1617.

Helms M, Ethelberg S, Molbak K, Group DS. International *Salmonella* Typhimurium DT104

infections, 1992–2001. Emerging Infectious Diseases 2005;11:9.

Homes C, Chillers T. National Antibiotic Resistance Monitoring System for Enteric Bacteria. Emerging Infectious Diseases 2005;10:20–61.

Hunter S, Vauterin P, Lambert-Fair M, et al. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. Journal of Clinical Microbiology 2005;43:1045–1050.

Hyma K, Lacher D, Nelson A, et al. Evolutionary genetics of a new pathogenic *Escherichia* species: *Escherichia albertii* and related *Shigella boydii* strains. Journal of Bacteriology 2005;187:619–628.

Kretsinger K, Sobel J, Tarkhashvili N, et al. *Helicobacter pylori,* Republic of Georgia. Emerging Infectious Diseases 2005;11:780–781.

Kubota K, Barrett L, Ackers M, Brachman P, Mintz E. Analysis of *Salmonella enterica* serotype Typhi pulsed-field gel electrophoresis patterns associated with international travel. Journal of Clinical Microbiology 2005;43:1205–1209.

Luby SP, Agboatwalla M, Feikin DP, Billheimer W, Altaf A, Hoekstra RM. Effect of handwashing on child health: a randomized controlled trial. Lancet 2005;366:225–233.

Lule J, Mermin J, et al. Effect of home based water chlorination and safe storage on diarrhea among persons with human immunodeficiency virus in Uganda. AJTMH 2005;73:926–933.

MacDonald PD, Whitwam R, Boggs J, et al. Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese. Clinical Infectious Disease 2005;40:677–682.

McLaughlin J, DePaola A, Bopp C, et al. Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. New England Journal of Medicine 2005;353:1463–1470.

Mead PS, Dunne E, Graves L, et al. Nationwide outbreak of listeriosis due to contaminated meat. Epidemiology and Infection 2005:1–8.

Mermin J, Lule J, Ekwaru J, et al. Effect of co-trimoxazole prophylaxis on morbidity, mortality, CD4-cell count, and viral load in HIV infection in rural Uganda. Lancet 2005;364:1428–1434.

Naugle A, Holt K, Levine P, Eckel R. Sustained decrease in the rate of *Escherichia coli* O157:H7 positive raw ground beef samples tested by the Food Safety and Inspection Service. Journal of Food Protection 2005;68:2504–2505.

Nelson J, Tauxe R, Angulo F. Reply to Cox. Journal of Infectious Diseases 2005;191:1566–1567.

Olsen S, Patrick M, Hunter S, et al. Multistate Outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. Clinical Infectious Disease 2005;40:962–967.

Rangel J, Sparling P, Crowe C, Griffin P, Swerdlow D. Epidemiology of *Escherichia coli* O157: H7 outbreaks United States, 1982–2002. Emerging Infectious Diseases 2005;11:603–609.

Rankin S, Whichard J, Joyce K, et al. Detection of a blaSHV extended-spectrum B-lactamase in *Salmonella* Newport MDR-AmpC. Journal of Clinical Microbiology 2005;43:5792–5793.

Raphael B, Pereira S, Flom G, Zhang Q, Ketley J, Konkel M. The *Campylobacter jejuni* response regulator, CbrR, modulates sodium deoxycholate resistance and chicken colonization. Journal of Bacteriology 2005;187:3662–3670.

Reller M, Nelson J, Molbak K, et al. A Large, Multiple-restaurant outbreak of infection with *Shigella flexneri* serotype 2a traced to tomatoes. Clinical Infectious Diseases 2005;42:163–169.

Sanchez TH, Brooks JT, Sullivan P, et al. Bacterial diarrhea in persons with HIV Infection, United States, 1992-2002. Clinical Infectious Diseases 2005;41:1621–1627.

Scallan E, Majowicz S, Hall G, et al. Prevalence of diarrhoea in the community in Australia, Canada, Ireland, and the United States. International Journal of Epidemiology 2005;34:454–460.

Schroeder C, Naugle A, Schlosser W, et al. Estimate of illnesses from *Salmonella* Enteritidis in eggs, United States, 2000. Emerging Infectious Diseases 2005;11:113–115.

Sobel J. Botulism. Clinical Infectious Diseases 2005;41:1167–1173.

Sobel J. Food and beverage sabotage. Encyclopedia of Bioterrorism Defense 2005:215–220.

Sobel J, Mixter C, Kolhe P, et al. Necrotizing enterocolitis associated with *Clostridium perfringens* type A in previously healthy North American adults. J Am Coll Surg 2005;201:48–56.

Sobel J, Painter J. Illnesses caused by marine toxins. Clinical Infectious Diseases 2005;41:1290–1296.

Srikantiah P, Bodager D, Toth B, et al. Web-based investigation of multistate salmonellosis outbreak. Emerging Infectious Diseases 2005;11:610–612.

Srikantiah P, Charles M, Reagan S, et al. SARS clinical features, United States, 2003. Emerging Infectious Diseases 2005;11:135–138.

Swaminathan B, Gerner-Smidt P, Barrett L. Foodborne disease trends and reports. Foodborne Pathogens and Disease 2005;2:189–191.

Tauxe R. Linking illnesses to foods: a conceptual framework. In: Hoffmann SA, Taylor MR, eds. Toward Safer Food Perspective on Risk and Priority Setting. Washington, DC, 2005:47–63.

Tauxe R, Khabbaz R, Cameron D, Feinman L. International Conference on Emerging Infectious Diseases. Emerging Infectious Diseases 2005;10:2037–2038.

Varma J, Greene K, Ovitt J, Barrett L, Medalla F, Angulo F. Hospitalization and antimicrobial resistance in *Salmonella* outbreaks, 1984 - 2002. Emerging Infectious Diseases 2005;11:4.

Varma J, Rossiter S, Hawkins M, et al. Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infection and hospitalization. Journal of Infectious Diseases 2005;19:554–561.

Vora G, Meador C, Bird M, Bopp C, Andreadis J, Stenger D. Microarray-based detection of genetic heterogeneity, antimicrobial resistance, and the viable but nonculturable state in human pathogenic *Vibrio* spp. PNAS 2005;102:19109–19114.

Whichard J, Joyce K, Fey P, Nelson J, Angulo F, Barrett T. B-lactam resistance and *Enterobacteriaceae*, United States. Emerging Infectious Diseases 2005;11:1464–1466.

Widdowson M, Sulka A, Bulens S, et al. Norovirus and foodborne disease, United States, 1991–2000. Emerging Infectious Diseases 2005;11:95–102.

Woodward D, Clark C, Caldeira R, et al. Identification and characterization of *Shigella boydii* 20 serovar nov., a new and emerging *Shigella* serotype. Journal of Medical Microbiology 2005;54:741–748.

Wright J, Tengelsen L, Smith K, et al. Multidrug-resistant *Salmonella* Typhimurium in four animal facilities. Emerging Infectious Diseases 2005;11:1235–1242.

CDC Internet sites relevant to Foodborne and Diarrheal Diseases

For additional information about foodborne disease, please visit any of the following web sites:

Case Definitions for Infectious Conditions under Public Health Surveillance
http://www.cdc.gov/EPO/DPHSI/casedef/case_definitions.htm

Causes of Foodborne Illness
http://www.cdc.gov/foodborneoutbreaks/foodborne_az.htm

Division of Bacterial and Mycotic Diseases
http://www.cdc.gov/ncidod/dbmd/

Division of Parasitic Diseases
http://www.cdc.gov/ncidod/dpd/

DPDx (Identification and Diagnosis of Parasites of Public Health Concern)
http://www.dpd.cdc.gov/dpdx/

Division of Viral and Rickettsial Diseases
http://www.cdc.gov/ncidod/dvrd/index.htm

Division of Viral Hepatitis
http://www.cdc.gov/ncidod/diseases/hepatitis/index.htm

Epidemiology Program Office, Division of Public Health Surveillance and Informatics
http://www.cdc.gov/epo/index.htm

Foodborne and Diarrheal Diseases Branch
http://www.cdc.gov/ /foodborne/

Foodborne and Diarrheal Diseases Branch, Outbreak Response and Surveillance Team
http://www.cdc.gov/foodborneoutbreaks/

FoodNet (Foodborne Diseases Active Surveillance Network)
http://www.cdc.gov/foodnet/

NARMS: Enteric Bacteria (National Antimicrobial Resistance Monitoring System)
http://www.cdc.gov/narms/

National Center for Infectious Diseases
http://www.cdc.gov/ncidod/

PHLIS (Public Health Laboratory Information System) Surveillance Data
http://www.cdc.gov/ncidod/dbmd/phlisdata/

PulseNet (National Molecular Subtyping Network for Foodborne Disease Surveillance)
http://www.cdc.gov/pulsenet/

Respiratory and Enteric Virus Branch
http://www.cdc.gov/ncidod/dvrd/revb/index.htm

Safe Water System
http://www.cdc.gov/safewater/

Bibliography

Botulism

Angulo FJ, St. Louis ME. Botulism. In: Evans AS, Brachman PS, eds. Bacterial Infections of Humans. New York: Plenum,1998:131–53.

CDC. Botulism in the United States, 1899–1996: Handbook for epidemiologists, clinicians, and laboratory workers. Atlanta, GA: US Department of Health and Human Services, Public Health Service, CDC, 1998. Available at http://www.cdc.gov/ncidod/dbmd/diseaseinfo/botulism_g.htm

CDC. Infant botulism—New York City, 2001–2002. MMWR 2003;52:21-4.

Shapiro RL, Hatheway C, Becher J, Swerdlow DL. Botulism surveillance and emergency response: a public health strategy for a global challenge. JAMA 1997;278:433–5.

Shapiro RL, Hatheway C, Swerdlow DL. Botulism in the United States: A clinical and epidemiologic review. Ann Intern Med 1998;129:221–8.

Sobel J, Tucker N, McLaughlin J, Maslanka S. Foodborne botulism in the United States, 1999–2000. Emerg Infect Dis 2004;10:1606–12.

Sobel J. Botulism. Clin Infect Dis 2005;41;1167–73.

Cholera

Mintz ED, Tauxe RV, Levine MM. The global resurgence of cholera. In: Noah ND, O'Mahony M, eds. Communicable disease epidemiology and control. Chichester, England: John Wiley & Sons, 1998:63–104.

Steinberg EB, Greene KD, Bopp CA, Cameron DN, Wells JG, Mintz ED. Cholera in the United States, 1995–2000: Trends at the end of the millennium. J Infect Dis 2001;184, 799–802.

Mahon BE, Mintz ED, Greene KD, Wells JG, Tauxe RV. Reported cholera in the United States, 1992-1994: a reflection of global changes in cholera epidemiology. JAMA 1996;276:307–12.

World Health Organization. Guidelines for cholera control. Geneva, Switzerland: World Health Organization, 1993.

Escherichia coli O157 and other Shiga toxin-producing *E. coli*

Bender JB, Hedberg CW, Besser JM, et al. Surveillance for *Escherichia coli* O157:H7 infections in Minnesota by molecular subtyping. N Engl J. Med 1997;337:388–94.

Brooks JT, Sowers EG, Wells JB, Greene KD, Griffin PM, et al. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. J Infect Dis 2005;192:1422–9.

Crump JA, Sulka AC, Langer AJ, et al. An outbreak of *Escherichia coli* O157:H7 among visitors to a dairy farm. N Engl J Med 2002;347:555–60.

Griffin PM, Mead PS, Sivapalasingam S. *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli*. Chapter 42. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL, eds. Infections of the gastrointestinal tract. Philadelphia: Lippincott Williams & Wilkins, 2002:627–42.

Mead PS, Griffin PM. *Escherichia coli* O157:H7. Lancet 1998;352:1207–12.

Hemolytic uremic syndrome

Banatvala N, Griffin PM, Greene KD, et al. The United States National Prospective Hemolytic Uremic Syndrome Study: microbiologic, serologic, clinical, and epidemiologic findings. J Infect Dis 2003;183(7):1063–70.

Mahon BE, Griffin PM, Mead PS, Tauxe RV. Hemolytic uremic syndrome surveillance to monitor trends in infection with *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* (Letter). Emerg Infect Dis 1997;3:409–12.

Listeria

Gottlieb SL, Newbern EC, Griffin PM, et al. Multistate outbreak of listeriosis linked to turkey deli meat and subsequent changes in US regulatory policy. Clin Infect Dis 2006;42:29–36.

Mead PS, Dunne EF, Graves L, Wiedmann M, Patrick M, et al. Nationwide outbreak of listeriosis due to contaminated meat. Epidemiol Infect 2006;134:744–51.

Mead PS, Slutsker L, Dietz V, McCaig L, Bresee J, Shapiro C, Griffin P, Tauxe R. Food-related illness and death in the United States. Emerg Infect Dis 1998;5(5):607–25.

Olsen SJ, Patrick M, Hunter SB, et al. Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. Clin Infect Dis 2005;40(7):962–7.

Slutsker L, Evans MC, Schuchat A. Listeriosis. In: Scheld WM, Craig WA, Hughes JM, eds. Emerging Infections, 4th ed. Washington DC; American Society for Microbiology, 2000:83–106.

Slutsker L, Schuchat A. Listeriosis in humans. In: Ryser ET and Marth EH, eds. *Listeria*, Listeriosis, and Food Safety, Second Edition. New York, NY: Marcel Dekker, Inc. Little, Brown and Company;1999:75–95.

Tappero J, Schuchat A, Deaver K, Mascola L, Wenger J, for the Listeriosis Study Group. Reduction in the incidence of human listeriosis in the United States: Effectiveness of prevention efforts. JAMA 1995;273(14):1118–22.

Salmonella

Braden CR. *Salmonella enterica* serotype Enteritidis and eggs: a national epidemic in the United States. Clin Infect Dis 2006;43;512–17.

Mahon BE, Slutsker L, Hutwagner L, et al. Consequences in Georgia of a nationwide outbreak of *Salmonella* infections: what you don't know might hurt you. Am J Public Health 1999;89:31–5.

Olsen SJ, Bishop R, Brenner FW, et al. The changing epidemiology of *Salmonella*: trends in serotypes isolated from humans in the U.S., 1987–1997. J Infect Dis 2001;183:756–61.

Voetsch AC, Van Gilder TJ, Angulo FJ, et al. FoodNet estimate of burden of illness caused by nontyphoidal *Salmonella* infections in the United States. Clin Infect Dis 2004;38(Suppl 3):S127–34.

Shigella

CDC. Shigellosis outbreak associated with an unchlorinated fill-and-drain wading pool, Iowa, 2001. MMWR, 2001;50:797–800.

CDC. Outbreaks of multidrug-resistant *Shigella sonnei* gastroenteritis associated with day care centers—Kansas, Kentucky, and Missouri, 2005. MMWR 2006;55:1068–71.

CDC. Outbreaks of *Shigella sonnei* infection associated with eating fresh parsley—United States and Canada, July–August 1998. MMWR 1999;48:285–9.

Gupta A, Polyak CS, Bishop RD, Sobel J, Mintz ED. Laboratory-confirmed shigellosis in the United States, 1989–2002: epidemiologic trends and patterns. Clin Infect Dis 2004;38:1372–7.

Mohle-Boetani JC, Stapleton M, Finger R, et al. Community-wide shigellosis: control of an outbreak and risk factors in child daycare centers. Am J Public Health 1995;85:812–6.

Shane A, Crump J, Tucker N, Painter J, Mintz E. Sharing *Shigella*: risk factors and costs of a multi-community outbreak of shigellosis. Arch Pediatr Adolesc Med 2003;157:601–3.

Sobel J, Cameron DN, Ismail J, et al. A prolonged outbreak of *Shigella sonnei* infections in traditionally observant Jewish communities in North America caused by a molecularly distinct bacterial subtype. J Infect Dis 1998;177:1405–8.

Typhoid

Ackers ML, Puhr ND, Tauxe RV, Mintz ED. Laboratory-based surveillance of *Salmonella* serotype Typhi infections in the United States: antimicrobial resistance on the rise. JAMA 2000;283:2668–73

Crump J, Barrett TJ, Nelson JT, Angulo FJ. Reevaluating fluoroquine breakpoints for *Salmonella enterica* serotype Typhi, and for non-Typhi *Salmonellae*. Clin Infect Dis 2003;37:75–81.

Mermin JH, Townes JM, Gerber M, Dolan N, Mintz ED, Tauxe RV. Typhoid fever in the United States, 1985–1994: changing risks of international travel and increasing antimicrobial resistance. Arch Intern Med 1998;158:633–8

Olsen SJ, Bleasdale SC, Magnano AR, et al. Outbreaks of typhoid fever in the United States, 1960–1999. Epidemiol Infect 2003;130:13–21.

Reller M, Olsen SJ, Kressel A. Sexual transmission of typhoid fever: a multi-state outbreak among men who have sex with men. Clin Infect Dis 2003;37:141–4.

Steinberg EB, Bishop RB, Dempsey AF, et al. Typhoid fever in travelers: who should be targeted for prevention? Clin Infect Dis 2004;39:186–91.

Vibrio Infections (non-cholera)

Daniels NA, Ray B, Easton A, Marano N, et al. Emergence of a new *Vibrio parahaemolyticus* serotype in raw oysters. A prevention quandary. JAMA 2000;284:1541–1545.

Daniels NA, MacKinnon L, Bishop R, Altekruse S, et al. *Vibrio parahaemolyticus* infections in the United States, 1973–1998. J Infect Dis 2000;181:1661–1666.

McLaughlin JB, DePaola A, Bopp CA, Martinek KA, Napolilli NP, Allison CG, Murray SL, Thompson EC, Bird MM, Middaugh JP. Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. N Engl J Med 2005;353(14):1463–70.

Shapiro RL, Altekruse S, Hutwagner L, et al. The role of gulf coast oysters harvested in warmer months in *Vibrio vulnificus* infections in the United States, 1988–1996. J Infect Dis 1998;178:752–759

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Laboratory-Confirmed Non-O157 Shiga Toxin-Producing *Escherichia coli* --- Connecticut, 2000--2005

Shiga toxin-producing *Escherichia coli* (STEC) infection causes diarrhea that is often bloody and can result in potentially life-threatening hemolytic uremic syndrome (HUS) (*1*). *Escherichia coli* O157:H7 is the most common cause of STEC infection in the United States, producing 73,000 illnesses annually, according to the last estimate in 1999 (*2*). Unlike O157, however, little is known about the incidence of non-O157 strains. Because STEC other than O157 are not commonly identified, the incidence, trends, and epidemiology of non-O157 STEC are not well understood. To assess trends in Shiga toxin enzyme immunoassay (Stx EIA) testing by local clinical laboratories, the Connecticut Department of Public Health (CTDPH) analyzed results of confirmatory testing conducted in the state laboratory during 2000--2005. The findings indicated that a total of 403 STEC infections were reported by clinical laboratories in Connecticut, including 207 identified as STEC by Stx EIA testing alone, and that the use of Stx EIA increased from 2000 to 2005. Use of Stx EIA without prompt culture confirmation can delay or prevent serotyping and subtyping of isolates and detection of both O157 and non-O157 STEC outbreaks. Public health authorities in all states should ensure that clinical laboratories forward Stx EIA-positive specimens to the state laboratory for isolation and identification of STEC, as recommended by the Association of Public Health Laboratories* and CDC (*3*).

Clinical laboratories typically use sorbitol-MacConkey (SMAC) agar, a culture method, to identify STEC O157, which cannot ferment sorbitol and therefore forms colorless colonies. Like other intestinal flora, most non-O157 STEC strains ferment sorbitol and form pink colonies; therefore, SMAC agar cannot be used to readily differentiate between sorbitol-fermenting non-O157 STEC strains and other sorbitol-fermenting intestinal flora growing on the plate. Rapid diagnostic EIAs capable of detecting Stx in stool specimens or culture broths are commercially available and used increasingly by clinical laboratories. These nonculture methods are capable of detecting both O157 and non-O157 STEC strains; however, these methods should not be considered as substitutes for culture.

Clinical laboratories in Connecticut have been required to report culture-confirmed STEC O157 infections to state public health officials since 1992 and Stx EIA-positive infections since 2000 (*4*). During 2000--2005, the number of clinical laboratories in Connecticut conducting Stx EIA testing increased from four (11%) of 35 laboratories to 10 (31%) of 32 laboratories. Because not all Stx EIA tests at these laboratories are confirmed by culture, clinical laboratories performing Stx EIA without culture confirmation have been required to submit the enrichment broth from all Stx-positive stool specimens to the CTDPH state laboratory since 2000.

At the CTDPH state laboratory, Stx-positive broths are plated on SMAC agar and SMAC agar enriched with cefixime-tellurite (CT-SMAC). Sorbitol-negative colonies are screened for the O157 antigen using a latex

agglutination test and, if positive, are tested for the H7 antigen. If the sorbitol-negative colonies are O157-negative, both sorbitol-positive and sorbitol-negative colonies are tested for Stx using EIA. In November 2002, the CTDPH state laboratory instituted the additional step of screening Stx-positive colonies for the six most common non-O157 STEC serogroups in the United States (O26, O45, O103, O111, O121, and O145), using commercial antisera. All non-O157 STEC isolates are forwarded to CDC for further characterization. To allow examination of the epidemiology of non-O157 STEC, in April 2004, CTDPH also began interviewing all patients with confirmed STEC cases using a standardized questionnaire that collects clinical and exposure information.

During 2000--2005, a total of 403 laboratory-confirmed STEC infections were reported in Connecticut. Of these, 196 (49%) were identified as STEC O157 at clinical laboratories using culture; the remaining 207 (51%) were identified as STEC at clinical laboratories using Stx EIA with no culture confirmation (Table). The percentage of STEC isolates identified initially by Stx EIA testing increased significantly ($p<0.001$) from 33% in 2000 to 59% in 2005. Similarly, the percentage of STEC O157 isolates identified as STEC initially by Stx EIA testing increased significantly ($p<0.01$) from 23% in 2000 to 40% in 2005. Among the Stx EIA-positive broths submitted to the CTDPH state laboratory, 82 (40%) yielded STEC O157 and 125 (60%) yielded non-O157 STEC. The percentage identified as non-O157 STEC has remained higher than 50% since 2001. Four serogroups accounted for 88 (70%) of the STEC non-O157 isolates: O103, 26 (21%) isolates; O111, 26 (21%) isolates; O26, 18 (14%) isolates; and O45, 18 (14%) isolates. The remaining 37 (30%) belonged to 15 other serogroups. During 2000--2005, the incidence of identified non-O157 STEC infections increased 50%, from 0.4 to 0.6 per 100,000 population.

Patients with non-O157 STEC infection were less likely than those with STEC O157 infection to have had bloody diarrhea (56% versus 90%, $p<0.001$), have been hospitalized (12% versus 45%, $p<0.001$), have developed HUS (zero versus 9%, $p<0.001$), or have eaten at a restaurant in the 7 days preceding illness onset (59% versus 88%, $p=0.01$). No differences were found in the proportion of patients who had eaten ground beef, had contact with farm animals, or visited a petting zoo in the 7 days before illness onset.

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Editorial Note:

Non-O157 STEC infections represent a substantial portion of laboratory-confirmed STEC cases in Connecticut, consistent with findings from studies in other states (*5*,*6*). The number of clinical laboratories in Connecticut conducting Stx EIA testing has been increasing, thus the identified increase in the incidence of non-O157 STEC infections likely is a reflection of increased Stx EIA testing in the state and subsequent required submission of Stx-positive broths to the state laboratory for further characterization. However, because only 31% of clinical laboratories tested for non-O157 STEC in 2005, the number of detected cases likely represents the minimum annual incidence in Connecticut for that year.

Overall, infections caused by non-O157 STEC were less severe than those caused by STEC O157. However, the severity of disease caused by STEC is related to the virulence profile of the infecting strain, and some non-O157 serotypes cause illness as severe as that caused by STEC O157 (*7*,*8*).

The sources of non-O157 STEC infections are not well described, although outbreak investigations indicate that some sources are similar to those of STEC O157 infections (*9*,*10*). Furthermore, the similar exposures of patients with STEC O157 and non-O157 STEC cases in Connecticut described in this report suggest that

many of the routes of transmission are similar.

The findings in this report are subject to at least three limitations. First, most clinical laboratories in Connecticut do not conduct Stx EIA testing; 22 (69%) of 32 laboratories use culture methods. As a result, the true number of non-O157 STEC infections remains undefined. Second, lack of uniformity exists among clinical laboratories regarding types of stool specimens that are cultured for STEC O157 or tested for Stx. Some laboratories culture or test all stool specimens, others only bloody stools, and others only on physician request. Finally, the numbers of each non-O157 STEC serogroup were too small to permit serogroup-specific analysis of disease severity and epidemiology.

In Connecticut, Stx EIA testing increasingly is replacing direct culture for STEC O157 in clinical laboratories. Connecticut has taken steps to ensure that all STEC isolates are further characterized, which can enable evaluation of the incidence and epidemiology of non-O157 STEC. Clinical laboratories in all states should forward Stx EIA-positive specimens to the public health laboratory for confirmation and characterization by culture methods to rule out false-positive EIA results and ensure accurate STEC surveillance (*3*).

References

1. Besser RE, Griffin PM, Slutsker L. *Escherichia coli* O157:H7 gastroenteritis and the hemolytic uremic syndrome: an emerging infectious disease. Annu Rev Med 1999;50:355--67.
2. Mead PS, Slutsker L, Dietz V. Food-related illness and death in the United States. Emerg Infect Dis 1999;5:607--25.
3. CDC. Importance of culture confirmation of Shiga toxin-producing *Escherichia coli* infection as illustrated by outbreaks of gastroenteritis---New York and North Carolina, 2005. MMWR 2006;55:1042--5.
4. Connecticut Department of Public Health. Reportable diseases and laboratory findings, 2000. Connecticut Epidemiologist 2000;20:4. Available at http://www.dph.state.ct.us/Publications/BCH/Infectious%20Diseases/vol20no1.pdf.
5. Fey PD, Wickert RS, Rupp ME, Safranek TJ, Hinrichs SH. Prevalence of non-O157:H7 Shiga-toxin-producing *Escherichia coli* in diarrheal stool samples from Nebraska. Emerg Infect Dis 2000;6:530--33.
6. Jelacic JK, Damrow T, Chen GS, et al. Shiga toxin-producing *Escherichia coli* in Montana: bacterial genotypes and clinical profiles. J Infect Dis 2003;188:719--29.
7. Ethelberg S, Olsen KE, Scheutz F, et al. Virulence factors for hemolytic uremic syndrome, Denmark. Emerg Infect Dis 2004;10:842--7.
8. Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. J Clin Microbiol 1999;37: 497--503.
9. McCarthy TA, Barrett NL, Hadler JL, et al. Hemolytic-uremic syndrome and *Escherichia coli* O121 at a lake in Connecticut, 1999. Pediatrics 2001;108:E59.
10. Brooks JT, Sowers EG, Wells JG, et al. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983--2002. J Infect Dis 2005;192:1422--9.

* Association of Public Health Laboratories. Guidelines for isolation and identification of Shiga toxin-producing *E. coli*, 2006. Available at http://www.aphl.org/programs/food_safety.

Table

TABLE. Results of Shiga toxin-producing *Escherichia coli* (STEC) culture confirmation tests, by year — Connecticut Department of Public Health, 2000–2005

Isolate characteristics	2000 No. (%)	2001 No. (%)	2002 No. (%)	2003 No. (%)	2004 No. (%)	2005 No. (%)	Total No. (%)
Total STEC isolates confirmed by state laboratory	97	63	62	63	55	63	**403**
Isolates initially identified by clinical laboratories as STEC by Stx EIA* testing	32 (33%)	33 (52%)	29 (47%)	41 (65%)	35 (64%)	37 (59%)	**207 (51%)**
Isolates confirmed by state laboratory as non-O157 by culture	13 (41%)	25 (76%)	17 (59%)	26 (63%)	24 (69%)	20 (54%)	**125 (60%)**
Total STEC O157 isolates confirmed by state laboratory	84	38	45	37	31	43	**278**
Isolates initially identified by clinical laboratories as STEC by Stx EIA testing	19 (23%)	8 (21%)	12 (27%)	15 (41%)	11 (35%)	17 (40%)	**82 (29%)**

* Shiga toxin enzyme immunoassay. During 2000–2005, the number of clinical laboratories in Connecticut conducting Stx EIA testing increased from four (11%) of 35 laboratories to 10 (31%) of 32 laboratories. Clinical laboratories performing Stx EIA without culture confirmation are required to submit the enrichment broth from Stx-positive stool specimens to the Connecticut Department of Public Health state laboratory.

Return to top.

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Escherichia coli O157:H7 Infection in Humans

Chinyu Su, MD, and Lawrence J. Brandt, MD

■ *Objective:* To review the clinical relevance of *Escherichia coli* O157:H7 infection, including the epidemiology of the infection and its clinical presentations, pathogenesis, microbiology, diagnosis, treatment, and prevention.

■ *Data Sources:* Articles on *E. coli* O157:H7 were identified through MEDLINE and the bibliographies of relevant articles.

■ *Study Selection:* All articles and case reports describing *E. coli* O157:H7 and its infection were selected.

■ *Data Extraction:* The data were abstracted without judgments about study design. Data quality and validity were assessed by independent author reviews.

■ *Data Synthesis:* Infection with *E. coli* O157:H7 presents with a wide spectrum of clinical manifestations, including asymptomatic carriage, nonbloody diarrhea, hemorrhagic colitis, the hemolytic–uremic syndrome, and thrombotic thrombocytopenic purpura. Not only is *E. coli* O157:H7 an important agent for hemorrhagic colitis, it is also one of the leading causes of bacterial diarrhea. Patients at extremes of age have an increased risk for infection and associated complications. Transmission of *E. coli* O157:H7 is primarily food-borne. Undercooked meat is the most common culprit, and secondary person-to-person spread is also important. The organism produces at least two Shiga-like toxins that differ antigenically, physicochemically, immunologically, and in their biological effects. These toxins are thought to have direct pathogenic significance in *E. coli* O157:H7 infection. This infection is usually diagnosed from a positive stool culture, from the presence of Shiga-like toxins, or both. Timely collection (within 7 days of illness onset) of a stool sample for culture is imperative for a high recovery rate. Treatment is primarily supportive and includes the management of complications as necessary. Antibiotic therapy has not been proved beneficial. Important public health measures include educating the public on the danger of eating undercooked meat, increasing physician awareness of *E. coli* 0157:H7 infection, and mandating case reporting.

■ *Conclusions:* Infection with *E. coli* O157:H7 presents with many clinical manifestations and should be included in the differential diagnosis for any patient with new-onset bloody diarrhea. Development of the hemolytic–uremic syndrome or thrombotic thrombocytopenic purpura should raise strong suspicion of *E. coli* O157:H7 infection and should lead to prompt evaluation. If infection is confirmed, it should be reported to public health officials.

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Escherichia coli serotype O157:H7 was first isolated in 1982, when 47 persons in Michigan and Oregon developed bloody diarrhea after eating hamburgers contaminated with the organism (1). Retrospective examination of more than 3000 *E. coli* cultures obtained between 1973 and 1982 has found only one isolation with serotype O157:H7; it was from a 50-year-old woman who had had an episode of acute, self-limited, grossly bloody diarrhea in 1975 (1). Since the initial reports, sporadic cases (2–9) and outbreaks (10–24) of *E. coli* O157:H7 infection have increasingly been reported, and surveillance and prospective studies to identify and characterize diseases associated with *E. coli* O157:H7 have been started in Canada, the United Kingdom, and the United States (3–9, 25, 26).

The first major step toward defining the pathogenic potential of *E. coli* O157:H7 was made in 1983, when isolates of this serotype were found to produce a toxin (27). This toxin has been called both "Shiga-like toxin," because of its close relation to Shiga toxin, and "Verotoxin," because of its toxicity to Vero (green monkey kidney) cells in tissue culture. Subsequent studies (28–34) have shown that *E. coli* O157:H7 produces at least two distinct Shiga-like toxins and that O157:H7 is only one serotype of Shiga-like toxin–producing *E. coli*. To distinguish their clinical and pathologic features from those of enterotoxigenic, enteroinvasive, and enteropathogenic *E. coli*, these Shiga-like toxin–producing strains of *E. coli*, which cause hemorrhagic colitis, have also been called enterohemorrhagic *E. coli* (35). Serotypes other than O157:H7 can cause illness similar to that caused by *E. coli* O157:H7, because many strains of *E. coli* can produce Shiga-like toxin. Moreover, many serotypes of Shiga-like toxin–producing *E. coli* other than O157:H7 have been isolated from patients with hemorrhagic colitis or the hemolytic– uremic syndrome. Although few studies have been done to determine the incidence of diarrheal illness caused by Shiga-like toxin–producing *E. coli* other than O157:H7, none of these organisms is a common cause of diarrhea. Thus, a 2-year survey in Canada reported that 0.7% of stools (36 of 5415) showed Shiga-like toxin–producing *E. coli* other than O157:H7; this rate was higher than that for shigellae, and the serotypes most often isolated were O26:H11 and O103:H2 (6). Serotype O157:H7 was also identified in 7 of these 36 stools, bringing into question the precise role of the non–O157:H7 serotype in causing the diarrhea. Shiga-like toxin–producing *E. coli* other than O157:H7, such as O2:H5, have also been isolated from patients with ulcerative colitis, but, again, their precise role in causing this disease or its exacerbations is unclear (36). What is clear is that many more studies are needed to define the roles of Shiga-like toxin–producing coliforms other than O157:H7 in causing human disease.

Many species of *E. coli* produce Shiga-like toxin, but we focus on *E. coli* O157:H7 because it is the most common.

The term "Shiga-like toxin" is preferred to "Verotoxin" because it emphasizes the common mechanism of action of the toxins produced by certain species of *E. coli* and the Shiga toxin elaborated by shigellae, as well as the wide target cell of injury, that is, cells with the surface receptor globotriaosyl ceramide.

Methods

Articles on *E. coli* O157:H7 were identified through MEDLINE and through review of bibliographies of relevant articles. All articles and case reports describing *E. coli* O157:H7 and its infection were selected. Data were abstracted without judgments about study design. Data quality and validity were assessed by independent author reviews.

Clinical Manifestations

Infection with *E. coli* O157:H7 presents with a wide spectrum of clinical manifestations, including severe abdominal cramps with little or no fever and watery diarrhea that often progresses to grossly bloody diarrhea (37). Infection can be asymptomatic (13, 15, 19) or can present with only nonbloody diarrhea (4, 5, 8, 10, 11, 13, 16, 20, 26). Extraintestinal involvement, including cardiac and neurologic manifestations, has been reported (38), and infection can be associated with the hemolytic–uremic syndrome (14, 16, 18, 19, 24) and thrombotic thrombocytopenic purpura (16, 39–41). The disease can be fatal (15, 16, 18, 19, 37).

Asymptomatic Infection and Nonbloody Diarrhea

Cases of asymptomatic *E. coli* O157:H7 infection have occasionally been detected in outbreaks (13, 15, 19), but the incidence rates are difficult to estimate because stool samples from asymptomatic persons are rarely obtained for culturing. In an outbreak in Canada that involved kindergarten children, 31% of those exposed to the implicated source (19 of 62) were asymptomatic (17). Fifty-three percent of the asymptomatic children (10 of 19) had laboratory evidence of *E. coli* O157:H7.

Nonbloody diarrhea without progression to hemorrhagic colitis has also been reported (4, 5, 8, 10, 11, 13, 16, 20, 26). It was noted in 18% of culture-confirmed cases (3 of 17) in a nursing home outbreak of *E. coli* O157:H7 infection (13) and in as many as two of four children with culture-positive stools in a day care center (20). Although cases of nonbloody diarrhea occur in outbreaks, the routine screening of all stool specimens for *E. coli* O157:H7 during one outbreak period showed that the number of these cases is probably small (16). Patients with nonbloody diarrhea have less severe disease, are less likely to develop the hemolytic–uremic syndrome, and are less likely to die than are patients with bloody stools (11). However, nonbloody diarrhea progressing to the hemolytic–uremic syndrome has been reported. In one 2-year prospective study, two of nine confirmed cases of *E. coli* O157:H7–associated hemolytic–uremic syndrome were preceded by nonbloody diarrhea (26). Patients with bloody stools have a longer duration of diarrhea and report abdominal cramps and vomiting more often than do those with nonbloody diarrhea (20).

Hemorrhagic Colitis

Hemorrhagic colitis caused by *E. coli* O157:H7 is a clinical syndrome that consists of abdominal cramps; diarrhea that progresses to become bloody; radiologic or endoscopic evidence of colonic mucosal edema, erosion, or hemorrhage; and the absence of conventional enteric organisms in the stool (42). This syndrome was first reported in 1971 (43), when five young adults developed reversible segmental colitis that rapidly resolved within 2 weeks without specific therapy. The term "evanescent colitis" was used to describe this entity as a clinical syndrome distinct from ulcerative, granulomatous, and ischemic colitis (43). After the outbreaks of hemorrhagic colitis in Michigan and Oregon (1) and subsequent similar outbreaks, *E. coli* O157:H7 came to be recognized as an important etiologic agent for hemorrhagic colitis.

Hemorrhagic colitis may be the only manifestation of *E. coli* O157:H7 infection, or it may herald the development of the hemolytic–uremic syndrome. Thirty-eight percent to 61% of such infections result in hemorrhagic colitis (44), and surveillance studies (8, 9) have found *E. coli* O157:H7 in 27% to 36% of sporadic cases of hemorrhagic colitis. Infection with *E. coli* O157:H7 usually begins with the sudden onset of severe abdominal cramps, which are followed within hours by watery diarrhea that progresses to grossly bloody stools (37). Upper gastrointestinal symptoms, such as nausea and vomiting, occur early and may be prominent. The incubation period ranges from 1 to 9 days (mean, 3.1 to 3.9 days) during community outbreaks (1, 14, 16) and from 1 to 14 days (mean, 4 to 8 days) in institutional settings (11, 13, 17, 19). Medical attention is usually sought 2 to 3 days after the onset of diarrhea or abdominal pain, primarily because of bloody diarrhea (5), which is the most common symptom of *E. coli* O157:H7 infection (37). The median duration of diarrhea is 3.0 to 7.5 days (range, 1 to >31 days) (10, 11, 37), and patients report a median of 10 to 11 bowel movements (range, 3 to >30 bowel movements) on the worst day of diarrhea (19, 37). Diarrhea lasts longer in children (mean, 9.1 ± 2.0 days) than in adults (mean, 6.6 ± 1.1 days) (5), and it lasts longer in persons with bloody stools (mean, 12.2 days) than in those with nonbloody stools (mean, 6.8 days) (20). Bloody stools develop a median of 0 to 1 days (range, 0 to 8 days) after the onset of diarrhea (11, 37) and last a median of 2 to 5 days (range, 1 to 22 days) (5, 10, 11, 37). The amount of blood in each stool ranges from streaks to more than 4 cups on the worst day (median, 4.5 tablespoons) (37). Most of the infected children (91%) in an outbreak in a day care center produced less than 1 tablespoon of blood per stool (20), whereas patients in other outbreaks have reported that bowel movements were essentially all blood with little fecal material (1, 5). Other symptoms related to *E. coli* O157:H7 infection include severe abdominal cramps, right lower quadrant pain, nausea, vomiting, fever, and chills (1, 4, 10, 26).

The incidence of fever ranges from 0% to 32% (1, 5, 10, 11, 16, 19, 20) and was as high as 64% among persons with bloody diarrhea in one day care center outbreak

(20). When present, fever is usually mild, unlike that seen with the bloody diarrhea of shigellosis, amebiasis, campylobacteriosis, or enteroinvasive *E. coli* infection (1). Abdominal distension and tenderness may be present, but the results of physical examination are usually normal (8, 19).

Laboratory studies usually show leukocytosis with moderate left shift and a mean leukocyte count of 13.0 to 14.0×10^9/L (range, 6.2 to 20.0×10^9/L) (1, 15, 19). Hematocrit is generally not significantly decreased despite the bloody nature of the stools. The results of other studies, including erythrocyte sedimentation rate, serum electrolyte concentrations, liver test results, prothrombin times, and urinalysis results, are typically normal (1). Mucus and leukocytes may be present in the stool but are usually seen in scant to moderate amounts (18, 19). In an outbreak that involved 19 patients, 13 patients (68%) had 0 or fewer than 1 polymorphonuclear leukocyte per high-power field in their stools; 2 patients had 1 to 5; 2 patients had 5 to 30; and 2 patients had more than 30 (37).

The results of roentgenologic and colonoscopic examinations often suggest a diagnosis of inflammatory bowel disease or ischemic colitis. Abdominal plain films show an ileus in 60% to 100% of patients; distension of the small bowel, cecum, and ascending colon with little gas in the left colon is common; and bowel obstruction is sometimes considered (19, 37). In barium enema studies, a pattern of submucosal edema resembling thumbprinting in the ascending and transverse colon was noted in six of seven patients in the index study (1) and in all three patients with bloody diarrhea in another study (19). The colonic mucosa, especially in the ascending colon, may have a shaggy appearance with thickened folds (18). Technetium–red blood cell scanning of the abdomen usually shows radiotracer within the right colon and extending into the transverse colon if bleeding is active (19). The results of sigmoidoscopic and colonoscopic examinations show an increasing frequency and severity of mucosal abnormalities from the rectum to the cecum (37, 45). Edema, erythema, and superficial ulceration, usually in a patchy distribution, are the most important features; other, less common findings include dusky-appearing mucosa and frank hemorrhage (37, 45). The most severe abnormalities occur in the cecum and the ascending colon; in one study (45), one or both of these sites were affected in all patients in whom the sites were examined. The rectosigmoid mucosa appears normal to moderately hyperemic and sometimes has superficial erosions (1, 5, 15, 19).

Mean durations of 4 to 8 days (range, 2 to 14 days) have been reported (1, 4, 8). Hospitalization rates (4, 14, 16, 18, 19) have ranged from 13% in a junior high school outbreak (10) and a nursing home outbreak (15) to 73% in a community outbreak in Oregon (1); the mean hospital stay is 6 to 14 days (4, 18, 19, 37). Most hospitalized patients had hemorrhagic colitis manifested by crampy abdominal pain, grossly bloody diarrhea, abdominal distention, and low-grade fever (10, 19). Most hospitalized patients with hemorrhagic colitis recovered completely within 1 week without specific therapy or complications (5). However, complications from hemorrhagic colitis associated with *E. coli* O157:H7 have been reported. In a nursing home outbreak, 3 of 34 residents had upper gastrointestinal bleeding documented during nasogastric suction to relieve abdominal distention; 2 of 34 residents had anemia with hematocrits less than 0.30; and 1 of 34 residents became hypotensive and subsequently had cerebrovascular ischemia (19).

In one outbreak, antibiotic treatment during the exposure period and before symptom onset was reported to be a risk factor for person-to-person transmission of *E. coli* O157:H7 (11). An association between antibiotic use after symptom onset and an increased case-fatality rate was also documented. Other factors that may increase susceptibility to *E. coli* O157:H7 include young and old age and gastrectomy; this finding suggests that gastric acidity may play a protective role in the pathogenesis of this infection (11).

The Hemolytic–Uremic Syndrome

The hemolytic–uremic syndrome is a distinct entity characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. Many factors have been implicated in the development of this syndrome, including genetics, pregnancy, drugs (such as oral contraceptives and estrogen therapy), toxins, chemicals, viruses, and bacteria (46–48). On the basis of epidemiologic, clinical, and laboratory data, two major subgroups of the syndrome have been identified: typical (or epidemic) and atypical (or sporadic) (47–49). The syndrome occurs predominantly in infants and young children and is the most common cause of acute renal failure in children (46); the mortality rate is 5% to 10%, and persistent disability occurs in one third of survivors (50).

Most patients with the hemolytic–uremic syndrome have gastrointestinal prodromes (48, 51), and nonbloody or bloody diarrhea is the most common presenting symptom. Other presenting symptoms include abdominal cramps, vomiting, fever, lethargy, seizure, pallor and respiratory distress; some patients have no distinct prodromes (52). The initial gastrointestinal manifestations have been confused with ulcerative, ischemic, and pseudomembranous colitis; cecal polyps; intussusception; and appendicitis (51, 53, 54). Radiographic abnormalities include bowel edema, transient segmental narrowing, and bowel stenosis (55), and sigmoidoscopy has shown friable mucosa, rectal ulceration, pseudomembranes, and diffuse colitis (51). The cecum and ascending colon are most commonly involved and often show hemorrhage and necrosis. Gastrointestinal complications of the hemolytic–uremic syndrome are common and include nonspecific inflammation (53), chronic ischemic colitis with colonic stricture (56), and full-thickness necrosis resulting in colonic perforation (51, 57). Rectal prolapse, toxic megacolon, and ascites have also been reported. Although central nervous system complications are not part of the classic triad of conditions associated with the hemolytic–uremic syndrome, they may occur in 30% to 50% of patients (22, 58–60). Common neurologic manifestations include irritability and lethargy, but serious complications, such as seizure and coma, can occur (59, 61). Decerebrate posturing, hemiparesis, and focal seizures have also been reported. Patients may sustain long-term neurologic sequelae, including generalized seizures, psychomotor or mental retardation, and cortical blindness (59, 61).

Escherichia coli O157:H7 has been implicated as an

important etiologic agent in the typical form of the hemolytic–uremic syndrome (11, 17, 20, 48, 62, 63), and it is the most common pathogen isolated from patients with this condition (26, 63–67). It was isolated from 46% of patients (13 of 26) in a 10-year, retrospective, population-based study of the hemolytic–uremic syndrome (52); from 58% of patients with the hemolytic–uremic syndrome (7 of 12) in a prospective study in the Pacific Northwest (63); and from 64% of children (9 of 14) with the hemolytic–uremic syndrome in British Columbia, Canada (26). Infection with *E. coli* O157:H7 has been associated with both sporadic cases (68) and outbreaks (69) of the hemolytic–uremic syndrome. In a family outbreak of the hemolytic–uremic syndrome that involved five siblings, Shiga-like toxin was isolated from each child's stool, and *E. coli* O157:H7 was isolated from the stools of two of the children (69). Similarly, evidence of Shiga-like toxin–producing *E. coli* infection was detected in 75% of children (30 of 40) with "idiopathic" hemolytic–uremic syndrome (62); 3 of the 12 isolates were serotype O157:H7. The occurrence of the hemolytic–uremic syndrome during outbreaks of *E. coli* O157:H7 infection is also well documented; as many as 30% of patients with hemorrhagic colitis associated with *E. coli* O157:H7 developed the hemolytic–uremic syndrome (70). Rates of the hemolytic–uremic syndrome in outbreaks of *E. coli* O157:H7 infection ranged from 3% to 53% among residents of two institutions for mentally retarded persons (11, 14, 16, 18–20). Indeed, the hemolytic–uremic syndrome is sometimes the clue to the recognition of *E. coli* O157:H7 outbreaks (17). When the results from four studies of sporadic cases of *E. coli* O157:H7 infection or hemorrhagic colitis were combined, it was found that 9% of patients had developed the hemolytic–uremic syndrome (3–5, 8, 71). Other studies of children with *E. coli* O157:H7 infection showed a progression rate of 6% to 8% (21–23). Considering the different study populations and the fact that some persons may not have bloody diarrhea, the true rate of progression to the hemolytic–uremic syndrome from *E. coli* O157:H7 infection is estimated to be 2% to 7% (71).

Escherichia coli O157:H7 accounts for most or a major part of cases of the hemolytic–uremic syndrome in North America, but, in studies of children in Argentina, where the incidence of the syndrome is among the highest in the world and where meat consumption is almost universal, investigators isolated *E. coli* O157:H7 in only 2% of children with the hemolytic–uremic syndrome (1 of 51), and 48% of these children (15 of 31) had evidence of free fecal toxin (72). This suggests that Shiga-like toxin–producing *E. coli* other than O157:H7 are an important cause of the hemolytic–uremic syndrome in Argentina. Combining data on seroconversion, free fecal toxin, and DNA-probe positivity, the frequency of Shiga-like toxin–associated diarrhea in Argentine children is 32% (14 of 44) (72). The high incidence of Shiga-like toxin–associated diarrhea in young Argentine children probably explains the high frequency and occurrence of the hemolytic–uremic syndrome seen in this age group. Although the syndrome is considered to be predominantly a disorder of childhood, its development after hemorrhagic colitis caused by *E. coli* O157:H7 has been reported in two young adults (25 and 36 years of age, respectively) (8, 73), in elderly patients (19), and in 22% of affected residents in one nursing home outbreak (11).

Studies of the hemolytic–uremic syndrome have been limited in their ability to ascertain temporal trends in the syndrome, but its incidence has clearly been increasing. A population-based study from King County in Washington State (74) documented an increase in the incidence of the hemolytic–uremic syndrome in that area during the 15-year study period. A 10-year, retrospective, population-based study in Minnesota (52) also reported a significant increase in the incidence of the syndrome; most of the increase was attributed to disease in young children. The increasing incidence of the syndrome coincided with the emergence of *E. coli* O157:H7 as an important pathogen, and the seasonal pattern of cases of the hemolytic–uremic syndrome was similar to that of *E. coli* O157:H7 infections (52), further strengthening the evidence for the association of the hemolytic–uremic syndrome with *E. coli* O157:H7.

Although most patients who have *E. coli* 0157:H7–associated hemolytic–uremic syndrome had bloody diarrhea before their illness, some had nonbloody diarrhea (26), and others had no prodromal illness (16). The hemolytic–uremic syndrome developed abruptly 5 to 10 days after the onset of diarrhea during outbreaks (18, 37), but the time between the onset of diarrhea and the diagnosis of the hemolytic–uremic syndrome varied (mean, 6.5 to 7.7 days) (63, 75). Leukocytosis with a left shift on presentation, high temperature (18), and extreme young or old age (18, 37, 52) appear to be indicators for progression to the hemolytic–uremic syndrome, and the initial leukocyte count correlates positively with adverse outcome (76). Conditions on day 3 of illness were also shown to be strongly predictive of progression to the hemolytic–uremic syndrome; persons who subsequently developed the syndrome were significantly more likely to have a documented fever or leukocytosis (leukocyte count, $> 12.0 \times 10^{9}$/L) on day 3 of illness (18). The prolonged use of antimotility or antidiarrheal agents has also been proposed as a risk factor (77, 78). The selective development of the hemolytic–uremic syndrome among patients infected with *E. coli* O157:H7 may be related to host susceptibility factors, such as preexisting immunity, inoculum size, virulence of the strain, or other unknown factors (10, 79, 80). The disproportionate number of cases of the hemolytic–uremic syndrome that occur in children younger than 5 years of age further suggests that host factors may be important (52). Strain characteristics may also play a role. One study (81) found that the risk for the hemolytic–uremic syndrome is increased when isolates contain only Shiga-like toxin II genes; this suggests that Shiga-like toxin II may be more virulent than Shiga-like toxin I. Although the case-fatality rate in childhood cases of the hemolytic–uremic syndrome is typically about 5% to 10% (46, 62), the case-fatality rate associated with the hemolytic–uremic syndrome during an outbreak of hemorrhagic colitis was 88% among elderly patients (11).

Thrombotic Thrombocytopenic Purpura

Thrombotic thrombocytopenic purpura consists of a pentad of findings: thrombocytopenia, microangiopathic hemolytic anemia, fever, renal failure, and neurologic

Table 1. Major Outbreaks of *Escherichia coli* 0157:H7 in the United States and Selected Outbreaks in Canada and Great Britain

Year	Location	Setting	Transmission Source	Study (Reference)
1982	Oregon	Community	Hamburger	Riley et al. (1)
1982	Michigan	Community	Hamburger	Riley et al. (1)
1982	Ontario	Nursing home	Hamburger	Laboratory Center for Disease Control (15)
1984	Nebraska	Nursing home	Hamburger	Ryan et al. (19)
1984	North Carolina	Day care center	Primary unknown and person-to-person	Spika et al. (20)
1985	Ontario	Nursing home	Cold sandwich and person-to-person	Carter et al. (11)
1985	Great Britain	Community	Raw potato	Morgan et al. (86)
1986	Washington	Community	Ground beef	Ostroff et al. (16)
1986	Ontario	School	Raw milk	Duncan et al. (13) Laboratory Center for Disease Control (17)
1987	Utah	Institution for the mentally handicapped	Ground beef and person-to-person	Pavia et al. (18)
1988	Minnesota	School	Precooked meat patties	Belongia et al. (10)
1989	Missouri	Community	Municipal water	Swerdlow et al. (24)
1990	North Dakota	Community	Roast beef	Centers for Disease Control (14)
1991	Oregon	Community	Swimming in lake water	Keene et al. (85)
1991	Massachusetts	Community	Fresh-pressed apple cider	Besser et al. (88)
1993	Washington, Idaho, California, and Nevada	Community	Hamburger	Bell et al. (84) Centers for Disease Control and Prevention (87)

symptoms. Thrombotic thrombocytopenic purpura is thought to represent a more extensive form of the clinical spectrum of vascular diseases that produces the hemolytic–uremic syndrome (47), and the criteria for diagnosis of thrombotic thrombocytopenic purpura are the same as those for the hemolytic–uremic syndrome, with the addition of fever and new-onset neurologic deficit. Postmortem examinations usually show widespread vascular lesions with platelet-fibrin thrombi (82). Many agents or conditions, including drugs, toxins, infectious agents, pregnancy, and underlying immunologic diseases, have been implicated as causes of thrombotic thrombocytopenic purpura (83).

Infection with *E. coli* O157:H7 has been implicated in some cases of thrombotic thrombocytopenic purpura (39–41), and this condition has served as a clue in the recognition of outbreaks of *E. coli* O157:H7 infection (16). Although such progression is rare, as many as 8% of patients (3 of 37) with hemorrhagic colitis associated with *E. coli* O157:H7 infection progressed to thrombotic thrombocytopenic purpura in one outbreak (16). All documented cases of thrombotic thrombocytopenic purpura associated with *E. coli* O157:H7 have occurred in adults, and progression to thrombotic thrombocytopenic purpura in children with *E. coli* O157:H7 infection has not been reported.

Death

Case-fatality rates for *E. coli* O157:H7 infection range from 3% to 36% among elderly residents of nursing homes (11, 15, 19) and residents of institutions for mental retardation (18). The risk for death is strongly related to age: Patients at extremes of age are at increased risk for *E. coli* O157:H7–associated diarrhea as well as for the hemolytic–uremic syndrome, thrombotic thrombocytopenic purpura, and death (37). In elderly persons, especially those with serious underlying diseases, infection can be particularly severe and often resembles ischemic colitis (19). Deaths in elderly persons appear to be caused by various events. In one outbreak, two elderly persons died (37). One was a 78-year-old woman with thrombotic thrombocytopenic purpura who had a grand mal seizure followed by severe hypotension and coma; she died on the 20th day of illness. The other was a 70-year-old woman with thrombotic thrombocytopenic purpura who developed bilateral pneumonitis and died on the 29th day of illness (37). In one nursing home outbreak in which four persons died, one patient died secondary to congestive heart failure from fluid overload; one had fever and *Clostridium perfringens* bacteremia; and two died after developing high fever (> 38.9 °C) with no other identifiable source of infection (19). In another large nursing home outbreak, 17 of 19 deaths were attributed directly or indirectly to *E. coli* infection, and the causes of death included colitis, pulmonary edema, pneumonia, myocardial infarction, and the hemolytic–uremic syndrome (11).

Epidemiology

Escherichia coli O157:H7 was first recognized as a cause of human illness in two separate outbreaks of hemorrhagic colitis in Michigan and Oregon in 1982 (1). The organisms were transmitted by the same source of undercooked beef, and Shiga-like toxin–producing strains of *E. coli* O157:H7 were isolated from the stools of the affected persons and from a sample of the implicated beef burgers but from no healthy controls. Increasing numbers of diseases related to *E. coli* O157:H7 have been reported since 1982; most have been sporadic (2–9), but many institutional and community-wide outbreaks (10–19, 24, 84–88) have occurred in nursing homes (11, 15, 19), schools (10,

17), and day care centers (20) or have been related to eating at fast food restaurants (1, 16, 84, 87), drinking untreated municipal water or fresh-pressed apple cider (24, 88), or swimming in lake water (85) (Table 1).

It is estimated that 0.6% to 2.4% of all cases of diarrhea (3, 6, 25, 26) and 15% to 36% of all cases of bloody diarrhea or hemorrhagic colitis (5, 7–9, 89) are associated with *E. coli* O157:H7 (Table 2). In a 1-year (1985–1986), population-based study in the Puget Sound area of Washington State (3), *E. coli* O157:H7 was the third most common cause of bacterial diarrhea. Among the 4539 patients who submitted stool specimens, *E. coli* O157:H7 was isolated in 25 cases (0.6%) and followed *Campylobacter* (165 cases, 3.6%) and *Salmonella* organisms (70 cases, 1.5%) in frequency. *Shigella* organisms were isolated slightly less frequently than *E. coli* O157:H7 (23 cases, 0.5%). The population-based incidence rates in the same study (3) were 8 cases per 100 000 person-years for *E. coli* O157:H7; 50 cases per 100 000 person years for *Campylobacter* organisms; 21 cases per 100 000 person-years for *Salmonella* organisms; and 7 per 100 000 person-years for *Shigella* organisms. Other prospective studies (6, 26) have found *E. coli* O157:H7 to be second to *Salmonella* organisms in areas of Canada (2.4%) and to *Campylobacter* organisms in Great Britain (1.9%) as the most common cause of bacterial diarrhea. In both studies (6, 26), *E. coli* O157:H7 was isolated more often than *Shigella*, *Yersinia*, or *Aeromonas* organisms. In a prospective study limited to persons with grossly bloody diarrhea in Calgary, Canada (5), *E. coli* O157:H7 was isolated from 15% of patients (19 of 125). A 21-month surveillance study in the United States, established after initial outbreaks, identified 103 cases of hemorrhagic colitis (8), 28 of which (27%) were associated with *E. coli* O157:H7. *Escherichia coli* O157:H7 was also found in 36% of sporadic cases (30 of 83) of hemorrhagic colitis in a British surveillance study (9). Thus, the frequency of *E. coli* O157:H7 in infectious diarrhea rivals that of other major bacterial organisms, and *E. coli* O157:H7 is an important cause of bloody diarrhea and hemorrhagic colitis.

The estimated incidences cited in the above studies, however, are problematic and probably underestimate the true incidence of *E. coli* O157:H7 infection. Certainly, the best way to examine the incidence of an organism is to do prospective, laboratory-based studies within defined populations, and such studies have been the major sources of data on the reported incidences of *E. coli* O157:H7. However, case reporting through a surveillance system is affected by many factors: the variety and severity of clinical manifestations; the number of infected persons seeking medical attention; whether a stool culture is ordered and its timing in relation to the onset of illness and possible use of antibiotics; whether the laboratory tests correctly identify the organism; and whether the results are reported to public health officials. Clinical laboratories are becoming increasingly familiar with the varied spectrum of illness produced by E. coli O157:H7, and the ability to screen for this organism is becoming more widespread.

The minimum estimated attack rates of *E. coli* O157:H7 among persons who consumed the suspected food product were 3.5% in a community outbreak (14) and 8% in a junior high school outbreak (10). These estimated attack rates include only cases in which patients had bloody diarrhea or a positive stool culture; thus, "possible" cases or those with milder symptoms were excluded. In a nursing home outbreak, the estimated attack rates from both food-borne and person-to-person transmission were 33% among the nursing home residents and 13% among the staff (11). The attack rate was reported to be as high as 67% (42 of 63 persons) in a kindergarten outbreak involving unpasteurized milk (17).

Table 2. Statistics on the Association of *Escherichia coli* 0157:H7 Infection with Diarrhea, Hemorrhagic Colitis, and the Hemolytic–Uremic Syndrome

Variable	Percentage
Incidence of *E. coli* O157:H7 in all cases of diarrhea	0.6–2.4
Incidence of *E. coli* O157:H7 in bloody diarrhea or hemorrhagic colitis	15–36
Development of hemorrhagic colitis in *E. coli* O157:H7 infection	38–61
Incidence of *E. coli* O157:H7 in the hemolytic–uremic syndrome	46–58
Progression of *E. coli* O157:H7 infection to the hemolytic–uremic syndrome	2–7

Most reported cases of *E. coli* O157:H7 infection have occurred in the United States, Canada, and Great Britain, but cases have also been documented in Japan (38), Australia (90), South Africa (91), Europe (92, 93), Argentina (72), and Chile (94). *Escherichia coli* O157:H7 has been detected in most areas of the United States; the largest numbers of isolations have been found in Washington State, Oregon, Minnesota, and Massachusetts (95). The geographical position of these states and of the two countries other than the United States (Canada and Great Britain) in which most reported cases have occurred suggests a predominance of infections in northern latitudes (95). However, the high number of reported cases in these regions may also reflect increased awareness among physicians in those areas and the fact that case reporting is required in some states.

Escherichia coli O157:H7 infections occur in all age groups, and the young are most often affected. In one study (4), the age-specific annual incidence rate was highest for children younger than 5 years of age (6.1 cases per 100 000 persons compared with an overall incidence rate of 2.1 cases per 100 000 persons). The lowest rate was for adults 50 to 59 years of age, who had an annual incidence rate of 0.5 per 100 000 persons (4). The trends in age-specific incidence of the hemolytic–uremic syndrome in the pediatric population parallel those in the incidence of *E. coli* O157:H7 infection. A 10-year retrospective, population-based study of the hemolytic–uremic syndrome in Minnesota reported a substantial increase in the incidence of the hemolytic–uremic syndrome during the study period, and a disproportionate number of cases occurred in children younger than 5 years of age (52). Although *E. coli* O157:H7 infections occur most often in young children and elderly persons, the elderly—especially those in institutional settings—have the highest morbidity and mortality rates (11, 19, 37). *Escherichia coli* O157:H7 generally affects both sexes equally, and no data are available on the ethnicity-specific incidence rate of infection; most

outbreaks seem to have affected patients with an ethnic distribution similar to that of the general population.

The rate of *E. coli* O157:H7 infection follows a seasonal pattern, with a peak incidence from June through September (4, 6, 70, 95). Sixty percent of *E. coli* O157:H7 infections and 73% of cases of the hemolytic–uremic syndrome and thrombotic thrombocytopenic purpura presented with bloody diarrhea between June and September, and patients affected during the summer months were younger than those seen during the rest of the year (4). In contrast to the pattern seen with *Salmonella* infection, the number of cases of *E. coli* infection does not increase after the December holiday period (70).

Food-borne transmission of *E. coli* O157:H7 is the most important means of infection. Transmission has primarily been linked to undercooked meat, and, during the 1982 outbreaks, the organism was cultured from a suspected lot of hamburger patties (1). Sources other than undercooked hamburger meat (15–19) that have been implicated in transmission of *E. coli* O157:H7 include heat-processed meat patties, which should be pathogen-free (10); roast beef (14, 96); ham, turkey, and cheese sandwiches (11); and potatoes (9). Unpasteurized milk has been implicated as the vehicle for two cases of the hemolytic–uremic syndrome (97) and for a kindergarten outbreak of *E. coli* O157:H7 infection (17); *E. coli* O157:H7 was isolated from the feces of healthy cows who had supplied raw milk consumed by the patients affected in the outbreak. Even fresh-pressed, unpreserved apple cider, a seemingly unlikely vehicle, was implicated in one outbreak (88). The transmission probably occurred through the pressing of apples contaminated on the ground or during the production process. The isolation of *E. coli* O157:H7 from milk and from the feces of healthy cattle (17, 97, 98) and the fact that hamburger is a major vehicle associated with food-borne outbreaks of *E. coli* O157:H7 infection (1, 15, 19) suggest that cattle are an important reservoir for the pathogen. *Escherichia coli* O157:H7 has been isolated more often from dairy than from beef cattle (97), but both beef and dairy cattle are thought to be principal domestic reservoirs for the organism. In one study (99), a particularly high rate of isolation of the organism from beef (31%) was found to correlate with the increasing incidence of human infection in the region studied. Because cattle are an important reservoir for *E. coli* O157:H7, the apparent increase in *E. coli* O157:H7 infections during the past several years suggests that an epizootic infection may be occurring in the animal reservoir (52). *Escherichia coli* O157:H7 has also been isolated from 1.5% to 3.7% of retail samples of beef, pork, poultry, and lamb from grocery stores in Canada and the United States (99). Because of the wide array of contaminated food products, the precise sources of organisms are often difficult to trace and thus remain unknown in most cases.

Non–food-borne vehicles have also been implicated in the spread of *E. coli* O157:H7. Water-borne transmission has been implicated in two outbreaks (12, 24), and transmission by person-to-person contact or by fomites has been suggested in sporadic cases (5, 100) and outbreaks (10, 11, 13, 19, 20). Secondary person-to-person contact can be an important method of spread in institutional settings, especially day care centers (20, 45) and nursing homes (11, 19), but it is less common in community-wide outbreaks. Nosocomial *E. coli* O157:H7 infections have also been reported (101, 102).

Pathology

Escherichia coli O157:H7 infection produces its most severe abnormalities in the ascending and transverse colon (103, 104); this is consistent with endoscopic and radiologic findings showing right-sided predominance (1, 8, 37). Colonic tissues show a spectrum of appearances ranging from normal to gross dilation with hyperemia of the involved segments (19). In one study (104), all specimens showed patchy, shallow mucosal ulcers with partial loss of normal mucosal folds, and many ulcers were covered by a thin layer of yellow or green exudate. Extreme submucosal edema, hemorrhage, and thickening of the bowel wall were present and, in one case, were so severe that the lumen of the ascending colon was almost obliterated.

Microscopically, no single histologic feature is diagnostic of *E. coli* O157:H7 infection, but the colonic pathology in colitis associated with *E. coli* O157:H7 often resembles a combination of ischemic colitis and infectious injury similar to that seen in toxin-mediated *Clostridium difficile*–associated colitis (103). Submucosal hemorrhage, edema, and fibrin exudation are the most prominent features; ulceration, hemorrhage, capillary thrombi, and mild neutrophil infiltration in the mucosa are less common (103, 104). Immunocytochemical examination showed that the submucosal plasma cells were primarily IgG, IgA, and IgM cells (104). In one study of 11 patients (103), all 20 colonic specimens showed variable hemorrhage and edema in the lamina propria. Nine patients had colonic pathology similar to the pattern of injury associated with acute ischemic colitis (103): focal coagulative necrosis, hemorrhage, and acute inflammation in the superficial mucosa and preservation of the deep colonic crypts. Five patients showed both neutrophilic infiltration of the lamina propria and crypts and formation of crypt abscesses, resembling the pattern of injury seen in infectious colitis (103). Pseudomembranous lesions similar to those in *C. difficile*–associated pseudomembranous colitis may also be present (40, 103–105). The ischemic and infectious patterns of injury can be seen alone or in combination (103); occasionally, normal specimens have also been described (5, 19, 40, 105, 106). In one case of nonbloody diarrhea, the ascending colon showed only patchy eosinophilic infiltrates (19). No single histologic feature is diagnostic of colitis associated with *E. coli* O157:H7, but the combination of infectious and ischemic patterns of injury, especially in association with capillary microthrombi and a compatible clinical picture, should suggest the diagnosis (103). Obtaining more than one biopsy specimen from any patient increases the likelihood of identifying an abnormality, because abnormalities are often patchy (103).

Light microscopy showed no evidence of bacterial adherence or invasion in either diseased areas or normal mucosa (19, 104). To date, immunocytochemical (104) or immunofluorescent (19) studies for *E. coli* O157 and H7 antigens have also failed to detect the organism in tissues. In a recent pilot study (107), immunohistochemical staining with peroxidase-labeled antibody to *E. coli* O157:H7 successfully detected the organism in biopsy or surgical

specimens from four patients known to have colitis associated with *E. coli* O157:H7 and from six patients with ischemic colitis (107).

Microbiology

With one exception (16), all *E. coli* O157:H7 isolates have been reported to produce Shiga-like toxins (5, 10, 11, 20, 27, 52, 108). These distinct toxins were first discovered in 1977 in certain *E. coli* strains associated with diarrheal disease (109), and they were termed "Shiga-like" because of their structural similarities to Shiga toxin. Shiga-like toxins have also been called Verotoxins because of their cytotoxic effect on Vero cells in tissue culture, but the toxin activity (and presumably the globotriaosyl ceramide receptor) extends to many other cell lines, including HeLa cells, intestinal villus cells, endothelial cells, and Burkitt lymphoma cells (110–114). Shiga-like toxins were isolated in England from stools of children with bloody diarrhea (115), but they were not linked to *E. coli* O157:H7 infection until 1982, when Shiga-like toxin was first isolated from an outbreak of hemorrhagic colitis in a Canadian institution for elderly patients (27).

Escherichia coli O157:H7 strains have been shown to produce at least two distinct Shiga-like toxins, Shiga-like toxin I and Shiga-like toxin II, that have different immunologic and physicochemical properties (27–32, 34). Shiga-like toxin I has many of the same biological properties as Shiga toxin, from which it is almost indistinguishable except at the nucleotide and protein level (29, 30, 116). Shiga-like toxin I has the same isoelectric point and relative heat stabilities as Shiga toxin, and it can be neutralized by antiserum to purified Shiga toxin (28–30, 117, 118). Shiga-like toxins have the same subunit structure as Shiga toxin; this consists of one active "A" subunit and five "B" binding subunits (30, 119). Nucleotide sequencing and deduced amino acid composition show that Shiga-like toxin I and Shiga toxin share a greater than 99% gene homology and that their structures differ in only one amino acid on subunit A (116, 120, 121). In contrast, Shiga-like toxin II (31–34) is genetically related to but antigenically distinct from Shiga-like toxin I. Shiga-like toxin II shares less than 60% of its DNA homology with Shiga toxin or Shiga-like toxin I (122, 123), and it lacks cross-neutralization with anti–Shiga-like toxin I or anti–Shiga toxin antibodies (31, 32, 34). Shiga-like toxin II has more sequence and antigenic variability than Shiga-like toxin I, and a growing number of closely related Shiga-like toxins have been identified that belong to the Shiga-like toxin II family (124).

Although different in their molecular sequences and immunologic properties, Shiga-like toxins I and II share the same cell receptor and the same intracellular mechanism of action in vitro. Both bind to the same surface membrane receptor, globotriaosyl ceramide, which is the major Shiga-like toxin–binding glycolipid in Vero cells (110, 111). Globotriaosyl ceramide is highly expressed in the cortex of human kidney (112) and is found in primary human endothelial cell cultures (111). Although it has not yet been identified, it is thought to be functional in human enterocytes (111). On binding to globotriaosyl ceramide receptors, Shiga-like toxins I and II inhibit protein synthesis by N-glycosidase cleavage at a specific site of an adenine residue on the 28s ribosomal subunit (125–128). In addition to sharing the same intracellular mechanism of action, Shiga-like toxins I and II are reported to have similar biological activities (34). Both have been shown to be cytotoxic to Vero and HeLa cells, enterotoxic to rabbit ileal loops, and paralytic-lethal for laboratory mice (30, 34, 129). It is unclear which toxin is more virulent, but given an equal amount of protein in cell lysates, Shiga-like toxin II is more lethal for mice and less cytotoxic than Shiga-like toxin I (34).

Shiga-like toxins I and II are both bacteriophage encoded, and toxin production appears to be a consequence of lysogenization with one or more toxin-converting phages (130–133). Some strains of *E. coli* O157:H7 produce only one Shiga-like toxin, and some produce both. Analysis of *E. coli* O157:H7 isolates obtained during outbreaks and from patients with the hemolytic–uremic syndrome has shown that most isolates produce either Shiga-like toxin II alone or Shiga-like toxins I and II (the numbers range from 93% of isolates producing only Shiga-like toxin II (16) to 100% of isolates producing Shiga-like toxins I and II in another outbreak [10]). In one study (108), Shiga-like toxin I alone was found in only 1 of 26 strains of *E. coli* O157:H7, and only 1 of 14 isolates in a community outbreak (16) had neither Shiga-like toxin I nor Shiga-like toxin II (16). However, the absence of Shiga-like toxin production may be due to the instability of Shiga-like toxin genes, which may result in the loss of toxin genes during repeated laboratory culturing (134).

Pathogenesis

Several animal models have been developed for use in the study of the pathogenesis of *E. coli* O157:H7 infection (135–141). Two studies (137, 139) have consistently induced nonbloody diarrhea in infant rabbits (5 to 10 days old in one study and 3 to 11 days old in the other) that were nasogastrically inoculated with *E. coli* O157:H7. The organism failed to produce diarrhea in older rabbits, 2-week-old guinea pigs, 3-week-old mice, and young rhesus monkeys (137). In another animal study (138), orally administered *E. coli* O157:H7 produced watery diarrhea in gnotobiotic pigs. Epithelial distortion and detachment, effacement or fusion of the intestinal microvilli, and projections or invaginations of the plasma membrane occurred at bacterial attachment sites (138). However, in none of these animal models were investigators able to reproduce grossly bloody diarrhea with a predominance of colonic disease as seen in humans. Such a pattern was seen in only one study (139), in which partially purified Shiga-like toxin from culture filtrates of *E. coli* O157:H7 was intragastrically administered to infant rabbits; one rabbit developed bloody diarrhea and extensive colonic pathology. In that study (139), rabbits given Shiga-like toxins alone and those inoculated with a high Shiga-like toxin–producing strain of *E. coli* O157:H7 produced the same histopathology, providing evidence to show that a toxin plays a role in pathogenesis. These histologic lesions included a predominance in the mid- and distal colon, characterized by apoptosis (defined as "individual cell death") in the surface epithelium, increased mitotic activity in the crypts, mucin depletion, and a mild to moderate infiltration of neutrophils in the lamina propria and epi-

thelium (139). Other intestinal morphologic changes include the premature expulsion of mature columnar absorptive epithelia of the rabbit intestinal villus in vivo as a result of the direct and selective action of Shiga-like toxin (114). The goblet mucus cells remain attached and the crypt epithelia proliferate to maintain epithelial integrity.

On the basis of animal models and evidence from clinical, microbiological, and epidemiologic studies, toxinemia has been implicated as the primary pathogenic event in the production of the spectrum of illnesses associated with *E. coli* O157:H7 infection (40, 142). Karmali and colleagues (142) proposed that Shiga-like toxin is the direct etiologic agent in the pathogenesis of both hemorrhagic colitis and the hemolytic–uremic syndrome. Observations that persons with diarrhea, hemorrhagic colitis, or the hemolytic–uremic syndrome produced high levels of Shiga-like toxins but that controls did not suggest that toxin production is important in pathogenesis (143). Evidence that Shiga-like toxins I and II are enterotoxic to rabbit ileal loops (34), that Shiga-like toxin I acts directly on the epithelium of the intestinal villus in rabbits (114), and that Shiga-like toxin may produce a specific cytotoxic effect on the colons of mice, leading to colonic hemorrhage (31), supports the hypothesis that Shiga-like toxin is causally involved in the pathogenesis of *E. coli* O157:H7 infection.

Escherichia coli O157:H7 has been closely linked with both hemorrhagic colitis and the hemolytic–uremic syndrome. The clinical and radiologic features of hemorrhagic colitis resemble the gastrointestinal prodrome of the hemolytic–uremic syndrome and suggest that these conditions have a common vasculitic process (142). The histopathologic lesions of platelet-fibrin thrombi in the microvasculature of various organs in the hemolytic–uremic syndrome are also consistent with systemic toxinemia. In vitro studies have shown that *E. coli* Shiga-like toxin I shows a direct cytotoxic response in vascular endothelial cells (144), and, when injected into rabbits, Shiga-like toxin I produces thrombotic microangiopathic lesions similar to those seen in humans with the hemolytic–uremic syndrome (105, 145). It has been proposed that microvascular thrombi form through a direct cytotoxic effect on vascular endothelium or a direct effect on platelet aggregation after infection with *E. coli* O157:H7 (40). It has been shown that *E. coli* O157:H7 Shiga-like toxins decrease prostacyclin synthesis (146) and that Shiga-like toxins incubated with platelet-poor plasma can lead to platelet aggregation (147). It is therefore reasonable to propose that, after infection with the organisms and release of toxins, damage to vascular endothelium is accompanied by a decreased synthesis of prostacyclin, an increased agglutination of platelets, and the exposure of subendothelium beneath the disrupted surface endothelium. A cascade of coagulative events is thus triggered, leading to intravascular thrombi formation. Ischemic changes precipitated by platelet-fibrin thrombi in the colonic microvasculature result in hemorrhagic colitis. Patients who develop the hemolytic–uremic syndrome or thrombotic thrombocytopenic purpura represent a clinical spectrum arising from the same underlying disease process, and they differ mainly in the distribution of their thrombotic lesions (40). Platelet-fibrin thrombi are predominantly located in the kidneys in patients with the hemolytic–uremic syndrome, but they appear to be more disseminated in persons with thrombotic thrombocytopenic purpura, with involvement in the pancreas, adrenal glands, heart, brain, and kidneys (82). The hypothesis of vascular ischemia secondary to thrombi formation in the pathogenesis of *E. coli* O157:H7 infection is supported by the report of a patient with hemorrhagic colitis and thrombotic thrombocytopenic purpura (40). Barium enema studies showed marked thickening of the mucosa and thumbprinting along the transverse and descending colon suggestive of ischemic colitis with submucosal edema or hemorrhage. Colonoscopy showed acute severe colitis, and biopsy showed focal ulceration of the mucosa and capillary-platelet thrombi in the submucosa (40). Furthermore, injecting Shiga-like toxin I into gnotobiotic pigs produces vascular damage and ischemic necrosis in the intestines and brain, resembling the lesions seen in humans with hemorrhagic colitis and thrombotic thrombocytopenic purpura (148). In short, current thinking on the pathogenesis of *E. coli* O157:H7 infection is that the organism releases its toxins in the bowel and that they are absorbed into the circulation, producing vascular endothelial damage with subsequent local intravascular coagulation and fibrin deposition and ultimately resulting in various clinical features of *E. coli* O157:H7 infection.

Patients with hemorrhagic colitis and the hemolytic–uremic syndrome have shown an increase in Shiga-like toxin–neutralizing antibody titers (62, 68, 72). This serologic finding further supports the idea that Shiga-like toxin is important in *E. coli* O157:H7 infection and suggests that antibodies to Shiga-like toxin may play a protective or pathogenic role. It has even been suggested that the more severe clinical courses seen among patients at extremes of age is caused by an absence of specific neutralizing antibodies (142).

Escherichia coli O157:H7 may possess a complex of virulence determinants other than Shiga-like toxins. An animal study of gnotobiotic pigs inoculated with two strains of *E. coli* O157:H7—one that produced high levels of Shiga-like toxin and one that produced only moderate levels of a different Shiga-like toxin—showed similar clinical manifestations and histopathology (141). This observation undermines the idea that Shiga-like toxins play a pathogenic role and suggests that at least one other virulent factor exists. Although *E. coli* O157:H7 does not invade epithelial cells, investigators have postulated that it colonizes the bowel through fimbrial adherence to the cells (149). Animal models have shown that it adheres to the luminal surface of the colon, cecum, gut-associated lymphoid tissues, and, to a lesser extent, the small intestinal epithelium of infected rabbits (139). A similar study with gnotobiotic pigs showed diffuse bacterial attachment to the epithelial surface of the cecum and colon and focal adherence to the ileum and rectum (138). Microscopically, these organisms produce lesions with a characteristic attaching and effacing pattern (150–153). It was subsequently found that, in tissue culture, fimbriated *E. coli* O157:H7 isolates adhered to Henle 407 cells, a human-derived intestinal cell line, and that a 60-Md plasmid present in most isolates (154) encoded expression of the fimbrial antigen of *E. coli* O157:H7 (149). Observations that plasmid-cured and thus nonfimbriated strains of

E. coli O157:H7 lost their ability to adhere to intestinal cells (149) suggest that the biological activity of cell adherence is mediated by the *E. coli* O157:H7 fimbria.

On the basis of several epidemiologic patterns, it was suggested that only a small inoculum was required to produce illness (71). First, unlike salmonella infection, which usually results from gross mishandling during food preparation, most cases of *E. coli* O157:H7 infection were traced to meat contaminated by only slight undercooking and not left at a warm temperature for a period of time to allow for bacterial overgrowth. Second, an appreciable rate of *E. coli* O157:H7 infection has been traced to vehicles such as raw milk and municipal water, which should be associated with only a small number of organisms because of their cold temperature and dilutional nature. Last, the appreciable rate of secondary person-to-person transmission is similar to that of shigellosis, which can be transmitted by a small inoculum. This low infectious dose makes it even more important to implement public health measures, including strict regulation of food processing (*see* below).

Table 3. Diagnosis of *Escherichia coli* O157:H7 or Shiga-like toxin–producing *E. coli*

Stool culture with sorbitol–MacConkey medium for *E. coli* O157:H7 strains
Serotyping for O157 or H7 antigens, or both
Detection of free toxin in stools or colonies with tissue cultures or enzyme-linked immunosorbent assays
Serology for antibodies to O157 lipopolysaccharides or Shiga-like toxins I and II
DNA probes for toxin genes in colonies
Polymerase chain reaction amplification to detect toxin genes

Diagnosis

Most patients with *E. coli* O157:H7 infection that occurs in epidemics are suspected of having infectious diarrhea. More laboratories are now screening for *E. coli* O157:H7, but infection with this organism is often unrecognized because most clinical laboratories still do not routinely test stool samples for this organism. Other differential diagnoses that have often been considered include inflammatory bowel disease, ischemic colitis, antibiotic-associated pseudomembranous colitis, intussusception, or various causes of an acute abdomen (16, 19, 37).

The strongest evidence for *E. coli* O157:H7 infection is the presence of organisms in stool culture, but diagnosis can also be supported by the presence of Shiga-like toxin, an increase in serum Shiga-like toxin antibody titers, or a host of new genotypic and phenotypic assays (Table 3). Stool culture for this organism requires a special growth medium, because *E. coli* O157:H7 ferments lactose rapidly and thus cannot be picked out from normal fecal flora when grown on a lactose-containing medium for routine stool cultures. However, serotype O157:H7 can be distinguished from most other strains of *E. coli* by its slow fermentation of sorbitol. When plated on MacConkey agar (indicator medium) and sorbitol agar (selective medium), *E. coli* O157:H7 appears sorbitol negative at 24 hours (155, 156). This MacConkey–sorbitol agar medium is 100% sensitive, 85% specific, and 86% accurate for detecting *E. coli* O157:H7 (156). Sorbitol-negative colonies can be picked and further tested by characterizing responses to other biochemical parameters (154, 157–160), serotyping using antisera to H7 and O157 antigens, or determining the presence of Shiga-like toxins. One limitation to this approach is that the rate of isolation decreases with delay in collection of stool samples; cultures obtained more than 6 days after the onset of illness or after the administration of antibiotics often produce negative results (20, 37, 75, 154). *Escherichia coli* O157:H7 was isolated from 75% to 100% of the stool samples obtained within 7 days of the onset of illness, but the recovery rates from samples collected after day 7 ranged from 0% to 33% (20, 37, 75, 154). In one study (75), the rate of positive stool culture decreased from 100% for samples collected within 2 days of the onset of diarrhea to 92% for samples collected on days 3 through 6 and to 33% for samples collected after day 7. The duration of carriage seems to be longer in children than in adults (5). Finally, there are sorbitol-fermenting *E. coli* O157 strains that have been reported to cause human disease (161), but their prevalence and significance are still unclear.

Screening specimens on sorbitol-containing MacConkey culture medium and then testing the non–sorbitol-fermenting colonies for *E. coli* O157:H7 by using biochemical parameters and by serotyping with O157 and H7 antisera (26, 37, 156, 162) can be laborious and time-consuming. Antisera to both H7 and O157 are now commercially available, so that after screening with sorbitol–MacConkey medium, the sorbitol-negative colonies can be rapidly confirmed with O serum and H serum in the slide agglutination test (163, 164). Investigators have shown that the commercially available latex slide agglutination tests for O157 serum are an efficient and reliable alternative to conventional serotyping with the standard-tube agglutination test, making rapid presumptive detection of *E. coli* O157:H7 possible (163, 164). However, colonies that agglutinate should be confirmed serologically, using agglutination or direct immunofluoresecent antibody tests (163–165). An alternative screening method was reported by Farmer and Davis (155), who devised an H7 antiserum–sorbitol fermentation medium as a single-tube screening medium; strains that were presumptive positives (negative for sorbitol fermentation and positive for H7 reaction) were then tested by slide or tube agglutination with *E. coli* O157 serum (155).

Another sensitive method of diagnosing *E. coli* O157:H7 infection is to look for Shiga-like toxins. These toxins have been detected in *E. coli* culture broth filtrate and in stool extracts (27, 29, 68). Demonstration of free fecal Shiga-like toxins can be made by tissue culture assays with neutralization by appropriate antisera (162, 166–168). The disadvantage of this approach is that classic tissue culture assays using HeLa or Vero cell culture cytotoxicity (109) require appropriate facilities and are slow and cumbersome. On the other hand, testing for Shiga-like toxin allows the detection not only of *E. coli* O157:H7 but of Shiga-like toxin–producing serotypes other than O157:H7, which may be increasing in importance as causes of human illness. Moreover, Shiga-like toxins have been found in fecal filtrates long after *E. coli* cannot be cultured from stools (62, 162): More than 4 to 9 days after an *E. coli* O157:H7 infection, the excretion of organisms into stools

usually decreases to an undetectable amount, but free fecal Shiga-like toxins may remain measurable for as long as 4 to 6 weeks. Free fecal Shiga-like toxin assay has been reported to be more sensitive than stool culture for the organism (11, 62). In a nursing home outbreak, the rate of isolation from stool samples was 34% and the detection rate for free fecal toxin was 50% (11). Although the organism has never been isolated without fecal Shiga-like toxin, the latter was often present even when stool culture was negative (62).

Other methods for detecting toxins include genetic probes and immunospecific assays, which are simpler and more sensitive than culture techniques, although some may be less practical for use in clinical laboratories. Deoxyribonucleic acid hybridization assays using synthetic nucleotides or fragments of structural genes specific for the toxins can also be used to detect Shiga-like toxin–producing *E. coli* (67, 89, 161, 169–173). Gene probes are sensitive and specific (169, 171). Using colony blot hybridization, only 2 of 102 strains were toxin-probe positive when toxin was not present (170), suggesting that the use of DNA probes to detect Shiga-like toxin production is as accurate as the use of toxin-specific antibodies. These specific DNA probes were able to detect colonies of Shiga-like toxin–producing *E. coli* present in numbers as small as 1 in 1200 colonies (67). In one study (169) that used synthetic oligonucleotides from selected sequences of genes encoding A-subunit of Shiga-like toxin I and B-subunit of Shiga-like toxin II at different degrees of stringency, the A-I probe had 92% sensitivity and 91% specificity for identifying Shiga-like toxin I–producing *E. coli*, and the B-II probe had 100% sensitivity and 97% specificity for identifying Shiga-like toxin II–producing *E. coli* (169). Both probes were able to identify strains that produce variants of Shiga-like toxins. Gene probes are diagnostically useful, but the cost and concern associated with radioactive safety have limited their widespread applicability. Various enzyme-linked immunosorbent assays using polyclonal and monoclonal antibodies against Shiga-like toxins I and II to detect the presence of toxins in culture or fecal extract have also been developed (174–177). On the basis of its specific binding to the globotriaosyl ceramide natural receptor, a modified enzyme-linked immunosorbent assay for the rapid detection of Shiga-like toxin I has been reported (178), in which toxin bound to the globotriaosyl ceramide receptor was detected by enzyme-linked immunosorbent assay with monoclonal antibodies against Shiga-like toxin I. Both techniques are highly sensitive and specific in detecting toxin production, and they promise to shorten the time to diagnosis of *E. coli* O157:H7 infection. Another genetic technique involves polymerase chain reaction (PCR) amplification to test for the presence of Shiga-like toxin genes (161, 179–182). Because PCR should detect organisms in low numbers, it can detect Shiga-like toxin production when culture fails (180). In addition, like other methods for detecting the presence of Shiga-like toxin, PCR can identify Shiga-like toxin–producing *E. coli* other than O157:H7. Techniques for the direct detection of Shiga-like toxin sequences in stool specimens have also been reported (180, 181), overcoming the difficulty of high-frequency loss of toxin genes with repeated cultures (134).

Additional phenotypic and genotypic assays have been developed to assist in epidemiologic studies, allowing investigators to determine the extent of outbreaks, trace human outbreaks to animal sources, and differentiate and analyze linkage between strains of *E. coli* O157:H7. These schemes include Shiga-like toxin genotyping (170, 183–185), plasmid DNA profiling (16, 27, 154, 185, 186), bacteriophage typing (11, 170, 183, 185–187), restriction digests of plasmid (154), restriction fragment length polymorphism with a bacteriophage lambda probe (185, 188), electrophoresis of plasmids and multilocus enzyme electrophoretic typing (189), pulsed-field gel electrophoresis of restriction fragment length polymorphism (190), and patterns of antibiotic susceptibilities (24).

Another useful diagnostic tool is serologic testing to detect antibodies to Shiga-like toxin or O157 lipopolysaccharides. Increases in serum Shiga-like toxin–neutralizing antibody titers during *E. coli* O157:H7 infections have been used to detect or support the diagnosis of infections (62, 68, 72). The antibody titers ranged from 4 to 80 in acute serum specimens collected between days 4 and 18 after the onset of illness; they ranged from 32 to 1280 in convalescent serum specimens collected between days 13 and 43 (62, 68). In one case (62), the acute and convalescent serum specimens yielded titers of 4096 and 32 000, respectively. In the same study, a fourfold or greater increase in Shiga-like toxin–neutralizing antibody titer was used to diagnose infection (62). Fifty-nine percent of patients (16 of 27) met the requirement, and this criterion was the only evidence of infection in 15% of those tested (62). This serologic test may be an alternative way to diagnose *E. coli* O157:H7 infection, especially during epidemics of this infection or when other methods fail to detect *E. coli* O157:H7. Similarly, serologic response to O157 lipopolysaccharides of *E. coli* O157:H7 has also been reported (191–195) and can be a useful adjunct for diagnosing *E. coli* O157:H7 infection. In one study (192), this serologic test detected evidence of *E. coli* O157:H7 infection in 73% of children with the hemolytic–uremic syndrome and was more sensitive than either isolation of the organism or the detection of fecal Shiga-like toxin. In studies involving patients with the hemolytic–uremic syndrome, the presence of antibodies to O157 lipopolysaccharide was able to provide evidence of *E. coli* O157:H7 infection when fecal bacteria or Shiga-like toxin activity could no longer be detected (192, 195). Most IgM antibodies became undetectable 2 to 3 months after the acute phase of the hemolytic–uremic syndrome (195). However, the interpretation of the serologic study for O157 lipopolysaccharide may be affected by possible cross-reactivity with other organisms and detection of nontoxigenic or non–H7 strains of *E. coli* O157.

In summary, the most common algorithm for diagnosing *E. coli* O157:H7 infection in current clinical practice is to culture stool specimens for the organisms using sorbitol–MacConkey agar; this can be done at local hospital laboratories. The sorbitol-negative colonies can be serotyped using commercially available anti-sera to O157 while the sample is sent to a reference laboratory. Presumptive diagnosis can also be made by biochemical testing. In either case, diagnosis is confirmed by the reference laboratory, where the O157 latex test or O157 direct fluorescent antibodies and H7 antisera are used to test for

O157:H7. In addition, DNA probes are used to detect Shiga-like toxin in stools at some reference laboratories. If the initial culture is negative but clinical suspicion is still high, stool samples can be sent to a reference laboratory, where more sophisticated techniques, such as PCR for toxin genes, can be used. In practice, serologic determination of Shiga-like toxin titers is used primarily as a diagnostic aid and is not done routinely. In areas where infection with Shiga-like toxin–producing *E. coli* is common, Shiga-like toxin titers on one serum specimen may be difficult to interpret.

Treatment

No specific treatment currently exists for *E. coli* O157:H7 infection other than supportive therapy and management of complications such as anemia and renal failure. Antimicrobial agents have not been shown to modify the illness, but few conclusive data are available on individual agents. Some studies (1, 4, 8) have shown that the duration of illness in persons treated with antibiotics did not differ significantly from that in untreated persons. Although the mean duration of diarrhea was similar in patients who did and did not receive antimicrobial therapy (4, 37), one study reported a significantly longer duration of bloody diarrhea in persons treated with antibiotics than in untreated persons (4). It has even been suggested that the use of antibiotics is a risk factor for infection and that an association exists between the use of antibiotics and increased mortality (11). It has been postulated that antibiotics can worsen the clinical course of *E. coli* O157:H7 infection through two mechanisms (196): 1) the elimination of competing bowel flora by antibiotics, leading to an overgrowth of *E. coli* O157:H7, and 2) lysis of or sublethal damage to the infecting organisms, with the subsequent liberation of Shiga-like toxins.

Most *E. coli* O157:H7 isolates are sensitive to most antimicrobial agents in vitro. Isolates of *E. coli* O157:H7 have been found to be uniformly susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, norfloxacin, sulfisoxazole, tetracycline, ticarcillin, tobramycin, trimethoprim, and trimethoprim–sulfamethoxazole (5, 37, 196). Isolates have been found to be resistant to erythromycin, metronidazole, and vancomycin (196), and some have been reported to be resistant to tetracycline (8, 24, 197). A strain of *E. coli* O157:H7 from a water-borne outbreak in 1989 was resistant to streptomycin, sulfisoxazole, and tetracycline (24). A study of antibiotic-resistant *E. coli* O157:H7 in Washington State showed an emergence of antibiotic resistance to streptomycin, sulfisoxazole, and tetracycline, from zero isolation (0 of 56) between 1984 and 1987 to 7.4% isolation (13 of 176) between 1989 and 1991 (197). Studies of an *E. coli* O157:H7 outbreak have suggested that patients receiving ampicillin and patients receiving placebo did not differ in durations of diarrhea or bloody diarrhea, number of stools per day, or hospitalization rate. In contrast, another study (18) showed that patients treated with trimethoprim–sulfamethoxazole had longer durations of diarrhea and bloody diarrhea and were more likely to develop the hemolytic–uremic syndrome. It has been suggested that trimethoprim–sulfamethoxazole and polymyxin B increase in vitro toxin concentration released by *E. coli* (167, 198, 199). This suggestion is based on a study (196) that showed a worse outcome with trimethoprim–sulfamethoxazole (18) and on the hypothesis that antibiotic therapy aggravates *E. coli* O157:H7 infection by sublethal damage or lysis of the infecting organism and the subsequent release of Shiga-like toxin into the gut lumen. However, without randomization, patients with more severe disease may be more likely to receive antibiotics, leading to bias in data interpretation (18). Other studies have found no association of trimethoprim–sulfamethoxazole (or other "appropriate" antibiotics) with progression to the hemolytic–uremic syndrome (77, 200). By decreasing Shiga-like toxin synthesis in vitro (176) and eliminating other enteric pathogens from the gut mucosa (201), it was postulated that ciprofloxacin may be useful for treating infection with this organism (36).

Use of antimotility agents has also been suggested as a risk factor for progression of *E. coli* O157:H7 infection to the hemolytic–uremic syndrome, because such use may allow more time for toxin absorption (77). A positive association has been found between the use of antimotility agents and the severity of *E. coli* O157:H7 infection in one study of four geriatric patients (19), although contradictory findings, which show no difference in duration of diarrhea and overall illness with antidiarrheal use, have been reported (4).

Prevention

The obvious health implications of *E. coli* O157:H7 infection and its complications, including the hemolytic–uremic syndrome and thrombotic thrombocytopenic purpura, warrant better educational and preventive measures. Several public health measures have been proposed (202), including improved case identification resulting from increased awareness of this infection; more widespread and frequent screening for the organism at health laboratory facilities; routine testing of all grossly bloody stool specimens for *E. coli* O157:H7; and establishment of an expanded and more active surveillance system, allowing timely reporting when cases are identified and prompt follow-up with appropriate investigation by public health officials. State-mandated reporting of *E. coli* O157:H7 infection has also been recommended; such a mandate has been shown to be critical for prompt outbreak recognition and control (84). To decrease primary transmission from animal sources, the public should be educated about the risks of consuming undercooked meats and unpasteurized milk. Both consumers and food service workers should be taught the proper techniques for handling and cooking meat, and it should be recommended that ground beef be cooked until its interior is no longer pink. The Food and Drug Administration has recommended a minimum internal temperature of 155 ° F (86.1 °C) for cooked hamburger (203). The implementation of regulatory standards on food processing can be expected to decrease the risk for cross-contamination. Other preventive measures include good hygienic practices when handling diapers at day care centers, to decrease secondary person-to-person transmission. Temporary exclusion of all children from a day care facility with presumptive evidence of ongoing *E. coli* O157:H7 transmission may be necessary (45). Enteric precaution for hospitalized patients with this infection

should be strictly followed to avoid nosocomial transmission.

Conclusion

Escherichia coli O157:H7 causes a wide spectrum of illnesses in humans, ranging from asymptomatic carriage to hemorrhagic colitis, and complications such as the hemolytic–uremic syndrome and thrombotic thrombocytopenic purpura can cause substantial morbidity and mortality. The increasing frequency of recognized cases may be due in part to heightened awareness of the organism, especially in regions with previous outbreaks; it may also reflect an epizootic infection among cattle, the principal animal reservoir for *E. coli* O157:H7. The potentially high morbidity and mortality associated with this infection warrant better preventive measures. Infection with *E. coli* O157:H7 should be included in the differential diagnosis for any patient presenting with bloody diarrhea. Furthermore, development of the hemolytic–uremic syndrome or thrombotic thrombocytopenic purpura after bloody diarrhea should raise strong suspicion of *E. coli* O157:H7 infection and should be an indication to report to public health officials so that disease occurrence and outbreaks can be monitored (52).

As the properties and actions of Shiga-like toxins and the pathogenesis of *E. coli* O157:H7 infection are being elucidated, many questions remain unanswered and provide areas for future research. Epidemiologic studies may evaluate the geographic variation in prevalence of *E. coli* O157:H7 infection and the significance of animal reservoirs with respect to the apparent increase in frequency of infection. Several Shiga-like toxin–producing *E. coli* other than those with the O157:H7 serotype have been associated with human diseases and may become increasingly important. It is also of clinical importance to determine host factors for infection with this organism, such as age and gastric activity, and to identify susceptibility for disease progression to the hemolytic–uremic syndrome using factors such as age, blood-group antigens (80), and antibiotic and antimotility therapy. Other factors, such as strain characteristics and inoculum size, may affect the outcome of the infection and require further examination. Toxin production is believed to be the primary etiologic event of *E. coli* O157:H7 infection, but detailed pathogenic sequences still need to be characterized. These include the intracellular mechanism for the inhibition of protein synthesis; receptor binding; and determinants mediating cellular adhesion and bowel colonization (such as an attachment–effacement mediated by a chromosomal-encoded *eae* gene product, an outer membrane protein called intimin) (204–208).

Rapid, convenient, and cost-effective diagnostic methods that are specific and sensitive for *E. coli* O157:H7 are necessary for prompt detection of infections. Such methods may involve serologic tests, immunologic assays, genetic tools, or PCR amplification. A large, multicenter trial with early randomization to minimize selection bias is needed to evaluate the effects of specific antibiotic therapy, whether beneficial or detrimental, especially for those persons at greatest risk for complications. Research could also address treatments other than antibiotics. Some nonconventional approaches mainly involve management of the hemolytic–uremic syndrome; these include plasma infusion, plasmapheresis, and intravenous immunoglobulin (209–214). Immunoglobulin preparations have been shown to contain anti–Shiga-like toxin I antibodies and to protect infant rabbits from diarrhea and death caused by intraperitoneal administered Shiga-like toxin I (215). They may also be therapeutically effective for infections progressing to the hemolytic–uremic syndrome or thrombotic thrombocytopenia purpura (79, 214). Some potential therapeutic avenues are thought to prevent progression of disease to more serious complications, specifically the hemolytic–uremic syndrome, and these include the neutralization of toxins by monoclonal antibodies and receptor blockade with binding subunit (216). Calcium channel blockers such as verapamil have been shown to inhibit the in vitro cytotoxicity of Shiga toxin (217) and to prevent cellular entry of Shiga-like toxin I, and they may have a potential therapeutic role in *E. coli* O157:H7 infection. Use of calcium channel blockers, antibodies, or receptor-binding subunit is only theoretical and has not yet entered clinical practice.

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References

1. **Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, et al.** Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med. 1983;308:681-5.
2. **Centers for Disease Control.** Isolation of *E. coli* O157:H7 from sporadic cases of hemorrhagic colitis—United States. MMWR Morb Mortal Wkly Rep. 1982;31:580, 585.
3. **MacDonald KL, O'Leary MJ, Cohen ML, Norris P, Wells JG, Noll E, et al.** *Escherichia coli* O157:H7, an emerging gastrointestinal pathogen. Results of a one-year, prospective, population-based study. JAMA. 1988;259:3567-70.
4. **Ostroff SM, Kobayashi JM, Lewis JH.** Infections with *Escherichia coli* O157:H7 in Washington State. The first year of statewide disease surveillance. JAMA. 1989;262:355-9.
5. **Pai CH, Gordon R, Sims HV, Bryan LE.** Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. Clinical, epidemiologic, and bacteriologic features. Ann Intern Med. 1984;101: 738-42.
6. **Pai CH, Ahmed N, Lior H, Johnson WM, Sims HV, Woods DE.** Epidemiology of sporadic diarrhea due to verocytotoxin-producing *Escherichia coli*: a two-year prospective study. J Infect Dis. 1988;157: 1054-7.
7. **Ratnam S, March S.** Sporadic occurrence of hemorrhagic colitis associated with *Escherichia coli* O157:H7 in Newfoundland. Can Med Assoc J. 1986;134:43-9.
8. **Remis RS, MacDonald KL, Riley LW, Puhr ND, Wells JG, Davis BR, et al.** Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. Ann Intern Med. 1984;101:624-6.
9. Report from the PHLS Communicable Disease Surveillance Centre. BMJ. 1987;295:1545-6.
10. **Belongia EA, MacDonald KL, Parham GL, White KE, Korlath JA, Lobato MN, et al.** An outbreak of *Escherichia coli* O157:H7 colitis associated with consumption of precooked meat patties. J Infect Dis. 1991;164:338-43.
11. **Carter AO, Borczyk AA, Carlson JA, Harvey B, Hockin JC, Karmali MA, et al.** A severe outbreak of *Escherichia coli* O157:H7–associated hemorrhagic colitis in a nursing home. N Engl J Med. 1987;316:1496-500.
12. **Dev VJ, Main M, Gould I.** Waterborne outbreak of *Escherichia coli* O157 [Letter]. Lancet. 1991;337:1412.
13. **Duncan L, Mai V, Carter A, Carlson JA, Borczyk AA, Karmali MA.** Outbreak of gastrointestinal disease in Sarnia, Ontario. Ontario Disease Surveillance Report. 1986;7:604-11.
14. **Centers for Disease Control.** Foodborne outbreak of gastroenteritis caused by *Escherichia coli* O157:H7–North Dakota, 1990. MMWR Morb Mortal Wkly Rep. 1991;40:265-7.
15. **Laboratory Center of Disease Control.** Hemorrhagic colitis in a home

for the aged—Ontario. Canada Diseases Weekly Report. 1983;9:29-32.
16. **Ostroff SM, Griffin PM, Tauxe RV, Shipman LD, Greene KD, Wells JG, et al.** A statewide outbreak of *Escherichia coli* O157:H7 infections in Washington State. Am J Epidemiol. 1990;132:239-47.
17. **Laboratory Center of Disease Control.** Outbreak of gastrointestinal disease—Ontario. Canada Diseases Weekly Report. 1987;13:5-8.
18. **Pavia AT, Nichols CR, Green DP, Tauxe RV, Mottice S, Greene KD, et al.** Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. J Pediatr. 1990;116:544-51.
19. **Ryan CA, Tauxe RV, Hosek GW, Wells JG, Stoesz PA, McFadden HW Jr, et al.** *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical, epidemiological, and pathological findings. J Infect Dis. 1986; 154:631-8.
20. **Spika JS, Parsons JE, Nordenberg D, Wells JG, Gunn RA, Blake PA.** Hemolytic uremic syndrome and diarrhea associated with *Escherichia coli* O157:H7 in a day care center. J Pediatr. 1986;109:287-91.
21. **Rowe PC, Walop W, Lior H, Mackenzie AM.** Haemolytic anaemia after childhood *Escherichia coli* O157:H7 infection: are females at increased risk? Epidemiol Infect. 1991;106:523-30.
22. **Rowe PC, Orrbine E, Wells GA, McLaine PN.** Epidemiology of hemolytic-uremic syndrome in Canadian children from 1986 to 1988. The Canadian Pediatric Kidney Disease Reference Centre. J Pediatr. 1991;119:218-24.
23. **Lior H.** Incidence of hemorrhagic colitis due to *Escherichia coli* in Canada. Can Med Assoc J. 1988;139:1073-4.
24. **Swerdlow DL, Woodruff BA, Brady RC, Griffin PM, Tippen S, Donnell HD Jr, et al.** A Waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann Intern Med. 1992;116:812-9.
25. **Cahoon FE, Thompson JS.** Frequency of *Escherichia coli* O157:H7 isolation from stool specimens. Can J Microbiol. 1987;33:914-5.
26. **Gransden WR, Damm MA, Anderson JD, Carter JE, Lior H.** Further evidence associating hemolytic–uremic syndrome with infection by Verotoxin-producing *Escherichia coli* O157:H7. J Infect Dis. 1986; 154:522-4.
27. **Johnson WM, Lior H, Bezanson GS.** Cytotoxic *Escherichia coli* O157:H7 associated with haemorrhagic colitis in Canada [Letter]. Lancet. 1983;1:76.
28. **Noda M, Yutsudo T, Nakabayashi N, Hirayama T, Takeda Y.** Purification and some properties of Shiga-like toxin from *Escherichia coli* O157:H7 that is immunologically identical to Shiga toxin. Microb Pathog. 1987;2:339-49.
29. **O'Brien AD, Lively TA, Chen ME, Rothman SW, Formal SB.** *Escherichia coli* O157:H7 strains associated with haemorrhagic colitis in the United States produce a *Shigella dysenteriae* 1 (SHIGA) like cytotoxin [Letter]. Lancet. 1983;1:702.
30. **O'Brien AD, Lively TA, Chang TW, Gorbach SL.** Purification of *Shigella dysenteriae* 1 (Shiga)-like toxin from *Escherichia coli* O157:H7 strain associated with haemorrhagic colitis [Letter]. Lancet. 1983;2: 573.
31. **Padhye VV, Beery JT, Kittell FB, Doyle MP.** Colonic hemorrhage produced in mice by a unique vero cell cytotoxin from an *Escherichia coli* strain that causes hemorrhagic colitis. J Infect Dis. 1987;155: 1249-53.
32. **Padhye VV, Kittell FB, Doyle MP.** Purification and physicochemical properties of a unique Vero cell cytotoxin from *Escherichia coli* O157:H7. Biochem Biophys Res Commun. 1986;139:424-30.
33. **Scotland SM, Smith HR, Rowe B.** Two distinct toxins active on Vero cells from *Escherichia coli* O157 [Letter]. Lancet. 1985;2:885-6.
34. **Strockbine NA, Marques LR, Newland JW, Smith HW, Holmes RK, O'Brien AD.** Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. Infect Immun. 1986;53:135-40.
35. **Levine MM.** *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J Infect Dis. 1987;155:377-89.
36. **von Wulffen H, Russman H, Karch H, Meyer T, Bitzan M, Kohrt TC, et al.** Verocytotoxin-producing *Escherichia coli* O2:H5 isolated from patients with ulcerative colitis [Letter]. Lancet. 1989;1:1449-50.
37. **Griffin PM, Ostroff SM, Tauxe RV, Greene KD, Wells JG, Lewis JH, et al.** Illnesses associated with *Escherichia coli* O157:H7 infections. A broad clinical spectrum. Ann Intern Med. 1988;109:705-12.
38. **Hamano S, Nakanishi Y, Nara T, Seki T, Ohtani T, Oishi T, et al.** Neurological manifestations of hemorrhagic colitis in the outbreak of *Escherichia coli* O157:H7 infection in Japan. Acta Paediatr. 1993;82: 454-8.
39. **Kovacs MJ, Roddy J, Gregoire S, Cameron W, Eidus L, Drouin J.** Thrombotic thrombocytopenic purpura following hemorrhagic colitis due to *Escherichia coli* O157:H7. Am J Med. 1990;88:177-9.
40. **Morrison DM, Tyrrell DL, Jewell LD.** Colonic biopsy in verotoxin-induced hemorrhagic colitis and thrombotic thrombocytopenic purpura (TTP). Am J Clin Pathol. 1986;86:108-12.
41. **Centers for Disease Control.** Thrombotic thrombocytopenic purpura associated with *Escherichia coli* O157:H7–Washington. MMWR Morb Mortal Wkly Rep. 1986;35:549-51.
42. **Riley LW.** The epidemiologic, clinical, and microbiologic features of hemorrhagic colitis. Annu Rev Microbiol. 1987;41:383-407.
43. **Miller WT, De Poto DW, Scholl HW, Raffensperger EC.** Evanescent colitis in the young adult: a new entity? Radiology. 1971;100:71-8.
44. **Edelman R, Karmali MA, Fleming PA.** From the National Institutes of Health. Summary of the International Symposium and Workshop on Infections due to Verocytotoxin (Shiga-like toxin)-producing *Escherichia coli*. J Infect Dis. 1988;157:1102-4.
45. **Belongia EA, Osterholm MT, Soler JT, Ammend DA, Braun JE, MacDonald KL.** Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. JAMA. 1993;269:883-8.
46. **Fong JS, de Chadarevian JP, Kaplan BS.** Hemolytic-uremic syndrome. Current concepts and management. Pediatr Clin North Am. 1982;29:835-56.
47. **Kaplan BS, Proesmans W.** The hemolytic-uremic syndrome of childhood and its variants. Semin Hematol. 1987;24:148-60.
48. **Pickering LK, Obrig TG, Stapleton FB.** Hemolytic-uremic syndrome and enterohemorrhagic *Escherichia coli*. Pediatr Infect Dis J. 1994;13: 459-75;quiz 476.
49. **Levin M, Walters MD, Barratt TM.** Hemolytic uremic syndrome. Adv Pediatr Infect Dis. 1989;4:51-81.
50. **Karmali MA.** Infection by verocytotoxin-producing *Escherichia coli*. Clin Microbiol Rev. 1989;2:15-38.
51. **Whitington PF, Friedman AL, Chesney RW.** Gastrointestinal disease in the hemolytic-uremic syndrome. Gastroenterology. 1979;76:728-33.
52. **Martin DL, MacDonald KL, White KE, Soler JT, Osterholm MT.** The epidemiology and clinical aspects of the hemolytic–uremic syndrome in Minnesota. N Engl J Med. 1990;323:1161-7.
53. **Bar-Ziv J, Ayoub JI, Fletcher BD.** Hemolytic uremic syndrome: a case presenting with acute colitis. Pediatr Radiol. 1974;2:203-6.
54. **Berman W Jr.** The hemolytic-uremic syndrome: initial clinical presentation mimicking ulcerative colitis. J Pediatr. 1972;81:275-8.
55. **Peterson RB, Meseroll WP, Shrago GG, Gooding CA.** Radiographic features of colitis associated with the hemolytic-uremic syndrome. Radiology. 1976;118:667-71.
56. **Sawaf H, Sharp MJ, Youn KJ, Jewell PA, Rabbani A.** Ischemic colitis and stricture after hemolytic-uremic syndrome. Pediatrics. 1978;61: 315-7.
57. **Schwartz DL, Becker JM, So HB, Schneider KM.** Segmental colonic gangrene: a surgical emergency in the hemolytic-uremic syndrome. Pediatrics. 1978;62:54-6.
58. **Cimolai N, Morrison BJ, Carter JE.** Risk factors for the central nervous system manifestations of gastroenteritis-associated hemolytic-uremic syndrome. Pediatrics. 1992;90:616-21.
59. **Bale JF Jr, Brasher C, Siegler RL.** CNS manifestations of the hemolytic-uremic syndrome. Relationship to metabolic alterations and prognosis. Am J Dis Child. 1980;134:869-72.
60. **Gianantonio CA, Vitacco M, Mendilaharzu F, Gallo GE, Sojo ET.** The hemolytic-uremic syndrome. Nephron. 1973;11:174-92.
61. **Schlieper A, Rowe PC, Orrbine E, Zoubek M, Clark W, Wolfish N, et al.** Sequalae of haemolytic uraemic syndrome. Arch Dis Child. 1992; 67:930-4.
62. **Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H.** The association between idiopathic hemolytic–uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J Infect Dis. 1985; 151:775-82.
63. **Neill MA, Tarr PI, Clausen CR, Christie DL, Hickman RO.** *Escherichia coli* O157:H7 as the predominant pathogen associated with the hemolytic–uremic syndrome: a prospective study in the Pacific Northwest. Pediatrics. 1987;80:37-40.
64. **Kleanthous H, Smith HR, Scotland SM, Gross RJ, Rowe B, Taylor CM, et al.** Haemolytic uraemic syndromes in the British Isles, 1985-8: association with verocytotoxin producing *Escherichia coli*. Part 2: Microbiological aspects. Arch Dis Child. 1990;65:722-7.
65. **Laboratory Centers for Disease Control.** Enterohemorrhagic *Escherichia coli* and the hemolytic–uremic syndrome–the Alberta experience. Canada Diseases Weekly Report. 1989;15:9-12.
66. **Neill MA, Tarr PI, Christie DL.** Clinical findings in hemorrhagic colitis with *Escherichia coli* O157:H7. Gastroenterology. 1987;92:1551.
67. **Scotland SM, Rowe B, Smith HR, Willshaw GA, Gross RJ.** Vero cytotoxin-producing strains of *Escherichia coli* from children with haemolytic uraemic syndrome and their detection by specific DNA probes. J Med Microbiol. 1988;25:237-43.
68. **Karmali MA, Steele BT, Petric M, Lim C.** Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet. 1983;1:619-20.
69. **Ish-Shalom N, Arbus GS, Karmali MA.** A family outbreak of hemolytic–uremic syndrome (hemolytic–uremic syndrome) affecting 5 siblings, associated with verotoxin (VT)-producing *Escherichia coli* (VTEC) O157:H7 [Abstract]. Pediatr Res. 1986;20:228A.
70. **Laboratory Center for Disease Control.** Hemorrhagic colitis and hemolytic–uremic syndrome caused by *Escherichia coli* O157:H7 in Canada. Canada Diseases Weekly Report. 1987;13:203-4.
71. **Griffin PM, Tauxe RV.** The epidemiology of infections caused by

Escherichia coli O157:H7, other enterohemorrhagic *Escherichia coli*, and the associated hemolytic–uremic syndrome. Epidemiol Rev. 1991;13:60-98.

72. **Lopez EL, Diaz M, Grinstein S, Devoto S, Mendilaharzu F, Murray BE, et al.** Hemolytic uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. J Infect Dis. 1989;160:469-75.
73. **Neill MA, Agosti J, Rosen H.** Hemorrhagic colitis with *Escherichia coli* O157:H7 preceding adult hemolytic–uremic syndrome. Arch Intern Med. 1985;145:2215-7.
74. **Tarr PI, Neill MA, Allen J, Siccardi CJ, Watkins SL, Hickman RO.** The increasing incidence of the hemolytic-uremic syndrome in King County, Washington: lack of evidence for ascertainment bias. Am J Epidemiol. 1989;129:582-6.
75. **Tarr PI, Neill MA, Clausen CR, Watkins SL, Christie DL, Hickman RO.** *Escherichia coli* O157:H7 and the hemolytic–uremic syndrome: importance of early cultures in establishing the etiology. J Infect Dis. 1990;162:553-6.
76. **Milford DV, Taylor CM, Guttridge B, Hall SM, Rowe B, Kleanthous H.** Haemolytic uraemic syndromes in the British Isles 1985-8: association with verocytotoxin producing *Escherichia coli*. Part 1: Clinical and epidemiological aspects. Arch Dis Child. 1990;65:716-21.
77. **Cimolai N, Carter JE, Morrison BJ, Anderson JD.** Risk factors for the progression of *Escherichia coli* O157:H7 enteritis to hemolytic-uremic syndrome. J Pediatr. 1990;116:589-92.
78. **Cimolai N, Carter JE.** Influence of antidiarrheal and antimicrobial medications on the hemorrhagic colitis associated with hemolytic-uremic syndrome. J Pediatr. 1990;116:676.
79. **Ashkenazi S, Cleary TG, Lopez E, Pickering LK.** Anticytotoxin-neutralizing antibodies in immune globulin preparations: potential use in hemolytic-uremic syndrome. J Pediatr. 1988;113:1008-14.
80. **Milford DV, Taylor CM, Rose PE, Roy TC, Rowe B.** Immunologic therapy for hemolytic–uremic syndrome [Letter]. J Pediatr. 1989;115: 502-4.
81. **Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM.** Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J Infect Dis. 1989;160:994-8.
82. **Kwaan HC.** Clinicopathologic features of thrombotic thrombocytopenic purpura. Semin Hematol. 1987;24:71-81.
83. **Ridolfi RL, Bell WR.** Thrombotic thrombocytopenic purpura. Report of 25 cases and review of the literature. Medicine (Baltimore). 1981; 60:413-28.
84. **Bell BP, Goldoft M, Griffin PM, Davis MA, Gordon DC, Tarr PI, et al.** A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic–uremic syndrome from hamburgers. JAMA. 1994;272:1349-53.
85. **Keene WE, McAnulty JM, Hoesly FC, Williams LP Jr, Hedberg K, Oxman GL, et al.** A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. N Engl J Med. 1994;331:579-84.
86. **Morgan GM, Newman C, Palmer SR, Allen JB, Shepherd W, Rampling AM, et al.** First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O157:H7 in the UK. Epidemiol Infect. 1988;101:83-91.
87. From the Centers for Disease Control and Prevention. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers—western United States, 1992-1993. JAMA. 1993;269:2194-6.
88. **Besser RE, Lett SM, Weber JT, Doyle MP, Barrett TJ, Wells JG, et al.** An outbreak of diarrhea and hemolytic–uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. JAMA. 1993; 269:2217-20.
89. **Smith HR, Rowe B, Gross RJ, Fry NK, Scotland SM.** Hemorrhagic colitis and Vero-cytotoxin-producing *Escherichia coli* in England and Wales. Lancet. 1987;1:1062-4.
90. **Albert MJ, Bettelheim KA.** Enterohaemorrhagic *E. coli* O157:H7 in central Australia [Letter]. J Diarrhoeal Dis Res. 1989;7:96-7.
91. **Browning NG, Botha JR, Sacho H, Moore PJ.** *Escherichia coli* O157:H7 haemorrhagic colitis. Report of the first South African case. S Afr J Surg. 1990;28:28-9.
92. **Caprioli A, Luzzi I, Rosmini F, Pasquini P, Cirrincione R, Gianviti A, et al.** Hemolytic-uremic syndrome and Vero cytotoxin-producing *Escherichia coli* infection in Italy. The HUS Italian Study Group. J Infect Dis. 1992;166:154-8.
93. **Sramkova L, Bielaszewska M, Janda J, Blahova K, Hausner O.** Vero cytotoxin-producing strains of *Escherichia coli* in children with haemolytic uraemic syndrome and diarrhoea in Czechoslovakia. Infection. 1990;18:204-9.
94. **Cordovez A, Prado V, Maggi L, Cordero J, Martinez J, Misraji A, et al.** Enterohemorrhagic *Escherichia coli* associated with hemolytic-uremic syndrome in Chilean children. J Clin Microbiol. 1992;30: 2153-7.
95. **Ostroff SM, Hopkins DP, Tauxe RV, Sowers EG, Strockbine NK.** Surveillance of *Escherichia coli* O157 isolation and confirmation, United States, 1988. MMWR CDC Surveill Summ. 1991;40:1-6.
96. **Outbreak of bloody diarrhea caused by** *Escherichia coli* O157:H7. Wisconsin Epidemiology Bulletin. 1988;10:1-2.
97. **Martin ML, Shipman LD, Wells JG, Potter ME, Hedberg K, Wachsmuth IK, et al.** Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome [Letter]. Lancet. 1986;2:1043.
98. **Wells JG, Shipman LD, Greene KD, Sowers EG, Green JH, Cameron DN, et al.** Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J Clin Microbiol. 1991;29:985-9.
99. **Doyle MP, Schoeni JL.** Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl Environ Microbiol. 1987;53: 2394-6.
100. **Ratnam S, March SB, Sprague WD.** Are humans a source of *Escherichia coli* O157:H7, the agent of hemorrhagic colitis? [Letter] N Engl J Med. 1986;315:1612-3.
101. **Karmali MA, Arbus GS, Petric M, Patrick ML, Roscoe M, Shaw J, et al.** Hospital-acquired *Escherichia coli* O157:H7 associated haemolytic uraemic syndrome in a nurse [Letter]. Lancet. 1988;1:526.
102. **Yannelli B, Domenico P, Cunha BA, Quadri SM.** Nosocomial *Escherichia coli* O157:H7 diarrhea [Letter]. Am J Infect Control. 1990;18: 341-2.
103. **Griffin PM, Olmstead LC, Petras RE.** *Escherichia coli* O157:H7-associated colitis. A clinical and histological study of 11 cases. Gastroenterology. 1990;99:142-9.
104. **Kelly J, Oryshak A, Wenetsek M, Grabiec J, Handy S.** The colonic pathology of *Escherichia coli* O157:H7 infection. Am J Surg Pathol. 1990;14:87-92.
105. **Richardson SE, Karmali MA, Becker LE, Smith CR.** The histopathology of the hemolytic–uremic syndrome associated with verocytotoxin-producing *Escherichia coli* infections. Hum Pathol. 1988;19: 1102-8.
106. **Dalal BI, Krishnan C, Laschuk B, Duff JH.** Sporadic hemorrhagic colitis associated with *Escherichia coli*, type O157:H7: unusual presentation mimicking ischemic colitis. Can J Surg. 1987;30:207-8.
107. **Brandt LJ, Su C, Alt E, Sigal S, Patterson K, Tarr PI.** Immunoperoxidase tissue staining to diagnose *E. coli* O157:H7 infection: a cause of ischemic colitis [Abstract]. Gastroenterology. 1995; 108:A787.
108. **Scotland SM, Willshaw GA, Smith HR, Rowe B.** Properties of strains of *Escherichia coli* belonging to serogroup O157 with special reference to production of Vero cytotoxins VT1 and VT2. Epidem Infect. 1987;99:613-24.
109. **Konowalchuk J, Speirs JI, Stavric S.** Vero response to a cytotoxin of *Escherichia coli*. Infect Immun. 1977;18:775-9.
110. **Lingwood CA, Law H, Richardson S, Petric M, Brunton JL, De Grandis S, et al.** Glycolipid binding of purified and recombinant *Escherichia coli* produced verotoxin in vitro. J Biol Chem. 1987;262: 8834-9.
111. **Waddell T, Head S, Petric M, Cohen A, Lingwood C.** Globotriosyl ceramide is specifically recognized by the *Escherichia coli* verocytotoxin 2. Biochem Biophys Res Commun. 1988;152:674-9.
112. **Boyd B, Lingwood C.** Verotoxin receptor glycolipid in human renal tissue. Nephron. 1989;51:207-10.
113. **Cohen A, Hannigan GE, Williams BR, Lingwood CA.** Roles of globotriosyl- and galabiosylceramide in verotoxin binding and high affinity interferon receptor. J Biol Chem. 1987;262:17088-91.
114. **Keenan KP, Sharpnack DD, Collins H, Formal SB, O'Brien AD.** Morphologic evaluation of the effects of Shiga toxin and *E coli* Shiga-like toxin on the rabbit intestine. Am J Pathol. 1986;125:69-80.
115. **Wade WG, Thom BT, Evans N.** Cytotoxic enteropathogenic *Escherichia coli*. Lancet. 1979;2:1235-6.
116. **Strockbine NA, Jackson MP, Sung LM, Holmes RK, O'Brien AD.** Cloning and sequencing of the genes for Shiga toxin from *Shigella dysenteriae* type 1. J Bacteriol. 1988;160:1116-22.
117. **O'Brien AD, LaVeck GD.** Purification and characterization of a *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. Infect Immun. 1983;40:675-83.
118. **O'Brien AD, LaVeck GD, Thompson MR, Formal SB.** Production of *Shigella dysenteriae* type 1-like cytotoxin by *Escherichia coli*. J Infect Dis. 1982;146:763-9.
119. **Takao T, Tanabe T, Hong YM, Shimonishi Y, Kurazono H, Yutsudo T, et al.** Identity of molecular structure of Shiga-like toxin (VT1) from *Escherichia coli* O157:H7 with that of Shiga toxin. Microb Pathog. 1988;5:57-69.
120. **Calderwood SB, Auclair F, Donohue-Rolfe A, Keusch GT, Mekalanos JJ.** Nucleotide sequence of the Shiga-like toxin genes of *Escherichia coli*. Proc Natl Acad Sci U S A. 1987;84:4364-8.
121. **Jackson MP, Newland JW, Holmes RK, O'Brien AD.** Nucleotide sequence analysis of the structural genes for Shiga-like toxin I encoded by bacteriophage 933J from *Escherichia coli*. Microb Pathog. 1987;2:147-53.
122. **Ito H, Terai A, Kurazono H, Takeda Y, Nishibuchi M.** Cloning and nucleotide sequencing of Vero toxin 2 variant genes from *Escherichia coli* O91:H21 isolated from a patient with hemolytic–uremic syndrome. Microb Pathog. 1990;8:47-60.
123. **Jackson MP, Neill RJ, O'Brien AD, Holmes RK, Newland JW.** Nucleotide sequence analysis and comparison of the structural genes for

Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophage from *Escherichia coli* 933. FEMS Microbiol Lett. 1987;44:109-14.

124. **Gyles CL.** *Escherichia coli* cytotoxins and enterotoxins. Can J Microbiol. 1992;38:734-46.
125. **Endo Y, Tsurugi K, Yutsudo T, Takeda Y, Ogasawara T, Igarashi K.** Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and Shiga toxin on eukaryotic ribosomes. RNA N-glycosidase activity of the toxins. Eur J Biochem. 1988;161:45-50.
126. **Furutani M, Ito K, Oku Y, Takeda Y, Igarashi K.** Demonstration of RNA N-glycosidase activity of a Vero toxin (VT2 variant) produced by *Escherichia coli* O91:H21 from a patient with the hemolytic–uremic syndrome. Microbiol Immunol. 1990;34:387-92.
127. **Igarashi K, Ogasawara T, Ito K, Yutsudo T, Takeda Y.** Inhibition of elongation factor 1-dependent aminoacyl-tRNA binding to ribosomes by Shiga-like toxin 1 (VT1) from *Escherichia coli* O157:H7 and by Shiga toxin. FEMS Microbiol Lett. 1987;44:91-4.
128. **Ogasawara T, Ito K, Igarashi K, Yutsudo T, Nakabayashi N, Takeda Y.** Inhibition of protein synthesis by a Vero toxin (VT2 or Shiga-like toxin II) produced by *Escherichia coli* O157:H7 at the level of elongation factor 1-dependent aminoacyl-tRNA binding to ribosomes. Microb Pathog. 1988;4:127-35.
129. **O'Brien AD, Holmes RK.** Shiga and Shiga-like toxins. Microbiol Rev. 1987;51:206-20.
130. **O'Brien AD, Newland JW, Miller SF Holmes RK.** Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science. 1984;226:694-6.
131. **Scotland SM, Smith HR, Willshaw GA, Rowe B.** Vero cytotoxin production in strain of *Escherichia coli* is determined by genes carried on bacteriophage [Letter]. Lancet. 1983;2:216.
132. **Smith HR, Day NP, Scotland SM, Gross RJ, Rowe B.** Phage-determined production of vero cytotoxin in strains of *Escherichia coli* serogroup O157 [Letter]. Lancet. 1984;1:1242-3.
133. **Smith HW, Green P, Parsell Z.** Vero cell toxins in *Escherichia coli* and related bacteria: transfer by phage and conjugation and toxic action in laboratory animals, chickens and pigs. J Gen Microbiol. 1983;129:3121-37.
134. **Garch H, Meyer T, Russmann H, Heesemann J.** Frequent loss of Shiga-like toxin genes in clinical isolates of *Escherichia coli* upon subcultivation. Infect Immun. 1992;60:3464-7.
135. **Beery JT, Doyle MP, Higley NA.** Cytotoxic activity of *Escherichia coli* O157:H7 culture filtrate on the mouse colon and kidney. Current Microbiology. 1983;11:335-42.
136. **Beery JT, Doyle MP, Schoeni JL.** Colonization of chicken cecae by *Escherichia coli* associated with hemorrhagic colitis. Appl Environ Microbiol. 1985;49:310-5.
137. **Farmer JJ 3d, Potter ME, Riley LW, Barrett TJ, Blake PA, Bopp CA, et al.** Animal models to study *Escherichia coli* O157:H7 isolated from patients with haemorrhagic colitis [Letter]. Lancet. 1983;1:702-3.
138. **Francis DH, Collins JE, Duimstra JR.** Infection of gnotobiotic pigs with an *Escherichia coli* O157:H7 strain associated with an outbreak of hemorrhagic colitis. Infect Immun. 1986;51:953-6.
139. **Pai CH, Kelly JK, Meyers GL.** Experimental infection of infant rabbits with verotoxin-producing *Escherichia coli*. Infect Immun. 1986;51:16-23.
140. **Potter ME, Kaufmann AF, Thomason BM, Blake PA, Farmer JJ 3d.** Diarrhea due to *Escherichia coli* O157:H7 in the infant rabbit. J Infect Dis. 1985;152:1341-3.
141. **Tzipori S, Wachsmuth IK, Chapman C, Birden R, Brittingham J, Jackson C, et al.** The pathogenesis of hemorrhagic colitis caused by *Escherichia coli* O157:H7 in gnotobiotic piglets. J Infect Dis. 1986; 154:712-6.
142. **Karmali MA, Petric M, Lim C, Fleming PC, Steele BT.** *Escherichia coli* cytotoxin, haemolytic-uraemic syndrome, and haemorrhagic colitis [Letter]. Lancet. 1983;2:1299-300.
143. **Marques LR, Moore MA, Wells JG, Wachsmuth IK, O'Brien AD.** Production of Shiga-like toxin by *Escherichia coli*. J Infect Dis. 1986; 154:338-41.
144. **Obrig TG, Del Vecchio PJ, Karmali MA, Petric M, Moran TP, Judge TK.** Pathogenesis of haemolytic uraemic syndrome [Letter]. Lancet. 1987;2:687.
145. **Richardson SE, Rotman TA, Jay V, Smith CR, Becker LE, Petric M, et al.** Experimental verocytotoxemia in rabbits. Infect Immun. 1992; 60:4154-67.
146. **Karch H, Bitzan M, Pietsch R, Stenger KO, von Wulffen H, Heesemann J, et al.** Purified verotoxins of *Escherichia coli* O157:H7 decrease prostacyclin synthesis by endothelial cells. Microb Pathog. 1988;5:215-21.
147. **Rose PE, Armour JA, Williams CE, Hill FG.** Verotoxin and neuraminidase induced platelet aggregating activity in plasma: their possible role in the pathogenesis of haemolytic uraemic syndrome. J Clin Pathol. 1985;38:438-41.
148. **Dykstra SA, Moxley RA, Janke BH, Nelson EA, Francis DH.** Clinical signs and lesions in gnotobiotic pigs inoculated with Shiga-like toxin I from *Escherichia coli*. Vet Pathol. 1993;30:410-7.
149. **Karch H, Heesemann J, Laufs R, O'Brien AD, Tacket CO, Levine MM.** A plasmid of enterohemorrhagic *Escherichia coli* O157:H7 is required for expression of a new fimbrial antigen and for adhesion to epithelial cells. Infect Immun. 1987;55:455-61.
150. **Sherman P, Soni R, Petric M, Karmali M.** Surface properties of the Vero cytotoxin-producing *Escherichia coli* O157:H7. Infect Immun. 1987;55:1824-9.
151. **Sherman PM, Soni R.** Adherence of Vero cytotoxin-producing *Escherichia coli* of serotype O157:H7 to human epithelial cells in tissue culture: role of outer membranes as bacterial adhesins. J Med Microbiol. 1988;26:11-7.
152. **Toth I, Cohen ML, Rumschlag HS, Riley LW, White EH, Carr JH, et al.** Influence of the 60-megadalton plasmid on adherence of *Escherichia coli* O157:H7 and genetic derivatives. Infect Immun. 1990;58: 1223-31.
153. **Tzipori S, Karch H, Wachsmuth KI, Robins-Browne RM, O'Brien AD, Lior H, et al.** Role of a 60-megadalton plasmid and Shiga-like toxins in the pathogenesis of infection caused by enterohemorrhagic *Escherichia coli* O157:H7 in gnotobiotic piglets. Infect Immun. 1987; 55:3117-25.
154. **Wells JG, Davis BR, Wachsmuth IK, Riley LW, Remis RS, Sokolow R, et al.** Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J Clin Microbiol. 1983;18:512-20.
155. **Farmer JJ 3d, Davis BR.** H7 antiserum-sorbitol fermentation medium: a single tube screening medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J Clin Microbiol. 1985; 22:620-5.
156. **March SB, Ratnam S.** Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J Clin Microbiol. 1986;23:869-72.
157. **Doyle MP, Schoeni JL.** Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl Environ Microbiol. 1984;48:855-6.
158. **Haldane DJ, Damm MA, Anderson JD.** Improved biochemical screening procedure for small clinical laboratories for vero (Shiga-like-)-toxin-producing strains of *Escherichia coli* O157:H7. J Clin Microbiol. 1986;24:652-3.
159. **Ratnam S, March SB, Ahmed R, Bezanson GS, Kasatiya S.** Characterization of *Escherichia coli* serotype O157:H7. J Clin Microbiol. 1988;26:2006-12.
160. **Thompson JS, Hodge DS, Borczyk AA.** Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157. J Clin Microbiol. 1990;28:2165-8.
161. **Gunzer F, Bohm H, Russman H, Bitzan M, Aleksic S, Karch H.** Molecular detection of sorbitol-fermenting *Escherichia coli* O157 in patients with hemolytic-uremic syndrome. J Clin Microbiol. 1992;30: 1807-10.
162. **Krishnan C, Fitzgerald VA, Dakin SJ, Behme RJ.** Laboratory investigation of outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7. J Clin Microbiol. 1987;25:1043-7.
163. **Chapman PA.** Evaluation of commercial latex slide test for identifying *Escherichia coli* O157. J Clin Pathol. 1989;42:1109-10.
164. **March SB, Ratnam S.** Latex agglutination test for detection of *Escherichia coli* serotype O157. J Clin Microbiol. 1989;27:1675-7.
165. **Tison DL.** Culture confirmation of *Escherichia coli* serotype O157:H7 by direct immunofluorescence. J Clin Microbiol. 1990;28:612-3.
166. **Downes FP, Barrett TJ, Green JH, Aloisio CH, Spika JS, Strockbine NA, et al.** Affinity purification and characterization of Shiga-like toxin II and production of toxin-specific monoclonal antibodies. Infect Immun. 1988;56:1926-33.
167. **Karmali MA, Petric M, Lim C, Cheung R, Arbus GS.** Sensitive method for detecting low numbers of verotoxin-producing *Escherichia coli* in mixed cultures by use of colony sweeps and polymyxin extraction of verotoxin. J Clin Microbiol. 1985;22:614-9.
168. **Strockbine NA, Marques LR, Holmes RK, O'Brien AD.** Characterization of monoclonal antibodies against Shiga-like toxin from *Escherichia coli*. Infect Immun. 1985;50:695-700.
169. **Brown JE, Sethabutr O, Jackson MP, Lolekha S, Echeverria P.** Hybridization of *Escherichia coli* producing Shiga-like toxin I, Shiga-like toxin II, and a variant of Shiga-like toxin II with synthetic oligonucleotide probes. Infect Immun. 1989;57:2811-4.
170. **Newland JW, Neill RJ.** DNA probes for Shiga-like toxins I and II and for toxin-converting bacteriophages. J Clin Microbiol. 1988;26:1292-7.
171. **Karch H, Meyer T.** Evaluation of oligonucleotide probes for identification of Shiga-like-toxin-producing *Escherichia coli*. J Clin Microbiol. 1989;27:1180-6.
172. **Seriwatana J, Brown JE, Echeverria P, Taylor DN, Suthienkul O, Newland J.** DNA probes to identify Shiga-like toxin I- and II-producing enteric bacterial pathogens isolated from patients with diarrhea in Thailand. J Clin Microbiol. 1988;26:1614-5.
173. **Willshaw GA, Smith HR, Scotland SM, Field AM, Rowe B.** Heterogeneity of *Escherichia coli* phages encoding Vero cytotoxins: comparison of cloned sequences determining VT1 and VT2 and development of specific gene probes. J Gen Microbiol. 1987;133:1309-17.
174. **Acheson DW, Keusch GT, Lightowlers M, Donohue-Rolfe A.** Enzyme-linked immunosorbent assay for Shiga toxin and Shiga-like toxin II using P1 glycoprotein from hydatid cysts. J Infect Dis. 1990;161:134-7.

175. **Ashkenazi S, Cleary TG.** A method for detecting Shiga toxin and Shiga-like toxin-I in pure and mixed culture. J Med Microbiol. 1990; 32:255-61.
176. **Downes FP, Green JH, Greene K, Strockbine N, Wells JG, Wachsmuth IK.** Development and evaluation of enzyme-linked immunosorbent assays for detection of shiga-like toxin I and shiga-like toxin II. J Clin Microbiol. 1989;27:1292-7.
177. **Perera LP, Marques LR, O'Brien AD.** Isolation and characterization of monoclonal antibodies to Shiga-like toxin II of enterohemorrhagic *Escherichia coli* and use of the monoclonal antibodies in a colony enzyme-linked immunosorbent assay. J Clin Microbiol. 1988;26:2127-31.
178. **Ashkenazi S, Cleary TG.** Rapid method to detect shiga toxin and shiga-like toxin 1 based on binding to globotriosyl ceramide (Gb_3), their natural receptor. J Clin Microbiol. 1989;27:1145-50.
179. **Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR.** Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J Clin Microbiol. 1990;28:540-5.
180. **Brian MJ, Frosolono M, Murray BE, Miranda A, Lopez EL, Gomez HF, et al.** Polymerase chain reaction for diagnosis of enterohemorrhagic *Escherichia coli* infection and hemolytic-uremic syndrome. J Clin Microbiol. 1992;30:1801-6.
181. **Paton AW, Paton JC, Goldwater PN, Manning PA.** Direct detection of *Escherichia coli* Shiga-like toxin genes in primary fecal cultures by polymerase chain reaction. J Clin Microbiol. 1993;31:3063-7.
182. **Karch H, Meyer T.** Single primer pair for amplifying segments of distinct Shiga-like-toxin genes by polymerase chain reaction. J Clin Microbiol. 1989;27:2751-7.
183. **Tarr PI, Neill MA, Clausen CR, Newland JW, Neill RJ, Moseley SL.** Genotypic variation in pathogenic *Escherichia coli* O157:H7 isolated from patients in Washington, 1984-1987. J Infect Dis. 1989;159:344-7.
184. **Thomas A, Smith HR, Rowe B.** Use of digoxigenin-labelled oligonucleotide DNA probes for VT2 and VT2 human variant genes to differentiate Vero cytotoxin-producing *Escherichia coli* strains of serogroup O157. J Clin Microbiol. 1993;31:1700-3.
185. **Paros M, Tarr PI, Kim H, Besser TE, Hancock DD.** A comparison of human and bovine *Escherichia coli* O157:H7 isolates by toxin genotype, plasmid profile, and bacteriophage lambda-restriction fragment length polymorphism profile. J Infect Dis. 1993;168:1300-3.
186. **Ahmed R, Bopp C, Borczyk A, Kasatiya S.** Phage-typing scheme for *Escherichia coli* O157:H7. J Infect Dis. 1987;155:806-9.
187. **Khakhria R, Duck D, Lior H.** Extended phage-typing scheme for *Escherichia coli* O157:H7. Epidemiol Infect. 1990;105:511-20.
188. **Samadpour M, Grimm LM, Desai B, Alfi D, Ongerth JE, Tarr PI.** Molecular epidemiology of *Escherichia coli* O157:H7 strains by bacteriophage lambda restriction fragment length polymorphism analysis: application to a multistate food-borne outbreak and a day-care center cluster. J Clin Microbiol. 1993;31:3179-83.
189. **Whittam TS, Wachsmuth IK, Wilson RA.** Genetic evidence of clonal descent of *Escherichia coli* O157:H7 associated with hemorrhagic colitis and hemolytic-uremic syndrome. J Infect Dis. 1988;157:1124-33.
190. **Bohm H, Karch H.** DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulsed-field gel electrophoresis. J Clin Microbiol. 1992;30: 2169-72.
191. **Chart H, Scotland SM, Smith HR, Rowe B.** Antibodies to *Escherichia coli* O157 in patients with hemorrhagic colitis and haemolytic uraemic syndrome. J Clin Pathol. 1989;42:973-6.
192. **Chart H, Smith HR, Scotland SM, Rowe B, Milford DV, Taylor CM.** Serological identification of *Escherichia coli* O157:H7 infection in haemolytic uraemic syndrome. Lancet. 1991;337:138-40.
193. **Padhye NV, Doyle MP.** Production and characterization of a monoclonal antibody specific for enterohemorrhagic *Escherichia coli* serotypes O157:H7 and O26:H11. J Clin Microbiol. 1991;29:99-103.
194. **Yamada S, Matsushita S, Kai A, Sasaki M, Tsuji A, Kanemitsu T, et al.** Detection of verocytotoxin from stool and serological testing of patients with diarrhea caused by *Escherichia coli* O157:H7. Microbiol Immunol. 1993;37:111-8.
195. **Bitzan M, Moebius E, Ludwig K, Muller-Wiefel DE, Heesemann J, Karch H.** High incidence of serum antibodies to *Escherichia coli* O157 lipopolysaccharide in children with hemolytic-uremic syndrome. J Pediatr. 1991;119:380-5.
196. **Tarr PI, Neill MA, Christie DL, Anderson DE.** *Escherichia coli* O157:H7 hemorrhagic colitis [Letter]. N Engl J Med. 1988;318:1697.
197. **Kim HH, Samadpour M, Grimm L, Clausen CR, Besser TE, Baylor M, et al.** Characteristics of antibiotic-resistant *Escherichia coli* O157:H7 in Washington State, 1984-1991. J Infect Dis. 1994;170:1606-9.
198. **Karch H, Strockbine NA, O'Brien AD.** Growth of *Escherichia coli* in the presence of trimethoprim-sulfamethoxazole facilitates detection of Shiga-like toxin producing strains by colony blot assay. FEMS Microbiol Lett. 1986;35:141-5.
199. **Walterspiel JN, Ashkenazi S, Morrow AL, Cleary TG.** Effect of subinhibitory concentrations of antibiotics on extracellular Shiga-like toxin I. Infection. 1992;20:25-9.
200. **Proulx F, Turgeon JP, Delage G, Lafleur L, Chicoine L.** Randomized, controlled trial of antibiotic therapy for *Escherichia coli* O157:H7 enteritis. J Pediatr. 1992;121:299-303.
201. **Pichler HE, Diridl G, Stickler K, Wolf D.** Clinical efficacy of ciprofloxacin compared with placebo in bacterial diarrhea. Am J Med. 1987;82:329-32.
202. **MacDonald KL, Osterholm MT.** The Emergence of *Escherichia coli* O157:H7 infection in the United States. The changing epidemiology of food-borne disease [Editorial]. JAMA. 1993;269:2264-6.
203. **Line JE, Fain A Jr, Moran AB.** Lethality of heat to *Escherichia coli* O157:H7: D-value and Z-value determinations in ground beef. Journal of Food Protection. 1991;54:762-6.
204. **Dytoc M, Soni R, Cockerill F 3d, De Azavedo J, Louie M, Brunton J, et al.** Multiple determinants of verotoxin-producing *Escherichia coli* O157:H7 attachment-effacement. Infect Immun. 1993;61:3382-91.
205. **Sherman P, Cockerill F 3d, Soni R, Brunton J.** Outer membranes are competitive inhibitors of *Escherichia coli* O157:H7 adherence to epithelial cells. Infect Immun. 1991;59:890-9.
206. **Yu J, Kaper JB.** Cloning and characterization of the *eae* gene of enterohaemorrhagic *Escherichia coli* O157:H7. Mol Microbiol. 1992; 6:411-7.
207. **Louie M, de Azavedo JC, Handelsman MY, Clark CG, Ally B, Dytoc M, et al.** Expression and characterization of the *eaeA* gene product of *Escherichia coli* serotype O157:H7. Infect Immun. 1993;61:4085-92.
208. **Schmidt H, Russmann H, Karch H.** Virulence determinants in nontoxinogenic *Escherichia coli* O157 strains that cause infantile diarrhea. Infect Immun. 1993;61:4894-8.
209. **Fitzpatrick MM, Dillon MJ.** Current views on aetiology and management of haemolytic uraemic syndrome. Postgrad Med J. 1991;67: 707-9.
210. **Loirat C, Sonsino E, Hinglais N, Jais JP, Landais P, Fermanian J.** Treatment of the childhood haemolytic uraemic syndrome with plasma. A multicentre randomized controlled trial. The French Society of Paediatric Nephrology. Pediatr Nephrol. 1988;2:279-85.
211. **Ogborn MR, Crocker JF, Barnard DR.** Plasma therapy for severe hemolytic-uremic syndrome in children in Atlantic Canada. Can Med Assoc J. 1990;143:1323-6.
212. **Rizzoni G, Claris-Appiani A, Edefonti A, Facchin P, Franchini F, Gusmano R, et al.** Plama infusion for hemolytic-uremic syndrome in children: results of a multicenter controlled trial. J Pediatr. 1988;112: 284-90.
213. **Robson WL, Fick GH, Jadavji T, Leung AK.** The use of intravenous gammaglobulin in the treatment of typical hemolytic-uremic syndrome. Pediatr Nephrol. 1991;5:289-92.
214. **Siegler RL.** Management of hemolytic-uremic syndrome. J Pediatr. 1988;112:1014-20.
215. **Havens PL, Dunne WM, Burd EM.** Effects of human intravenous immune globulin on diarrhea caused by Shiga-like toxin I and Shiga-like toxin II in infant rabbits. Microbiol Immunol. 1992;36:1077-85.
216. **Voelker R.** New strategies aimed at *E* O157:H7. JAMA. 1994;272: 503.
217. **Sandvig K, Brown JE.** Ionic requirements for entry of Shiga toxin for *Shigella dysenteriae* 1 into cells. Infect Immun. 1987;55:298-303.

COMPANY NEWS; Jack in the Box's Worst Nightmare

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Correction Appended

Since the outbreak of food-poisoning from hamburger sold at Jack in the Box outlets here in mid-January left two children dead, the stock of the chain's parent company, Foodmaker Inc., has dropped more than 30 percent. The 60 Jack in the Box restaurants in the state have been barraged by anonymous telephone callers accusing them of being baby killers. Customers are scarce. And local newspapers have carried advertisements by lawyers offering to represent poisoning victims.

"In the last 10 years, we've sold 400 million pounds of hamburger safely and without incident," said Robert Nugent, president of Jack in the Box, the nation's fifth-largest hamburger chain, with 1,170 outlets in 13 states in the West. "Then bang, it hits you. It's your worst, worst nightmare."

Since Jan. 13, nearly 400 people in the Pacific Northwest and a handful in other Western states have been stricken with E. coli 0157:H7, a bacterium that can be fatal, particularly in children. Most of the cases have been linked to hamburgers sold at Jack in the Box restaurants. Tougher State Procedure

The finger pointing has been intense. The San Diego-based chain blamed its supplier, the Vons Companies of Arcadia, Calif., for supplying tainted meat and filed a lawsuit against Vons on Thursday. The meat was contaminated at the slaughterhouse, according to Washington State health officials; Jack in the Box acknowledges that its contract did not call for Vons to test the meat.

Meanwhile, the United States Agriculture Department and state health officials say the bacteria could have been killed if Jack in the Box had cooked the hamburgers at 155 degrees as required by the state, rather than at the Federal standard of 140 degrees. The company has disputed that argument and said it did not know the state increased the required cooking standard in May.

Analysts worry whether Jack in the Box will be able to recover. Public relations specialists say the chain had acted correctly in offering to cover the medical expenses of victims, in setting up a special telephone hotline, in making a generous contribution to help find a cure for the E. coli infection and in replacing Vons and letting the public know through an advertising campaign. But they agree that the company's initial reaction was damaging.

"Jack in the Box got off to a bad start because they first said they had no comment," said Michael Brennen, vice president of DeLauney Phillips Inc., a Seattle public relations firm that has followed the situation. "Then, they attempted to pass the blame to Vons. I would have advised them to step right up and accept responsibility. But they were acting from a legal standpoint of not wanting to accept the fault." Slow Sales and Lawsuits

Sales have been falling, though the company will not say by how much. Four lawsuits have been filed. And it is unclear how much Jack in the Box will have to pay to cover medical expenses or whether it will offer to pay expenses for victims of secondary transmission who become ill from contact with a primary carrier.

One New York stock analyst said that Washington represents only about 6 percent of Jack

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in the Box's cash flow, but what happens in California, the home of about half of the chain's outlets, will be the most significant factor.

At the least, the situation could stall the ambitious expansion of the 42-year-old company, which opened 63 restaurants last year and planned to open another 70 in 1993, including 12 in Washington.

"We may now have to adjust this," Mr. Nugent said. "Our overall business situation may be negatively impacted, which would impact cash flow and available capital for expansion."

Jack in the Box accounts for two-thirds of the $1.22 billion in 1992 sales of Foodmaker, which also owns Chi-Chi's Mexican food restaurants.

The crisis started on Jan. 13, when Children's Hospital, a pediatrics referral center in Seattle, alerted the Washington Health Department that its doctors were treating an unusually large number of children with E. coli infections.

Mr. Nugent said the company first became aware of the outbreak late on Friday, Jan. 15, when a member of his staff found an "F.Y.I." message from the health department pointing to the apparent link between the outbreak and Jack in the Box. An executive group met early the following Sunday and sent a team to Seattle to assess the situation.

Jack in the Box's name was not mentioned publicly until the afternoon of Jan. 18, when the health department said it was convinced that the illness was linked to the fast-food chain. Seven children were on kidney dialysis at Children's Hospital and the illnesses continued to mount.

On that Monday, Jack in the Box took down promotions for hamburgers and stopped selling them in Washington. By midday the next day, it replaced its patties chainwide with 28,000 pounds of new meat; burgers went on sale again. The chain said that while its previous cooking procedures met Federal regulations, it was switching to the state standard of 155 degrees.

As the number of people stricken continued to rise, 2-year-old Michael Nole of Tacoma died on Jan. 22. Shares of Foodmaker plunged, and the Securities and Exchange Commission suspended trading in the stock, reopening it the following week. Foodmaker lost 50 cents yesterday, closing at $9.50 on the New York Stock Exchange. It was trading at $13.625 on Jan. 18.

A second child has since died from an E. coli infection. Although he did not eat at Jack in the Box, secondary infection is a possibility.

In its investigation, the state said it had found contamination in 2 of 10 ground beef samples from Seattle-area restaurants. The most likely source, it said, was meat contaminated with feces at the time of slaughter. The Centers for Disease Control and Prevention in Atlanta is still trying to determine which of three slaughterhouses that furnished Vons with meat for the Jack in the Box account was the source.

Mr. Nugent has traced the contaminated hamburger to a Nov. 19 production run of 193 cases. Some 164 cases of frozen patties were shipped to the chain's Seattle distribution center. Of these, 100 cases were consumed and 64 recovered. The other 29 cases were shipped to the company's Commerce, Calif., distribution center, which supplies Southern California, Nevada and Hawaii. Only six were recovered and the company is still trying to trace the paths of the rest. The outbreak spread to Nevada, hitting four children who ate at the chain's restaurants there.

Health officials said tests indicated there was no mishandling or refrigeration problems in the processing or transportation of the beef to Jack in the Box distribution centers.

Stock in Vons, a $5.3 billion company that owns Vons Stores, the nation's ninth-largest supermarket chain, closed at $22.75 yesterday, down $2 on the Big Board.

Photo: The Jack in the Box restaurant in Tacoma, Wash., where a 2-year-old boy ate before

dying of food poisoning last month. The company, whose business and stock have been hurt, is trying to regain the public's trust. (Therese Frare for The New York Times) Graph shows daily closing stock prices for Foodmaker, Inc., parent of the Jack in the Box fast-food restaurant chain, Jan. 4 - Feb. 1, 1993. (Source: Datastream) (pg. 37)

Correction: February 7, 1993, Sunday Because of an editing error, an article in Business Day yesterday about the outbreak of food poisoning in the Northwest described one child's death incorrectly in one passage. Although the child died from an E. coli infection, the kind associated with the food-poisoning cases, the child did not eat at a Jack in the Box restaurant, and there is no direct evidence that hamburgers sold there caused the death. As the article noted, secondary infection -- by touching an infected person, for example -- is a possibility.

A version of this article appeared in print on Saturday, February 6, 1993, on section 1 page 35 of the New York edition.

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Public Health Importance of Non-O157 Shiga Toxin-Producing *Escherichia coli* (non-O157 STEC) in the US Food Supply

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Tables and Figures

Summary

Non-O157 Shiga toxin-producing *Escherichia coli* (non-O157 STEC) have emerged as a significant public health issue. Some non-O157 STEC possess the same range of virulence factors as *Escherichia coli* (*E. coli*) O157:H7, including the locus of enterocyte effacement (LEE), production of Shiga toxin, and other plasmid mediated factors, and are capable of causing serious illnesses, or death. Numerous serotypes, including O26, O103, O111 and O145 have been identified as agents of food borne disease. Historically, most *E. coli* O157:H7 STEC outbreaks have been associated with consumption of ground beef. Non-O157 STEC have also been found in ground beef and on cattle hides and feces at levels comparable to those for *E. coli* O157. Bovine feces may be a source of environmental contamination, (e.g., soil or water) which can lead to secondary contamination of produce growing in fields. *E. coli* O157:H7 was implicated in a large outbreak associated with spinach in 2006, and non-O157 STEC have been isolated from produce.

It is difficult to distinguish pathogenic non-O157 STEC strains from non-pathogenic *E. coli* because the former rarely possess any distinguishing phenotypic or biochemical characteristics from the latter. The lack of reliable and validated laboratory methods for testing various food matrices has meant that food is not routinely tested for non-O157 STEC and research is needed to support the development of new and better targeted detection methods. This report describes: the microbiological and molecular characteristics of non-O157 STEC; presents food, animal, environmental, clinical and epidemiologic data; and outlines the laboratory challenges and methodological limitations and capabilities for their detection.

Introduction

Escherichia coli (*E. coli*) was first associated with human illness in the early 1940s, when it was linked to infant diarrhea (Bray and Beavan, 1948). Since then, many pathogenic *E. coli* strains have been identified. These are classified on the basis of their virulence properties, mechanisms of pathogenicity, clinical symptoms, and the presence of distinct O and H antigens (Doyle *et al.*, 1997). Groupings include; enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), entero-aggregative *E. coli* (EAggEC), and Shiga-toxin producing *E. coli* (STEC)[1]. Of these groupings, STEC organisms have the potential to cause the most severe clinical symptoms.

The first association of STEC with human disease was made in 1982 in the New England region of the United States (Riley *et al.*, 1983). The emergence of this human pathogen, identified as *Escherichia coli* O157:H7 (*E. coli* O157:H7), spurred much interest in the clinical and public health research communities, due to the severity of the ensuing illnesses. The food safety research community became interested in this organism due to its foodborne transmission and apparent ability to survive food-processing procedures that had hitherto assured food safety. In 1994, following a large foodborne outbreak caused by the consumption of under-cooked hamburgers (Centers for Disease Control and Prevention (CDC), 1993), the Food Safety and Inspection Service (FSIS) declared that *E. coli* O157:H7 and *E. coli* O157: non motile (hereafter *E. coli* O157) were to be regarded as an adulterant in raw ground beef, and established a zero-tolerance policy for this pathogen in this food product. As such, FSIS would request a recall if the product had entered commerce. Any raw ground beef found to contain *E. coli* O157 must be disposed of, or sent for further processing involving a lethality step. In that same year FSIS instituted testing of ground beef for the presence of *E. coli* O157.

The current focus of FSIS STEC monitoring remains solely on *E. coli* O157. However, there is growing evidence that some non-O157 STEC are foodborne pathogens. Clinical studies were the first to identify certain non-O157 STEC as causative organisms in illnesses and targeted studies have confirmed the presence of non-O157 STEC in the same reservoirs as *E. coli* O157, with

[1] The commonly-used terms enterohemorrhagic *E. coli* (EHEC) and verotoxigenic *E. coli* (VTEC) refer to STEC serotypes with the same clinical, pathogenic, and epidemiologic features as *E. coli* O157.

similar survival characteristics. However, the main focus of the food safety research community has remained on *E. coli* O157. While *E. coli* O157 is the STEC most commonly linked to human illness in the United States, other STEC serogroups have also caused cases and outbreaks of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), and in some countries, including Australia, Argentina, Canada, and European Union (EU) nations, non-O157 STEC infections are as prevalent, or more so, than O157 infections. There is increasing evidence to support a focus on the elimination from the US food supply of all pathogenic STEC, rather than just *E. coli* O157.

This report will serve as a comprehensive reference for interested stakeholders by summarizing current scientific literature on the characteristics of non-O157 STEC, the relevant epidemiology, and the laboratory challenges for the detection of these pathogens

Section I. Characterization of the Organism

i. Distribution of non-O157 STEC in food and the environment[2].

Ruminants, primarily cattle, but also sheep and goats[3], are the primary source of transmission of STEC to humans (Bettelheim, 2000). In addition to ground beef and unpasteurized milk (Doyle, 1991, Allerburger *et al.*, 2001), highly acidic ready-to-eat foods such as fermented meat (Tilden *et al.*, 1996) and apple cider (Besser *et al.*, 1993) have long been identified as significant sources of STEC foodborne illness (Griffin and Tauxe, 1991; Keene *et al.*, 1997). Other infection routes include manure-contaminated vegetables (Cieslak *et al.*, 1993), person-to-person contact (Reida *et al.*, 1994), animal to person contact (Crump *et al.*, 2002), contaminated water (Keene *et al.*, 1994; Yatsuyanagi *et al.*, 2002) and visiting dairy farms or petting zoos (Zhao *et al.*, 1995; Crump *et al.*, 2002).

There is a relative paucity of studies on the prevalence and distribution of non-O157 STEC in food and in the environment in comparison to the wealth of published research on *E. coli* O157. The non-O157 STEC studies that do exist generally fall into one of two categories; targeted studies that aim to characterize these organisms present in the food supply and the environment, and epidemiological investigations designed to identify causes and routes of infection (Section II). This section will review the former studies, with particular emphasis on meat and poultry products under FSIS jurisdiction.

More STEC outbreaks have been traced to the consumption of ground beef than to any other food (Dean-Nystrom *et al.*, 1997; Hussein and Sakuma, 2005). In the US, 12-19% of ground beef is produced from dairy cattle culled because of health, age, or production reasons (Wilkus, 2007). Studies of US dairy cattle have reported non-O157 STEC prevalence from 0% to 19% (Wachsmuth *et al.*, 1991; Wells *et al.*, 1991; Cray *et al.*, 1996; Thran *et al.*, 2001). Barkocy-Gallagher *et al.*, 2003 reported a prevalence of non-O157 STEC in beef cattle feces at 19.4% and on hides at 56.3%. Pathogen prevalence on hides may reflect several sources of contamination, such as soil, feces from other animals, and the environment (Barkocy-Gallagher *et al.*, 2003). Blanco *et al.*, (2003) studied the dairy farm environment and detected STEC in calf-and cow-feeders, and in both calf-barn surfaces and

[2] The section focuses on US data only: findings from other countries are addressed elsewhere (Section II, vi).

[3] Data on sheep and goats primarily come from outside the US, and so are outside the focus of this section.

cow-barn surfaces, and proposed that farm environments could remain as sources of STEC for several months.

Barkocy-Gallagher *et al.*, (2003) reported that the prevalence of non-O157 STEC on pre-evisceration carcasses was 58%, dropping to 9% post-processing. Similarly, Arthur *et al.*, (2002) reported that 53.9% of beef carcasses in large processing plants carried at least one type of non-O157 STEC prior to evisceration, but that the prevalence could be reduced to 8.3% with various intervention strategies. A recent retail study by Samadpour *et al.* (2006) reported non-O157 STEC in 2.3% of 1,750 retail raw ground beef samples, compared to *E. coli* O157, found in 1.1% of samples tested. Little information exists on the prevalence of pathogenic non-O157 STEC in FSIS-regulated products other than beef in the US. Doyle and Schoeni (1987) isolated *E. coli* O157 from 6 (3.7%) of 164 beef, 4 (1.5%) of 264 pork, 4 (1.5%) of 263 poultry, and 4 (2.0%) of 205 lamb samples in their survey. Samadpour *et al* (1994), found non-O157 STEC in 9 (18%) of 51 pork samples, 10 (48%) of 21 lamb samples, 5 (63%) of 8 veal samples, 4 (12%) of 33 chicken samples, 1 (7%) of 15 turkey samples, 6 (10%) of 62 fish samples, and 2 (5%) of 44 shellfish samples tested. Fratamico *et al.*, (2004) determined that 70% of 687 swine fecal samples tested positive for the presence of Shiga toxin, and found that most of the serogroups isolated have been associated with human illness. These authors concluded that swine could be a potential reservoir of STEC strains that cause human illness, but conceded that the extent to which swine play a role in the epidemiology of human infection needs further investigation. In general, pigs, poultry and other non-ruminants are not considered to be a source of STEC and sporadic reports such as these may derive from inadvertent exposure to infected ruminants (Caprioli *et al.*, 2005).

While it might seem reasonable to assume a link between the presence of STEC in food animals and subsequent foodborne illness, not all non-O157 STEC are pathogenic to humans (Section I, ii), and the proportion of non-O157 STEC that can cause disease in humans has not been established. Therefore the implications of prevalence data for these organisms in food must be carefully considered.

ii. Virulence characteristics of STEC.

Pathogenesis of STEC is a multi-step process (Paton and Paton, 1998), starting with the acid resistance of the strain, which enables the organism to

survive in low-pH foods and in the acid environment of the stomach. The organism must then adhere to and colonize the intestine, invade epithelial cells, and produce toxin. Not all serotypes of STEC are equally pathogenic - there is much evidence of genetic diversity within serotypes, which can affect virulence determinants and, ultimately, pathogenicity (Nataro and Kaper, 1998). Such differences can be manifested, for example, in the infectious dose of the organism (typically 5 - 50 cells (Tilden *et al.*, 1996)), the level and type of toxin produced, the extent of gastrointestinal colonization, the rate of toxin delivery to the endothelial cells and/or the severity of ensuing disease.

Although the set of virulence factors necessary to cause STEC-related disease has not been completely defined, association between the carriage of certain genes and the ability to cause severe disease in humans has been made. Non-O157 STEC typically possess the same range of virulence factors as *E. coli* O157, including the locus of enterocyte effacement (LEE), Shiga toxin production, and other plasmid mediated factors.

Locus for enterocyte effacement (LEE) The majority of STEC are capable of colonizing the intestine with a characteristic attaching and effacing (A/E) cytopathology. The A/E lesion is characterized by effacement of microvilli and intimate adherence between the bacteria and the epithelial cell membrane, with accumulation of polymerized actin beneath the adherent bacteria (Nataro and Kaper, 1998). This ability is encoded by a number of genes present on a 'pathogenicity island' referred to as the locus for enterocyte effacement (LEE). The LEE encodes for intimin (an eaeA gene product), an outer membrane protein involved in the intimate attachment of bacteria to enterocytes in the gut, and the intimin receptor Tir (encoded by tir). Several different intimin types have been identified. STEC most commonly produce intimin γ and ε (Pelayo et al., 1999).

The LEE also encodes for a type III secretion system that exports LEE effector molecules (including *espA*, *espB* and *espD*) directly into the epithelial cell. Karch *et al.,* (1997) identified a high incidence of *eaeA* positive STEC in HUS patients, particularly children (Beutin *et al.*, 1998; Pradel *et al.*, 2000) suggesting that the presence of this gene is associated with increased virulence in STEC.

The *eaeA* gene is not a universal requirement for virulence (Wieler *et al.*, 1996), and pathogenic strains associated with serious clinical outcomes have been isolated that do not possess this gene (Keskimäki *et al.*, 1997; Pradel *et al.*, 2000; Eklund *et al.*, 2001). Paton *et al.*, 2001 reported the presence of the *saa* gene in an LEE-negative STEC (*E. coli* O113:H21) strain responsible for a HUS outbreak. This gene encodes for an auto-agglutinating adhesion designated Saa (STEC autoagglutinating adhesion). Subsequent investigation by these researchers found homologues of *saa* in several LEE-negative STEC serotypes associated with HUS patients.

Shiga toxin (Stx)[4] Konowalchuk *et al.* (1977) were the first to recognize that a toxin produced by some *E. coli* bacteria displayed cytotoxicity against green monkey kidney cells (vero cells). This toxin was initially termed verotoxin, later Shiga toxin (due to its similarity to the toxin produced by *Shigella dysenteriae*), and is now recognized as a primary virulence factor associated with STEC. The vero-cell assay developed by Konowalchuk *et al.* (1977) is still recognized as the 'gold standard' for the confirmation of STEC. Karmali *et al.* (1983) recognized that production of Shiga toxin by *E. coli* O157 was a crucial factor in the pathogenicity of this organism.

STEC serotypes are diverse in their properties, and produce immunologically distinct Shiga toxin (encoded by the Shiga toxin 1 gene (stx_1) and the Shiga toxin 2 gene (stx_2)) (World Health Organization (WHO), 1998; Bower, 1999). Shiga toxins are multimeric cytotoxins consisting of 1 A and 5 B subunits. Cellular binding of Shiga toxin is coordinated through the B subunits, while the A subunit inhibits cellular protein synthesis (Bower, 1999). The cytotoxic effect of Shiga toxin on intestinal epithelial cells causes the characteristic bloody diarrhea associated with STEC infection. The type and/or amount of Shiga toxin produced will determine the capacity of the organism to cause human disease. The stx_2 gene can produce a number of variants, termed Shiga toxin 2c, Shiga toxin 2d, Shiga toxin 2e and Shiga toxin 2f, of varying toxicity to humans (Paton and Paton, 1998; Schurman *et al.*, 2000; Bertin *et al.*, 2001). Shiga toxin 2 and Shiga toxin 2c has been cited as 1000 times more cytotoxic than Shiga toxin 1 towards human renal cells and has been more commonly associated with the development of HUS than Shiga toxin 1

[4] Other bacteria, including *Citrobacter freundii*, *Enterobacter cloacae* and of course *Shigella dysenteriae* can also produce Shiga toxin. However, STEC have emerged as the most significant cause of sporadic human illness associated with this toxin (Acheson and Keusch, 1996).

(Bertin *et al.*, 2001). However, strains producing Shiga toxin 1 only have also been associated with human illness, including HUS (Eklund *et al.*, 2001). As mentioned, not all non-O157 STEC strains that produce Shiga toxin cause HUS. This variability in virulence may have led to an underestimate of the pathogenicity of this diverse set of strains. However, based on data from Europe and Australia, a subset of non-O157 STEC strains are as virulent as *E. coli* O157 (Wickham *et al.*, 2006) with epidemiological evidence of similar incubation periods, symptom onsets, symptom profiles, and comparable proportions of case-patients who develop HUS. Related outbreaks are often indistinguishable from *E. coli* O157:H7 outbreaks (Brooks *et al*, 2004; Brooks *et al.*, 2005)

Plasmid-mediated factors Many STEC possess a highly conserved 97-kb plasmid (pO157), which encodes for several putative virulence factors, including a serine protease (espP), a bifunctional catalase peroxidase (KatP), enterohemolysin (ehx), an immunomodulator (lif), and secretion proteins (etp) (McNally et al., 2001). Studies have suggested an association between the carriage of the eaeA gene and enterohemolysin production (Eklund et al., 2001). The precise role of these genes in the virulence of STEC has not been fully elucidated, though pathogenesis is certainly complex, with many contributory factors (McNally et al., 2001).

In theory, an investigator should be able to pinpoint the cause of illness by identifying the presence of one or more of these virulence factors. However, for practically every virulence factor identified, there is an example of an illness caused by an isolate lacking the gene coding for this trait. Pradel *et al.* (2000) surveyed STEC isolated from cows, children and food (meat and cheese), characterizing the Shiga toxin types of each isolate, and whether or not they contained the *eaeA* gene. They observed a wide diversity of strains and noted that in general the strains isolated from the children were dissimilar to those isolated from the animals in terms of their genetic profile. McNally *et al.* (2001) reported that the comparatively low incidence of human disease attributable to STEC, given its relatively high incidence in cattle, could be attributed to inherent differences between the strains isolated from cattle and from humans. These researchers observed significant differences between human- and bovine-derived strains, and their production of certain LEE-encoded virulence factors, and proposed the possibility of different STEC lineages in cattle and humans. It may be that STEC from bovine sources

exhibit reduced resistance to environmental stresses, and therefore cannot survive food processing and/or digestion.

Because the presence of STEC in food is not a marker for human illness, testing procedures for pathogenic STEC in food must include other screening criteria in addition to Shiga toxin testing. More work is needed to determine the range and scope of distribution of Shiga toxin genes among *E. coli* serotypes, the ease of transfer of these genes among strains in the environment, their distribution in nature, their mode of entry into the food chain, and their potential pathogenicity (Bollinger *et al.*, 2005; Samadpour *et al.*, 2006). The field of STEC virulence and pathogenicity of STEC is an area of much uncertainty, and research is ongoing to identify properties that can be utilized to reliably distinguish pathogenic STEC from non-pathogenic strains.

Section II. Public Health Impacts

i. Diseases caused by non-O157 STEC, and their associated morbidity and mortality.

STEC infection causes symptoms ranging from mild non-bloody diarrhea in healthy adults to more significant health outcomes, sometimes proving fatal, in young, old or immunocompromised individuals. In such susceptible individuals, STEC infection generally causes diarrhea and abdominal cramps, with little or no fever, and resolves itself in 5 to 10 days. However, in some instances, more serious sequelae including hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura can develop.

Hemorrhagic colitis (HC) The classic paper entitled "Hemorrhagic colitis associated with a rare *Escherichia coli* serotype" was published by Riley *et al.* in *The New England Journal of Medicine* in 1983 when knowledge of STEC was in its infancy. This paper was the first to propose a link between an STEC (*E. coli* O157) and a significant human disease. Hemorrhagic colitis (HC) is a form of gastroenteritis in which STEC attach to the large intestine and secrete Shiga toxin, leading to bloody diarrhea as a result of damage to the lining of the large intestine. If the toxins are subsequently absorbed into the bloodstream, they can also affect other organs, such as the kidneys. HC can occur in people of all ages but is most common in children and the elderly. Symptoms include the sudden onset of severe abdominal cramps along with watery diarrhea that typically becomes bloody within 24 hours. The diarrhea usually lasts 1 to 8 days. Fever is usually absent or mild but occasionally can exceed 102° F (38.9° C). The prognosis for this disease is good; rarely, death may occur in elderly patients.

Hemolytic uremic syndrome (HUS) The term HUS was coined in the 1950s to describe an acute, often fatal syndrome in children characterized by hemolytic anemia (caused by the destruction of red blood cells), thrombocytopenia (a low platelet count), and severe renal failure. About 5% of HC patients, generally children younger than 5 years and the elderly, go on to develop hemolytic uremic syndrome (HUS) (Banatvala *et al.*, 2001). Some HUS patients develop complications of the nervous system or brain damage.

In children, 90% of HUS cases follow an infectious disease; STEC have been identified as the primary cause (up to 90%) of HUS in temperate climates[5]. Less commonly *Shigella*, *Salmonella*, *Yersinia*, and *Campylobacter* have been implicated. The CDC initiated an active surveillance for this condition in 1997, and Dunne *et al.* (2000) reported an annual incidence of 10.6 cases per million for 1997 through 1999 for children under 16. During 2003, a total of 178 cases of HUS were reported from 32 US States; of these, 118 (66%) occurred among children aged <10 years (CDC, 2005[6]). Lynn *et al.*, (2005) reported an increase in pediatric HUS cases in England and Wales from 50 in 1985, to 1087 in 1997. The increased incidence was attributed to improved laboratory techniques, increased reporting, and recognition of the importance of elucidating the causes of diarrheal disease.
HUS is the most common cause of long-term renal failure in children in the US and Britain (Boyce *et al.*, 1995; Chapman, 1995), and has a reported mortality rate of 5-10% in the US (Corrigan and Boineau, 2001). Approximately 85% of children with classic HUS recover completely with supportive therapy; however; 15-20% of children may develop hypertension 3-5 years after the onset of disease. Adult patients with HUS have a lower mortality rate; however, the renal prognosis is poor in patients who are not treated. Up to 80% of adults with HUS will ultimately require long-term dialysis or renal transplantation.

Thrombotic thrombocytopenic purpura (TTP) TTP is a blood disorder characterized by fever, a low platelet count, low red blood cell count (caused by premature breakdown of the cells), compromised kidney function, and neurological abnormalities. Whereas HUS more commonly affects children and the elderly, TTP is more commonly found in non-elderly adults. Symptoms include fluctuating neurological symptoms, such as bizarre behavior, altered mental status, stroke or headaches, kidney failure, fever, thrombocytopenia (low platelet count) leading to bruising or frank purpura[7], and microangiopathic hemolytic anemia. The mortality rate associated with TTP approached 100% until the 1980s; today, the survival rate is 80-90% with early diagnosis and treatment with plasma infusion and plasma exchange, however, mortality remains at approximately 95% for untreated

[5]The remaining 10% of cases are generally associated with an upper respiratory infection.
[6] The patients reported in national notifiable diseases surveillance (CDC, 2005) include only those with antecedent diarrheal illness.
[7] Purpura is the appearance of purple discolorations on the skin caused by bleeding underneath the skin.

cases. Up to one third of patients who survive the initial episode experience a relapse within the following 10 years (Elkins *et al.*, 1996).

ii. Recognized sources of infection

There is limited information on the prevalence of non-O157 STEC in the US food supply. However, it is widely recognized that ruminants, primarily cattle, are the natural reservoirs of STEC (Chapman *et al.*, 1993; Rasmussen *et al.*, 1993; Armstrong *et al.*, 1996). Rangel *et al.* (2005) reviewed CDC *E. coli* O157 outbreak data (1982 through 2002) and reported that ground beef accounted for 75 of the 183 *E. coli* O157 foodborne outbreaks identified. Therefore an understanding of the dissemination and persistence of STEC among cattle is particularly important.

Ground beef. Approximately 30% of all cattle are asymptomatic carriers of *E. coli* O157 and other STEC (Elder *et al.*, 2000). *E. coli* O157 and non-O157 STEC alike asymptomatically reside in the intestines of cattle, and are regularly shed in feces. Meat can become contaminated during slaughter, and organisms can be mixed into beef when it is ground. Sanitation efforts after slaughter have been shown to reduce the contamination of carcasses with *E. coli* O157 (Elder *et al.*, 2000); however, the ability of these organisms to withstand processing environments, as evidenced by the continuing occurrence of outbreaks linked to the consumption of undercooked ground beef, suggests that additional control measures must be identified and implemented. Methods that focus on reducing STEC populations in food animals before entry to the food chain, and on the elimination of these pathogens by appropriate food processing and handling, will contribute to further reductions in human illnesses.

Other bovine sources. Hussein and Sakuma (2005) published a review on the prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products. The prevalence of STEC in US dairy cattle ranged from 0.2 to 8.4% (studies performed through the 1990s), with numbers in heifers ranging from 1.6 to 19.0%, and numbers in calves ranging from 0.4 to 40%. Some of the studies reviewed had isolated non-O157 STEC from raw milk,

cheese and milk filters; however, most of the isolated serotypes had never been linked to human illness.

Other known sources of infection, which can often be linked to secondary contamination from bovine sources, include:

Produce. The Rangel *et al.* (2005) study found that 38 of 183 *E. coli* O157 foodborne outbreaks identified (1982 through 2002) were attributable to produce, with 26 outbreaks traced to leafy green vegetables. The high-profile "spinach outbreak" in 2006, followed by two lettuce-linked outbreaks, has heightened consumer awareness of the potential for foodborne illness associated with produce. Contamination can arise from seeds, irrigation water, or the use of untreated animal manure harboring the pathogen. Due to the nature of the contamination, the pathogen may become established in the structure of the plant during its development, making it impossible to eliminate by washing (Itoh *et al.*, 1998). Pathogens can exceed 10^7 per gram of sprouts produced from inoculated seeds during sprout production, without adversely affecting appearance. Treating seeds and sprouts with chlorinated water or other disinfectants fails to eliminate the pathogens (Taormina *et al.*, 1999), leading the FDA to make the recommendation that raw alfalfa sprouts should be considered potentially contaminated and avoided by persons at high-risk such as the young, elderly and immunocompromised.

Fermented meats. Traditionally, fermented meats were not subjected to a heat-lethality process; instead the safety of such products was assured by the low pH achieved during the fermentation process. However the emergence of STEC, which exhibit significant acid-resistance and can thereby survive the fermentation process if present in the raw meat ingredients, meant that traditional fermentation processes were no longer sufficient to assure the safety of such products (Riordan *et al.*, 1998). Today, most fermented meat production processes include a heat-lethality step.

Unpasteurized milk. Bacteria present on the cow's udders, hide or on equipment may contaminate raw milk. Raw milk consumption has been

associated with brucellosis, campylobacteriosis, cryptosporidiosis, *E. coli* O157, listeriosis, salmonellosis, staphylococcal enterotoxin poisoning, tuberculosis, and yersiniosis (Potter *et al.*, 1984). The sale of raw milk is prohibited or strictly limited in most US states (Bren, 2004); however, cow-sharing programs and other such initiatives have led to increased availability in some jurisdictions.

Unpasteurized juice. Fruit used for juice is typically of poorer quality than that sold as eating-quality fruit. It has been demonstrated that *E. coli* O157 can grow on dropped, damaged or blemished fruit (Dingman, 2000), thereby providing an avenue for pathogen entry (Riordan *et al.,* 2000). In addition, dropped fruit potentially contaminated with manure may be used in juice-making, and though all produce is washed prior to processing, it is very difficult to completely remove pathogens from fruit with traditional washing procedures (Annous *et al.*, 2001). The low pH associated with products such as apple juice, which had traditionally assured their safety, is insufficient to eliminate surviving STEC. A number of outbreaks linked to the consumption of unpasteurized juice (e.g. Besser *et al.*, 1993) led the FDA in 2001 to release Juice HACCP regulations, essentially requiring all juice to be either pasteurized or sold with a warning label.

Swimming in or drinking sewage-contaminated water. The very low reported infectious dose of STEC, less than 10 cells in some instances (Tilden *et al.,* 1996), means that manure or fecal contamination of large volumes of water can cause illness in susceptible individuals.

Person-to-person contact. The low infectious dose of these pathogens means that bacteria present in the stools of infected persons can be passed from one person to another if hygiene or handwashing habits are inadequate. This is particularly likely among toddlers who are not toilet trained. Family members and playmates of these children are at high risk of becoming infected.

iii. Foodborne outbreaks of non-O157 STEC in the United States

Acheson and Keusch stated in 1996 that 'we cannot let ourselves be complacent in thinking that *E. coli* O157 is the only Shiga toxin producing bacteria that can cause problems'. Evidence from targeted clinical studies suggests that new pathogenic forms of non-O157 STEC continue to emerge, and that testing food for *E. coli* O157 alone is insufficient. Worldwide, the list of non-O157 STEC associated with human illnesses consists of over 100 different serotypes (Eklund *et al.*, 2001). Targeted clinical studies are increasing report evidence of sporadic cases of non-O157 STEC infection in the US. For example, Fey *et al.*, (2000) reported data from clinical studies characterizing STEC isolates from several US locations. Their studies involved testing stools submitted to clinical labs for bacterial culture, and cited numerous sporadic cases of illness associated with non-O157 STEC serotypes. Acheson (2001) reported an incidence of STEC positive stools of 0.75%; 54% *E. coli* O157 serotypes, and 46% non-O157 STEC. Several different non-O157 STEC serotypes were detected, including O26, O103, O121, O111, O145, O165, O69, OX25, O6, OX3, O45, O8, O38, O25, O55, O2 and O1, in addition to a previously undetected outbreak associated with serotype O153 (Fey *et al.*, 2000; Acheson, 2001). These researchers concluded that, overall, the non-O157 STEC, in particular strains O26, O55, O103, O111, and O145, are just as prevalent and clinically significant as *E. coli* O157 in the US.

Since 1990, 13 outbreaks of non-O157 STEC have been reported in the US (Table 1). *E. coli* O111 was the most frequently reported serogroup, followed by *E. coli* O121. Multiple pathogens were identified in three of the outbreaks. In particular, two non-O157 STEC serogroups were isolated from one outbreak in 2001 (O111 and O51). Of the non-O157 STEC outbreaks reported, none were attributed to FSIS-regulated products although the exposure or vehicle identified in the majority of the outbreaks was generally unconfirmed.

The first reported outbreak of non-O157 STEC in the US occurred in 1994, and was reported by CDC in 1995. In total, 16 of 18 confirmed and

suspected cases developed bloody stools, diarrhea, and abdominal cramps. Three case-patient isolates were identified as *E. coli* O104:H21. The source of the bacteria was thought to be post-pasteurization contamination of milk at a nearby dairy farm.

In June of 1999, 55 of 521 attendees of a cheerleading camp developed gastroenteritis and two were hospitalized after developing HUS (Brooks *et al.*, 2004). The etiologic agent was determined to be *E. coli* O111:H8. This outbreak was clinically indistinguishable from outbreaks caused by *E. coli* O157. The potential vehicle was narrowed down to a particular lunch meal. In August 2006, Utah health officials reported an outbreak involving lettuce from a fast food restaurant (Berger, 2006). Four people became ill, including three who developed kidney failure. The serotype was identified as *E. coli* O121:H19.

It is important to note the limitations of such outbreak investigations. The ability to detect outbreaks depends upon the identification and reporting of diseases in a timely manner. Because diseases are generally underreported, foodborne outbreaks that are recognized and reported only represent a small proportion of all such outbreaks occurring in the US. State laboratories with the ability to serogroup and/or serotype isolates in-house will be better equipped to identify clusters of non-O157 STEC illnesses. However, not all state laboratories have such resources.

iv. USDA-FSIS investigation of non-O157 STEC illnesses

In 2006, the Foodborne Disease Investigations Branch (FDIB) of the Office of Public Health Science (OPHS) investigated a case-patient ill with *E. coli* O103 infection who had consumed an undercooked ground beef patty one day prior to illness. The state laboratory tested samples from the patient and leftover uncooked ground beef patties and determined them to be indistinguishable by pulsed-field gel electrophoresis (PFGE). However, FSIS was unable to take further action because of possible cross-contamination in the meat

market grinder between product types and because records were not adequate to determine a specific production.

Also in 2006, FDIB investigated a case-patient ill with *E. coli* O157. Leftover ground beef products were collected and tested by the state department of agriculture. The ground beef samples tested positive for Shiga-toxin but were negative for *E. coli* O157, and the ground beef could not be confirmed as the source of the human illness. Nonetheless, product samples were submitted to the CDC for characterization and *E. coli* O6:H34 was subsequently identified.

v. Non-O157 STEC epidemiology in the US

National Foodborne Outbreak Surveillance. *Escherichia* coli O157 has been nationally notifiable since 1994 (Mead and Griffin, 1998). A decade ago, approximately 50% of clinical labs in the US tested bloody stools only for the most common STEC serotype, *E. coli* O157 (Tarr and Neill, 1996). CDC monitors outbreaks of foodborne disease, including outbreaks caused by STEC, and publishes an annual report[8]. Each year, state and territorial epidemiologists report the results of outbreak investigations to CDC. While outbreaks account for a small percentage of the total number of illnesses that occur each year, these reports provide valuable information about sources of foodborne infection and often highlight important prevention opportunities.

Although the true incidence of non-O157 STEC infection in the US population remains unclear, several studies have attempted to quantify the prevalence of these organisms in symptomatic patients. A Nebraska study assessing the prevalence of non-O157 STEC in diarrheal samples positive for STEC identified five different serotypes (Fey *et al.*, 2000). The conclusions of the authors were that non-O157 STEC serotypes were at least as prevalent as serogroup O157 in this small sample.

A study by Klein (2002) examined 1,851 stool samples taken from pediatric patients in an emergency room of a private clinic over a three-year period. The authors found that 2.1% of stool samples contained non-O157 STEC. They found no cases of HUS associated with non-O157 STEC. Although they concluded that *E. coli* O157 was the predominant STEC in this population,

[8] http://www.cdc.gov/mmwr/summary.html

they recommended a combined diagnostic approach using both SMAC for *E. coli* O157 and Enzyme Immunoassay (EIA) for the presence of Shiga toxin since both tests could miss the potentially causative organisms in some cases.

The most extensive assessment to date was published by Brooks *et al.* in 2005, who summarized data from a convenience sample of sporadic cases confirmed by the CDC. The CDC received 940 isolates from a 20-year-period (1983 to 2002). The top six serogroups identified (O26, O45, O103, O111, O121, O145) accounted for 71% of all the isolates while O26, O103, and O111 accounted for more than 50% of isolates. The non-O157 STEC isolates, as a group, were similar to *E. coli* O157 in seasonality, presence in children, and distribution of Shiga toxin genes. However, serogroup O111 was the only serogroup associated with HUS. Interestingly, it is also the most common serotype found in Australia and Germany (Gerber *et al.*, 2002; Elliott *et al.*, 2001).

Active Surveillance in FoodNet sites. In 1997 CDC initiated HUS surveillance as part of CDC's Foodborne Diseases Active Surveillance Network (FoodNet). FoodNet is a collaborative project of the CDC, ten Emerging Infections Program sites (CA, CO, CT, GA, MD, MN, NM, NY, OR, and TN), USDA, and FDA. The project consists of active surveillance for foodborne diseases and related epidemiological studies. The core of FoodNet is laboratory-based active surveillance at over 650 clinical laboratories that test stool samples in the ten FoodNet sites. In active surveillance, the laboratories in the catchment areas are contacted regularly by collaborating FoodNet investigators to collect information on all of the laboratory-confirmed cases of diarrheal illness (CDC, 2006b).
In the HUS surveillance study, pediatric nephrologists in catchment areas for sites are regularly surveyed, while adult cases are reported in a passive system, as are cases outside of catchment areas. Thus, HUS caused by non-O157 STEC is more routinely identified (Table 2).
In 2000, the Council for State and Territorial Epidemiologists passed a resolution under which all Shiga toxin-producing *E. coli* were made nationally notifiable under the name Enterohemorrhagic *E. coli* (EHEC); the CDC currently defines Enterohemorrhagic *Escherichia coli* as (a) Enterohemorrhagic *Escherichia coli* O157: H7 or (b) Enterohemorrhagic *Escherichia coli S*higa toxin positive (not serogrouped) or (c)

Enterohemorrhagic *Escherichia coli* Shiga toxin positive (serogroup non-O157). Information on the actual serotype (other than *E. coli* O157) is not routinely collected, however, and the CDC stated in its summary of notifiable diseases for 2001 (CDC, 2003) that "the number of cases reported for EHEC should be interpreted as an underestimate in a maturing surveillance system", because few stool specimens are tested in a way that would identify Shiga toxin-producing *E. coli* other than *E. coli* O157. National surveillance for all STEC under this definition began in 2001. Surveillance for laboratory-confirmed cases of *E. coli* O157 has been conducted since FoodNet was established in 1996. In 2000, FoodNet began surveillance for laboratory-confirmed cases of non-O157 STEC infections (Figure 1). From 2000-2006, FoodNet reported 626 laboratory-confirmed cases of non-O157 STEC infection; ranging from 35 in 2002 to 209 cases in 2006 (Figure 2). O-antigen information was available for 488 of the 626 (78%) laboratory-confirmed non-O157 STEC infections. A total of 33 different O antigens were documented; O111 (27%), O103 (21%), and O26 (21%) were the most commonly identified non-O157 serogroups (Table 3). An additional 85 STEC cases with O-antigen undetermined were ascertained during this time period. The majority of cases are identified during the summer months (Figure 3). Information on age was available for 401 (96%) cases; of these, 28% were less than 5 years old (Table 4); 51 (14%) of all cases were hospitalized. Between 2000 and 2005, one death was associated with a non-O157 STEC infection.

Survey of non-FoodNet sites. FSIS OPHS epidemiologists examined surveillance data on non-O157 STEC illnesses from 2004 through 2005 was examined to evaluate the public health impact of these organisms, and to determine potential exposures by serogroup, thereby providing additional information on the impact of FSIS-regulated products on illnesses. Foodborne disease epidemiologists in selected states were asked to provide information on the number of *E. coli* O157 and non-O157 STEC case-patients over the study period, any available serotype information on the isolated non-O157 STEC, and any consumption/exposure history. Thirteen sites responded, and eight sites provided exposure histories.

Of all STEC reported 79% were *E. coli* O157 and 18% were non-O157 STEC. Of the non-O157 STEC, the most commonly reported serogroups were O26 (30%), O103 (18%), O111 (12%), O121 (12%), and O45 (13%). The largest

percentages of non-O157 STEC were reported in Virginia (53%), followed by Utah (40%) and Wyoming (32%).

Live animal exposure was cited as a potential vehicle in 55% of cases, including pet dogs and cats, farm animals, birds, reptiles, and even a giraffe. Sixty-three percent of case-patients with STEC serotype O26 reported live animal exposures. Thirty-three percent of non-O157 STEC case-patients reported beef consumption, and 34% reported consumption of other meats. However, evaluation of the food consumption data was difficult: only sparse food history information was available, and individual case-patients often reported multiple food exposures.

It is important to reiterate that the data presented here are incomplete, and do not provide a reliable estimate of the total burden of non-O157 STEC in the US. Several factors influence surveillance data, including laboratory practices, and these are known to vary across sites from which these data were collected.

vi. Non-O157 STEC illnesses in other countries

Worldwide, the list of non-O157 STEC associated with human illnesses consists of over 100 different serotypes (Eklund *et al.*, 2001). While *E. coli* O157 is the principal STEC strain isolated from implicated food and clinical isolates in the US, non-O157 STEC predominate in other countries including Australia, Brazil, Canada, Germany and the UK, among others (Beutin *et al.*, 1998; Dekoninck *et al.*, 1998; Keskimäki *et al.*, 1998; Pradel *et al.*, 2000; Baffone *et al.*, 2001; Guth *et al.*, 2003) (Table 5). Many studies have been performed throughout the world to assess the prevalence of *E. coli* O157 and other STEC in retails foods, in order to determine the public health risk posed by these organisms. Limited comparisons can be made, however, due to the variety of sampling and testing methods, and study designs used.

Section III. Methods

i. Difficulties in distinguishing non-O157 STEC from non-pathogenic *E. coli*.

Samadpour *et al.*, (1994) were among the first to speculate that the apparent predominance of *E. coli* O157:H7 among STEC in the US was probably a result of its relative ease of isolation, and stated that the lower perceived incidence of non-O157 STEC in comparison to *E. coli* O157:H7 was likely a consequence of the detection and isolation methods used, which selectively excluded most non-O157 STEC. As already stated, the detection of toxin or toxin genes, the single 'universal' virulence factor among STEC, is not sufficient to formulate a definite association with illness as some strains may produce toxin but lack other necessary virulence factors (as seems to be the case in many non-bovine ruminant strains). Mounting data on virulence factors associated with STEC has led to the development of targeted molecular techniques for their detection while concurrently demonstrating the genetic heterogeneity of these organisms. The ongoing development of new technologies has provided an expanded capability for testing isolates, using toxin and/or molecular based technologies, which do not focus on phenotypic characteristics.

The difficulty in distinguishing pathogenic non-O157 STEC strains from non-pathogenic commensal *E. coli* obscures the true clinical significance of non-O157 STEC in the US. The perceived lack of a problem with non-O157 STEC has resulted in little commercial or research interest in developing reliable 'routine' detection methods for these organisms, thereby contributing to the fact that foods are generally not routinely tested for non-O157 STEC, and the magnitude of the problems associated with non-O157 STEC remains largely unrecognized. However, data from targeted epidemiological investigations, clinical studies, lab diagnoses in individual cases, and the increased incidence of HUS demonstrate that the increasing significance of non-O157 STEC becomes apparent when diagnostic methods that can detect these serotypes are employed (Keskimäki *et al.*, 1998). This has resulted in an increasing awareness among the scientific community of the proliferation of non-O157 STEC in the environment and in cases of human gastrointestinal illness.

ii. Considerations when choosing an analytical method for non-O157 STEC.

Goldwater and Bettelheim (2000) stated that "the current heavy reliance on very specific tests based on either the use of specific nucleic acid sequences or monoclonal antibodies will become a severe disadvantage in detecting …newly emerging pathogens." It was precisely such selective procedures, in a more low-tech format, i.e. the development of the highly selective SMAC medium in 1986 by March and Ratnam, which contributed to the delay in recognition of the prevalence and importance of non-O157 STEC. Finally, and perhaps most significantly for most laboratories, it may be necessary to use a specific analytical method, or one deemed equivalent to some specified standard, e.g. an AOAC approved method.

In order to establish routine testing of food for non-O157 STEC, regulatory Agencies would be faced with the task of choosing the most appropriate method for non-O157 STEC detection for their purposes, mindful of the above considerations, with the exception that there are as yet no existing standards to meet. Indeed, the choices such agencies might make in regards to this pathogen would likely influence future research into this organism.

iii. Testing for *E. coli* O157 and non-O157 STEC.

The difficulties inherent in the isolation and identification of non-O157 STEC are well recognized. When reporting on the 1995 mettwurst STEC outbreak in Australia, which was ultimately linked to *E. coli* O111, Goldwater and Bettelheim (1996) stated that when investigating the cause of this outbreak, "if (their) laboratory had to rely on conventional microbiologic culture procedures, including sorbitol-MacConkey agar, strains of serogroup O157 would have been identified from three patients, as well as from the epidemiologically incriminated mettwurst. The laboratory would not have found the O111 strains because they all fermented sorbitol readily and would have been discarded as normal flora as would the other enterohemorrhagic *E. coli* serotypes". Some years later Bettelheim (2003) stated that the relatively easy identification of non-O157 STEC, in the same manner as *E. coli* O157 "may never be achievable". As already mentioned, the problem is the lack of a single distinctive phenotypic characteristic common to all pathogenic non-O157 STEC that can be reliably harnessed to some selective medium, biochemical test, or other procedure. Existing technologies for the

detection of *E. coli* O157 could potentially be adapted by manufacturers of diagnostics products for the development of non-O157 STEC detection methods, if they perceived a commercial market for these products. An unscientific poll of exhibitors at the 2007 International Association for Food Protection (IAFP) Annual Meeting revealed that while the manufacturers are aware of the existence of non-O157 STEC and the associated problems, as yet there is little impetus for them to develop commercial kits for the detection of these organisms, because there is no market for them: food producers, even those with large *E. coli* O157 testing programs, generally do not test their products for these organisms. Without a drive to push development of non-O157 STEC methods, the introduction of regulatory testing requirements might result in the adoption of sub-optimal technology by the industry, in the initial rush to introduce testing programs.

Commercial testing systems for *E. coli* O157 and non-O157 STEC detection and identification are presented here. *E. coli* O157 test methods are detailed because it is quite likely that non-O157 STEC-specific tests will be developed as an extension of an *E. coli* O157 test.

iv. Method Components.

The basic routine for pathogen detection can be broken down into three stages: enrichment, screening and confirmation.

Enrichment. When choosing an enrichment medium appropriate for the isolation of a target organism in a substrate such as raw ground beef (or any material commonly host to heterogeneous microflora), it is important to strike the right balance between providing sufficient nutrients for the target organism to be resuscitated (if necessary) and grow, while restricting the growth of non-target organisms. An ideal medium will have high productivity without compromising selectivity, and will allow the target organism to proliferate while suppressing background flora. For non-O157 STEC (including serotypes O26 and O111) this has been achieved by enrichment in media that have lower nutrient levels (e.g. buffered peptone water (BPW)) than other, richer media (e.g. tryptone soy broth (TSB)), and incubating at 41-42°C rather than at 37°C (Catarame *et al.*, 2003; Drysdale *et al.*, 2004). Addition of vancomycin (8mg/l) has been reported as optimal for non-O157 STEC recovery (Drysdale *et al.*, 2004). Many researchers have found that using mEC+N (i.e. *E. coli* broth with 1.12 g/l bile salts, and 20mg novobiocin/l), the enrichment medium for *E. coli* O157 detection used by

FSIS, results in significantly reduced recovery of non-O157 STEC (e.g. Drysdale *et al.*, 2004). Many of the *E. coli* O157 screening methods listed below have attendant suggested enrichment broths: it is likely that development of screening methods specific for non-O157 STEC would include the development of optimal enrichment broths.

Screening. Few commercially available screening kits have been developed for non-O157 STEC, for reasons already discussed. Those on the market include Denka Seiken's EHEC Immunomagnetic Separation (IMS) kits for *E. coli* O157, *E. coli* O26 and *E. coli* O111; and Dynal Biotech's Dynabeads® for *E. coli* serotypes O26, O103, O111, O145 and O157, which also use IMS. Another commercial test for non-O157 STEC is the Oxoid Dryspot™ *E. coli* Seroscreen, a single-screen latex agglutination system that can detect serotypes O26, O91, O103, O111, O128, O145 and O157. Screening tools for *E. coli* O157 are presented below:

o PCR-based assays

PCR assays can be completed in as few as 2 to 4 hours post-enrichment, acting as an effective, rapid and very specific screening tool. Conventional PCR assays require amplification of one specific target gene in a thermocycler, separation of PCR products by gel electrophoresis, followed by visualization and analysis of the resultant electrophoretic patterns. The Marshfield Clinic *E. coli* O157 Test Method[9], which can detect the *eae* gene present in *E. coli* O157 and other EHEC strains with a confirmed positive or negative result in less than 12 h, is an example of such an assay.

Multiplex PCR assays increase the specificity of the test by allowing multiple genes to be targeted at once. A carefully designed multiplex PCR can be used as a screen to test composite samples as it allows rapid detection of any STEC carrying any individual or combination of the target genes (Sharma *et al.*, 1999). Fratamico *et al.* developed the first multiplex PCR for *E. coli* O157 in 1995, and since then gene clusters specific to many clinically significant non-O157 STEC serotypes including *E. coli* O26 (D'Souza *et al.*, 2002), *E. coli* O103 (Fratamico *et al.*, 2005), *E. coli* O111 (Wang *et al.*, 1998), *E. coli* O121 (Fratamico *et al.*, 2003), *E. coli* O145 (Perelle *et al.*, 2003), among others (Paton and Paton, 1998; Sharma *et al.*, 1999;

[9] AOAC Performance Tested Method 070502

Fratamico *et al.*, 2000; DebRoy *et al.*, 2004; Perelle *et al.*, 2004) have been identified and incorporated into multiplex PCRs.

Commercially available kits that use PCR for the detection of *E. coli* O157 in raw ground beef include Applied Biosystems' TaqMan® *Escherichia coli* O157:H7 Detection Kit, BioControl's Genetic Detection System[10] O157 Assay and Shiga-toxin gene assay, the Dupont BAX system[11], (currently used by FSIS to screen for *E. coli* O157), and Warnex Diagnostics' Genevision™ Rapid Pathogen Detection *Escherichia coli* O157 and *E. coli* O157:H7 kits.[12]

o Shiga toxin tests

The vero cell assay has been regarded as the "gold standard" for Shiga toxin detection since the discovery of this toxin family by Konowalchuk *et al.* in 1977. However, the constraints inherent in tissue culture, as well as the associated prolonged turnaround time for results and lack of specificity with this procedure, mean that this method is not routinely used for the screening of STEC (Rahn *et al.*, 1996), though it can be used for confirmation purposes.

Commercial kits for Shiga toxin detection include the Antex Biologica VeroTest, the Diffchamb Transia Plate Verotoxin kit, the Merck Duopath Verotoxin test[13], Meridian Diagnostics' Premier™ EHEC, Oxoid's VTEC-RPLA (Reversed Passive Latex Agglutination) for VT1 and VT2, and r-Biopharm's RidaScreen Verotoxin test. Most tests require an isolated colony, however, thereby significantly increasing the time required for a result.

o Immunoassay based methods

There is a wide variety of immunoassay-based methods available, each typically involving a pre-enrichment (from <8h to 24+h) of the test sample followed by specific detection of the cellular antigen in either a lateral flow device or by immunomagnetic capture (10-45 min post-enrichment). Commercially available lateral flow systems include Strategic Diagnostic's RapidChek® system[14], Diffchamb's Transia™ Card *E. coli* O157:H7[15] (both of which are listed as *E. coli* O157 screening tools in Chapter 5 of the FSIS

[10] AOAC Official MethodsAOAC 2005.04 and AOAC 2005.05 respectively
[11] AOAC Performance Tested Method Nos. 010401 and 050501.
[12] AOAC Performance Tested Methods 010408 and 010409 respectively.
[13] AOAC Performance Tested Method 020402
[14] AOAC Performance Tested Method 030301
[15] AOAC Performance Tested Method 010402

Microbiology Laboratory Guidebook), BioControl's VIP for EHEC[16], the Centrus International Envisio system, DuPont's™ Lateral Flow System™[17], Meridian Diagnostics Immuno*Card* STAT!® *E. coli* O157 Plus, Merck's Singlepath® *E. coli* O157[18], and the Neogen Reveal® system[19] (formerly used by FSIS to screen for *E. coli* O157).

Some non-lateral flow immunoassay-based tests include BioControl Systems' Assurance EHEC EIA[20], the bioMérieux VIDAS® *E. coli* O157 (ECO) test with O157:H7 ID Agar[21], Diffchamb's Transia Plate *E. coli* O157[22], IGEN International's PATH*IGEN E. coli* O157 test[23], the Matrix Bioscience PATHATRIX *E. coli* O157 test system[24], Neogen's GeneQuence™ *E. coli* O157, and TECRA's *E. coli* O157 VIA[25].

Several immuno-latex agglutination kits for *E. coli* O157 are available, including the Denka Seiken *EHEC* O157 kit already mentioned, Microgen Bioproducts Ltd. Microscreen *E. coli* O157, Pro-Lab Diagnostics' Prolex™ *E. coli* O157 with *E. coli* H7 Flagellar Antigen Latex Reagent test, Remel's RIM *E. coli* O157:H7 Latex Test, and the Wellcolex *E. coli* O157 and O157:H7 tests.

o Other Methods

Neogen's ISO-GRID™ for enumeration of total *E. coli* and *E. coli* O157:H7[26] utilizes hydrophobic grid membrane filter technology to detect and quantify target organisms. The BioControl Assurance GDS™ *E coli* O157:H7 assay[27] and Shiga Toxin gene assay[28] combine technologies by incorporating immunomagnetic separation and highly specific primers in their system for the detection of *E. coli* O157 and the Shiga toxin gene respectively.

Confirmation. A positive test result for *E. coli* O157 by any of the above screening tests is generally confirmed by a PCR for the H7 antigen in industry testing, where time is of the essence. Confirmation of the presence of Shiga

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[16] AOAC Official Method 996.09.
[17] AOAC Performance Tested Method 010401
[18] AOAC Performance Tested Method 010407)
[19] AOAC Official Method 2000.13 (8h enrichment) and AOAC Official Method 2000.14 (20h enrichment)
[20] AOAC Official Method 996.10
[21] AOAC Performance Tested Method 010502
[22] AOAC Performance Tested Method 040401
[23] AOAC Performance Tested Method 010301
[24] AOAC Performance Tested Method 030202
[25] AOAC Performance Tested Method 001101
[26] AOAC Official Method 997.11
[27] AOAC Official Method 2005.04
[28] AOAC Official Method 2005.05

toxin and additional cultural methods are required for FSIS test samples. Initially, STEC culture media development was based on the phenotypic characteristics of *E. coli* O157. In 1986, March and Ratnam developed sorbitol MacConkey agar (SMAC) as a selective agar for the detection of *E. coli* O157, based on the inability of most *E. coli* O157 to ferment sorbitol, and although sorbitol-fermenting *E. coli* O157 have subsequently been identified that do ferment sorbitol (Karch *et al.*, 1997; Bielaszewska *et al.*, 1998) SMAC remains the selective medium of choice for identifying this organism. Potassium tellurite and cefixime have been added to SMAC to decrease the numbers of background flora (Zadik *et al.*, 1993). In addition, a number of selective agar supplements have been designed based on the inability of *E. coli* O157 to produce β-Glucuronidase (Doyle and Schoeni, 1984), though a small number of *E. coli* O157 have subsequently been identified as β-Glucuronidase-positive (Keskimäki *et al.*, 1998). Other selective media for *E. coli* O157 include Rainbow Agar™ (Bettelheim, 1998a), CHROMagar™ (Bettelheim, 1998b), and bioMérieux' O157:H7 ID; all chromogenic media that rely on reactions that distinguish target colonies from background flora by color changes. Unfortunately, most of these media will not permit distinction of non-O157 STEC from background flora, and, as yet there is not one medium for non-O157 STEC that is as widespread and as validated as SMAC agar is for *E. coli* O157. However, media have been developed to exploit the enterohemoylitic characteristic of STEC (Sugiyama *et al.*, 2001). Such media are composed of blood agar with various additives, e.g. BVCC (blood agar with vancomycin cefixime cefsulodin) and WMBA (washed sheep blood agar with mitomycin C). The addition of rhamnose to MacConkey agar (RMAC) results in a medium selective for *E. coli* O26, with target colonies growing a distinctive brown color (Hiramatsu *et al.*, 2002). Research is ongoing in this field to increase the specificity of selective media for non-O157 STEC.

Section IV. Conclusion

Examination of FoodNet (2001 – 2006) and national surveillance data (2004-2005) shows a continued increase in the number of reported non O157 STEC infections. Of the five most commonly reported serogroups from both FoodNet sites and non-FoodNet sites, four are shared between both groups (although ranking varies): O26, O45, O103, and O111. The fifth most common serogroup for FoodNet sites is O145, for non-FoodNet sites it is O121. This is consistent with an analysis of non-O157 STEC isolates submitted to CDC, which documented these six as the most commonly isolated serogroups (Brooks *et al.*, 2005). The number of reported cases in FoodNet sites increased 256% from 2000 to 2005, while national surveillance data have documented a 193% increase from 2001 to 2005. Additionally, national surveillance data have shown a 1935% increase in EHEC that has not been serogrouped over the same time-period, while *E. coli* O157 decreased 20%. This demonstrates that although the incidence of *E. coli* O157:H7 infection decreased over this time period, the incidence of infection with non-O157 STEC serotypes did not follow the same trend.

The Healthy People 2010 objective for the incidence of *E. coli* O157:H7 infection was set as 1.00 infections per 100,000 population; no national health objective has been set for non-O157 STEC infections. The preliminary overall 2006 incidence of non-O157 STEC infection in FoodNet sites was 0.46 per 100,000 population, ranging from 0.12 per 100,000 in Tennessee to 1.19 per 100,000 in New Mexico. Several non-FoodNet sites had 2005 non-O157 STEC incidence rates above 1.00 per 100,000; for example, Idaho with 1.26 per 100,000 and Utah with 1.21 per 100,000, reflecting incidences similar to those seen in FoodNet sites in recent years, and those for STEC O157 infection. Furthermore, Virginia reported more cases of non-O157 STEC than *E. coli* O157:H7. This suggests not only regional differences in incidence, but a higher burden of illness than was previously thought.

Efforts by the research community to identify factors associated with the presence and persistence of non-O157 STEC will facilitate development of targeted, successful mitigation strategies, as has been the case for *E. coli* O157:H7. However, this will likely not be an easy task: Schurman *et al.* (2000) stated that there is 'no one area in the food chain where stringent intervention will eliminate STEC as a concern. Highly controlled slaughter is crucial to the production of safe food, but not to the exclusion of other critical

control points in the food chain, beginning on the farm and ending on the consumers table.'

FSIS is unique among the world's public health agencies in the way it routinely tests raw ground beef and raw ground beef components for *E. coli* O157, and in its ability to request a recall of any product found to be contaminated with this organism. This has led to significant changes in the raw ground beef production industry since *E. coli* O157 was declared an adulterant in 1994. Manufacturers, in their desire to produce a safe product, have implemented many targeted food safety interventions that have been proven to significantly reduce the numbers of ground beef samples testing positive for *E. coli* O157 (Naugle *et al.*, 2005). Non-O157 STEC pose just as great a risk to public health as *E. coli* O157. However, the introduction of a new regulatory requirement to combat these organisms will not be undertaken lightly. Even with the overwhelming evidence of the inherent dangers of non-O157 STEC, and the availability of options presented for their detection and identification in food, the cost and other practicalities of the introduction of control measures must be carefully considered.

References

1. Acheson DWK, Keusch GT. 1996 Which Shiga toxin-producing types of *E. coli* are important? *ASM News* 62 (6) 302-306.
2. Acheson DWK. 2001. Unpublished report.
3. Allerberger FM, Wagner P, Schweiger H-P, Rammer A, Resch MP, Dierich A, Friedrich W, Karch H. 2001. *Escherichia coli* O157 infections and unpasteurised milk. *Eurosurveillance* 6(10):147-151
4. Annous BA, Sapers GM, Mattrazzo AM, Riordan DCR. 2001. Efficacy of washing with a commercial flat-bed brush washer, using conventional and experimental washing agents, in reducing populations of *Escherichia coli* on artificially inoculated apples. *Journal of Food Protection* 64(2): 159-163.
5. Armstrong GL, Hollingsworth J, Morris JG Jr. 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiologic Reviews* 18(1):29-51.
6. Arthur TM, Barkocy-Gallagher GA, Rivera-Betancourt M, Koohmaraie M. 2002. Prevalence and characterization of non-O157 Shiga toxin-producing Escherichia coli on carcasses in commercial beef cattle processing plants. *Applied and Environmental Microbiology* 68(10):4847-52.
7. Baffone W, Ciaschini G, Pianetti G, Casaroli A, Bruscolini F. 2001. Detection of *Escherichia coli* O157:H7 and other intestinal pathogens in patients with diarrhoeal disease. *European Journal of Epidemiology* 17: 97-99.
8. Banatvala N, Griffin PM, Greene KD, Barrett TJ, Bibb WF, Green JH, Wells JG; Hemolytic Uremic Syndrome Study Collaborators. 2001. The United States National Prospective Hemolytic Uremic Syndrome Study: microbiologic, serologic, clinical, and epidemiologic findings. *Journal of Infectious Diseases*. 1; 183(7):1063-70.
9. Barkocy-Gallagher GA, Arthur TM, Rivera-Betancourt M, Nou X, Shackelford SD, Wheeler TL, Koohmaraie M. 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *Journal of Food Protection* 66(11):1978-86.
10. Berger S. *E. coli* VTEC non – O157, Lettuce - USA (Utah). Accessed from http://list.uvm.edu/cgi-bin/wa?A2=ind0609a&L=safety&P=3507 September 5, 2007.
11. Bertin Y, Boukhors K, Pradel N, Livrelli V, Martin C. 2001. Stx2 subtyping of Shiga toxin-producing *Escherichia coli* isolated from cattle in France: detection of a new Stx2 subtype and correlation with additional virulence factors. *Journal of Clinical Microbiology* 39 (9): 3060-3065.
12. Besser RE, Lett SM, Weber JT, Doyle MP, Barrett TJ, Wells JG, Griffin PM. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *Journal of the American Medical Association* 5; 269(17): 2217-20.
13. Bettelheim KA. 1998a. Studies of *Escherichia coli* cultured on Rainbow Agar O157 with particular reference to enterohaemorrhagic *Escherichia coli* (EHEC). *Microbiology and Immunology* 42(4):265-9.
14. Bettelheim KA. 1998b. Reliability of CHROMagar O157 for the detection of enterohaemorrhagic *Escherichia coli* (EHEC) O157 but not EHEC belonging to other serogroups. *Journal of Applied Microbiology* 85(3):425-8.
15. Bettelheim KA. 2000. Role of non-O157 VTEC. *Symposium Series Society for Applied Microbiology* (29):38S-50S.

16. Bettelheim KA. 2003. Non-O157 verotoxin-producing *Escherichia coli*: a problem, paradox, and paradigm. *Experimental Biology and Medicine* 228(4):333-44.
17. Beutin L, Zimmermann S, Gleier K. 1998. Human infections with Shiga toxin-producing *Escherichia coli* other than serogroup O157 in Germany. *Emerging Infectious Diseases* 4 (4): 635-63
18. Bielaszewska M, Schmidt H, Karmali M, Khakhria R, Janda J, Bláhová K, Karch H. 1998. Isolation and characterization of sorbitol-fermenting Shiga toxin (verocytotoxin)-producing *Escherichia coli* O157:H- strains in the Czech republic. *Journal of Clinical Microbiology* 36 (7): 2135-2137.
19. Blanco J, Blanco M, Blanco JE, Mora A, Gonzalez EA, Bernardes MI, Alonso MP, Coira A, Rodriguez A, Rey J, Alonso AM, Usera AM. 2003. Verotoxin-producing *Escherichia coli* in Spain: Prevalence, serotypes and virulence genes of O157:H7 and non-O157 VTEC in ruminants, raw beef products, and humans. *The Society for Experimental Biology and Medicine*. Supplement pp.345-351.
20. Bollinger LM, Hussein HS, Sakuma T, Hall MR, Atwill ER. 2005. Prevalence of Shiga-toxin – producing *Escherichia coli* in beef cattle. *Proceedings, Western Section, American Society of Animal Science* 56:222-225.
21. Bower JR. 1999. Foodborne diseases: Shiga toxin producing *E. coli* (STEC). *The Pediatric Infectious Disease Journal.* 18(10):909-10.
22. Boyce TG, Swerdlow DL, Griffin PM. 1995. *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. *New England Journal of Medicine* 333(6):364-8.
23. Bray J, Beavan TED. 1948. Slide agglutination of *Bacterium coli* var. *neapolitanum* in summer diarrhœa. *Journal of Pathology* 60: 395-401.
24. Bren L. 2004. Got milk? Make sure it's pasteurized. *FDA Consumer Magazine*. 38(5):29-31.
25. Brooks JT, Bergmire-Sweat D, Kennedy M. 2004. Outbreak of Shiga toxin–producing *Escherichia coli* O111:H8 infections among attendees of a high school cheerleading camp. Clin Infect Dis; 38: 190-198.
26. Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA. 2005. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. *Journal of Infectious Diseases* 192(8):1422-9.
27. Caprioli A, Morabito S, Brugereb H, Oswald E. 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Veterinary Research*. 36(3):289-311.
28. Catarame TM, O'Hanlon KA, Duffy G, Sheridan JJ, Blair IS, McDowell DA. 2003. Optimization of enrichment and plating procedures for the recovery of *Escherichia coli* O111 and O26 from minced beef. *Journal of Applied Microbiology* 95(5):949-57.
29. Centers for Disease Control and Prevention. 2005. Summary of notifiable diseases - United States, 2003. *Morbidity and Mortality Weekly Reports* 52(54); 1-85.
30. Centers for Disease Control and Prevention. November 12, 2003 memo: STEC: Case and outbreak surveillance from 2002 and recommendations on reporting: http://www.cdc.gov/foodborneoutbreaks/ecoli/CSTE2002.pdf Accessed September 5, 2007.
31. Centers for Disease Control and Prevention. 1993. Update: Multistate Outbreak of Escherichia coli O157:H7 Infections from Hamburgers -- Western United States, 1992-1993. *Morbidity and Mortality Weekly Reports* 42 (14): 258-263.

32. Centers for Disease Control and Prevention. 1995. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21 – Helena, Montana, 1994. *Morbidity and Mortality Weekly Reports* 44 (27): 501-503.
33. Centers for Disease Control and Prevention. 2000. *Escherichia coli* O111:H8 Outbreak among Teenage Campers - Texas. *Morbidity and Mortality Weekly Reports* 49(15); 321-4.
34. Centers for Disease Control and Prevention. 2003. Summary of notifiable diseases - United States, 2003. *Morbidity and Mortality Weekly Reports* 50(53); 1-108.
35. Centers for Disease Control and Prevention. 2006a. Summary of Notifiable Diseases --- United States, 2004. *Morbidity and Mortality Weekly Reports* 53: 1-79
36. Centers for Disease Control and Prevention. 2006b. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 States, United States, 2005. *Morbidity and Mortality Weekly Reports*; 55: 392-5.
37. Chapman PA, Siddons CA, Cerdan Malo AT, Harkin MA. 2000. A one year study of *Escherichia coli* O157 in raw beef and lamb products. *Epidemiology and Infection*. 124(2):207-13.
38. Chapman PA, Siddons CA, Wright DJ, Norman P, Fox J, Crick E. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiology and Infection* 111(3):439-47.
39. Chapman PA. 1995. Verocytotoxin-producing *Escherichia coli*: An overview with emphasis on the epidemiology and prospects for control of *E. coli* O157:H7. *Food Control* 6 (4):187-193.
40. Chinen I, Tanaro JD, Miliwebsky E, Lound LH, Chillemi G, Ledri S, Baschkier A, Scarpin M, Manfredi E, Rivas M. 2001. Isolation and characterization of *Escherichia coli* O157:H7 from retail meats in Argentina. *Journal of Food Protection* 64(9):1346-51.
41. Cieslak PR, Barrett TJ, Griffin PM, Gensheimer KF, Beckett G, Buffington J, Smith MG. 1993. *Escherichia coli* O157:H7 infection from a manured garden. *The Lancet* 342(8867):367.
42. Conedera G, Dalvit P, Martini M, Galiero G, Gramaglia M, Goffredo E, Loffredo G, Morabito S, Ottaviani D, Paterlini F, Pezzotti G, Pisanu M, Semprini P, Caprioli A. 2004. Verocytotoxin-producing *Escherichia coli* O157 in minced beef and dairy products in Italy. *International Journal of Food Microbiology*. 96(1):67-73.
43. Corrigan JJ Jr, Boineau FG. 2001. Hemolytic-uremic syndrome. Pediatric Review. (11):365-9.
44. Cray WC Jr, Thomas LA, Schneider RA, Moon HW. 1996. Virulence attributes of *Escherichia coli* isolated from dairy heifer feces. *Veterinary Microbiology*. 53(3-4):369-74.
45. Crump JA, Sulka AC, Langer AJ, Schaben C, Crielly AS, Gage R, Baysinger M, Moll M, Withers G, Toney DM, Hunter SB, Hoekstra RM, Wong SK, Griffin PM, Van Gilder TJ. 2002. An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. *New England Journal of Medicine*. 347(8):555-60.
46. Dean-Nystrom EA, Bosworth BT, Cray WC Jr, Moon HW. 1997. Pathogenicity of *Escherichia coli* O157:H7 in the intestines of neonatal calves. *Infection and Immunity* 65(5):1842-8.
47. DebRoy C, Roberts E, Kundrat J, Davis MA, Briggs CE, Fratamico PM. 2004. Detection of *Escherichia coli* serogroups O26 and O113 by PCR amplification of the wzx and wzy genes. *Applied and Environmental Microbiology* 70(3):1830-2.
48. Dekoninck, A., Blaton, B., Lontie, M. and Verhaegen, J. 1998. Prevalence of *Escherichia coli* O157:H7. *Acta Clinica Belgica* 53 (1): 55-56.
49. Dingman DW. 2000. Growth of *Escherichia coli* O157:H7 in bruised apple (*Malus domestica*) tissue as influenced by cultivar, date of harvest, and source. *Applied and Environmental Microbiology* 66(3):1077-83.

50. Doyle MP, Schoeni JL. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. *Applied and Environmental Microbiology* 48 (4): 855-6.
51. Doyle MP, Schoeni JL. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. *Applied and Environmental Microbiology* 53(10):2394-6.
52. Doyle MP, Zhao T, Meng J, and Zhao, S. 1997. *Escherichia coli* O157:H7. In '*Food Microbiology: Fundamentals and Frontiers*' pp. 171-191. M.P. Doyle, L.R. Beuchat and T. Montville eds. ASM Press, Washington DC.
53. Doyle MP. 1991. *Escherichia coli* O157:H7 and its significance in foods. *International Journal of Food Microbiology*. 12(4):289-301.
54. Drysdale M, MacRae M, Strachan NJ, Reid TM, Ogden ID. 2004. The detection of non-O157 *E. coli* in food by immunomagnetic separation. *Journal of Applied Microbiology* 97(1):220-4.
55. D'Souza JM, Wang L, Reeves P. 1997. Sequence of the *Escherichia coli* O26 O antigen gene cluster and identification of O26 specific genes. *Gene*. 297(1-2):123-7.
56. Dunne E, Wallace D, Bender J, Dembek Z, Davis MA, Gilbert L, Zansky S, Shiferaw B, Carter M, Griffin P, Mead P, FoodNet Working Group. 2000. Results from three years of active surveillance for Hemolytic Uremic Syndrome (HUS) through FoodNet, United States, 199701999. Abstracts of the Infectious Diseases Society of America 38th Annual Meeting. New Orleans, LA.
57. Eklund M, Scheutz F, Siitonen A. 2001. Clinical isolates of non-O157 Shiga toxin producing *Escherichia coli*: serotypes, virulence characteristics, and molecular profiles of strains of the same serotype. *Journal of Clinical Microbiology* 39 (8): 2829-2834.
58. Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M, Laegreid WW. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proceedings of the National Academies of Science* USA. 28; 97(7):2999-3003.
59. Elkins SL, Wilson PP Jr, Files JC, Morrison FS. 1996. Thrombotic thrombocytopenic purpura: evolution across 15 years. *Journal of Clinical Apheresis* 11(4):173-5.
60. Elliott EJ, Robins-Browne RM, O'Loughlin EV. 2001. Nationwide study of haemolytic uraemic syndrome: clinical, microbiological, and epidemiological features. *Archive of Childhood Diseases*. 85: 125-131.
61. Fey PD, Wickert RS, Rupp ME, Safranek TJ, Hinrichs SH. 2000. Prevalence of non-O157 Shiga toxin-producing *Escherichia coli* in diarrheal stool samples from Nebraska. *Emerging Infectious Diseases* 6 (5): 530-533.
62. Fratamico PM, Bagi LK, Bush EJ, Solow BT. 2004. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* in swine feces recovered in the National Animal Health Monitoring System's Swine 2000 study. *Applied and Environmental Microbiology* 70(12):7173-8.
63. Fratamico PM, Bagi LK, Pepe T. 2000. A multiplex polymerase chain reaction assay for rapid detection and identification of *Escherichia coli* O157:H7 in foods and bovine feces. *Journal of Food Protection* 63(8):1032-7.
64. Fratamico PM, Briggs CE, Needle D, Chen CY, DebRoy C. 2003. Sequence of the *Escherichia coli* O121 O-antigen gene cluster and detection of enterohemorrhagic *E. coli* O121 by PCR amplification of the wzx and wzy genes. *Journal of Clinical Microbiology* 41(7):3379-83

65. Fratamico PM, Debroy C, Strobaugh Jr TP, Chen CY. 2005. DNA sequence of the *Escherichia coli* O103 O antigen gene cluster and detection of enterohemorrhagic *E. coli* O103 by PCR amplification of the wzx and wzy genes. *Canadian Journal of Microbiology* 51(6):515-22.
66. Fratamico PM, Sackitey SK, Wiedmann M, Deng MY. 1995. Detection of *Escherichia coli* O157:H7 by multiplex PCR. *Journal of Clinical Microbiology* 33(8):2188-91.
67. Gerber A, Karch H, Allerberger F. 2002. Clinical course and the role of Shiga toxin–producing *Escherichia coli* infection in the hemolytic-uremic syndrome in pediatric patients, 1997–2000, in Germany and Austria: A prospective study. *Journal of Infectious Diseases*. 186: 493-500.
68. Goldwater PN, Bettelheim KA. 1996. An outbreak of Hemolytic Uremic Syndrome due to *Escherichia coli* O157:H-: or was it? *Emerging Infectious Diseases* 2(2): 153-154.
69. Goldwater PN, Bettelheim KA. 2000. *Escherichia coli* 'O' group serology of a haemolytic uraemic syndrome (HUS) epidemic. *Scandinavian Journal of Infectious Diseases* 32(4):385-94.
70. Griffin PM, Tauxe RV. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiologic Reviews* 13:60-98.
71. Guth BE, Chinen I, Miliwebsky E, Cerqueira AM, Chillemi G, Andrade JR, Baschkier A, Rivas M. 2003. Serotypes and Shiga toxin genotypes among *Escherichia coli* isolated from animals and food in Argentina and Brazil. *Veterinary Microbiology*. 29; 92(4):335-49.
72. Hiramatsu R, Matsumoto M, Miwa Y, Suzuki Y, Saito M, Miyazaki Y. 2002. Characterization of Shiga toxin-producing *Escherichia coli* O26 strains and establishment of selective isolation media for these strains. *Journal of Clinical Microbiology* 40(3):922-5.
73. Hussein HS, Sakuma T. 2005. Prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products. *Journal of Dairy Science* 88(2):450-65.
74. Itoh, Y, Sugita-Konishi Y, Kasuga F, Iwaki M, Hara-Kudo Y, Saito N, Noguchi Y, Konuma H, Kumagai S. 1998. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts *Applied and Environmental Microbiology* 64(4)**:** 1532–1535.
75. Karch H, Huppertz H-I, Bockemühl J, Schmidt H, Schwarzkopf A. Lissner R. 1997. Shiga toxin-producing *Escherichia coli* infections in Germany. *Journal of Food Protection* 60 (11): 1454-1457.
76. Karmali MA, Steele BT, Petric M, Lim C. 1983. Sporadic cases of hemolytic uremic syndrome associated with fecal cytotoxin and cytotoxin producing *Escherichia coli*. *The Lancet* i: 619-620.
77. Keene WE, Hedberg K, Herriott DE, Hancock DD, McKay RW, Barrett TJ, Fleming DW. 1997. A prolonged outbreak of *Escherichia coli* O157:H7 infections caused by commercially distributed raw milk. *Journal of Infectious Diseases* 176(3):815-8.
78. Keene WE, McAnulty JM, Hoesly FC, Williams LP Jr, Hedberg K, Oxman GL, Barrett TJ, Pfaller MA, Fleming DW. 1994. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. *New England Journal of Medicine* 331(9):579-84.
79. Keskimäki M, Ikäheimo R, Kärkkäinen P, Scheutz F, Ratiner Y, Puohineimi P, Siitonen A. 1997. Shiga toxin-producing *Escherichia coli* serotype OX3:H21 as a cause of hemolytic-uremic syndrome. *Clinical Infectious Diseases* 24: 1278-1279.
80. Keskimäki M, Saari M, Heiskanen T, Siitonen A. 1998. Shiga toxin-producing *Escherichia coli* in Finland from 1990 through 1997: Prevalence and characteristics of isolates. *Journal of Clinical Microbiology* 36 (12): 3641-3646.

81. Konowalchuk J, Speirs JI, Stavric S. 1977 Vero response to a cytotoxin of *Escherichia coli*. *Infection and Immunity* 18: 775-779.
82. Lindqvist R, Antonsson AK, Norling B, Persson L, Ekström ACL, Fäger U, Eriksson E, Löfdahl S, Norberg P. 1998. The prevalence of verocytotoxin-producing *Escherichia coli* (VTEC) and *E. coli* O157:H7 in beef in Sweden determined by PCR assays and an immuno-magnetic serparation (IMS) method. *Food Microbiology* 15, 591-601.
83. Magwira CA, Gashe BA, Collison EK. 2005. Prevalence and antibiotic resistance profiles of *Escherichia coli* O157:H7 in beef products from retail outlets in Gaborone, Botswana. *Journal of Food Protection* 68(2):403-6.
84. March SB, Ratnam S. 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *Journal of Clinical Microbiology* 23: 869-872.
85. McNally A, Roe AJ, Simpson S, Thompson-Carter FM, Hoey DE, Currie C, Chakraborty T, Smith DGE, Gally DL. 2001. Differences in levels of secreted locus of enterocyte effacement proteins between human disease-associated and bovine *Escherichia coli* O157. *Infection and Immunity* 69 (8): 5107-5114.
86. Mead PS, Griffin PM. 1998. *Escherichia coli* O157:H7. *The Lancet* 352(9135):1207-12.
87. Nataro JP, Kaper JB. 1998. Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews* 11(1):142-201.
88. Naugle AL, Holt KG, Levine P, Eckel R. 2005. Food safety and inspection service regulatory testing program for *Escherichia coli* O157:H7 in raw ground beef. *Journal of Food Protection* 68(3):462-8
89. Paton AW, Srimanote P, Woodrow MC, Paton JC. 2001. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli* strains that are virulent for humans. *Infection and Immunity* 69(11):6999-7009.
90. Paton JC, Paton AW. 1998. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clinical Microbiology Reviews* 11 (3): 450-479.
91. Pelayo JS, Scaletsky IC, Pedroso MZ, Sperandio V, Giron JA, Frankel G, Trabulsi LR. 1999. Virulence properties of atypical EPEC strains. *Journal of Medical Microbiology* 48(1):41-9.
92. Perelle S, Dilasser F, Grout J, Fach P. 2003. Development of a 5'-nuclease PCR assay for detecting Shiga toxin-producing *Escherichia coli* O145 based on the identification of an 'O-island 29' homologue. *Journal of Applied Microbiology* 94(4):587-94.
93. Perelle S, Dilasser F, Grout J, Fach P. 2004. Detection by 5'-nuclease PCR of Shiga-toxin producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases. *Molecular and Cellular Probes*. 18(3):185-92.
94. Piérard D, Van Damme L, Moriau L, Stevens D, Lauwers S. 1997. Virulence factors of verocytotoxin-producing *Escherichia coli* isolated from raw meats. *Applied and Environmental Microbiology* 63(11):4585-7.
95. Potter ME, Kaufmann AF, Blake PA, Feldman RA. 1984. Unpasteurized milk. The hazards of a health fetish. *Journal of the American Medical Association* 252(15):2048-52.
96. Pradel N, Livrelli V, De Champs C, Palcoux JB, Reynaud A, Scheutz F, Sirot J, Joly B, Forestier C. 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *Journal of Clinical Microbiology* 38(3):1023-31.

97. Pradel N, Livrelli V, De Champs C, Palcoux JB, Reynaud A, Scheutz F, Sirot J, Joly B, Forestier C. 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *Journal of Clinical Microbiology* 38(3):1023-31.
98. Rahn K, Wilson JB, McFadden KA, Read SC, Ellis AG, Renwick SA, Clarke RC Johnson RP. 1996. Comparison of Vero cell assay and PCR as indicators of the presence of verocytotoxigenic *Escherichia coli* in bovine and human fecal samples. *Applied and Environmental Microbiology* 62 (12): 4314-4317.
99. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerging Infectious Diseases* 11 (4): 603-610.
100. Rasmussen MA, Cray WC Jr, Casey TA, Whipp SC. 1993. Rumen contents as a reservoir of enterohemorrhagic *Escherichia coli*. *FEMS Microbiology Letters* 114(1):79-84.
101. Reida P, Wolff M, Pohls HW, Kuhlmann W, Lehmacher A, Aleksic S, Karch H, Bockemuhl J. 1994. An outbreak due to enterohaemorrhagic *Escherichia coli* O157:H7 in a children day care centre characterized by person-to-person transmission and environmental contamination. *Zentralblatt fur Bakteriologie*. 281(4):534-43.
102. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine* 308(12):681-5.
103. Riordan DCR, Duffy G, Sheridan JJ, Eblen BS, Whiting RC, Blair IS, McDowell DA. 1998. Survival of *Escherichia coli* O157:H7 during the manufacture of pepperoni. *Journal of Food Protection* 61(2): 146-151.
104. Riordan DCR, Sapers GM, Annous BA. 2000. The survival of *Escherichia coli* O157:H7 in the presence of *Penicillium expansum* and *Glomerella cingulata* in wounds on apple surfaces. *Journal of Food Protection* 63(12): 1637-1642.
105. Samadpour M, Barbour MW, Nguyen T, Cao TM, Buck F, Depavia GA, Mazengia E, Yang P, Alfi D, Lopes M, Stopforth JD. 2006.Incidence of enterohemorrhagic *Escherichia coli*, *Escherichia coli* O157, *Salmonella*, and *Listeria monocytogenes* in retail fresh ground beef, sprouts, and mushrooms. *Journal of Food Protection* 69(2):441-3.
106. Samadpour M, Ongerth JE, Liston J, Tran N, Nguyen D, Whittam TS, Wilson RA, Tarr PI. 1994. Occurrence of Shiga-like toxin-producing *Escherichia coli* in retail fresh seafood, beef, lamb, pork, and poultry from grocery stores in Seattle, Washington. *Applied and Environmental Microbiology* 60 (3): 1038-1040.
107. Sanath Kumar H, Otta SK, Karunasagar I, Karunasagar I. 2001. Detection of Shiga-toxigenic *Escherichia coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. *Letters in Applied Microbiology* 33(5):334-8.
108. Schurman RD, Hariharan H, Heaney SB, Rahn K. 2000. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* in beef cattle slaughtered on Prince Edward Island. *Journal of Food Protection* 63 (11): 1583-1586.
109. Sharma VK, Dean-Nystrom EA, Casey TA. 1999. Semi-automated fluorogenic PCR assays (TaqMan) for rapid detection of *Escherichia coli* O157:H7 and other Shiga toxigenic *E. coli*. *Molecular and Cellular Probes*. 13(4):291-302.
110. Sugiyama K, Inoue K, Sakazaki R. 2001. Mitomycin-supplemented washed blood agar for the isolation of Shiga toxin-producing *Escherichia coli* other than O157:H7. *Letters in Applied Microbiology*. 33(3):193-5.

111. Taormina PJ, Beuchat LR, Slutsker L. 1999. Infections associated with eating seed sprouts: an international concern. *Emerging Infectious Diseases*. 5(5):626-34.
112. Tarr PI, Neill MA. 1996. Perspective: The problem of non-O157:H7 Shiga toxin (verocytotoxin)- producing *Escherichia coli*. *The Journal of Infectious Diseases* 174: 1136-1139.
113. Thran BH, Hussein HS, Hall MR, Khaiboullina SF. 2001. Shiga toxin-producing *Escherichia coli* in beef heifers grazing an irrigated pasture. *Journal of Food Protection* 64(10):1613-6.
114. Tilden J Jr., Young W, McNamara AM, Custer CM, Boesel B, Lambert-Fair MA, Majkowski J, Vugia D, Werner SB, Hollingsworth J, Morris JG Jr. 1996. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. *American Journal of Public Health* 86 (8 Pt 1):1142-5.
115. Vernozy-Rozand C, Ray-Gueniot S, Ragot C, Bavai C, Mazuy C, Montet MP, Bouvet J, Richard Y. 2002. Prevalence of *Escherichia coli* O157:H7 in industrial minced beef. *Letters in Applied Microbiology* 35(1):7-11.
116. Wachsmuth IK, Griffin PM, Wells JG. 1991. *Escherichia coli* O157:H7, a cause of hemorrhagic colitis and hemolytic uremic syndrome. *Acta Paediatrica Japonica*. 33(5):603-12.
117. Wang L, Curd H, Qu W, Reeves PR. 1998. Sequencing of *Escherichia coli* O111 O-antigen gene cluster and identification of O111-specific genes. *Journal of Clinical Microbiology* 36(11):3182-
118. Wells JG, Shipman LD, Greene KD, Sowers EG, Green JH, Cameron DN, Downes FP, Martin ML, Griffin PM, Ostroff SM, *et al.* 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. *Journal of Clinical Microbiology* 29(5):985-
119. Wickham ME, Lupp C, Mascarenhas M, Vazquez A, Coombes BK, Brown N., Coburn BA, Deng W, Puente JL, Karmali MA, Finlay BB. 2006. Bacterial genetic determinants of nNon-O157 STEC outbreaks and hemolytic-uremic syndrome after infection. *Journal of Infectious Diseases* 194: 819-828.
120. Wieler LH, Vieler E, Erpenstein C, Schlapp T, Steinruck H, Bauerfeind R, Byomi A, Baljer G. 1996. Shiga toxin-producing *Escherichia coli* strains from bovines: association of adhesion with carriage of *eae* and other genes. *Journal of Clinical Microbiology* 34(12):2980-4.
121. Wilkus, J. Personal communication.
122. World Health Organization. 1998. Zoonotic non-O157 Shiga toxin-producing *Escherichia coli* (STEC). Report of a WHO Scientific working group meeting. Berlin, Germany 23-26 June.
123. Yatsuyanagi J, Saito S, Ito I. 2002. A case of hemolytic-uremic syndrome associated with Shiga toxin 2-producing *Escherichia coli* O121 infection caused by drinking water contaminated with bovine feces. *Japanese Journal of Infectious Diseases* 55(5):174-6.
124. Yongmin, B. 2004. The challenges for food safety in China. *China Perspectives* n°53, May - June 2004, p4.
125. Zadik PM, Chapman PA, Siddons CA. 1993. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. *Journal of Medical Microbiology*. 39(2):155-8.
126. Zhao T, Doyle MP, Shere J, Garber L. 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Applied and Environmental Microbiology* 61(4):1290-3.

Table 1. Outbreaks of Shiga toxin-producing *E. coli* non-O157 infections in the United States, 1990-2006[*]

Year	Serogroup	State	Number of Ill Persons	Serologically Confirmed	Suspected Exposure or Vehicle	HUS Reported	Exposure or Vehicle Confirmed
1990	O111[†]	Ohio	5	Yes	Unknown	Yes	No
1994	O104[†]	Montana	18	Yes	Milk	No	No
1998	O121[‡]	Montana	40	Unknown	Unknown	Unknown	Unknown
1999	O111[†]	Texas	56	Yes	Salad Bar	Yes	No
	O121[†]	Connecticut	11	Yes	Lake Water	Yes	No
2000	O103[†]	Washington	18	Yes	Punch	Yes	No
	O111[§]	Minnesota	59[□]	Yes	Calves	No	No
2001	O26[†]	Minnesota	4	No	Lake Water	No	No
	O111[§] O51[¶]	Minnesota	25[¶]	Yes	Calves	No	Yes, Calves
	O111[†]	South Dakota	3	No	Daycare	No	No
2004	O111[‡]	New York North Carolina[**]	212[††]	Yes	Unpasteurized Apple Cider	No[**]	Yes, Environmental Sample
2005	O45[‡‡]	New York	52[§§]	Yes	Ill Food Worker	No	No
2006	O121[□□]	Utah	4	Yes	Lettuce	Yes	No

Table adapted from Brooks *et al*, 2005.

[*]Provisional data from January 1, 2006 to June 30, 2006

[†] Brooks, JT, *et al.* Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. *J Infect Dis.* 2005; 192:1426.

[‡]CDC outbreak surveillance data

[§]Minnesota Department of Health Disease Control Newsletter, Volume 33, Number 2 (pages 13-20), March/April 2005

[□] Smith KE, *et al.* Outbreaks of enteric infections caused by multiple pathogens associated with calves at a farm day camp. *Pediatr Infect Dis J*. 2004; 23:1098-104; two case-patients tested positive for O111 as part of a camp outbreak, C*ryptosporidium parvum*, *Campylobacter jejuni*, and *Salmonella* Typhimurium were also identified among other confirmed case-patients

[¶] Smith KE, *et al.* Outbreaks of enteric infections caused by multiple pathogens associated with calves at a farm day camp. *Pediatr Infect Dis J.* 2004; 23:1098-104; two case-patients tested positive for O111 and two tested positive for O rough:H11, which was concluded to be indistinguishable from O51:H11, which was isolated from calves, as part of a camp outbreak, *E. coli* O157:H7 and C*ryptosporidium parvum* were also identified among other confirmed case-patients

[**]CDC Electronic Foodborne Outbreak Reporting System (eFORS)

[††]*Escherichia coli* O111 and *Cryptosporidium parvum* were identified

‡‡ CDC. Importance of Culture Confirmation of Shiga Toxin-producing *Escherichia coli* Infection as Illustrated by Outbreaks of Gastroenteritis --- New York and North Carolina, 2005. MMWR Weekly. 2006; 55:1042-1045

§§Sixteen samples were stx1 positive by PCR; three samples sent to the CDC were positive for O45:NM

□□Weber-Morgan Health Department August 7, 2006 *E. coli* News Release

Table 2. Non-O157 STEC infections by serogroups, 2000-2005[29]

	Year							
	2000	**2001**	**2002**	**2003**	**2004**	**2005**	**2000-2005**	
Serogroup	***n***	***n***	***n***	***n***	***n***	***n***	***n***	**%**
0	0	3	0	0	0	0	3	0.8
8	1	0	0	0	0	0	1	0.3
22	0	0	0	0	2	1	3	0.8
26	5	17	9	7	12	31	81	21.2
33	0	0	0	1	0	0	1	0.3
45	3	8	3	4	6	7	31	8.1
51	0	1	0	0	0	1	2	0.5
76	0	0	0	1	1	2	4	1.0
84	0	0	0	1	0	0	1	0.3
87	0	0	0	0	0	1	1	0.3
88	1	1	0	0	1	0	3	0.8
91	1	1	1	0	0	3	6	1.6
103	6	13	6	9	15	30	79	20.7
110	0	0	0	0	1	0	1	0.3
111	10	11	9	13	41	20	104	27.2
113	0	0	0	1	0	0	1	0.3
117	0	0	0	0	0	1	1	0.3
118	1	1	0	2	1	1	6	1.6
119	1	0	0	0	0	0	1	0.3
121	2	0	2	3	0	8	15	3.9
126	0	0	0	0	1	0	1	0.3
128	0	1	0	0	0	0	1	0.3
132	0	0	0	0	1	0	1	0.3
145	3	2	2	3	5	3	18	4.7
146	0	0	2	0	0	1	3	0.8
153	0	0	1	0	1	1	3	0.8
159	1	0	0	0	0	0	1	0.3
163	1	0	0	0	0	0	1	0.3
165	0	1	0	2	0	0	3	0.8
174	0	1	0	0	2	0	3	0.8
177	0	0	0	0	0	1	1	0.3
181	0	0	0	0	0	1	1	0.3
Total serogroups	**36**	**61**	**35**	**47**	**90**	**113**	**382**	100.0
Missing serogroups	*0*	*0*	*0*	*0*	*20*	*15*	*35*	

[29] Source: CDC, 2006. Personal communication.

Table 3. Number of laboratory-confirmed non-O157 STEC infections ascertained in FoodNet, by age group, 2000-2005[30]

	Non-O157 STEC	
Age Categories	***n***	**%**
<5	112	27.9
5-9	50	12.5
10-19	104	25.9
20-29	41	10.2
30-39	25	6.2
40-49	18	4.5
50-59	16	4.0
60-69	15	3.7
70+	20	5.0
Total responses	**401**	**100.0**
Missing responses	*16*	

[30] Source: CDC, 2006. Personal communication.

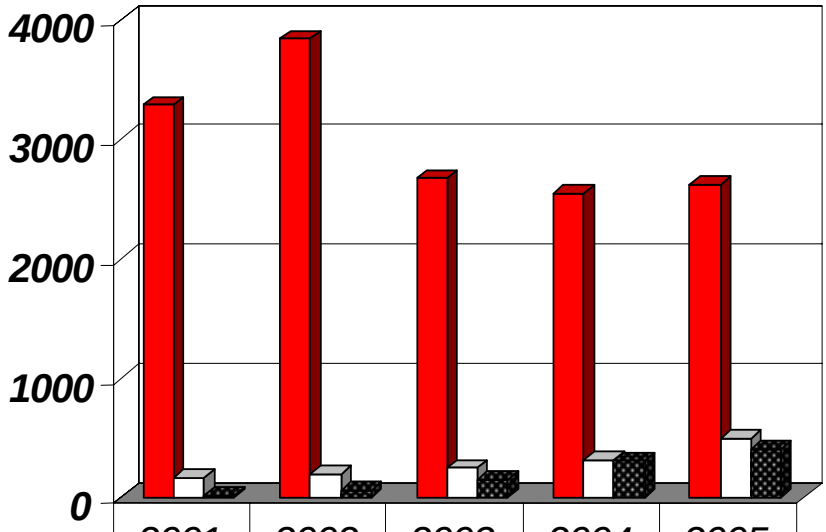
Table 4. Shiga-toxin *E. coli* O157 and non-O157 in 13 non-FoodNet states, United States, 2004-July 2006[*31]															
	CO	**ID**	**FL**	**MI**	**MO**	**NC**	**OK**	**PA**	**SD**	**UT**	**VA**	**WA**	**WY**		
***E.coli* O157**	145	129	153	196	200	135	70	150	67	99	113	361	28	1846	78.5%
non-O157 STEC	19	39	14	19	42	20	6	51	2	58	130	6	13	419	17.8%
O4	1													1	0.4%
O8								1						1	0.4%
O11	2													2	0.7%
O19					1									1	0.4%
O26	10	15		1	8	2	1	8		26	9	3		83	30.4%
O28		1												1	0.4%
O33					1									1	0.4%
O45				5		7	1	16		1	5			35	12.8%
O49				1										1	0.4%
O51							1							1	0.4%
O63					1									1	0.4%
O77								1						1	0.4%
O84								1						1	0.4%
O91					2					1	1			4	1.5%
O103	2	3		2	9	6		13		4	10		1	50	18.3%
O111	2	7		2	2	2		4	1	9	1			30	11.7%
O112	1													1	0.4%
O116								1						1	0.4%
O118								1						1	0.4%
O121		10				2		1	1	15		1	2	32	11.7%
O128		1												1	0.4%
O156	1													1	0.4%
O145		1		2	2	1		2		1	1		1	11	4.0%
O158											1			1	0.4%
O165		1			2			2						5	1.8%
O174												1		1	0.4%
O177											1	1		2	0.7%
O179					1									1	0.4%
O181					1									1	0.4%
missing	*0*	*0*	*14*	*6*	*12*	*0*	*3*	*0*	*0*	*1*	*101*	*0*	*9*	*146*	
STEC O undet	2	6	53	2	0	0	2	8	5	1	3	0	4	86	3.7%

[31] Source: FSIS Human Health Sciences Division (2006) Internal report.

Table 5. Prevalence of STEC in retail foods in non-US countries

Country	Organism	Reported prevalence	Reference
Argentina	*E. coli* O157:H7	4.8% of fresh sausages; 3.8% of raw ground beef; 3.3% of dry sausages	Chinen *et al.*, 2001
Belgium	All STEC	4.6% of raw meat samples (beef, mutton and venison)	Pierard *et al.*, 1997
Botswana	*E. coli* O157:H7	5.2% of meat cubes; 3.8% of raw ground beef; 2.3% of fresh sausages	Magwira *et al.*, 2005
England	*E. coli* O157:H7	2.9% of lamb products; 1.1% of beef products	Chapman *et al.*, 2000
France	All STEC	11% of beef; 10% of cheese	Pradel *et al.*, 2000
France	*E. coli* O157:H7	0.1% of raw ground beef	Vernozy-Rozand *et al.*, 2002
India	non-O157 STEC	2/60 fish samples; 3/48 clam samples	Sanath Kumar *et al.*, 2001
Italy	*E. coli* O157:H7	0.4% of raw ground beef	Conedera *et al.*, 2004
New Zealand	All STEC	17% of lamb; 12% of beef; 4% of pork; 0% of chicken	Brooks *et al.*, 2001
Sweden	*E. coli* O157:H7 All STEC	0.06-0.5% of raw ground beef 4% of raw ground beef	Lindqvist *et al.*, 1998

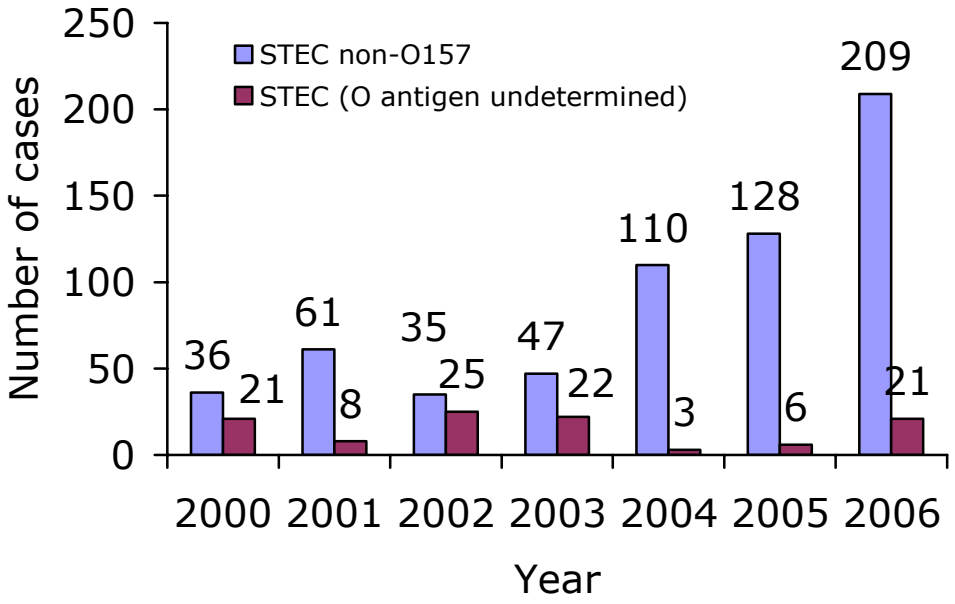
Figure 1. Cases of enterohemorrhagic *E. coli* reported in the United States, 2001-2005[32]



	2001	2002	2003	2004	2005
E. coli O157	3287	3840	2671	2544	2621
E. coli Non-O157 *STEC*	171	194	252	316	501
E. coli not serogrouped	20	60	156	308	407

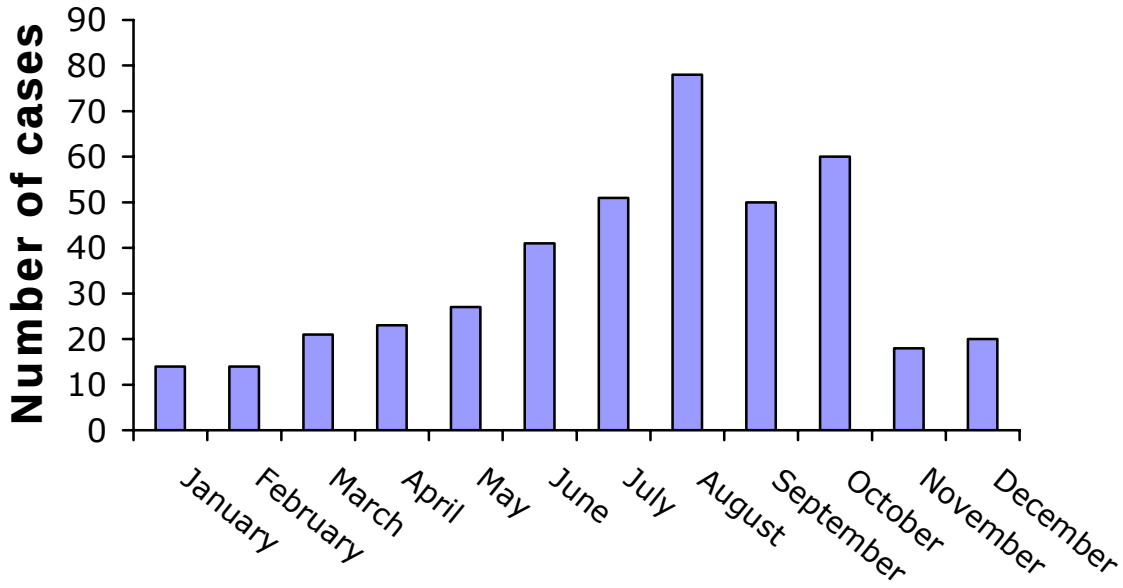
[32] Source: CDC (2006) Summary of Notifiable Diseases, 2001-2005. *MMWR*

Figure 2. Laboratory-confirmed non-O157 STEC and O-antigen undetermined infections ascertained in FoodNet, 2000-2006[33]



[33] 2006 – preliminary data

Figure 3. Laboratory-confirmed non-O157 STEC infections ascertained in FoodNet by month, 2000-2005[34]



[34] Source: CDC, 2006. Personal communication.

Paperwork Reduction Act

The **Federal Register** information collection notice was published in the proposed rule on September 29, 1998 (63 FR 51864). A revised information collections package was submitted to the Office of Management and Budget and approved under OMB control number 0560–0148.

Discussion of Comments

Five comments, all in favor of the proposed change, were received from tobacco importers and brokers in response to the proposed rule which was published in the **Federal Register** at 63 FR 51864 (September 29, 1998). There were no unfavorable comments. Accordingly, for the reasons given when the proposed rule was published, it has been determined to adopt the proposed rule as a final rule.

List of Subjects in 7 CFR Part 1464

Imports, Loan programs—agriculture, Tobacco.

For the reasons set forth in the preamble, 7 CFR 1464 is amended as follows:

PART 1464—TOBACCO [Amended]

1. The authority citation for 7 CFR 1464 continues to read as follows:

Authority: 7 U.S.C. 1421, 1423, 1441, 1445, 1445–1 and 1445–2; 15 U.S.C. 714b, 714c.

2. Section 1464.101(b) is amended by revising the definition of ''de minimis special entries'' to read as follows:

§ 1464.101 Definitions.

* * * * *

(b) Terms. * * *

De minimis special entries. Imports of unmanufactured tobacco when the total importation at any time or on any date is 100 kilograms or less and such tobacco is imported segregated from other tobacco for use as samples, for research, or other use approved by the Director.

* * * * *

Signed at Washington, DC, on January 11, 1999.

Keith Kelly,

Executive Vice President, Commodity Credit Corporation.

[FR Doc. 99–1134 Filed 1–15–99; 8:45 am]

BILLING CODE 3410–05–P

DEPARTMENT OF AGRICULTURE

Food Safety and Inspection Service

9 CFR Chapter III

[Docket No. 97–068N]

Beef Products Contaminated With *Escherichia Coli* O157:H7

AGENCY: Food Safety and Inspection Service, USDA.

ACTION: Policy on beef products contaminated with E. coli O157:H7.

SUMMARY: In 1994, the Food Safety and Inspection Service (FSIS) notified the public that raw ground beef products contaminated with the pathogen *Escherichia coli* O157:H7 are adulterated under the Federal Meat Inspection Act unless the ground beef is further processed to destroy this pathogen. FSIS is publishing this notice to provide the public with information about its policy regarding beef products contaminated with *Escherichia coli* O157:H7 and to afford the public an opportunity to submit comments and recommendations relevant to the Agency's policy, and any regulatory requirements that may be appropriate to prevent the distribution of beef products adulterated with this pathogen.

DATES: Comments must be received by March 22, 1999.

ADDRESSES: Submit one original and two copies of written comments to FSIS Docket Clerk, Docket No. 97–068N, U.S. Department of Agriculture, Food Safety and Inspection Service, Room 102, Cotton Annex, 300 12th Street, SW, Washington, DC 20250–3700. All comments submitted in response to this notice will be available for public inspection in the Docket Clerk's office between 8:30 a.m. and 4:30 p.m., Monday through Friday.

FOR FURTHER INFORMATION CONTACT: Patricia F. Stolfa, Assistant Deputy Administrator, Regulations and Inspection Methods, Food Safety and Inspection Service, Washington, DC 20250–3700; (202) 205–0699.

SUPPLEMENTARY INFORMATION:

Introduction

The Food Safety and Inspection Service (FSIS) administers a regulatory program under the Federal Meat Inspection Act (FMIA) (21 U.S.C. 601 *et seq.*) to protect the health and welfare of consumers by preventing the distribution of meat and meat food products that are unwholesome, adulterated, or misbranded. This notice explains the Agency's policy governing beef products that contain the pathogen *Escherichia coli* O157:H7 (*E. coli* O157:H7). Interested parties are encouraged to submit their views, relevant information, and suggestions regarding this policy or any regulatory requirements that the commenters believe may be appropriate to prevent the distribution of products contaminated with *E. coli* O157:H7.

Beef Products of Concern

In 1994, FSIS notified the public that raw ground beef products contaminated with *E. coli* O157:H7 are adulterated within the meaning of the FMIA unless the ground beef is further processed to destroy this pathogen. Exposure to *E. coli* O157:H7 has been linked with serious, life-threatening human illnesses (hemorrhagic colitis and hemolytic uremic syndrome). Raw ground beef products present a significant public health risk because they are frequently consumed after preparation (*e.g.*, cooking hamburger to a rare or medium rare state) that does not destroy *E. coli* O157:H7 organisms that have been introduced below the product's surface by chopping or grinding (*e.g.*, ground beef, veal patties, and beef pattie mix).

The public health risk presented by beef products contaminated with *E. coli* O157:H7 is not limited, however, to raw ground beef products. Given the low infectious dose of *E. coli* O157:H7 associated with foodborne disease outbreaks and the very severe consequences of an *E. coli* O157:H7 infection, the Agency believes that the status under the FMIA of beef products contaminated with *E. coli* O157:H7 must depend on whether there is adequate assurance that subsequent handling of the product will result in food that is not contaminated when consumed.

In evaluating the public health risk presented by *E. coli* O157:H7-contaminated beef products, FSIS has carefully considered the deliberations of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) and its Meat and Poultry Subcommittee. Last year, the Food and Drug Administration (FDA) requested recommendations, for use in the 1999 edition of its Food Code, on appropriate cooking temperatures for, among other foods, intact beef steaks for the control of vegetative enteric pathogens. In discussing intact product, the Committee stated that:

Due to a low probability of pathogenic bacteria being present in or migrating from the external surface to the interior of beef muscle, cuts of intact muscle (steaks) should be safe if the external surfaces are exposed

to temperatures sufficient to effect a cooked color change. In addition, the cut (exposed) surfaces must receive additional heat to effect a complete sear across the cut surfaces. . . .

The Committee's definition of ''Intact Beef Steak'' limited the applicability of this conclusion to ''[a] cut of whole muscle(s) that has not been injected, mechanically tenderized, or reconstructed.'' [1] For purposes of FDA's current Food Code (1997, Subpart 1–201.10(B)(41)), ''injected'' means:

manipulating a MEAT so that infectious or toxigenic microorganisms may be introduced from its surface to its interior through tenderizing with deep penetration or injecting the MEAT such as with juices which may be referred to as ''injecting,'' ''pinning,'' or ''stitch pumping.'' [2]

FSIS believes that in evaluating beef products contaminated with *E. coli* O157:H7, intact cuts of muscle that are to be distributed for consumption as intact cuts should be distinguished from non-intact products, as well as from intact cuts of muscle that are to be further processed into non-intact product prior to distribution for consumption. Intact beef cuts of muscle include steaks, roasts, and other intact cuts (*e.g.*, briskets, stew beef, and beef ''cubes for stew,'' [3] as well as thin-sliced strips of beef for stir-frying) in which the meat interior remains protected from pathogens migrating below the exterior surface).

Non-intact beef products include beef that has been injected with solutions, mechanically tenderized by needling, cubing,[4] Frenching, or pounding devices, or reconstructed into formed entrees (*e.g.*, beef that has been scored to incorporate a marinade, beef that has a solution of proteolytic enzymes applied to or injected into the cut of meat, or a formed and shaped product such as beef gyros). Pathogens may be introduced below the surface of these products as a result of the processes by which they are made. In addition, non-intact beef products include those beef products in which pathogens may be introduced below the surface by a comminution process such as chopping, grinding, flaking, or mincing (*e.g.*, fresh veal sausage and fabricated beef steak).

Intact cuts of beef that are to be further processed into non-intact cuts prior to distribution for consumption must be treated in the same manner as non-intact cuts of beef, since pathogens may be introduced below the surface of these products when they are further processed into non-intact products. Manufacturing trimmings (*i.e.*, pieces of meat remaining after steaks, roasts, and other intact cuts are removed) are an example of this type of product. Although manufacturing trimmings may be intact, they are generally further processed into non-intact products.

The Agency believes that with the exception of beef products that are intact cuts of muscle that are to be distributed for consumption as intact cuts, an *E. coli* O157:H7-contaminated beef product must not be distributed until it has been processed into a ready-to-eat product—*i.e.*, a food product that may be consumed safely without any further cooking or other preparation. Otherwise, such products (*i.e.*, non-intact products and intact cuts of muscle that are to be further processed into non-intact products prior to distribution for consumption) must be deemed adulterated. Intact steaks and roasts and other intact cuts of muscle with surface contamination are customarily cooked in a manner that ensures that these products are not contaminated with *E. coli* O157:H7 when consumed. Consequently, such intact products that are to be distributed for consumption as intact cuts are not deemed adulterated.

E. coli O157:H7 Sampling and Testing Program

FSIS currently samples and tests various raw ground beef products (including veal products) for *E. coli* O157:H7.[5] The program sampling is done at inspected establishments and retail stores. The Agency has limited the sampling and testing program to beef products because foodborne illness from *E. coli* O157:H7 has not been associated, to date, with other types of livestock or poultry subject to federal inspection.

The sampling and testing program does not cover intermediate products, such as beef derived from advanced meat/bone separation machinery and recovery systems, since these products are generally further processed to formulate products such as hamburger, but they are not themselves distributed to consumers. Additionally, the sampling and testing program does not cover multi-ingredient products that contain beef, as well as other livestock or poultry ingredients (*e.g.*, sausage that contains both fresh beef and pork).

If FSIS confirms the presence of *E. coli* O157:H7 in a raw ground beef product sampled in the sampling and testing program, it takes regulatory action (coordinating with State officials for products found at retail). The action taken by FSIS is based on the facts of the particular case (*e.g.*, the quantity of product that the sample represents; whether the product is associated with an outbreak of foodborne illness), but in all cases it reflects the Agency's determination that, unless further processed in a manner that destroys this pathogen (*e.g.*, into ready-to-eat beef patties), the product involved that is contaminated with *E. coli* O157:H7 is adulterated.

At this time, FSIS is not expanding its sampling and testing program to include all types of non-intact beef products or intact cuts of muscle that are to be further processed into non-intact products prior to distribution. The Agency may reconsider its sampling and testing program, as well as the scope of products deemed adulterated, in response to any comments received on the Agency's position regarding application of the FMIA's adulteration standards.

Other FSIS Activities

FSIS's effort to reduce the risk of foodborne illness associated with beef products has included development of a guidance document to assist processors of ground beef in developing procedures to minimize the risk of *E. coli* O157:H7, and other pathogens, in their products. Draft Agency guidance, along with materials developed by two trade associations, was made available to the public and was the subject of an April 22, 1998, public meeting (63 FR 13618, March 20, 1998).[6] The Agency has reviewed the comments received on the draft materials and is publishing a notice of the availability of the revised guidance in this issue of the **Federal Register**.

FSIS is participating in a risk assessment regarding *E. coli* O157:H7. A public meeting regarding the risk assessment was announced in an earlier

[1] The NACMCF-adopted minutes of the Subcommittee on Meat and Poultry are available for viewing in the FSIS docket room.

[2] A copy of the 1997 FDA Food Code is available for viewing in the FSIS docket room. In addition, an electronic version of the Code is linked on line through the FSIS web page located at http://www.fsis.usda.gov.

[3] The phrase ''cubes for stew'' generally refers to meat hand-cut into uniform squares.

[4] The term ''cubing'' generally refers to the process of flattening and knitting together meat into cutlet size products by means of a machine.

[5] For the Agency's current sampling and testing program instructions, see FSIS Directive 10,010.1, Microbiological Testing Program for *Escherichia coli* O157:H7 in Raw Ground Beef, February 1, 1998. A copy of this document is available for viewing in the FSIS docket room.

[6] Copies of the comments received on the guidance document (Docket #98–004N), along with the transcript of the public meeting and the draft guidance document are available for viewing in the FSIS docket room. In addition, an electronic version of the FSIS and industry guidance documents are available on line through the FSIS web page located at http://www.fsis.usda.gov (see the link for HACCP guidance documents).

Federal Register notice and was held on October 28, 1998 (63 FR 4432, August 18, 1998).[7]

FSIS is now reviewing its regulations to determine what changes the Agency should make to increase consumer protection against meat and poultry products adulterated with *E. coli* O157:H7, or other pathogens. Therefore, FSIS is soliciting input from the public about regulatory requirements that may be appropriate to prevent the distribution of products adulterated with *E. coli* O157:H7. Any changes that the Agency would make in the regulations would have to be consistent with the Agency's view expressed in this notice that beef products, other than surface-contaminated intact cuts that are to be distributed for consumption as intact products, that contain *E. coli* O157:H7 are adulterated unless conditions of transportation and other handling ensure that they will not be distributed until they have been processed into ready-to-eat products.

Because FDA has amended its regulations to permit the use of ionizing radiation for refrigerated or frozen uncooked meat, meat byproducts, and certain meat food products to control foodborne pathogens (62 FR 64107, December 3, 1997), FSIS is preparing a proposed rule on procedural and labeling requirements for irradiated products. Interested persons will have the opportunity, in that rulemaking, to submit comments to the Agency on irradiation treatment of *E. coli* O157:H7-contaminated products as an option for effectively eliminating this one specific pathogen.

Done at Washington, DC, on January 13, 1999.

Thomas J. Billy,

Administrator.

[FR Doc. 99–1123 Filed 1–15–99; 8:45 am]

BILLING CODE 3410–DM–P

[7] Copies of the comments received on the risk assessment process (Docket #98–037N), the transcript of the risk assessment public meeting, and a preliminary scoping document are available for viewing in the FSIS docket room. In addition, an electronic version of the preliminary scoping document is available on line through the FSIS web page located at http://www.fsis.usda.gov (see the link for the Office of Public Health and Science, *E. coli* risk).

DEPARTMENT OF THE TREASURY

Office of Thrift Supervision

12 CFR Parts 563, 563b

[No. 99–1]

RIN 1550–AA72

Capital Distributions

AGENCY: Office of Thrift Supervision, Treasury.

ACTION: Final rule.

SUMMARY: The Office of Thrift Supervision (OTS) is issuing a final rule revising its capital distribution regulation. Today's rule updates, simplifies, and streamlines this regulation to reflect OTS's implementation of the system of prompt corrective action (PCA) established under the Federal Deposit Insurance Corporation Improvement Act of 1991 (FDICIA). The final rule also conforms OTS's capital distribution requirements more closely to those of the other banking agencies.

EFFECTIVE DATE: April 1, 1999.

FOR FURTHER INFORMATION CONTACT: Edward J. O'Connell, III, Project Manager, (202) 906–5694; Evelyne Bonhomme, Counsel (Banking and Finance), (202) 906–7052; Karen Osterloh, Assistant Chief Counsel, (202) 906–6639, Regulations and Legislation Division, Chief Counsel's Office, Office of Thrift Supervision, 1700 G Street NW., Washington, D.C. 20552.

SUPPLEMENTARY INFORMATION:

I. Background

On January 7, 1998, the OTS published a proposed rule adding a new subpart E to part 563 to govern capital distributions by savings associations.[1] The proposal was intended to update, simplify, and streamline the existing capital distribution rule to reflect OTS's implementation of the system of prompt corrective action (PCA) established under the Federal Deposit Insurance Corporation Improvement Act of 1991 (FDICIA). Consistent with section 303 of the Community Development and Regulatory Improvement Act of 1994 (CDRIA), the proposed rule was also designed to conform the OTS capital distribution regulation to the rules of the other banking agencies, to the extent possible.

[1] 63 FR 1044 (Jan. 7, 1998).

II. Summary of Comments and Description of Final Rule

A. General Discussion of the Comments

The public comment period on the proposed rule closed on March 9, 1998. Four commenters responded: one federal savings bank, one savings and loan holding company, one law firm representing a federal savings bank, and one trade association. Two commenters supported the proposed rule with certain modifications and clarifications. One commenter, the savings and loan holding company, opposed the proposed changes. Another commenter addressed coverage of capital distributions by operating subsidiaries. The issues raised by the commenters are addressed in the section-by-section analysis below.

B. Section-by-Section Analysis

Proposed § 563.140—What Does this Subpart Cover?

Section 563.140 of the proposed rule described the scope of the regulation. Proposed subpart E would apply to all capital distributions by savings associations. The OTS specifically requested comment on whether the capital distribution rule should also apply to capital distributions by operating subsidiaries of savings associations. This issue is addressed below under § 563.141.

Proposed § 563.141—What is a Capital Distribution?

Proposed § 563.141 defined the term ''capital distribution'' as a distribution of cash or other property to a savings association's owners, made on account of their ownership. The proposed definition, at § 563.141(a), excluded dividends consisting only of a savings association's shares or rights to purchase shares, and excluded payments that a mutual savings association is required to make under the terms of a deposit instrument.

Capital distributions would also include a savings association's payment to repurchase, redeem, retire, or otherwise acquire any of its shares or other ownership interests, any payment to repurchase, redeem, or otherwise acquire debt instruments included in total capital, and any extension of credit to finance an affiliate's acquisition of those shares or interests. Proposed § 563.141(b). Additionally, a capital distribution would include any direct or indirect payment of cash or other property to owners or affiliates made in connection with a corporate

or parts of cattle develop written procedures for the removal, segregation, and disposition of specified risk materials (SRMs). Establishments are also required by FSIS to maintain daily records sufficient to document the implementation and monitoring of their procedures for the removal, segregation, and disposition of SRMs, and any corrective actions taken to ensure that such procedures are effective.

Need and Use of the Information: FSIS will collect information from establishments to ensure that cattle slaughtered for meat product are free from Bovine Spongiform Encephalopathy.

Description of Respondents: Business or other for-profit.

Number of Respondents: 3,512.

Frequency of Responses: Recordkeeping; Reporting: On occasion.

Total Burden Hours: 123,216.

Food Safety and Inspection Service

Title: Advanced Meat Recovery Systems.

OMB Control Number: 0583–0130.

Summary of Collection: The Food Safety and Inspection Service (FSIS) has been delegated the authority to exercise the functions of the Secretary as provided in the Federal Meat Inspection Act (FMIA) (21 U.S.C. 601 *et seq.*). This statute mandates that FSIS protect the public by ensuring that meat and poultry products are safe, wholesome, unadulterated, and properly labeled and packaged. FSIS requires that official establishments that produce meat from Advanced Meat Recovery (AMR) systems ensure that bones used for AMR systems do not contain brain, trigeminal ganglia, or spinal cord, to test for calcium, iron, spinal cord, and dorsal root ganglia, to document their testing protocols, to assess the age of cattle product used in the AMR system, and to document their procedures for handling product in a manner that does not cause product to be misbranded or adulterated, and to maintain records of their documentation and test results.

Need and Use of the Information: FSIS will collect information from establishments to ensure that the meat product produced by the use of AMR systems is free from Bovine Spongiform Encephalopathy.

Description of Respondents: Business or other for-profit.

Number of Respondents: 56.

Frequency of Responses: Recordkeeping; Reporting: On occasion.

Total Burden Hours: 25,209.

Ruth Brown,

Departmental Information Collection Clearance Officer.

[FR Doc. E7–19758 Filed 10–5–07; 8:45 am]

BILLING CODE 3410–DM–P

DEPARTMENT OF AGRICULTURE

Food Safety and Inspection Service

[Docket No. FSIS–2007–0041]

Non-Escherichia coli O157:H7 Shiga Toxin-Producing E. coli

AGENCY: Food Safety and Inspection Service, USDA.

ACTION: Notice of public meeting.

SUMMARY: This notice is announcing that the U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS), the Food and Drug Administration's Center for Food Safety and Applied Nutrition (FDA CFSAN), and the National Centers for Disease Control and Prevention (CDC) will co-sponsor a public meeting on October 17, 2007. The purpose of the meeting is to consider the public health significance of non-*Escherichia coli* (*E. coli*) O157:H7 Shiga toxin-producing *E. coli*.

DATES: The public meeting will be held on Wednesday, October 17, 2007, 8:30 a.m. to 3:30 p.m.

ADDRESSES: The meeting will be held at the Arlington campus of George Mason University, 3401 N. Fairfax Drive, Room 244, Arlington, VA 22201.

Registration

Pre-registration for this meeting is encouraged. To pre-register to attend in person or via teleconference, access the FSIS Web site, *http://www.fsis.usda.gov*. Contact Sheila Johnson for more information on logistics at 202–690–6498 or via e-mail at *Sheila.johnson@fsis.usda.gov*.

All documents related to the meeting will be available for public inspection in the FSIS Docket Room, 1400 Independence Avenue, SW., Room 2534 South Building, Washington, DC 20250, between 8:30 a.m. and 4:30 p.m., Monday through Friday, as soon as they become available.

FSIS will finalize an agenda on or before the meeting date and post it on the FSIS Web page at: *http://www.fsis.usda.gov/News/Meetings_&_Events/*. Also, when it becomes available, the official transcript of the meeting will be kept in the FSIS Docket Room at the above address and will also be posted on the Agency Web site, *http://www.fsis.usda.gov*.

FOR FURTHER INFORMATION CONTACT: Denise Eblen, phone (202) 690–6238, fax (202) 690–6334, e-mail: *Denise.eblen@fsis.usda.gov* or at the mail address: U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, 1400 Independence Avenue, SW., 357 Aerospace Center, Washington, DC 20250–3766.

Persons requiring a sign language interpreter or other special accommodations should notify Dr. Eblen by October 10, 2007.

SUPPLEMENTARY INFORMATION:

Background

Shiga toxin-producing *E. coli* (STEC) was first identified in the early 1980s in North America as the cause of outbreaks of bloody diarrhea, often leading to severe and fatal illness. These outbreaks were associated with ground beef consumption, and *E. coli* O157:H7 was the STEC identified as causing the illnesses. In 1994, FSIS notified the public that raw ground beef contaminated with *E. coli* O157:H7 is adulterated under the FMIA unless the ground beef is processed to destroy this pathogen. Also in 1994, FSIS began sampling and testing ground beef for *E. coli* O157:H7.

On January 19, 1999, FSIS published a policy statement in the **Federal Register** that explained that if non-intact raw beef products or intact raw beef products that are to be processed into non-intact product prior to distribution for consumption are found to be contaminated with *E. coli* O157:H7, they will be deemed to be adulterated if not processed to destroy the pathogen (64 FR 2803).

Shiga toxins are produced by other *E. coli* serotypes in addition to *E. coli* O157:H7. While many STEC strains have been found in ruminant feces, not all of these STECs are pathogenic. The scientific community believes that the STECs that are pathogenic not only contain the Shiga toxin but also additional virulence determinants that, together with the toxin, cause illnesses similar to those caused by *E. coli* O157:H7. The subset of STECs that contain both the toxin and these additional virulence determinants, including *E. coli* O157:H7, is known as enterohemorrhagic *E. coli* (EHEC).

In the United States, there is growing awareness that STECs other than *E. coli* O157:H7 (non-O157:H7 STECs) cause sporadic and outbreak-associated illnesses. This awareness is attributable in part to the increasing availability of laboratory reagents that can be used to diagnose illnesses and to detect strains of STECs in food and other

environmental samples. The number of non-O157:H7 STEC infections reported to the CDC from 2000 to 2005 increased from 171 to 501 cases, suggesting a higher burden of illness than previously thought.

Outbreaks associated with non-O157:H7 STECs have been reported worldwide, including thirteen in the United States from 1990 to 2006. The 2006 data is still preliminary. Many outbreaks were attributed to consumption of fresh produce; none were attributed to ground beef consumption. However, in 2006, non-O157:H7 STEC illness was diagnosed in a patient in New York who had consumed ground beef shortly before illness onset. The same STEC strain, indistinguishable by pulsed field gel electrophoresis, was detected in the patient's stool and in leftover ground beef that the patient had consumed. In this case, FSIS was unable to take further action because the product could not be definitively traced to a production lot.

FSIS, FDA CFSAN, and CDC will hold a public meeting on October 17, 2007, to solicit input from industry, consumers, academia, and other public health and regulatory agencies on the issue of whether non-O157:H7 STECs should also be considered to be adulterants. This meeting will rely on relevant data in addressing the most important questions that underlie this issue, including:

- What is the epidemiology of non-O157:H7 STEC illness?
- What can be done to enhance the surveillance and reporting of non-O157:H7 STEC illnesses?
- What is the prevalence of non-O157:H7 STEC in livestock and in finished product? Are species other than cattle, such as sheep, goats, and swine, important sources of non-O157:H7 STECs?
- What are the best methods for detecting pathogenic non-O157:H7 STECs in food? What are the most relevant markers for pathogenic STECs?
- Are interventions designed to remove or destroy *E. coli* O157:H7 in foods or raw products effective against non-O157:H7 STECs as well?
- How should regulatory agencies define, monitor, and control pathogenic non-O157:H7 STECs in food or raw products?

All interested parties are welcome to attend the meeting and to submit written comments and suggestions through October 15, 2007 to Dr. Eblen by phone (202) 690–6238, fax (202) 690–6334, e-mail: *Denise.eblen@fsis.usda.gov*, or at the mail address: U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, 1400 Independence Avenue, SW., 357 Aerospace Center, Washington, DC 20250–3766. Individuals who do not wish FSIS to post their personal contact information—mailing address, e-mail address, telephone number—on the Internet may leave the information off their comments.

The comments and the official transcript of the meeting, when they become available, will be posted on the agency's Web site at *http://www.fsis.usda.gov*.

Additional Public Notification

Public awareness of all segments of rulemaking and policy development is important. Consequently, in an effort to ensure that minorities, women, and persons with disabilities are aware of this notice, FSIS will announce it online through the FSIS Web page located at *http://www.fsis.usda.gov/regulations/2007_Notices_Index/*. FSIS will also make copies of this **Federal Register** publication available through the FSIS Constituent Update, which is used to provide information regarding FSIS policies, procedures, regulations, **Federal Register** notices, FSIS public meetings, and other types of information that could affect or would be of interest to constituents and stakeholders. The Update is communicated via Listserv, a free electronic mail subscription service for industry, trade groups, consumer interest groups, health professionals, and other individuals who have asked to be included. The Update is also available on the FSIS Web page. Through Listserv and the Web page, FSIS is able to provide information to a much broader and more diverse audience. In addition, FSIS offers an electronic mail subscription service which provides automatic and customized access to selected food safety news and information. This service is available at *http://www.fsis.usda.gov/news_and_events/email_subscription/*. Options range from recalls to export information to regulations, directives and notices. Customers can add or delete subscriptions themselves, and have the option to password protect their accounts.

Done at Washington, DC, on: October 4, 2007.

Alfred V. Almanza,

Administrator.

[FR Doc. 07–4975 Filed 10–4–07; 1:45 pm]

BILLING CODE 3410–DM–P

DEPARTMENT OF AGRICULTURE

Forest Service

Notice of New Recreation Fee Site; Federal Lands Recreation Enhancement Act, (Title VIII, Pub. L. 108–447)

AGENCY: Daniel Boone National Forest, USDA Forest Service.

ACTION: Notice of new recreation fee site.

SUMMARY: The Daniel Boone National Forest will begin charging a $25 group day use rental fee for the Alpine Picnic Area picnic shelter, the Natural Arch Scenic Area picnic shelter and the Natural Arch Scenic Area amphitheater. These facilities are currently only available on a first come first serve basis. Rentals of other picnic shelters on the Daniel Boone National Forest have shown that groups would like an option to reserve the shelters for their use. Shelter rentals allow public groups to plan activities in advance with the guarantee the shelter will be available for their use. The facilities will continue to be available on a first come first serve basis if not reserved. Fee revenue will be used to help cover the administrative cost of reserving and preparing the facilities for group rentals.

DATES: The fee is scheduled for implementation in May of 2008.

ADDRESSES: Recreation Fee Program Coordinator, Daniel Boone National Forest, 1700 Bypass Road, Winchester, KY 40391.

FOR FURTHER INFORMATION CONTACT: Myra Williamson, Recreation Fee Coordinator, 859–745–3154.

SUPPLEMENTARY INFORMATION: The Federal Recreation Lands Enhancement Act (Title VIII, Pub. L. 108–447) directed the Secretary of Agriculture to publish advance notice in the **Federal Register** whenever new recreation fee areas are established. This new fee will be reviewed by a Recreation Resource Advisory Committee prior to a final decision and implementation. The Daniel Boone National Forest currently charges $25 group use rental fees for two other picnic shelters under the authority of the Federal Recreation Lands Enhancement Act.

Dated: October 1, 2007.

Jerome E. Perez,

Daniel Boone National Forest Supervisor.

[FR Doc. 07–4964 Filed 10–5–07; 8:45 am]

BILLING CODE 3410–52–M

USDA

Food Safety and Inspection Service

FY 2008 - 2013

STRATEGIC PLAN

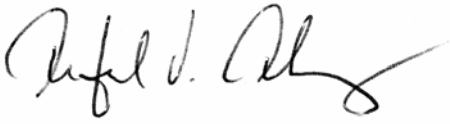
"Foundation document for both the long range and day to day operations of the Agency."

Letter from the Administrator

The Food Safety and Inspection Service (FSIS) and its employees are very proud to commemorate over 100 years of protecting the food supply under the Federal Meat Inspection Act (FMIA). FSIS is the public health regulatory agency that ensures the safety and security of the U.S. meat, poultry, and processed egg products supply. For the past century under FMIA, FSIS and its predecessors have ensured that meat products are safe to consume by carrying out continuous inspections at slaughter and food processing establishments.

The vital services of FSIS have touched the lives of almost every citizen, every day in America. FSIS is accountable for protecting the lives and well-being of 295 million U.S. citizens and millions more around the world. The Agency's 9,500 employees include approximately 7,800 inspection program personnel, who are assigned to approximately 6,200 Federal slaughter, food processing, and import establishments.

To meet future realities of food safety and public health challenges, FSIS requires a strong Strategic Plan. This Plan encompasses our strategic intentions over the next five years, and will serve as a foundation document for both the long range and day to day operations of the Agency. The Strategic Planning process is, and shall be, one of many tools that will ensure that we are prepared for food safety challenges in the next century.



Alfred Almanza

Administrator - FSIS

Executive Summary

The Food Safety and Inspection Service (FSIS) Management Council and their designated representatives on the Strategic Planning and Reporting Team created the Fiscal Year (FY) 2008-2013 Strategic Plan in order to prepare for important new challenges and opportunities that are likely to arise in the coming years. The Plan outlines six goals which reflect the Agency's public health responsibilities. The following goals will help Agency leadership and staffs focus on the day-to-day activities as the entire Agency works toward a vision of the future:

- **Goal 1** - Enhance inspection and enforcement systems and operations to protect public health.
- **Goal 2** - Enhance the use of risk analysis and vulnerability assessments in FSIS' approach to protecting public health.
- **Goal 3** - Enhance the development of science and risk-based policies and systems.
- **Goal 4** - Enhance the development and maintenance of an integrated and robust data collection and analysis system to verify the effectiveness and efficiency of Agency programs.
- **Goal 5** - Enhance the development and maintenance of an innovative infrastructure to support the Agency's mission and programs.
- **Goal 6** - Enhance the effectiveness of Agency outreach and communications to achieve public health goals.

FSIS used the Office of Management and Budget (OMB)'s Circular A-11 as a guide in developing the Strategic Plan. This Strategic Plan addresses the key components of the circular. OMB developed sections of the A-11 in order for agencies to maintain compliance with the Strategic Planning requirements of the Government Performance and Results Act of 1993 (GPRA).

Congress enacted GPRA in an effort to focus on government management, performance, and results. GPRA establishes requirements for Strategic Planning, performance goals, and ultimately the measurement of success in meeting those goals. Strategic plans, annual performance-based budgets, and annual performance reports comprise the main elements of GPRA. Together, these elements create a recurring cycle of planning, programming, budgeting, accounting, financial management, and reporting.

The Agency formulated its strategic goals and vision around the FSIS Administrator's priorities and the National Academy of Sciences model for a public health regulatory agency. The three areas of emphasis in the model are Assessment, Policy Development, and Assurance. The areas are not unrelated and independent; they are interdependent. The ongoing activities in one have an effect on the other two, and form a "feedback loop" involving all FSIS offices.

FSIS will focus on six Administrator priorities over the next five years. These priorities will drive policies, goals, and actions. The priorities are: Continued Evolution of Inspection and Enforcement, Data and Risk Analysis, Food Defense, Communications, Management Controls and Efficiency, and Training, Education, and Outreach.

Table of Contents

1. OVERVIEW

INTRODUCTION

FSIS, a public health regulatory agency within the U.S. Department of Agriculture (USDA), is responsible for ensuring that the commercial supply of meat, poultry, and processed egg products moving in interstate commerce or exported to other countries is safe, secure, wholesome, and correctly labeled and packaged. Legislative mandates provide FSIS with the authority to conduct its public health mission.

Ensuring the safety of meat, poultry, and processed egg products requires a strong infrastructure. Approximately 7,800 inspection personnel stationed in about 6,200 federally inspected meat, poultry, and processed egg products plants verify that the processing of tens of billions of pounds of red meat and poultry, and billions of pounds of liquid egg products comply with statutory requirements. In addition, billions of pounds of red meat, poultry, and liquid egg products are presented for import inspection at U.S. ports and borders from countries that FSIS has determined to have inspection systems equivalent to Federal inspection systems. Ensuring that these products are safe, secure, and wholesome is a serious responsibility.

Everyone in the food chain, from farmer through consumer, has a responsibility in keeping the food supply safe. Meat, poultry, and processed egg products can be contaminated with bacteria at any point during production, distribution, and consumption. FSIS works closely with other Federal agencies that have some role in the regulation of meat, poultry, and processed egg products along the farm to table continuum. To ensure food safety along this continuum, it is vital that all of FSIS' stakeholders – including other Federal, State, and local governments, producers, the industry, food handlers, and consumers – participate in promoting food safety. Toward this end, FSIS will secure the involvement of stakeholders to achieve its goals.

Most importantly, FSIS' plans for a more robust risk-based system must rely heavily on data to promote proactive decisions affecting food safety and public health. FSIS must, and will, enhance data management and delivery via information technology tools to quickly respond to indications of risk to human health and food defense and do so with utmost efficiency and effectiveness.

FSIS MISSION

Protect consumers by ensuring that meat, poultry, and egg products are safe, secure, wholesome and correctly labeled and packaged.

FSIS will carry out its public health mission through six strategic goals. FSIS' mission is embodied in the staff's dedication to the latest science and public health protection, as well as the mandates established by law. In addition, FSIS will ensure full compliance with all applicable Equal Employment Opportunity Commission regulations, orders, and other written instructions to provide, promote, and maintain equality of opportunity for all USDA employees, applicants for employment, and customers. FSIS prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or a part of an individual's income is derived from any public assistance program.

FSIS VISION

Provide superior public health protection through risk-based food safety programs that are verified and validated through data systems.

2. PLANNING AND PERFORMANCE FRAMEWORK

FIVE-YEAR PLANNING

The six strategic goals contained in this plan describe FSIS' major programmatic policies and intentions. Each strategic goal will have a brief description or explanation, a discussion of the means and strategies to attain the goal and a stated outcome.

The Strategic Plan also contains several corporate measures that were negotiated with USDA and OMB. These measures act as representative measures for USDA's food safety and food defense efforts. Each measure specifies baseline information and long-term performance targets. In addition, a list of key factors is included to highlight possible challenges to FSIS' stated goals along with a description of how FSIS uses program evaluations and controls to verify that its strategic intentions are being carried out.

The Agency approach to creating this Strategic Plan began with Agency leaders and senior managers establishing initial goals based on the attributes of a National Academy of Sciences public health model. The FSIS Management Council then provided the goals to FSIS' Strategic Planning and Reporting Team in order to identify outcomes that would benefit the public and to contribute to the general narrative throughout the document. The team was composed of representatives selected by each program manager in FSIS. This was done in order to ensure the inclusion of the appropriate decision-makers and subject matter experts during the Strategic Plan's formulation. During the formulation period, Agency leaders and senior management were given the opportunity to review, discuss, and approve the ideas and management concepts in the draft through periodic presentations, status checks, and various drafts. As a result, this process forms not only the nucleus of the FSIS Strategic Plan, but also the food safety portion of the USDA Strategic Plan (2005-2010) and all FSIS budget submissions, program plans, and performance management efforts. The Agency also has action plans to carry out various Federal management initiatives that detail agency approaches for annual improvement in human capital, information technology and eGovernment, financial management, and budget and performance integration. These initiatives are subject to their own metrics and reporting systems.

ADMINISTRATOR'S PRIORITIES

FSIS is holding itself accountable for improving public health. Over the next five years, FSIS will build upon its priorities and continue to improve the Agency's infrastructure with greater attention to risk so that it can then improve overall performance under the public health model. The development and use of an integrated and results driven data collection and analysis system supports achievement of goals in these priority areas:

The Continued Evolution of Inspection and Enforcement

FSIS' policies and practices will continue the evolution of inspection and enforcement for both domestic and imported products. A risk-based approach, encompassing the Agency's actions combined with the Agency's scientific commitment, will facilitate FSIS' ability to combat ever changing threats to public health.

FSIS continues to strengthen its data communication and response infrastructure that protects public health as well as the integrity of the food and agricultural system. An early success to aggregate data across disparate systems has been the development of a data warehouse, which aggregates performance data from domestic and import inspection programs. The user interface for the data warehouse was accomplished through the development of the Enterprise Reporting System (ERS).

The development of ERS has consolidated the existing stove-pipe reporting applications. The implementation of this project set the foundation for the methodology and framework for new system development projects, utilizing the Service Oriented Architecture as the basis for USDA-FSIS system modernization. AssuranceNet, a management control performance monitoring system, utilizes information from the data warehouse, as well, to monitor and alert management. AssuranceNet is currently being expanded to monitor key management control performance measures for all FSIS program areas.

Data and Risk Analysis

FSIS is committed to emphasizing science in the development of food safety policies. A scientific approach to food safety that incorporates risk analysis is critical to FSIS' ability to combat the ever changing threats to public health. Thus, another priority is risk analysis, which includes risk assessment, risk management, and risk communication. In addition to providing regulatory agencies with a solid foundation for policy changes, science-based risk analysis is necessary to help the Agency better predict and respond to food safety threats by allowing staff to focus Agency resources on hazards that pose the greatest threat to public health. Analysis of FSIS regulatory sampling data, as well as other sources of data, including baseline studies, helps the Agency detect trends and identify connections between persistence, prevalence, and other factors such as practices employed by plants, seasonal variations, and establishment size. The Agency's data is being consolidated into a data warehouse and data store to provide a more complete picture of food safety threats from domestic and imported products and to provide traceability for reports to better protect public health.

Food Defense

FSIS has accomplished much in the area of food defense, making a strong system even stronger. The Agency designed its existing science-based food safety and defense verification system, with Hazard Analysis and Critical Control Point (HACCP) as the foundation, to prevent and control contamination of the food supply during processing, regardless of whether the contamination is naturally occurring or introduced intentionally.

Communications

The Agency has embarked on a comprehensive effort to ensure that all levels and means of communications both within the Agency and with external constituents are as efficient, effective, and rapid as possible. FSIS recognizes that as a public health regulatory agency, the organization is only as effective as the communication systems it has in place. FSIS continuously explores and utilizes a variety of methods to reach its different audiences. The Agency has won awards for its web site and uses leading edge technologies, customer satisfaction surveys, and usability testing to provide easy-to-find, always available quality public health and defense information to keep up with its customers' needs and to better protect public health.

Management Controls and Efficiency

FSIS is seeking to achieve its operational goals and objectives through the most effective means possible. In order to better focus Agency resources, FSIS is establishing a more fully documented management control program. In 2006, the Agency launched its AssuranceNet management control system. Initially launched with over 50 domestic inspection performance measures, version 2.0 launched in early 2007 and increased the monitored performance measures to over 100 including import inspection measures. AssuranceNet is a web-based application that transforms near real-time performance data into valuable decision-making information for managers. It currently extrapolates information from FSIS inspection and import databases plus employee responses to key questions in these areas, as well as administrative duties, to perform complex calculations that graphically display inspection performance indicators in a standard and traceable manner.

The system will be enhanced again in 2008 to include the In-Commerce System for compliance and investigations data entry and performance measures, adding additional inspection measures, integrating a case management system and geographic mapping information, and adding measures for policy. The goal of the system is to have all program areas integrated by the end of 2009. The automated system flags areas that fall below established standards to redirect Agency staff from tedious and intensive data mining efforts to analyzing and acting on the information to better protect public health.

One of the system's goals is to provide a way for analysts and managers to spend less time aggregating and reviewing data and more time spotting issues and trends to target improvements more quickly. Managers can see their results in easily read reports that automatically display measures that exceed their target as red to indicate that further research is warranted. The system also graphically displays standard and custom reports as well as dashboard performance metrics. The system leverages USDA's Enterprise Shared Services (WebSphere and eAuthentication), provides training on AgLearn, follows the web-based applications requirements from version 2 of the USDA Style Guide, and has its front end hosted at the National Information Technology Center (NITC). In 2009, AssuranceNet will expand this performance and management tool to its remaining program areas and the system will become part of the Public Health Information Consolidation Project (PHICP) business case.

Management controls are the organization environment, policies, and procedures used to reasonably ensure that:

- programs achieve their intended results;
- resources are used consistent with Agency mission;
- programs and resources are protected from waste, fraud, and mismanagement;
- laws and regulations are followed; and
- reliable and timely information is obtained, maintained, reported and used for decision making.

Management authority, responsibility, and accountability are more clearly defined and delegated when the Agency documents its management controls. In addition, program performance is routinely analyzed, policies and procedures are regularly updated, management decisions are transparent and traceable, documentation is accurately maintained, and supervision is appropriate and continuous.

Training, Education and Outreach

Training and education of the FSIS workforce is a cornerstone of public health protection. Training enables inspection program personnel to make sound and effective regulatory decisions based on appropriate scientific and public health principles. One of the Agency's top priorities, therefore, is to aggressively train and educate our workforce.

To accomplish this, FSIS is implementing an ongoing strategy to provide employees with a challenging program of initial training when they report to their first assignment; follow-up training that reinforces acquired skills; and advanced skills training to prepare the employee for performing complex public health protection duties. This enables FSIS to maintain a well-trained workforce as the Agency's policies and programs continue to advance to meet the ever-changing needs of public health.

FSIS recognizes the importance of State and local food protection agencies to its food safety-public health mission. That mission demands controls from the farm to the table, often by commercial operations that are regulated primarily by State and local governments. In addition to the 27 State meat and poultry inspection programs, States may assert jurisdiction over the raising and handling of livestock, custom slaughter operations, food warehouses, food transport, and most prominently, retail stores and restaurants.

Over the years, FSIS has sought to support and enhance State and local agencies' capabilities in dealing with food safety issues. Most notably, it has funded a variety of cooperative agreements (discretionary food safety grants) for projects with and for its State and local partners. Although FSIS has not been able to fund such projects the past few years (other than for the Food Emergency Response Network (FERN) food laboratory project), the recent completion of cooperative agreements from FY 2005 and earlier has resulted in valuable work products now being available from FSIS and/or the State and local partners.

Among FSIS' many responsibilities, the Agency inspects "Small and Very Small" meat and poultry slaughter and processing plants. The businesses that fall into this category have a particular need for current and frequent food safety information because they generally lack the resources to monitor food safety developments from the Agency, academia or trade associations. To address this challenge, FSIS has initiated efforts to work with Small and Very Small plants, including another 2,400 (approximately) under state inspections, to overcome these issues. FSIS has implemented an action plan to deliver outreach assistance to promote risk-based food safety and food defense systems for Small and Very Small plants. The reaction to these initial steps has been very positive. However, data from FSAs and recalls show that additional effort is needed. FSIS plans to take further steps to address this challenge by educating, as well as regulating, industry to improve public health and safety. This will ensure rapid and consistent delivery of key Agency services on emerging issues to better serve the needs of Small and Very Small plants. It will also promote an understanding of the scientific, technical, and regulatory information needed domestically and internationally by Small and Very Small plants to develop food safety and food defense systems fully capable of addressing existing and emerging threats to public health.

Among the recommendations of the President's Inter-agency Working Group on Import Safety was enhanced outreach to trading partners to assure that government inspection officials in foreign countries understood the requirements for exporting to the United States. FSIS participates with the Foreign Agricultural Service and other government agencies to provide technical assistance that enhances the ability for foreign manufacturers to produce safe food for export to the United States. FSIS is also embarking on a program to increase the transparency of import requirements to foreign countries and the regulated industry through conversion of the Import Inspection Manual of Procedures to FSIS Directives.

Another area of emphasis for FSIS has been the strengthening of its training for inspection personnel for export verification and certification duties in order to meet both domestic requirements and to meet additional requirements imposed by foreign countries. In addition, the Agency continues to conduct inspection seminars for foreign food safety officials to better familiarize them with the FSIS inspection system, requirements, and accomplishments.

PUBLIC HEALTH MODEL

Though FSIS is already highly active in the areas discussed, it is developing plans to broaden its responsibilities and improve its effectiveness. The Agency is organizing its efforts around the attributes of the National Academy of Sciences (NAS) model for a public health regulatory agency. Three major areas of emphasis are widely recognized and accepted by both the Federal and State sectors as defining a public health regulatory institution. These areas are Assessment, Policy Development, and Assurance. These three activity areas, Assessment, Policy Development, and Assurance, are interdependent. The ongoing activities in one area have an effect on the other two, and form a "feedback loop" for all FSIS offices.

PUBLIC HEALTH MODEL

Assessment

The first area, "assessment," is the activity by which known or potential public health problems are identified and accurately assessed with respect to magnitude of the problem and potential impact on public health. Assessment is carried out using the latest surveillance and testing methods to gather data for conducting the cutting-edge analyses, including quantitative risk assessments, forecasting models, data-mining and trend analysis. Assessment is a continuous and ongoing activity because the nature of the threat to public health is constantly evolving.

In order to institutionalize effective assessment, the Agency must establish and continuously upgrade a first class scientific program that is well designed, funded, and staffed. The scientific program and its professional, technical staff are the very epicenter of a public health regulatory agency and the capabilities of the scientific program define the success of the organization. As an example, to assess the potential threat to public health, FSIS conducts an annual surveillance program through its sampling program to measure the prevalence of *Salmonella.* The surveillance program includes serotyping (confirmatory tests) that identifies the strains of *Salmonella* that cause human illness. Additionally, FSIS has developed models estimating the public health impact of its programs based on pathogen prevalence and other factors. FSIS also continuously analyzes data from domestic and import inspection results, recalls, food safety assessments, and policy questions to determine if FSIS needs to strengthen or clarify its public health policies and procedures.

Policy Development

The second public health model area is "regulatory policy development." Policy development is defined as the process by which society makes decisions about problems, chooses goals and the proper means to reach them, handles conflicting views about what should be done, and allocates resources to deploy those policies.

Regulatory policy development is the guidance system in a public health regulatory agency. It translates the issues affecting public health into a course of action that addresses problems such as the health consequences associated with pathogens.

Through its regulatory policy development activity, FSIS develops and implements policies to reduce the risk of foodborne illnesses. These activities are firmly based on sound science and decisions are based on weighing public health benefits with societal costs and the technical feasibility for implementing solutions. In the broadest organizational sense, policy development activities include establishing Agency priorities, setting Agency direction, Strategic Planning, issuing regulations, directives, and other policy vehicles, mobilizing resources, training, constituency building, distributing public information, and promoting and coordinating public and private cooperation and outreach.

Assurance

The third area is “assurance”. Assurance is the activity that verifies FSIS performance measures and targets and then validates that the Agency is effective in achieving the desired results. This is the function of providing services and implementing Agency policies and procedures to meet public health needs. One aspect of this is done through policy evaluation and the enforcement of established statutory and regulatory responsibilities which holds industry and the Agency accountable for ensuring that meat, poultry, and processed egg products are safe, secure, wholesome, and accurately labeled. FSIS assurance also occurs through domestic and import inspection activities and verification testing. Some examples of other assurance activities include reviewing the results of the discard rates of samples and addressing the issues that cause sample discards to occur, ensuring that establishments are scheduled for pathogen testing according to program design, and training employees on public health policies and procedures. Today, FSIS inspection activities are increasingly guided by science. Specifically, risk assessments are used to evaluate the public health benefit of allocating inspection resources and guiding inspection activities. The result is more effective risk-based inspection programs that provide improved public health assurance that the meat, poultry, and processed egg products are safe.

AssuranceNet: AssuranceNet is a web-based application that transforms near real-time performance data into valuable decision-making information for managers. It currently extrapolates information from FSIS inspection and import databases plus employee responses to key questions in these areas, as well as administrative duties, to perform complex calculations that graphically display inspection performance indicators in a standard and traceable manner.

CORPORATE PERFORMANCE MEASURES

Through technical conferences and policy deliberations, FSIS continuously examines the Nation's changing food safety system and practices, and articulates a long-term view in regard to the Agency performance and the benefits to public health. Therefore, the following three measures have been selected to represent FSIS as corporate performance measures in both USDA's and OMB's performance management efforts. (Projections as of March 2008 – subject to change)

USDA Strategic Objective 4.1.1: Reduce overall public exposure to generic ***Salmonella*** from broiler carcasses using existing scientific standards

	FSIS Verification Test Results		Performance Objectives[1]					
	FY 2006	FY 2007	FY 2008	FY 2009	FY 2010	FY 2011	FY 2012	FY 2013
Percent of Establishments in Category I	45%	73%	80%	85%	90%	92%	94%	95%
Volume Adjusted Percent Positive Rate[2]	11.15%	7.37%	7.2%	7.1%	6.9%	6.8%	6.8%	6.8%
Not Volume Adjusted Percent Positive Rate[2,3]	12.6%	9.04%	8.8%	8.7%	8.5%	8.4%	8.4%	8.4%

1 Performance objectives were established by assuming a continuous decrease to meet the FY 2010 goal of 90 percent of establishments in Category 1 and FY 2013 goal of 95 percent of establishments in Category 1.

2 Projections are based on the assumption that the average 8.2 percent positive for Category I establishments and average 11.3 percent for Category II and III establishments combined achieved in 2007 remain constant, while the number of establishments in Category I increases.

3 Adjusting for production volume provides measures and objectives that are more representative of FSIS' progress towards preventing cases of human illness.

Salmonella: *Salmonella* bacteria are the most frequently reported cause of foodborne illness. The bacterium lives in the intestinal track of humans and other animals, including birds. *Salmonella* present on and in raw meat and poultry can survive if the product is not cooked to a safe minimum internal temperature, as measured with a food thermometer.

As of June 2006, FSIS began employing a "category" system to measure establishments' *Salmonella* performance due to a change in how the establishments were selected for testing. FSIS compares how many establishments are in "Category 1" from one quarter to the next and from one year to the next. Category 1 represents establishments that have achieved 50 percent or less of the performance standard or baseline guidance, for two consecutive FSIS test sets. Category 2 represents establishments that have achieved greater than 50 percent on at least one of the two most recent FSIS test sets without exceeding the performance standard or baseline guidance. Category 3 represents establishments that

have exceeded the performance standard or baseline guidance on either or both of the two more recent FSIS test sets. For example, for broiler slaughter establishments, the performance standard is constructed such that the standard is met if there are 13 or fewer positive samples in 51 daily tests. Consequently, a Category 1 establishment would have six or fewer positive results in the two most recent 51 sample sets.

As more establishments reach Category 1 status, fewer people will be exposed to *Salmonella* from raw classes of product regulated by FSIS. FSIS set a goal of having 90 percent of establishments achieve Category 1 status by 2010. By then, FSIS will have completed one or more new baseline studies. The results of these new baselines would be to establish new performance standards or baseline guidance and to re-set Category 1, Category 2, and Category 3 criteria.

The Healthy People 2010 goal for *Salmonella* illnesses is 6.8 cases per 100,000. FSIS estimates based upon its public health attribution work that the Health People 2010 goal for Salmonella illnesses from broilers is 0.68 cases per 100,000 or a percent positive rate of 8.5.

USDA Strategic Objective 4.1.2: Decrease the overall-percent-positive rate for ***Listeria monocytogenes*** in ready-to-eat products through the use of Food Safety Assessments

	FSIS Verification Test Results		**Performance Objectives[1,2]**					
	FY 2006	FY 2007	FY 2008	FY 2009	FY 2010	FY 2011	FY 2012	FY 2013
Volume Adjusted Percent Positive Rate[2]	0.33%	0.29%	0.27%	0.25%	0.24%	0.24%	0.24%	0.23%
Not Volume Adjusted Percent Positive Rate[2]	0.59%	0.37%	0.35%	0.33%	0.30%	0.30%	0.29%	0.29%

1The FY 2013 performance objective was established by assuming a 1 percent reduction from FY 2007.

2 By Executive Order, the Healthy People 2010 goal for *Lm* was to have been met by 2005.

3 Prior to 2008, percent positives for *Lm* were calculated by dividing the total number of samples positive for *Lm* by the total number of samples tested. That method, however, is not representative of the potential exposure to the pathogen, because it does not take into account differences in production volume across the establishments being sampled. For example, an *Lm* positive at a production facility producing a small amount of ready-to-eat products cause fewer *Lm* cases than a positive at a large production facility. Adjusting for production volume provides measures and objectives that are more representative of FSIS' progress towards preventing cases of human illness.

Listeria: *Listeria monocytogenes* (*Lm*) is a bacterium that is recognized as an important public health problem in the United States. The disease listeriosis affects primarily pregnant women, newborns, and adults with weakened immune systems. The bacterium has been found in a variety of raw foods, such as uncooked meats, as well as in processed foods that become contaminated after processing, such as cold cuts at the deli counter.

FSIS combats *Listeria* through the use of Food Safety Assessments (FSAs). An FSA is a comprehensive evaluation of an establishment's food-safety system, including its sanitation controls, its compliance with microbiological performance criteria, the adequacy of slaughterhouse and processing plant Hazard Analysis and Critical Control Point (HACCP) systems, the design and operation of its prerequisite programs and its response to food-safety control deviations.

FSIS conducts regulatory sampling of Ready-To-Eat (RTE) products for the presence of *Lm*. *Lm* is the best indicator of sanitary operations for the RTE processing environment at retail. Percent positives indicate the finding of *Lm* in the samples. Therefore, higher percent-positives is a probable indication of higher *Lm* in the food supply regulated by FSIS.

The Healthy People 2010 goal for illnesses dues to *Listeria monocytogenes* is 0.24 cases per 100,000. FSIS estimates based upon its public health attribution work that the Healthy People 2010 goal for Listeriosis illnesses from RTE products is 0.14 cases per 100,000 or percent positive rate of 0.30. FSIS met the Healthy People 2010 goal for Listeriosis illnesses from RTE products in FY 2007. The FY 2013 performance objective was calculated by assuming a one percent annual decrease from the FY 2007 percent positive rate.

USDA Strategic Objective 4.1.3: Reduce the overall public exposure of ***E. coli* O157:H7** in ground beef

	FSIS Verification Test Results		**Performance Objectives[1]**					
	FY 2006	FY 2007	FY 2008	FY 2009	FY 2010	FY 2011	FY 2012	FY 2013
Volume Adjusted Percent Positive Rate	0.40%	0.28%	0.23%	0.22%	0.20%	0.18%	0.17%	0.15%
Not Volume Adjusted[2] Percent Positive Rate	0.17%	0.20%	0.20%	0.20%	0.19%	0.19%	0.19%	0.19%

1 Performance objectives assume a continuous decrease to meet the FY 2013 goal.

2 The FY 2013 performance objective was calculated by halving the Healthy People 2010 goal for *E.coli* O157:H7 in ground beef.

2 Prior to 2008, percent positives for *E. coli* were calculated by dividing the total number of samples positive for *E.coli* by the total number of samples tested. That method, however, is not representative of the potential exposure to the pathogen, because it does not take into account differences in production volume across the establishments being sampled. For example, an O157:H7 positive at a production facility producing a small amount of ground beef would cause fewer O157:H7 cases than a positive at a large production facility. Adjusting for production volume provides measures and objectives that are more representative of FSIS' progress towards preventing cases of human illness.

***E. coli* O157:H7:** *E. coli* O157:H7 is one of hundreds of strains of the bacterium *Escherichia coli*. Although most strains are harmless, this particular strain produces a powerful toxin that can cause severe illness. *E. coli* O157:H7, in fact, is a leading cause of severe foodborne illness. The organism can be found on most cattle farms. Meat can become contaminated during slaughter, and organisms can be accidentally mixed into meat when it is ground, or blade/needle tenderized. Eating meat, especially ground beef that has not been cooked sufficiently to kill *E. coli* O157:H7, can cause infection. Contaminated meat looks and smells normal. The number of organisms required to cause disease is very small.

In the wake of an increase in the number of *E. coli* O157:H7-positive samples collected in June 2007 by FSIS, and an increase in recalls and illnesses associated with this pathogen, FSIS implemented several risk management initiatives. This strategy is emblematic of FSIS taking into account a broader, more complete range of evidence when evaluating whether to seek a recall or take regulatory action. It is also indicative of FSIS' commitment to building upon its science- and risk-based activities to enhance public health protection and maintain consumer confidence in the safety of the nation's food supply.

The Healthy People 2010 goal for illnesses due to *E. coli* O157:H7 is 1.0 cases per 100,000. FSIS estimates are based upon its public health attribution work (that the Healthy People 2010 goal for illnesses from *E.coli* O157:H7 in ground beef is 0.34 cases per 100,000 or percent positive rate of 0.32). FSIS met this Healthy People 2010 goal for in FY 2006. The FY 2013 performance objective was calculated by halving the Healthy People 2010 goal for *E.coli* O157:H7 in ground beef.

BUDGET AND PERFORMANCE INTEGRATION

One of the five initiatives on the President's Management Agenda (PMA) is Budget and Performance Integration (BPI). This initiative builds on the Government Performance and Results Act of 1993 (GPRA) and previous efforts to identify program goals and performance measures and to link them with the budget process. The PMA BPI initiative outlines the criteria that agencies must comply within the performance of its mission, strategic goals, related business processes and major activities. BPI has two primary goals, which are:

- To use performance information in budgeting; and
- To improve program performance and efficiency.

In addition, FSIS' BPI objectives focus on performance-based budgeting; results-focused program performance assessment; accountability for results and program resources; strategic, organizational, and program level alignment and integration of budget, performance, and accounting information; and alignment of costs. These objectives stress:

- Increased accountability, effectiveness, and efficiency by implementing multi-year plans designed to improve the management and performance of a program;
- Data systems and process integration that provide reliable cost and performance information for planning, programming and budget decision making;
- Investment in high pay-off or high priority activities, which focus mostly on programs that can achieve demonstrably greater results for the same or less cost; and
- Applying the findings of program evaluations, such as OMB's Program Assessment Rating Tool (PART) to identify and address program strengths and weaknesses.

FSIS is committed to developing and improving programs that are focused on producing meaningful results for the taxpayer while at the same time protecting public health. FSIS in partnership with USDA's Farm Service Agency (FSA) procured a Commercial Off the Shelf (COTS) software solution that will enable the integration of budget and performance data to improve the visibility, timeliness, reliability and accuracy of management information for improved decision-making support. This solution for improved performance and accountability is called the Budget and Performance Management System (BPMS) which has been selected as the official budgeting system for USDA by the Departmental Chief Financial Officer. FSIS and FSA are in the process of creating data models to integrate Agency-wide performance and accountability capabilities, processes and systems.

The BPMS framework will facilitate FSIS' transformation to a results-focused, performance-based organization and the implementation of the PMA BPI initiative.

BPMS consists of the following major components:

- Framework for Budget and Performance Integration
- Salary and Benefits Projections
- Budget Formulation
- Budget Execution
- Cost Management
- Cost per Unit Metrics
- Activity Based and Reimbursables Cost Models
- Activity Reporting System (with NFC solution)
- Performance Improvement
- Scorecarding

The BPMS solution will support the following intermediate objectives:

- To reduce the time spent performing manual data entry tasks within the Chief Financial Officer organization;
- To reduce the time spent compiling, verifying and validating performance reporting information;
- To improve the quality, timeliness and controls for budget and cost information through use of a common framework and data source;
- To fully cost 100 percent of FSIS programs within five years, and align these programs to the Strategic Planning framework in support of Department and Office of Management and Budget (OMB) reporting and analysis requirements;
- To allow budgeting information to be accessible outside of the budget organization through web-enabled access to budget, cost, and performance information; and
- To increase insights into cost and performance via linkages between cost, operational efficiency, and strategic performance as outlined in the Strategic Plan framework.

OPERATING ENVIRONMENT AND EXTERNAL FACTORS

A number of environmental and external factors can impact the Agency's priorities and goals. These include the following:

Additional Major Outbreaks/New or Emerging Pathogens

Even with a comprehensive in-plant and import inspection system, major outbreaks of foodborne illness can occur, depending on the handling and preparation of meat, poultry, and processed egg products by commercial establishments and individual consumers. Ongoing research may also identify new and emerging strains of organisms that can cause foodborne illness.

Industry Demand

FSIS sees ever increasing demand for meat, poultry, and processed egg products both here and abroad. The projected growth in demand for meat and poultry products requires an ever-adapting front-line of inspection personnel.

When FSIS received its final appropriation from Congress in February 2007, the Agency had already begun an aggressive effort to hire a significant number of new inspectors and reduce vacancy rates. By the end of September 2007, FSIS had already hired more than 600 new in-plant personnel. After accounting for those who retired or left FSIS, the Agency has achieved a net gain of approximately 160 in-plant positions filled for FY 2007. FSIS expects to achieve its goal of hiring a net increase of 184 inspectors, and an additional 12 new inspectors in New Mexico. In FY 2007, New Mexico voluntarily turned its State program over to FSIS. Since hiring employees takes an average of 12 weeks under the most ideal conditions, and may extend anywhere from five to six months for positions in hard-to-fill locations, it is evident that the Agency worked very hard to meet hiring goals and reduce vacant positions. FSIS has pioneered the aggressive use of existing and new staffing authorities to fill mission-critical positions, especially for in-plant and front-line positions, where 85 percent of FSIS employees are located.

Research and Surveillance

Because FSIS does not conduct research, it must rely on other organizations to conduct the research it needs to support its public health mission. These other organizations have included the Agricultural Research Service (ARS), Cooperative State Research, Education, and Extension Service (CSREES), Economic Research Service (ERS), the Food and Drug Administration (FDA), The Centers for Disease Control (CDC), as well as academia and other private sources who conduct the research needed to fill data gaps that are necessary to conduct risk assessments and make risk management decisions.

Growth in the Agency's food safety and food defense responsibilities is reflected not merely in the volume of product inspected and shipped, but also dramatically in the need to cover complex public health issues associated with the handling of meat, poultry, and processed egg products outside of the federally inspected establishments. These responsibilities include surveillance of the transportation, storage, and distribution of inspected products for intentional and non-intentional chemical, biological and physical contamination of inspected products; conducting investigations to detect, prosecute and deter criminal violations; performing food defense activities including assessment and emergency response; covering and follow-up of recalls; conducting illness outbreak and consumer complaint investigations; and auditing and reviewing of State and foreign inspection programs.

To meet these responsibilities, the Office of Program Evaluation, Enforcement and Review (OPEER) is developing an In-Commerce system to improve the Agency's ability to prioritize, analyze, and focus its resources on targeted food safety and food defense in-commerce activities. The In-Commerce system will collect data to push to the Agency data warehouse and retrieve its own and other related data from the data warehouse, enabling improved response time to events that warrant immediate action and in the alerting of Agency executives. The investigative staff will also provide FSIS with the ability to synthesize findings from case studies to identify common trends in plant performance and inspection program operations, root causes of development problems, and options for proactive solutions.

Risk-Based Inspection

FSIS is designing procedures to replace traditional inspection systems for slaughter and processing operations. Under an optimal risk-based inspection system, the type and intensity of inspection activity at each establishment would be determined by an analytical process that permits inspectors to anticipate problems and focus their efforts on those processes and establishments most likely to have control issues and pose a public health risk. FSIS recognizes each step taken toward risk-based systems must further protect public health.

In FY 2008, FSIS will continue its major focus on the design and implementation of this more robust risk-based inspection system. While FSIS maintains that its current system is strong, the Agency must adapt to the ever-changing realities of food safety and public health. The Agency envisions a number of advantages offered by a more robust risk-based system. In particular, a risk-based system can be fluid, rapidly adapting to emerging hazards. It can more easily identify problems that have occurred and anticipate problems to minimize risk. This more robust system will allow the Agency to align resources with the corresponding level of risk posed by specific hazards, products, and processes.

FSIS has already made progress toward a risk-based approach to food safety, especially in the risk-based approach to pathogen control. An example is the FSIS verification sampling program for *Listeria monocytogenes*. Under this initiative, FSIS structures its verification activities to the interventions that plants choose to adopt and to the potential for *Listeria* growth in their products. In other words, FSIS conducts less sampling in those plants that have in place the best control programs for *Listeria* and more sampling, as well as in-depth Food Safety Assessments, in plants that adopt less vigorous programs.

FSIS' goal is to further enhance and strengthen this risk-based system. Based on the Agency's progress with *Listeria*, FSIS has been developing a risk-based verification system for *E. coli* O157:H7, and announced in February 2006 an 11-step strategy for *Salmonella*. FSIS will take this risk-based approach further by using inspection data and other information to determine the hazard from product type and plant performance to determine the intensity of inspection at processing plants and import inspection facilities.

The Agency will apply the risk-based focus to imported products as well. Using information from on-site audits, port-of-entry inspections and other sources, decisions can be made as to the level and scope of enforcement needed in order to assure that imported products continue to meet U.S. food safety requirements.

Threats and Acts of Terrorism Directed at the Nation's Food Supply

As the possibility of purposeful food contamination for political and ideological gain has grown, bio-terrorism and bio-security are terms that have made their way into the dialogue on food safety. Expansion in the bio-security area may result in a different allocation of resources.

Food contamination, animal and plant diseases, and infestations can have catastrophic effects on human health and the economy. USDA, the Department of Health and Human Services, and the Department of Homeland Security are working together to create a comprehensive food and agriculture policy that will improve the Government's ability to respond to the dangers of disease, pests and poisons, whether foreign or domestic in origin and whether naturally or intentionally introduced.

Legislative Action and Federal Management Mandates

The mission and programs of FSIS are grounded in legislative mandates. Changes in Federal mandates and legislation could affect what the Agency does and how it does it.

Budget Constraints/Balanced Budget

Limited budgets and workforce size could unfavorably impact the Agency's implementation of program change and innovation, as well as the achievement of current inspection goals.

Unionized Labor

As a major stakeholder in FSIS programs, unionized labor could alter conditions under which changes are made.

Consumer and Industry Organizations (i.e., special interest groups)

Our stakeholders advocate modification of USDA's food safety activities and methods, sometimes resulting in different program expectations and priorities.

New Scientific Technologies

New in-plant equipment and processes could impact program objectives through faster processing times and create a need for more product testing and sampling, resulting in a realignment of resources.

Public Opinion

The media shapes and molds public opinion, and therefore food safety goals and objectives could be modified based on media views, representations, and pressure resulting in a different application of resources, both human and capital.

Political Imperatives

Legislative or administration priorities could impact Departmental and Agency leadership, which could result in new missions, programs, and goals.

Codex Alimentarius

FSIS works through the Codex Alimentarius Commission to help develop international food safety standards. Member countries are encouraged to accept and implement Codex-approved standards nationally, but they are not obligated to do so. Adopting Codex standards could result in a different allocation of resources.

Trade Issues

As a member of the World Trade Organization, the United States is party to agreements that establish rights and obligations in international trade. In addition, internal and external transportation or trade

issues could impact Agency goals and objectives through trade barriers or conflicting standards which could result in product delays and affect markets.

The United States continues to struggle for market access in important Asian markets after the initial discovery of Bovine Spongiform Encephalopathy (BSE) in December 2003. However, U.S. beef exports are forecast to climb over 19 percent in 2008 to around 775,000 tons due to increased opportunities in the North America Free Trade Agreement (NAFTA) area. U.S. beef maintains strong sales to Canada and Mexico, while slowly making inroads in the Japanese market despite being impeded by a 20 month or younger age restriction and the age-verification process. The USDA Secretary has urged Japan, as well as all U.S. trading partners, to implement import requirements for beef and beef products as soon as possible that are based on science and consistent with international guidelines, including those of the World Organization for Animal Health (OIE).

In addition to challenging import requirements, the U.S. beef industry has experienced difficulties with Asian countries rejecting shipments due to errors made during the assembly of lots for export. These "mispack" problems have been seized upon by Asian importing countries as a means to further restrict trade with U.S. beef producers even though no food safety hazards are involved. The U.S. industry and government regulatory agencies have continuing programs in place to minimize commercial errors that have adversely affected beef trade. These programs, combined with robust trade negotiation policies, have resulted in significant renewals of U.S. market access for beef products that were formerly banned due to BSE concerns.

New Scientific Advances

Newly developed understanding of the epidemiology of foodborne diseases and the association between animal pathogens and human foodborne illness could result in significant reallocation of resources. Risk assessments based on increased scientific knowledge could identify new points in the farm-to-table continuum where risk reduction mitigations could be applied to decrease the level of human foodborne illness.

STRATEGIC GOAL ONE

Enhance inspection and enforcement systems and operations to protect public health.

PUBLIC OUTCOMES

- FSIS achieves improving levels of performance in daily food safety and food defense operations.
- FSIS has a workforce of proficient personnel, who know their duties and responsibilities.
- Meat and poultry inspection is concentrated at points of greatest risk.
- Oversight of imported products is targeted at areas of greatest risk.
- The Nation enjoys safer, more secure domestic and imported food products.
- Effective response procedures for non-routine (emergency) incidents are in place.
- Monitoring and surveillance systems to support food defense preparedness activities are in place.
- Effectively monitored foodborne illness report data, which is utilized to reassess FSIS-regulated plants.
- Progressive studies designed to attribute illness to specific foods are available.

OBJECTIVES

- Expanded use of performance-based management controls to verify risk-based inspection.
- Effectively enabled teams of inspectors to carry out risk-based inspection.
- More informed food safety and defense actions and interventions deployed.
- Increased document analyses and on-site audits of Federal and State inspection systems and establishments.
- Enhanced data collection and integration to strengthen oversight of foreign inspection systems.
- Developed, launched, and maintained automated system to support Agency's response to non-routine (emergency) incidents.
- A surveillance system which integrates inter-Agency and national information to improve situational awareness and early detection.
- Rigorous enforcement actions and sanctions against violations of food safety laws and regulations.
- Enhanced Agency food safety and defense IT systems.
- Enhanced IT data coordination activities with other Federal agencies.
- Strengthened public health, scientific, and technical skills of the Agency workforce.

MEANS AND STRATEGIES

FSIS has the statutory authority and responsibility for protecting the public health by assuring that meat, poultry, and processed egg products are wholesome, not adulterated, and properly labeled. The governing statutes give the Agency the authority to protect the health and welfare of consumers by promulgating regulations governing the production, import and distribution of these products and to

enforce the laws through food safety and food defense inspection, surveillance, and assurance. FSIS also has the authority to control product, temporarily stop operations, and to refuse or withdraw inspection, and to implement administrative, criminal, and civil action. The Agency carries out this mission with an in-plant workforce of approximately 7,800 inspection program personnel.

Using authorities and resources at its disposal, FSIS will ensure the safety and defense of the nation's domestic and imported food supply through the:

- allocation of in-plant inspection resources on the basis of the risk inherent in products, processes, and producers;
- ongoing use of Food Safety Assessments to
    - provide evaluations of establishment food safety systems;
    - provide guidance and baseline information that plants can use to improve their systems; and
    - verify industry's compliance with risk-based strategies for pathogens;
- implementation and continuous improvement of performance-based management control systems;
- implementation and use of team inspection to evaluate and assure plant compliance with food safety statutes;
- coordination of the use of investigative resources at ports-of-entry and in food warehousing, distribution, and retail channels;
- use of strong, timely, and effective enforcement actions; and audits of domestic and State foreign inspection systems and establishments. Investigation and enforcement strategies and actions will be based on risk through enhanced data collection and analysis, and development and application of risk-based measures; and
- use of a wide range of integrated data gathered through port-of-entry inspection, on-site audits and other sources to evaluate the continued equivalence of inspection systems in foreign countries exporting to the United States.

Enhanced Agency data systems and effective integration of technology: These efforts will improve the timeliness and effectiveness of Agency public health protection (i.e., regulatory verification, investigation and enforcement activities) and ensure immediate and effective food safety and defense incident response. Information technology (IT) modernization efforts will provide critical support to ongoing initiatives to consolidate Agency data for predictive analytics and integration, identify data gaps, and modernize and facilitate sample collection data reporting. Participation in the Department of Homeland Security's (DHS) Automated Commercial Environment/International Trade Data System (ACE/ITDS) will effectively coordinate FSIS food inspection and protection activities and FSIS import and export IT systems with 27 Federal agencies and enhance food safety and food defense protection from threats from imported foods. FSIS will also create a new, innovative risk-based, web-based inspection IT system to integrate Agency risk-based policies with Agency domestic and foreign inspection systems. The system will collect and analyze information from the Agency's data warehouse. The In-Commerce System will support the Agency's directives on surveillance and compliance, support food defense requirements in the field, provide a case management system, and graphically display area management controls and performance measures, alerting managers when targets have been exceeded.

Codex: FSIS hosts the U.S. Codex office, which manages the participation of FSIS and other U.S. government regulatory officials and non-governmental organizations in the work of the Codex Commission and its Committees. FSIS is actively involved in the Committee on Food Import and Export Inspection and Certification Systems (CCFICS) which develops principles and guidance for food import and export certification and inspection, as well as the Committee on Food Hygiene, which develops guidance on food hygiene principles and microbiological risk analysis. FSIS will continue active involvement in assuring that scientific principles are the basis for the international standards

developed by the Commission.

Workforce training and education: Training and education of the FSIS workforce is a cornerstone of public health protection. Training enables inspection program personnel to make sound and effective regulatory decisions based on appropriate scientific and public health principles. One of the Agency's top priorities, therefore, is to aggressively train and educate our workforce. To accomplish this, FSIS is implementing an ongoing strategy to provide employees with a challenging program of initial training when they report to their first assignment; follow-up training that reinforces acquired skills; and advanced skills training to prepare the employee for performing complex public health protection duties. This enables FSIS to maintain a well-trained workforce as the Agency's policies and programs continue to advance to meet the ever-changing needs of public health.

STRATEGIC GOAL TWO

Enhance the use of risk analysis and vulnerability assessments in FSIS' approach to protecting public health.

PUBLIC OUTCOMES

- Risk-based measures strengthen regulatory verification and enforcement activities on behalf of the consumer.
- Assessments are used to identify physical, chemical, biological, and radiological hazards for domestic and imported products.
- Plants are continuously reassessed based on foodborne illness data.
- A research agenda, developed with ARS, is in place to support food safety and food defense initiatives.

OBJECTIVES

- Increased effectiveness of risk-based regulatory and enforcement activities.
- Improved linkages between homeland and food defense policies and systems.
- Increased risk assessments.
- Rapidly identified and addressed vulnerabilities in food defense, program integrity, and resource management.
- Increased number of sources that dispense public health information.
- Increased number of FSIS- regulated establishments with developed and implemented functional food defense plans.

MEANS AND STRATEGIES

To achieve this goal, FSIS has adopted the well-recognized scientific approach of Risk Analysis in its efforts towards applying resources in a prudent manner. The Risk Analysis approach contains three aspects:

- **Risk Assessment:** the process of estimating the severity and likelihood of harm to human health or the environment occurring from exposure to a substance or activity that, under plausible circumstances, can cause harm to human health or the environment;
- **Risk Management:** the process of evaluating policy alternatives in view of the results of risk assessment and selecting and implementing appropriate options to protect public health. Risk management determines what action to take to reduce, eliminate, or control risks. This includes establishing risk assessment policies, regulations, procedures, and a framework for decision making based on risk; and
- **Risk Communication**: the process of exchanging information among risk assessors, risk managers, other stakeholders, and the public about levels of health or environmental risk, the significance and meaning of those risks, and the decisions, actions, or policies aimed at managing or controlling the risks.

Using the risk analysis approach, FSIS will then use the Public Health Model (i.e., assessment, policy development, and assurance) to ensure that the risk mitigation strategies are working as intended. Assessment will include collecting and analyzing relevant data to determine whether inspection activities are protecting public health. Policy development will include developing enhanced methods to better ensure that inspection activities are best protecting public health. Finally, assurance will include evidence-based analysis of data to demonstrate that public health policy is effective and applied as intended.

FSIS will develop and apply real-time measures of how well slaughter and processing establishments, exempt facilities (operations that qualify under exceptions specified in Section 623 of the Federal Meat Inspection Act, Section 464 of the Poultry Products Act, and 9 CFR § 303 of the livestock regulations and 9 CFR § 831 Subpart C of the poultry regulations), food warehouses, distributors, importers, food handlers, and others control the biological, chemical and physical hazards, and food defense risks inherent in or attendant to their operations.

FSIS will also develop Food Safety Assessments (FSA) to provide information contributing to the design of an establishment's food safety system. The assessments identify gaps and weaknesses in the establishment's HACCP plan and how well the plant addresses the biological, chemical, and physical hazards in its system as well as in its implementation of its system.

Together, these FSIS program functions will enhance risk and vulnerability assessments, decision making, and Agency regulatory verification and enforcement. FSIS will ensure open communication throughout these processes.

The manager of the U.S. Codex Office, as head of the U.S. Delegation to the Codex Committee on General Principles (CCGP), continues to act as the lead advocate for U.S. interests in the development of documents such as *Working Principles for Risk Analysis for Food Safety for Application by Governments,* which seeks to establish risk analysis as a key element of a national food safety system, and to encourage its use among the 172 countries that are members of the Codex Alimentarius Commission.

STRATEGIC GOAL THREE

Enhance the development of science and risk-based policies and systems.

PUBLIC OUTCOMES

- The Nation has a fully implemented risk-based inspection system.
- Highly regarded science-based regulatory policies and guidance documents are available to food safety educators, State partners, industry stakeholders, and foreign trading partners.

OBJECTIVES

- Increased public health policies backed by risk assessments, epidemiological data, evaluations, and other data.
- Increased policy development and outreach activities prioritized based on their impact on public health.
- Increased food defense policies, programs, and interventions developed to address systemic vulnerabilities found in assessments.
- Strengthened risk-based inspection system based on the findings of program evaluations and other studies.
- Integrated information technology and policy development applied to the risk-based inspection system nationwide.
- Reduced *Salmonella* in Ready-to-eat (RTE) and Not Ready-to-eat (NRTE) products consistent with Healthy People 2010 and Healthy People 2020 goals through development and implementation of policy.
- Reduced *E. coli* 0157:H7 and other Shiga toxin-producing *E. coli* (STEC) consistent with Healthy People 2010 and Healthy People 2020 goals through development and implementation of policy.
- Reduced *Listeria monocytogenes* in RTE and NRTE products consistent with Healthy People 2010 and Healthy People 2020 goals through development and implementation of policy.

MEANS AND STRATEGIES

To ensure food safety from farm-to-table, it is vital that all of FSIS' stakeholders understand the Agency's mission and, at a minimum, have the knowledge and information necessary to comply with regulatory requirements. Because of its food safety and defense responsibilities and its presence in plants, FSIS depends upon a large and dedicated workforce to inspect the Nation's commercial supply of meat, poultry, and processed egg products. Since meat, poultry, and processed egg products are of animal origin, they are not sterile and can be contaminated with bacteria at any point during production, distribution, and consumption. Due to the enormity of the task, science-based policy development is necessary to achieve more positive and measurable public health outcomes. Consumer food handling recommendations will be increasingly based on scientific and risk research, social marketing concepts, and evaluative research.

With the implementation of the Pathogen Reduction/HACCP systems rule, FSIS has already made significant advances in improving the inspection system. FSIS will further enhance this system to

anticipate and quickly respond to food safety challenges before they adversely affect public health. FSIS will use measures of inherent product risk, exposure, and establishment risk control to better allocate resources for protecting the public health. FSIS will continually collect and analyze inspection, microbiological, enforcement and other types of data to better target higher risk operations. FSIS will capture this information in the inspection system, which will integrate Agency risk-based inspection policies and data with other Agency IT systems.

FSIS already collects data on imported products and the results of FSIS inspection at the port of entry. This information, along with the results of on-site and document audits, will be used to develop measures of risk presented by individual foreign inspection systems so that FSIS can assure the appropriate frequency and scope of its oversight.

FSIS is also dedicated to participating in the Healthy People 2010 and Healthy People 2020 initiatives. These comprehensive, cross-cutting public health studies outline two decades of national health goals, and define critical measures that the U.S. must undertake to promote healthy behaviors, achieve improved health outcomes, reduce risk factors, and assure access to preventive strategies and services that aspire to improve the health of all Americans.

In addition, the U.S. Codex Office will continue to coordinate an inter-Agency effort that ensures the incorporation of the U.S. national objectives for science and risk-based policies and systems into the positions that are presented by U.S. delegations at meetings of the Codex Alimentarius Commission and the Commission's various subsidiary bodies.

STRATEGIC GOAL FOUR

Enhance the development and maintenance of an integrated and robust data collection and analysis system to verify the effectiveness and efficiency of Agency programs.

PUBLIC OUTCOMES

- Continuous monitoring, assessment, and enhancement of public health protection activities are carried out each day by enforcement and field inspection personnel throughout the United States.
- Improved data analyses infrastructure supporting science-based polices and initiatives on food safety and food defense.
- Enhanced Agency systems providing timely data for planning, evaluating, decision making, and policy development.
- Integrated data systems with other Federal agencies to provide seamless oversight of imported products.
- Enhanced Agency system for issuing export certificates that integrates domestic and foreign country requirements.

OBJECTIVES

- Integrated IT linking outreach, inspection, compliance, and enforcement efforts.
- Effective, real-time monitoring and assessment of public health regulatory activity.
- Improved scientific tools and techniques to reduce or eliminate hazards.
- Improved association of program outcomes to public health surveillance data.
- Identified key control activities and established performance measures for program activity.
- Expanded use of data analysis to determine the effectiveness and efficiency of Agency programs.
- Linked AssuranceNet with the Agency's data warehouse so that Agency goals and objectives are met (Agency data warehouse is where multiple sources of data are fed so Agency programs can easily access it.)
- Developed and launched Enterprise Reporting System to provide a more holistic view of the Agency's data for analysis.
- Established integrated data analysis infrastructure to identify early trends or indicators in order to intervene and/or develop science-based policies in inspection and enforcement systems.
- Developed automated export certification system that incorporates all domestic and foreign country requirements to strengthen security and assurances that exported shipments will move unhampered in international trade.

MEANS AND STRATEGIES

FSIS has established and documented a management control system. Management control is a process designed to provide reasonable assurance regarding the achievement of the effectiveness

and efficiency of operations and programs; reliability of financial reporting; and compliance with applicable laws and regulations. Program areas continuously monitor their management controls to demonstrate the effectiveness of their activities and to evaluate their impact to the Agency's public health mission and food defense activities. Management controls use performance measures as a means to quantitatively determine their effectiveness.

AssuranceNet is the Agency's automated management control and performance measure monitoring system to help managers make informed decisions for program improvement. Using real-time data, the system generates standard reports for managers on the effectiveness of specific activities. Currently, the Office of Field Operations and Office of International Affairs are using AssuranceNet, and other program areas will follow in a phased approach. The Agency will expand the capabilities of AssuranceNet as other program areas are phased in.

FSIS is leveraging AssuranceNet for the Agency's In-Commerce System. The In-Commerce System will improve the Agency's ability to schedule, analyze, and focus its resources on high-risk food safety and food defense in-commerce activities. It will provide all program offices with a central repository for reporting, managing and analyzing in-commerce data collected both within and outside of FSIS. These data will also be incorporated in the public health data infrastructure for analysis with other Agency data.

To support data-driven decision-making, FSIS has developed the first phase of a data warehouse to centralize and integrate Agency data into a single, reliable source of Agency knowledge. The data warehouse will continue to grow and expand to contain all corporate food safety and food defense data, both within the Agency and from external partners.

FSIS has developed phase one of the Enterprise Reporting System (ERS) to provide analysts and managers access to corporate data for making efficient and effective public health decisions. The development of ERS sets the groundwork for the architectural direction and technology to be used for the future FSIS systems, specifically the development of the Public Health Information System (PHIS) under the Public Health Information Consolidation Project (PHICP). The PHICP incorporates the replacement for PBIS – the Domestic Inspection System, the replacement for AIIS 3 (the Automated Import Information System), the replacement/re-scoping of the Food and Agriculture Bio-surveillance Information System (FABIS) into the Food and Agriculture Safety and Public Health Analytical System. It also provides enhancements for the Consumer Complaint Monitoring System II that will allow state programs to share their public health data directly with the Agency.

Both ERS and AssuranceNet will be integrated into the newly developed FSIS technical architecture under PHICP. FSIS has developed, and will continue to enhance AssuranceNet, the Agency's automated management control system, to analyze the effectiveness of Agency policy and procedures. AssuranceNet makes use of the Enterprise Reporting System for its reports, as will the PHIS.

FSIS will employ data analysis as a management verification measure in determining the success of Agency strategies to combat threats to food safety and defense and to help ensure that program components are effective in meeting public health goals and objectives. FSIS will use regulatory verification, compliance, enforcement, sampling, and other data and information to discern trends, causes, and outcomes to determine whether the Agency's inspection, verification, enforcement, compliance, and stakeholder outreach are effective in protecting the public health.

In addition, FSIS continually evaluates the effectiveness of its messages to meet the needs of its stakeholders. The communication programs within FSIS do more than provide clear and focused messages about public health through food safety. They move beyond their message development activities to monitor the actual impact that they are having on the public health. FSIS educational campaigns continue to use social marketing and educational principles to identify barriers and target audiences.

STRATEGIC GOAL FIVE

Enhance the development and maintenance of an innovative infrastructure to support the Agency's mission and programs.

PUBLIC OUTCOMES

- Comprehensive, accurate, real-time information for decision-making.
- Effective Agency-wide system of management controls.
- An effective and efficient IT data collection system that meets all Federal IT security requirements.
- Effective Agency strategic management of human capital to carry out food safety mission.
- Effective recruitment of the most qualified inspection personnel and support staffs in filling Agency mission critical positions.
- Effective Agency budget and performance integration to efficiently carry out food safety mission.

OBJECTIVES

- Improved management controls and operating procedures for all FSIS programs.
- Eliminated barriers that impede free and open competition in the workplace by ensuring that all policies, processes and procedures provide all individuals the opportunity to develop, participate and compete equitably.
- Utilized best-practices in human capital management to structure and deploy a competitive, highly skilled workforce, representative of America's great diversity that can more effectively meet Agency staffing challenges.
- Informed decision-making through improved fiscal management and through the implementation of budget and performance integration.
- Focused accountability of FSIS management through Strategic Planning, budget planning, and program planning.
- Enhanced delivery of program services by building a more robust logistical and acquisition infrastructure.
- Effective high-speed Internet access for inspection program personnel.
- Protected FSIS Mission Critical IT systems and essential infrastructure.
- Effective data systems and process integration that provide reliable cost and performance information for planning, programming, and budget decision making.
- Maximized high pay-off or high priority activities, which focus mostly on programs that can achieve demonstrably greater results for the same or less cost.
- Applied findings of program evaluations, such as OMB's Program Assessment Rating Tool (PART) in order to identify and address program strengths and weaknesses.
- A positive workplace environment that is free of unlawful discrimination, harassment, retaliation, and accessible to individuals with disabilities.

- Recognized, appreciated and valued diversity, thereby creating and maintaining an organizational culture based on mutual respect, trust, and inclusiveness.
- Equal opportunity in employment through the enforcement of the Federal civil rights laws and through education and technical assistance.
- A model Equal Employment Opportunity (EEO) Program through the robust achievement of model Title VII and Rehabilitation Act programs.

MEANS AND STRATEGIES

Over the next five years, the enhancement of the Agency's administrative infrastructure means broadening the use of management controls, strengthening financial reporting, more closely managing human capital, expanding information technology systems, and implementing performance-based budgeting.

FSIS will continue the implementation of an integrated, Agency-wide Management Control System. Portions of this system have already been implemented with the development and fielding of AssuranceNet. Currently, the Office of Field Operations and the Office of International Affairs use AssuranceNet to monitor their daily public health regulatory activities, and soon all remaining FSIS Program areas and functions will be incorporated into this system.

FSIS is dedicated to improving its Management Control system, which highlights the standards and organizational responsibilities for the accountable and efficient use of resources. The Management Control system consists of five interrelated components (control environment, risk assessment, control activities, information and communication, and monitoring.) Management Controls, when effectively applied by FSIS program leadership, will serve to assure that the Agency maintains its course toward achievement of its strategic goals and objectives.

FSIS will continue to improve its control system by evaluating and applying audit findings from outside entities. The FSIS Management Control system complies with OMB Circular A-123 requirements. The Agency has initiated Management Control audits to test the effectiveness of program areas' Management Controls and verify that they are achieving program objectives. Protocols for a new control audit to systematically assess, verify, and test Agency-wide Management Controls have also been developed. This audit will improve operational and administrative management efficiencies, cost effectiveness accountability, and managerial oversight in support of the FSIS efforts to achieve a Green rating on the PMA, Human Capital Management, and USDA Strategic Plan goals.

FSIS will seek creative ways to improve the Agency's communications program, both internally and externally. These communication activities will provide greater support to the Agency's public health and food defense initiatives.

The Agency will continue to provide an effective IT infrastructure that meets all Federal IT security requirements to support mission-critical activities. FSIS personnel use IT systems to record and report information about their daily food safety, food defense, and humane handling verification and enforcement activities. FSIS IT systems permit enforcement inspection and in-commerce personnel to electronically access and retrieve documents that contain information that they need to properly perform their inspection duties.

Agency IT systems also permit enforcement and inspection program personnel to participate in computer-based on-line training and allows them to obtain timely updates to enforcement and inspection-related computer applications. To ensure that FSIS' IT systems continue to be effective and efficient in assisting enforcement and inspection personnel to conduct their inspection activities, FSIS continues to implement a plan to upgrade its IT systems and provide its in-commerce and inspection assignments with high-speed Internet access. FSIS will also continue its IT modernization efforts to consolidate Agency data and modernize and facilitate sample collection for data reporting.

The IT Public Health Data Communication Infrastructure System: A public health communications infrastructure was proposed by FSIS in May 2006. This system will integrate the needs of the Agency's internal and external customers and protect public health by providing reliable, up-to-date and securely accessible information and analysis for decision-makers, especially to support risk-based inspection, food defense, and predictive (rather than solely reactive) analysis. The infrastructure will also support timely, consistent and reliable internal and external communications with stakeholders and use current and approved business needs to drive technology decisions.

The FSIS Office of the Chief Information Officer (OCIO) is working to improve current IT data and information for its customers by instituting an enterprise architecture (EA) governance process that will enable the Agency to support its current state, as well as transition to its target environment. This EA governance process, which includes the Capital Planning and Investment Control (CPIC) processes, Strategic Planning processes, and FSIS Systems Development Life Cycle (SDLC) methodologies, is a significant component of the public health communications infrastructure. The FSIS EA documentation describes governance and program management areas, organizational structure and management controls, baseline and target architectures, and a transition plan by which FSIS seeks to continue to achieve its overarching mission, goals, and objectives in support of the Federal government and citizens at-large. The Agency is also shifting toward documented management controls, performance measures, the President's Management Agenda (PMA), and defining business needs to drive IT systems to support strategic decision-making, planning and communications.

The Public Health Information Consolidation Practices: The Public Health Information Consolidation Projects (PHICP) investment will incorporate the development of three major applications that were previously separate major and minor investments: Performance Based Inspection System (PBIS), Automated Import Information System (AIIS), and a re-scoped Food and Agriculture Bio-surveillance Information System (FABIS) into the FSIS Public Health Information System (PHIS). This consolidation effort will also ensure the streamlining of the three major areas into a single system with various modules to address each business function. The new development effort will result in the creation of the PHIS. This will include the following major business functions\modules:

- Domestic Products
- Imported Products
- Exported Products
- Predictive Analytics

The Domestic, Imported, and Exported products all contain common functions that will be incorporated into each of the modules. Some of the common functions include:

- Inspection
- Surveillance
- Enforcement
- Scheduling

The development effort will span an initial development lifecycle of three to five years for the full implementation of the PHIS; along with the integration of existing FSIS systems.

Contracting with Small Businesses: The Agency will further increase an already aggressive commitment to maximizing the promotion and publicizing of small business program initiatives/opportunities, and its award of eligible contracts to small/small-disadvantaged business concerns. The Agency's impressive small business goal achievement extends over multiple fiscal years, and efforts will continue toward achieving results that exceed goals established by USDA. FSIS will particularly focus on increasing use of Service-Disabled Veteran-Owned businesses (SDVO) through internal Agency-wide promotion of SDVO sources. Efforts will also continue in expanding its

list of available SDVO vendors to maximize opportunities in considering SDVO's as a source to meeting future Agency requirements for goods and/or services.

Civil Rights, Equal Employment Opportunity, Diversity, and Inclusion: The ability of FSIS to meet the complex needs of our Nation's food safety and public health obligations and the expectations of the American people rests squarely on the Agency's dedicated and hard-working employees. FSIS must position itself to attract, develop and retain a top-quality workforce that can deliver results and ensure the safety and security of the U.S. meat, poultry, and processed egg products supply.

Equal employment opportunity is key to accomplishing this goal. In order to develop a competitive, highly qualified workforce, FSIS must fully utilize all employees' talents, without regard to non-merit factors such as race, sex, national origin, disability, age, color, religion, and similarly protected categories. While the promise of workplace equality is a legal right afforded all of our Nation's workers, equal opportunity, diversity, and inclusion is more than a matter of social justice. It is a national economic imperative. FSIS will continue to make full use of America's human capital by deploying workplace practices that promote opportunities for the best and brightest talent available. The Agency will continue to be committed to ensuring all FSIS employees and applicants for employment have equal access and opportunity to achieve their fullest potential, and that employees are valued, trusted, and respected.

FSIS will take proactive steps to ensure equal employment opportunity for all their employees and applicants for employment. The Agency will continue to build and advance an infrastructure to promote and sustain strategic, comprehensive diversity and inclusion initiatives that integrate EEO into the Agency's strategic mission and leverage the diversity of the FSIS workforce. The Agency will continue to regularly evaluate its employment practices to identify barriers to equality of opportunity for all individuals; and, where such barriers are identified, FSIS will take measures to eliminate those barriers to equal access and opportunity.

Policies and practices that impede equitable and open competition cost the Agency each year. The most obvious are out-of-pocket costs borne by the Agency and FSIS employees in connection with workplace disputes. Perhaps less obvious – but just as expensive – are costs associated with decreased morale and productivity and the ineffective and inefficient use of human capital resources. FSIS can and will act to avoid these costs. FSIS will continue its firm commitment to the principles of equal employment opportunity, diversity, and inclusiveness, and make those principles a fundamental part of FSIS culture. With these steps, FSIS will ensure that all persons are provided opportunities to participate in the full range of employment opportunities and achieve to their fullest potential. Through an aggressive and continuing effort to achieve and sustain the essential elements of model Title VII and Rehabilitation Act programs, FSIS will work to proactively prevent potential discrimination before it occurs and establish systems to monitor responsiveness and legal compliance. The Agency will promote equal opportunity and leverage diversity and inclusion to create excellence in FSIS employees and Agency mission performance.

Budget/Finance: FSIS will, with crucial funding, maintain and improve upon its long history of protecting public health. The Agency will continue to seek financial support for its dedicated staff of scientific and technical employees, in order to foster a workforce that serves as the foundation of public health and performs the work needed to lower rates of foodborne illness. FSIS' plans for a more robust risk-based system must rely heavily on data to allow proactive decisions affecting food safety and public health. Funding must help deliver the data via information technology tools needed by the workforce to quickly respond to indications of risk to human health and food defense and do so with utmost efficiency and effectiveness. The Agency will continue to request funding that supports its long term vision. The Agency will allocate funds on the basis of the Agency's needs in an efficient and effective manner to provide financial data for informed-decision making. Management decisions will be made by utilizing financial data provided by the Foundation Financial Management System (USDA's accounting system) as well as a modified software package labeled the Budget and Performance Management System (BPMS). FSIS intends on using BPMS to accurately forecast salary and benefit requirements and other costs while increasing the precision of cost data to

effectively utilize resources and provide better data for informed decision-making. Spending patterns and types of spending will be analyzed to find greater efficiencies. These activities will include the management of field resources to provide guidance to Agency staffs on expenditures for travel and relocation. The BPMS will eventually be maintained in the newly implemented accounting system redesigned to track costs by the Agency's performance measures. Expenditure of funds will be continuously monitored and effective management controls will guide the oversight of expenditures.

Human Resources

- **Recruitment:** FSIS will continue to strengthen Agency relationships with the veterinary and science colleges and universities on the Recruitment Schedule by participating in on-site campus career fairs, serving as guest lecturers in classroom settings, and ultimately hiring students and new graduates for Agency positions. FSIS will continue to utilize and expand recruitment incentives and hiring flexibilities for hard-to-fill positions by offering recruitment bonuses, first post of duty moves, and student loan repayments.
- **Reform:** The Agency's mission has expanded to include responsibility for food defense, biosecurity, and public health science. As a result, the Agency must take steps towards establishing a more results-oriented Human Resource (HR) system that helps FSIS protect public health by better managing, developing and rewarding employees serving the American public. Through the use of a Demonstration Project, the Agency will implement the Public Health Human Resources System (PHRRS) which will establish a contribution-based pay system to effectively address the challenges of getting the right people with the right skills in the right locations. This new system has pay flexibilities to set an employee's pay at appointment anywhere in the pay band based on qualifications and labor market factors. In addition, results-based performance ratings linked to pay increases and recognition for contributions will be key to reaching Agency goals.

  Other initiatives to aid in attracting and retaining employees include:

  - aggressive pursuit of additional staffing authority to fill mission-critical positions;
  - a modernized automated application process which allows the Agency to hire more quickly;
  - continued expansion of outreach activities targeting a number of richly diverse universities and professional organizations to ensure the recruitment of a high quality and diverse workforce; and
  - a succession plan in which leadership training and development programs are robust and active.

STRATEGIC GOAL SIX

Enhance the effectiveness of Agency outreach and communications to achieve public health goals.

PUBLIC OUTCOMES

- The Nation has better informed consumers of meat, poultry, and processed egg products.
- Food safety educators, State partners, industry stakeholders, and foreign trading partners receive and act upon scientifically sound information and guidelines.
- The public, including previously under-served groups, has even greater access to food safety information and messages.
- The public and all FSIS employees are dedicated to the Agency mission and its corporate priorities and goals.
- Reduced public exposure to foodborne illness from products under FSIS inspection.
- Small and Very Small plants have one location to obtain information and assistance pertaining to improved food safety systems.

OBJECTIVES

- Widely disseminated outreach programs to industry and foreign countries to assist them in meeting regulatory requirements and food defense programs.
- Widely disseminated outreach and education programs to industry, consumers, and food handlers to encourage the maintenance of food safety and food defense during production and in-distribution security.
- Identified key research needs to work with public/private entities to shape a research agenda.
- Enhanced internal and external communications in regards to public health priorities and food defense initiatives.
- Increased effectiveness and application of public messages.
- Continued collection and action on information in the public web site's customer satisfaction survey, and continue to lead the Department in satisfaction scores.
- Instituted leading edge, web-based tools (such as AskKaren, askFSIS, and the email subscription service) to provide immediate, accurate, 24/7 access to reliable and approved Agency information to better protect public health.
- Delivered targeted information for the Agency's customers, particularly businesses and partners as well as consumers and educators.

MEANS AND STRATEGIES

Outreach and Communication with Stakeholders: FSIS promotes stakeholder understanding and support of the Agency's public health mission through a variety of outreach efforts. Outreach to Agency stakeholders takes place in the numerous public meetings and scientific symposia that FSIS conducts each year. Through these public forums, FSIS maintains a dialogue with industry, academia, scientific, and consumer communities on various Agency priorities. FSIS conducts separate monthly meetings with industry associations and consumer representatives and, as a need arises, will work one-on-one with any stakeholder to provide information or answer questions in a timely manner.

In addition, activities are in place to meet the specific and diverse needs of Small and Very Small establishments, American Indians, Native Alaskans, Hispanics, and others. FSIS uses its Agency Web site, which includes a compilation of regulations and other Agency topics, to share information with stakeholders. A dedicated page on the Web site specifically addresses the needs of Small and Very Small establishment owners. The Agency employs other methods of reaching its stakeholders, including workshops, meetings, training sessions, press releases, constituent updates, and publications including many translated into Spanish.

FSIS also works closely with the National Advisory Committee on Meat and Poultry Inspection (NACMPI) and the National Advisory Committee on Microbiological Criteria in Foods (NACMCF) to address major Agency initiatives such as HACCP, outreach to Small and Very Small plants, Risk-Based Inspection, and other public health issues.

FSIS works closely with USDA in reaching out to Agency stakeholders in addition to maintaining a working relationship with many industry associations and States and local governments. FSIS also provides employees with up-to-date information through Agency publications and employee town hall meetings.

Through its public Web site, www.fsis.usda.gov, FSIS reaches a large, diverse, worldwide audience. Visitors to the Web site include consumers, educators, scientists and researchers, businesses, government partners, and owners/operators of small meat and poultry plants. Visitors to the public site may browse by audience as well as by subject. FSIS offers an email subscription service to notify subscribers when key pages they select have been updated; this allows members of each stakeholder group to quickly find content of greatest interest to them. The site offers two interactive question-and-answer tools, one geared toward consumers with food handling questions (Ask Karen) and the other is designed to answer technical and policy questions from a business audience. The Agency also offers a secure intranet, InsideFSIS, where FSIS employees can access career, training, work and home life balance, travel and commuting, and organizational information resources.

For FSIS' more robust risk-based inspection system to be successful, all plants must have well-designed, food-safety and defense systems, and the FSIS workforce must be well-trained to perform their public health duties. FSIS has a vital role in educating, as well as regulating, industry to improve public health and safety. FSIS provides outreach to Small and Very Small plants for more rapid and consistent delivery of key Agency services on emerging issues. The outreach services provide uniform responses to Small and Very Small plant questions, and supplies information to support risk based systems. FSIS collaborates with the Foreign Agricultural Service to provide technical assistance and education for foreign government officials to assist them in meeting U.S. inspection requirements. These efforts will promote an understanding of the scientific, technical, and regulatory information needed domestically and internationally by meat, poultry, and egg product plants to develop risk-based food safety and food defense systems fully capable of addressing existing and emerging threats to public health.

Outreach and Communication with Consumers: The Agency's goal is to protect public health through food safety. While the Agency makes every effort to ensure a safe product leaves meat and poultry plants, the Agency recognizes that the handling and cooking of meat play a role, too. As a result, the Agency continues to seek to educate consumers on food safety issues through a variety of means, both traditional and non-traditional, to affect positive behavior changes in order to reduce the risk of foodborne illness.

FSIS continues to expand education campaigns and distribute new publications. A highly successful Food Safety Education Conference, held in 2006 in Denver, Colorado, has laid a solid foundation that the Agency continues to build upon by working through partnerships to expand outreach to at-risk audiences.

FSIS also continues its outreach to the under-served population which includes: African Americans, Asian Americans, Native American Indians and Alaskan Natives, and the visually-impaired through events and Food Safety Message Cards: “What You Need to Know About Foodborne Illness”. These cards are prepared, distributed and posted on the FSIS Web site for African Americans, American Indians and Alaskan Natives, Asian Americans, Hispanics, and in various languages.

These programs continue to foster safe handling of meat, poultry, and processed egg products among the general public, those who face increased risks from foodborne illness: the very young, older adults, pregnant women, people with chronic diseases, and people with compromised immune systems, as well as underserved populations.

3. EVALUATION AND CONTROLS FRAMEWORK

INTRODUCTION

FSIS employs substantial, ongoing management assurances and controls to ensure the Agency is successful in fulfilling its public health mission. The Agency conducts well-designed, objective, and timely analyses, audits, and evaluations of Agency programs and activities to ensure the Agency is meeting its strategic goals and objectives; effectively and efficiently applying human capital and budgetary resources; and, preventing waste, fraud, abuse, and mismanagement. These actions assure the presence, integrity, and effectiveness of Agency management controls and systems to meet FSIS' public health objectives.

FSIS MANAGEMENT CONTROLS

FSIS continually seeks ways to better achieve its mission and program results and to improve accountability. A key factor in achieving such outcomes and minimizing operational problems is the implementation of appropriate and effective management controls. As programs change and the Agency strives to improve operational processes and implement new technological developments, management continually assesses and evaluates management controls to assure that they are effective and updated when necessary.

The Federal Managers' Financial Integrity Act of 1982 (FMFIA) requires the Government Accountability Office (GAO) to issue standards for management controls in the government. FSIS administers these standards and other regulations to provide the overall framework for establishing and maintaining management controls and for identifying and addressing major performance and management challenges and areas at greatest risk of fraud, waste, abuse, and mismanagement. Management controls are designed to provide ongoing feedback regarding the achievement of an organization's objectives. FSIS has sought to achieve management assurances by complying with the five standard interrelated components of an effective management control program, which are control environment, risk assessment, control activities, information and communication, and monitoring.

- The control environment is the control consciousness (e.g., values, ethics, accountability, etc.) of an organization -- the atmosphere in which people conduct their activities and carry out their control responsibilities. The control environment is greatly influenced by the extent to which individuals recognize that they will be held accountable.
- Risk assessment is the identification and analysis of risks associated with the achievement of operations, financial reporting, and compliance goals and objectives. This, in turn, forms a basis for determining how those risks should be managed.
- Control activities are actions, supported by policies and procedures that, when carried out properly and in a timely manner, manage or reduce risks. Examples include preventative measures such as written policies and procedures, limits to authority, or supporting documentation, and detective measures such as verifying charges in the general ledger to file copies of approved invoices.
- Information and communication are essential to effecting control. Information about an organization's plans, control environment, risks, control activities, and performance must be communicated up, down, and across an organization. Reliable and relevant information from both internal and external sources must be identified, captured, processed, and communicated to the people who need it, in a form and timeframe that is useful.

- Monitoring is the assessment of management control performance over time. It is accomplished by ongoing monitoring activities and by separate evaluations of internal control such as self-assessments, peer reviews, and internal audits. The purpose of monitoring is to determine whether internal control is adequately designed, properly executed, and effective.

Toward that end, FSIS has embedded management controls into its overall management process so that it may best achieve its strategic goals and objectives, and can assure the Agency maintains its course toward achievement of its food safety mission. FSIS has established a more fully documented management control program that ensures meeting our strategic goals and objectives. With fully documented management controls, legal authority, individual responsibility, and programmatic accountability are more clearly defined and delegated. In addition, program performance is routinely analyzed, policies and procedures are regularly updated, management decisions are transparent and traceable, documentation is accurately maintained, and supervision is appropriate and continuous.

To facilitate successful implementation across the Agency, each program area within FSIS has a liaison for management controls. The management control liaisons assist in the development of management control policy and procedures; assist program managers in identifying and implementing management control processes and performance measures; assist in scheduling and planning management control audits; communicate management control-related guidance and directions to the programs; coordinate, compile and submit program reporting requirements; and, assist with educating and instructing employees on management control principles. This preventative approach assures an effective and efficient organization.

Agency leadership is ultimately responsible for improving the accountability and effectiveness of programs and operations by establishing, assessing, correcting, and reporting on management control. They accomplish this by taking systematic and proactive measures to:

- develop and implement appropriate, cost-effective management controls for results-oriented management;
- assess the adequacy of management control in programs and operations;
- separately assess and document management controls;
- identify needed improvements;
- take corresponding corrective action; and
- report annually on management controls through management assurance statements.

AssuranceNet: AssuranceNet is a web-based application that transforms near real-time performance data into valuable decision-making information for managers. It currently extrapolates information from FSIS inspection and import databases plus employee responses to key questions in these areas, as well as administrative duties, to perform complex calculations that graphically display inspection performance indicators in a standard and traceable manner.

INVESTIGATIONS

FSIS has established investigative functions for operational and personnel performance issues to assure that the Agency's activities achieve the highest operational, ethical and managerial standards. When operational or performance deviations occur, FSIS responds quickly to ascertain the root causes. FSIS often investigates whether its systems and process controls maintain program integrity and authority, and whether it administers its programs in the most effective, efficient and economical manner possible.

Program investigations sometimes look at how Agency management conducts its responsibilities. This is based on the concept that to properly administer a program, management must provide the

organization, policy guidance, planning, supervision and controls necessary to assure that its programs stay on track and progress.

Lastly, maintaining the public's trust is critical to FSIS improving food safety and food defense of meat, poultry, and processed egg products. FSIS conducts thorough and timely employee investigations in accordance with the Whistleblower Protection Act of 1989, the Inspector General Act of 1978, and the Inspector General Act Amendments of 1988. These investigations ensure transparency in Agency operations and maintain the public's trust.

PROGRAM EVALUATION

Improving FSIS programs and policies is critical in the effort to increase the safety and security of meat, poultry, and processed egg products. Improvements that make FSIS activities more effective and/or more efficient improve public health by reducing morbidity and mortality from the consumption of adulterated food products, and improve public welfare by reducing the mislabeling and misbranding of food products.

Evaluation is a formal, structured process of gathering and analyzing objective information needed to assess a program or policy. Evaluations provide practical information to assist decision makers who implement programs and policies, document program success or show how to improve efforts, and supply information to enhance the credibility of programs. Thus, evaluation indirectly improves public health and welfare.

FSIS has a dedicated evaluation staff whose work is an important component of the FSIS management assurance program. FSIS annually performs numerous evaluations, analytical reports, and other types of data analyses at the request of the FSIS Management Council or specific Agency offices. These evaluations focus on three areas:

- ensuring that programs are effective at improving public health by ensuring a safe and secure food supply;
- ensuring that Agency resources are used consistently with and efficiently towards accomplishing the Agency's public health mission; and
- improving accountability through the collection, maintenance, and use of timely information.

PROGRAM AUDIT

The concept of accountability for public resources is key in FSIS' processes. Stakeholders want to know whether Agency services are being provided efficiently, effectively, and in compliance with laws and regulations; and whether FSIS programs are achieving their objectives and desired outcomes, and at what cost. Therefore, the Agency conducts audits in order to provide stakeholders with confidence in the information that FSIS reports on regarding the results of programs or operations, as well as in the related systems of management controls. Audits ultimately lead to improving decisions, oversight, accountability, and management. Thus, auditing is a key element of FSIS management assurance and controls for fulfilling the Agency's Strategic Plans, and its duty to be accountable to the public.

FSIS conducts program audits to provide managers with information intended to enhance their programs. The audits include a methodical examination, review, and verification of a specific program element, activity, or event. The process involves a review of program background and supporting documentation, and a thorough check and analysis of the program's activities. Upon completing the audit, a written audit report is developed that addresses the findings and recommendations made for enhancing the program.

Program audits are an important feature of FSIS' management assurance program. FSIS formulates audit plans and conducts audits of existing programs, program components, and program activities. These audits focus on ensuring that: programs are effective; the food supply is safe and secure; resources are being used in the most efficient manner to accomplish the Agency's mission and objectives; and, reliable and timely information is obtained, reported, and used for Agency decision making. These audits provide the Agency with continued opportunity to ensure effective program operations. Examples of audit activities conducted in support of the Strategic Plan include: administrative audits; management control audits; compliance, investigation, and enforcement audits; and recovery audits.

Administrative audits are reviews against standards to determine whether Agency management discharges or conducts its responsibilities in accordance with established requirements. These audits are designed to ensure consistency, effectiveness, and efficiency in programs and activities. If FSIS administrative audits identify potential areas of inconsistency or other results, the Agency takes corrective action to ensure program efficiency and effectiveness. Follow-up audits are then conducted to ensure that programs have implemented and completed appropriate corrective action plans and corrective actions are completed in a timely manner.

Management control audits, which fulfill the monitoring function of the FSIS Management Control Program, are systematic audits of each individual program area's and/or division's management control system. The audits help to ensure authorities, responsibilities, and accountabilities are clearly defined and the program functions are effectively implemented. In general, management control audits have three objectives:

- to verify the development and implementation of the program's Management Control System;
- to test the effectiveness of the program's management controls in achieving program objectives; and
- to verify the establishment and attainment of each program's performance measures.

FSIS also conducts program audits of compliance, investigation, and enforcement activities. These audits are designed to ensure consistency and effectiveness in compliance, investigation, and enforcement activities; effective use of resources; consistency and effectiveness in the application of FSIS enforcement authorities and sanctions to punish violators; and adherence to Agency operating protocols.

Finally, FSIS conducts recovery audits. The PMA includes an initiative to reduce erroneous payments made by Federal government agencies that enter into contracts in excess of $500 million per fiscal year. Departments, such as USDA, that meet this requirement must implement a recovery audit program for amounts potentially paid erroneously to contractors. USDA implements this requirement at the mission area/Agency level. Therefore FSIS, as a part of USDA, conducts audits for contracts that are deemed to have potential recovery cost savings benefits.

Civil Rights Compliance Assistance, Review and Evaluation (CARE) Program:

In accordance with 29 Code of Federal Regulation Part 1614 and the Equal Employment Opportunity Commission (EEOC) Management Directive 715 (MD-715), the Agency performs routine audits to evaluate the Agency's compliance with the requirements of Federal equal employment opportunity (EEO) laws, regulations, and program guidance. The Compliance Assistance, Review and Evaluation (CARE) Program is one of two primary tools used to assess the Agency's performance with respect to its obligations under Title VI, Title VII, and Rehabilitation Act programs and to evaluate FSIS' responsiveness and legal compliance. The other primary means of measuring the Agency's performance is the annual self-assessment required by EEOC MD-715 and reported to EEOC through the Department as part of the Agency's Annual Equal Employment Opportunity Program Status Report (EEOC Form 715-01). The Agency continues to focus on making both these assessment and evaluation processes increasingly more effective and efficient in the timely and responsive delivery of performance management data and analyses to FSIS key human capital decision makes. Through

continual process improvements in these compliance management processes the Agency's capacity to take proactive, preventive measures that reduce or mitigate the opportunities for unlawful employment discrimination continues to grow and improve.

DOMESTIC, STATE, AND FOREIGN AUDITS

The Agency has standard policy and procedures for auditing domestic, State, and Foreign programs. In addition to these standard audits, FSIS, on occasion, conducts systematic and special audits to assess targeted program operations.

FSIS conducts audits of domestic inspection programs to ensure the application of inspection procedures and protocols in federally inspected meat, poultry, and processed egg products establishments in the United States. These audits are conducted to closely examine the food safety systems established and maintained by establishments, and to determine if inspection requirements are being uniformly applied by FSIS employees nationwide.

FSIS also conducts comprehensive fiscal reviews of State inspection programs, and conducts periodic onsite fiscal reviews of each State's cooperative Meat, Poultry and Inspection (MPI) program on a triennial basis. The purpose of the fiscal review is for the Agency to determine the "at least equal to" status of the existing State Agencies (SA) inspection programs. Under Federal regulations, States operating meat and/or poultry inspection programs are required to operate these programs in a manner that is "at least equal to" the Federal program. FSIS has developed a comprehensive protocol to conduct comprehensive onsite fiscal reviews of the overall operations conducted by these state programs in order to verify that their programs and laws are at least equal to those applied by FSIS. In addition, the onsite review assures that costs claimed by the SA are allowable and properly accounted and that appropriate internal control mechanisms comply with guidelines.

FSIS also conducts audits of the meat, poultry, and processed egg products inspection systems of foreign countries that have been evaluated as having an inspection system in place that is "equivalent" to the U.S. system. These comprehensive audits cover all aspects of the inspection system, including staffing, funding of inspectors, government oversight, and laboratory procedures. These audits, in conjunction port-of-entry inspections, provide information that FSIS uses to assess the country's ability to maintain an equivalent inspection system that assures that products destined for consumption by the U.S. public are safe. As part of the foreign inspection system audits, FSIS auditors convey information regarding food defense and transportation security. During each audit, FSIS incorporates a "focus" area and requires the country to show what measures have been implemented to address both food defense at the inspected establishments and transportation security of the products between the inspected establishments and the ports from which the products are shipped to the United States.

EXTERNAL OVERSIGHT

Agency management assurances and controls also include external audits and evaluations of Agency programs by independent bodies. The General Accounting Office (GAO) and the USDA Office of the Inspector General (OIG) both provide external, independent oversight to ensure that effective management controls are in place in all program, financial, and administrative activities. Through evaluations and audits of Federal programs and expenditures, they advise executive agencies about ways to make government more effective and responsive in order to improve performance, reduce program vulnerabilities, enhance program integrity, increase efficiency and effectiveness, and ensure the accountability of the Federal government. In addition, these activities ensure that appropriate corrective action is taken to resolve program weaknesses or deficient areas.

While evaluations and audits are being conducted, FSIS works closely with GAO and OIG program officials to coordinate oversight activities and provide logistical support. Upon completion of these

activities, FSIS uses these reports and associated findings and recommendations generated by GAO and OIG to identify both program strengths and potential areas for improvement. FSIS also uses these audit reports and recommendations to analyze risks, if any, associated with the achievement of FSIS operations, in order to determine whether management control enhancements would be effective in managing these risks.

Upon completion of a GAO and OIG evaluation and/or audit, FSIS is presented with a comprehensive report which details the GAO and OIG general understanding of the program being reviewed, findings of weaknesses or deficient areas within that program, and recommendations for corrective actions and/or management controls. FSIS provides a response detailing any inaccuracies or disputed findings and information on improvements/management controls that have either already been initiated or management is committing to initiate.

In responding to findings of weaknesses or deficient areas detailed in these reports, FSIS analyzes the findings, and determines appropriate control actions that can be implemented to mitigate risks. FSIS also prepares periodic status reports, monitors activities, and analyzes the program's intended and corrective actions in relation to previously agreed-upon management commitments to assess management control performance over time and determine whether management controls are adequately designed properly executed, and effective.

DATA ASSESSMENT AND ANALYSIS

FSIS has strengthened both its data collection and analysis activities to ensure valid, timely data is collected, carefully analyzed, and continually reported in a user-friendly manner. FSIS also employs assessment and analysis of public health data to ensure that the Agency is meeting its strategic goals and objectives. An effective gauge of how FSIS policies are working is looking at how public health is impacted. Analysis of data obtained from FSIS' regulatory verification activities, compliance and enforcement activities, sampling, as well as other sources of data, over time, provide the Agency with evidence that shows whether or not our approach is working. FSIS therefore employs data analysis as a management verification measure in determining the success of our strategies to combat threats to food safety and defense and to help ensure that program components are effective in meeting our public health goals and objectives.

In addition to updating and upgrading its data processing systems, two new groups have been formed in the Agency to ensure that it is analyzing its data in a coordinated and efficient manner. The two groups are the Data Analysis and Integration Group (DAIG) and the Data Coordination Committee (DCC). The DAIG consists of a staff dedicated to working with all program areas on data analysis issues to ensure data analyses are consistent and of high-quality; ensure data analyses are relevant to program offices' business processes and the Agency mission; provide assistance in data analysis; and provide a new level of sophistication for data analysis. The DCC is comprised of senior level staff from each of FSIS' program areas who coordinate data-related activities within the Agency and who act as liaisons between the DAIG and their program areas.

The DAIG has developed information sheets to describe the data streams within the Agency. The sheets provide detailed information on the data streams, including how the information is collected, its limitations, the reports generated from the data, and the audience for dissemination of those reports. The DAIG has also developed a summary table – the FSIS Data Analysis and Reports Project Matrix – of all data analysis and reports that are being conducted by the Agency. This documentation of the Agency's data and the analysis and reports being conducted or developed by the Agency provide a clearer picture of what data are available and what is currently being done with the data to avoid redundancies.

Using data analysis as a verification tool, the Agency is able to analyze trends and conclude whether improvements in regulatory oversight and training have been effective. This data analysis also will serve as a verification of whether our Agency's strong, science-based policies aimed at reducing pathogens in America's meat, poultry, and processed egg products are effective in helping the Agency

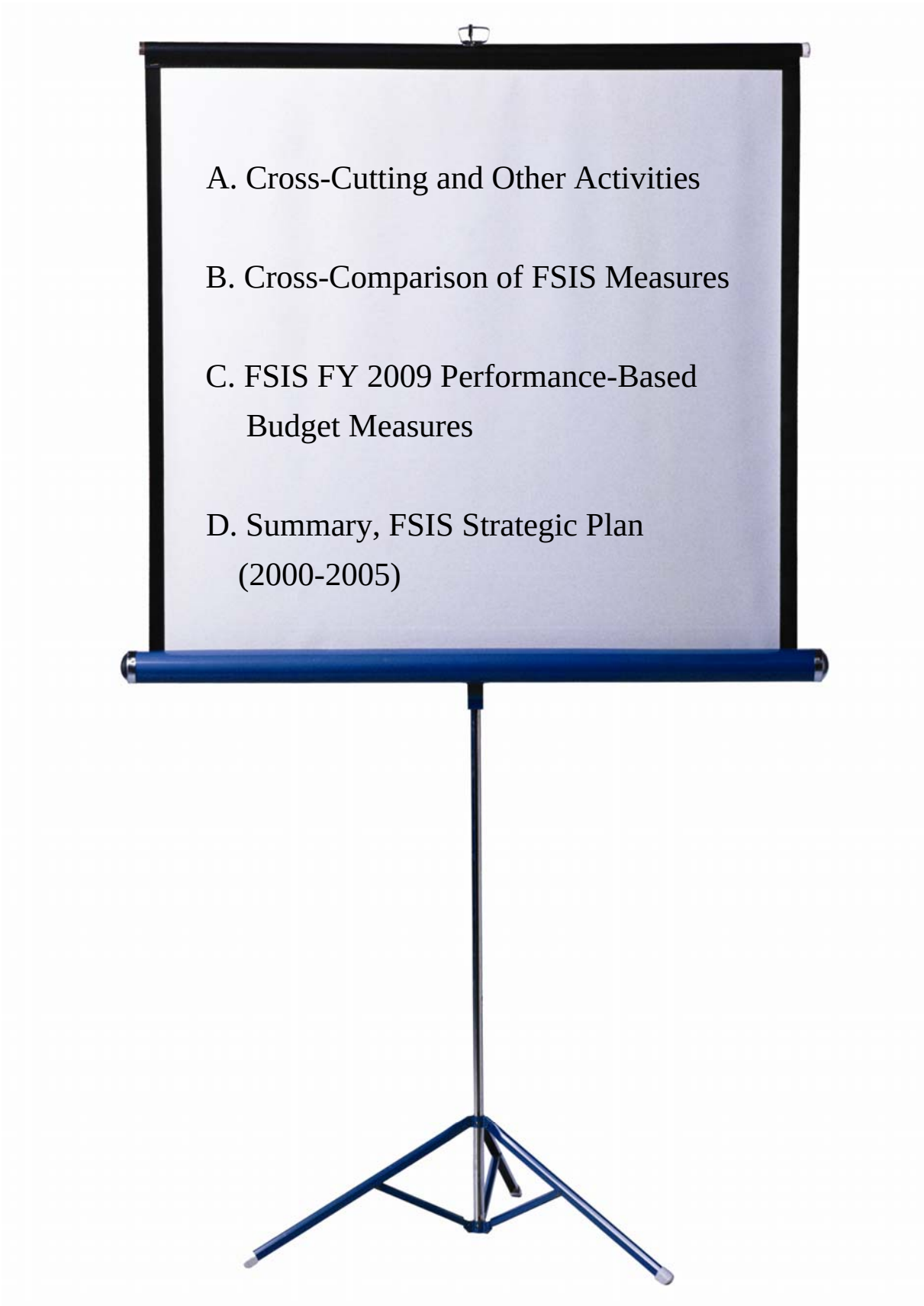
meet its public health goals. As a beginning to this effort, the Agency has developed and launched Phase 1 of its Enterprise Reporting System to replace each inspection, import/export and selected lab systems' separate readers to provide a more holistic view of the Agency's data for analysis.

FSIS uses regulatory verification, compliance, enforcement, sampling, and other data and information to discern trends, causes, and outcomes to validate that the Agency's inspection, verification, enforcement, compliance, and stakeholder outreach are effective in protecting the public health. Data to be considered can include: analysis of product recalls, detentions and seizures; analysis of compliance data such as the PBIS database of in-plant inspection activities and compliance rates; CDC FoodNet data; FSIS' regulatory sampling data for foodborne pathogens such as *E. coli* O157:H7 and *Salmonella*; and enforcement actions such as suspensions, in-plant Notices of Intended Enforcement (NOIEs), prosecutions, and administrative enforcement actions. Analysis of this data aids FSIS in gauging how effective we have been in meeting our strategic goals of establishing public health systems and policies that ensure products are safe and secure, and in protecting the health and welfare of consumers.

SUMMARY

Through application of this system of management controls, FSIS ensures the Agency is effective and efficient in fulfilling its public health mission. The wide variety of sound, objective, and critical analyses, audits, and evaluations of FSIS programs, conducted by Agency officials and outside authorities such as GAO and OIG ensure FSIS is: meeting its strategic goals and objectives; effectively and efficiently applying human capital and budgetary resources; and, preventing waste, fraud, abuse, and mismanagement. These on-going activities assure the effectiveness of Agency management controls and systems to ensure the public health through food safety and defense.

Addendum


A. Cross-Cutting and Other Activities
B. Cross-Comparison of FSIS Measures
C. FSIS FY 2009 Performance-Based Budget Measures
D. Summary, FSIS Strategic Plan (2000-2005)

Addendum A

CROSS-CUTTING AND OTHER ACTIVITIES

FSIS is involved in a variety of forward thinking intergovernmental activities involving Federal, State and local beneficiaries. A sampling of these activities are:

- Food Safety Risk Assessment Committee;
- Food Emergency Response Network (FERN);
- Association of Food and Drug Officials (AFDO) Partnerships; and
- Codex Alimentarius Commission.

The vision for future intergovernmental activities is to include a variety of outreach programs that coordinate with other agencies on common goals to be prepared for any food safety and food defense issues.

Risk Assessment Committee

USDA established a Food Safety Risk Assessment Committee in July 2003 to enhance coordination and communication among various USDA agencies in planning and conducting activities related to risk assessments. The committee has representatives from FSIS, Agricultural Marketing Service, Agricultural Research Service, Animal and Plant Health Inspection Service, Cooperative State Research, Education and Extension Service, Economic Research Service, Food and Nutrition Service, and the Office of Risk Assessment and Cost Benefit Analysis. (This is an office in the Department's Office of the Chief Economist. See Web site: http://www.riskworld.com/websites/webfiles/ws5aa011.htm)

The risk assessment committee combines the expertise of USDA agencies to build a solid scientific basis on which to base regulatory and policy decisions. The committee:

- Prioritizes risk assessments, identify research needs and identify needs for modeling techniques, methods or data;
- Provides guidance related to carrying out risk assessments, including addressing issues such as data quality and peer review; and
- Identifies outside experts and/or universities to assist in the development of risk assessments.

Food Emergency Response Network

FERN consists of Federal, State, and local governmental laboratories responsible for protecting citizens and the American food supply from intentional biological, chemical, and radiological terrorism. The goal of the FERN is to (1) have a robust food testing laboratory network with the surge capacity capable of collecting data in order to respond to an event involving the intentional or accidental contamination of the food supply or even a hoax, (2) maintain U.S. agricultural and industrial economic stability by rapid identification if an event occurs, and (3) ensure/restore consumer confidence in the safety of the Nation's food supply by the rapid response of the Network.

While FSIS' initial goal was to have 100 FERN laboratories participate in FSIS Microbiology Cooperative Agreement Program, the Agency developed plans in FY 2006 towards restructuring FERN. In this new approach, FSIS will limit labs participating in the Microbiology Cooperative Agreement Program to a total of 25 FERN labs that will provide full U.S. geographic representation for microbiological testing. Currently, 21 labs have been designated FERN labs, for USDA purposes.

The 25 FERN labs will provide National coverage, by region, with the expertise needed to meet the overall mission of FERN. All 25 labs will be capable of providing screening microbial tests and results for the ten priority threat agents in all food matrices. Approximately 15 of these 25 labs nationwide will be funded as Regional Reference Labs. In addition to the screening capacity, these Regional Reference Labs will also serve as technical transfer labs, sharing knowledge and expertise. If necessary, these labs will conduct specific projects, as needed. All 25 FERN labs will be funded to participate in screening projects, method validation studies, and field trials of new methods for other threat agents. Once fully funded, the public health infrastructure will be far better prepared to respond to a contaminated food supply, and will benefit the physical and financial health of the Nation.

The Food and Drug Administration (FDA) is a federal partner in FERN. FDA staffs a National Program Office that administers the following support programs for FERN: (1) houses and technically supports eLEXNET (FERN laboratory data capture program); (2) funds and administers a Cooperative Agreement Program to eight FERN Chemistry and five Radiological laboratories; (3) administers the FERN Proficiency Testing Program through the Center for Food Safety & Nutrition (CFSAN); (4) provides training support to the FERN Microbiological, Chemistry and Radiology training sessions through the Office of Regulatory Affairs' University (ORA U); and (5) staffs the five Regional Coordination Centers with staff officers to provide outreach and coordination to all of the FERN federal, state and local partner laboratories within the five FERN regions.

Association of Food and Drug Officials

Association of Food and Drug Officials (AFDO) is the association through which state and local food safety officials work together to address common problems. In cooperation with USDA, AFDO developed a HACCP-based course of instruction on meat and poultry processing at retail. Another partnership with AFDO resulted in guidelines for effective state regulation of slaughter facilities exempt from mandatory inspection under Federal law. A third partnership is for coordinating state and local governments in a national effort to link government food laboratories at all levels of government into a national network. FSIS has worked with AFDO to coordinate annual meetings of Federal, state, and local food laboratory directors and promoted uniformity and reliability among state and local labs in food analyses and information sharing. The June 2003, meeting of the nation's state laboratory directors for the first time included environmental labs, veterinary diagnostic labs, and clinical labs as well as food labs, in recognition of the fact that many laboratory communities may need to work together under many food safety and food defense scenarios.

FSIS Virtual Representative —"Ask Karen"

"Ask Karen," the Agency's virtual representative (vRep) has been asked many thousands of questions from customers world-wide. This Web-based, automated response system is available 24 hours a day, seven days a week. "Ask Karen" responds to inquiries from the public about the safe handling, preparation and storage of meat, poultry, and processed egg products from an extensive database of food safety information. This innovative communication tool is another means of supporting the Agency's public health focus and educating consumers, one-on-one, about safe food handling behaviors in order to reduce the risk of foodborne illness.

USDA's Meat and Poultry Hotline

FSIS handles tens of thousands of telephone hotline calls. Calls include requests from newspapers, magazines, radio, television, and book authors for live interviews with radio and television stations. The Hotline also serves Spanish-speaking callers. Additionally, with increased promotion of the use of the Hotline e-mail address, consumers are now assisted through e-mail.

Codex Alimentarius Commission

The U.S. Codex Office coordinates all U.S. government and non-government participation in the Codex Alimentarius Commission, which sets safety standards for all foods in international trade. U.S. Codex hosts numerous international committee meetings, represents the U.S. at major international meetings, and participates in results oriented training to facilitate international safe handling processes. The U.S. Codex office provides leadership for world-wide outreach to meet a broad range of stakeholder goals, sharing common approaches and building strategic partnerships for the long-term success of U.S. policy. The U.S. Codex office hosts the U.S. Codex Policy Steering Committee, chaired by the Under Secretary of Food Safety, which provides overall strategic and tactical guidance for these efforts.

Addendum B

CROSS-COMPARISON OF FSIS CORPORATE PERFORMANCE MEASURES

USDA Strategic Goal 4:

Enhance Protection and Safety of the Nation's Agriculture and Food Supply.

USDA Objective 4.1:

Reduce the Incidence of Foodborne Illnesses Related to Meat, Poultry, and Egg Products in the United States.

USDA-OCFO Departmental Strategic Plan (2005-2010) Performance Measures	USDA-OBPA FY 2008 Annual Performance Plan/Report Measures	FSIS Performance-Budget Measures from FY 2009 Explanatory Notes
4.1.1 Reduce overall public exposure to generic *Salmonella* from broiler carcasses using existing scientific standards.	4.1.1 Reduce overall public exposure to generic *Salmonella* from broiler carcasses using existing scientific standards.	Reduce overall public exposure to generic *Salmonella* from broiler carcasses using existing scientific standards.
4.1.2 Decrease the overall-percent-positive rate for *Listeria monocytogenes* in ready-to-eat products through the use of Food Safety Assessments.	4.1.2 Decrease the overall-percent-positive rate for *Listeria monocytogenes* in ready-to-eat products through the use of Food Safety Assessments.	Decrease the overall-percent-positive rate for *Listeria monocytogenes* in ready-to-eat products through the use of Food Safety Assessments.
4.1.3 Reduce the overall public exposure of *E. coli* O157:H7 in ground beef.	4.1.3 Reduce the overall public exposure of *E. coli* O157:H7 in ground beef.	Reduce the overall public exposure of *E. coli* O157:H7 in ground beef.

Addendum C

FOOD SAFETY AND INSPECTION SERVICE

2009 Explanatory Notes

SUMMARY OF BUDGET PERFORMANCE

Key Performance Outcomes and Measures

Agency Mission: Protect consumers by ensuring that meat, poultry, and egg products are safe, secure, wholesome and correctly labeled and packaged.

Key Outcomes: Reduction in foodborne illness associated with the consumption of meat, poultry, and egg products. FSIS' key outcome restates USDA's Strategic Objective 4.1: *Reduce the incidence of foodborne illnesses related to meat, poultry, and egg products in the U.S.*

Enhance International Competitiveness of American Agriculture through coordination of all U.S. government and non-government participation in the sanitary and phytosanitary standards-setting activities of the Codex Alimentarius Commission. This key outcome relates to USDA's Strategic Objective 1.3: *Improve sanitary and phytosanitary (SPS) system to facilitate agricultural trade.*

Key Performance Measures: The continued mission of FSIS is to protect consumers by ensuring that the commercial supply of meat, poultry, and egg products are safe, secure, wholesome and correctly labeled and packaged.

FSIS agency goals embody USDA's Strategic Goal 4: *Enhance Protection and Safety of the Nation's Agriculture and Food Supply*, and specifically Objective 4.1 – *Reduce the Incidence of Foodborne Illnesses Related to Meat, Poultry, and Egg Products in the U.S.*

FSIS programs also contribute to USDA Strategic Goal 1: *Enhance International Competitiveness of American Agriculture*. FSIS contributes to USDA Objective 1.3 *Improved Sanitary and Phytosanitary (SPS) System to Facilitate Agricultural Trade*. In addition to FSIS' unique work with the Codex Alimentarius committees, FSIS houses the U.S. Codex Alimentarius office, whose principal purpose is the setting of international sanitary and phytosanitary standards.

FSIS' FY 2009 budget request is targeted at these core food safety strategies:

- Base program decisions and policy development on science;
- Apply the public health and technical skills of our workforce to foodborne hazards;
- Defend the food supply from intentional contamination;
- Manage the inspection program effectively and economically; and
- Continue effective public health outreach and education.

The FSIS FY 2009 budget request includes initiatives to build up the infrastructure of its public health information system, including efforts to enhance the electronic exchange of export-import data; to prepare for future risk-based inspection; to defend the security of the food supply; to manage its human capital wisely; and to promote consumer protection standards at home and in the world arena.

Key Performance Targets:

	2004 actual	2005 actual	2006 actual	2007 actual	2008 target	2009 target
Pathogen Reduction						
Reduce overall public exposure to generic *Salmonella* from broiler carcasses using existing scientific standards*	n/a	n/a	45%*	71%*	80%*	85%*
Decrease the overall percent positive rate for *Listeria monocytogenes* in ready-to-eat products through the use of Food Safety Assessments	0.89%	0.70%	0.60%	0.31%	0.29%	0.28%
Reduce the prevalence of *E. coli* O157:H7 on ground beef	0.19%	0.17%	0.16%	0.23%	0.24%	0.20%
Pathogen Reduction Costs ($000)	785,557	815,064	837,756	892,136	930,120	951,946

* Prior to June 2006, FSIS reported the percent-positive findings of *Salmonella* on raw product tested, similar to the measurement of *Listeria monocytogenes* (*Lm*) and *E. coli* O157:H7. However, as of June 2006, FSIS no longer compares the percent positives from one year to the next due to a change in how the establishments are selected for testing. FSIS is now employing a "category" system to measure establishments' performance. FSIS compares how many establishments are in "Category 1" from one quarter to the next and from one year to the next. Category 1 represents establishments that have achieved 50 percent or less of the performance standard or baseline guidance, for two consecutive FSIS test sets. Category 2 represents establishments that have achieved greater than 50 percent on at least one of the two most recent FSIS test sets without exceeding the performance standard or baseline guidance. Category 3 represents establishments that have exceeded the performance standard or baseline guidance on either or both of the two more recent FSIS test sets. For example, for broiler slaughter establishments, the performance standard is constructed such that the standard is met if there are 13 or fewer positive samples in 51 daily tests. Consequently, a Category 1 establishment would have six or fewer positive results in the two most recent 51 sample sets.

As more establishments reach Category 1 status, fewer people will be exposed to *Salmonella* from raw classes of product regulated by FSIS. Consequently, as more establishments gain greater control of this pathogen, the likelihood of achieving the Healthy People 2010 goal of halving the number of people per 100,000 becoming infected with *Salmonella* from all food sources, including meat and poultry products, is more likely to result. FSIS set a goal of having 90 percent of establishments achieve Category 1 status by 2010. By then, FSIS will have completed one or more new baseline studies. The results of these new baselines would be to establish new performance standards or baseline guidance and to re-set Category 1, Category 2, and Category 3 criteria.

Addendum D

SUMMARY, FSIS STRATEGIC PLAN (2000 – 2005)

This is a summary of the Agency's previous official plan. This document can be found on the FSIS Homepage.

Mission

FSIS ensures that the Nation's commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged, as required by the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act.

Strategic Goal

Protect the public health by significantly reducing the prevalence of foodborne hazards from meat, poultry, and egg products.

The goal reflects the Agency's public health responsibilities embodied in its Mission Statement and required by its legislative mandates.

The **outcome** of this goal is a further reduction of 25 percent in the number of foodborne illnesses associated with meat, poultry, and egg products by the year 2005, using a baseline year of 1997. The CDC baseline numbers for foodborne illnesses and deaths attributable to all foods are estimated to be 76 million and 5,000 respectively.

Objectives

1. Provide worldwide leadership towards the creation and utilization of risk assessment capacity for meat, poultry, and egg products that is supported by the latest research and technology.
2. Create a coordinated national and international food safety risk management system for meat, poultry, and egg products from farm-to-table.
3. Conduct a comprehensive national and international risk communication program that is an open exchange of information and opinion about risk among risk assessors, risk managers, and the public to reduce risk.
4. Create and maintain an FSIS infrastructure to support Risk Assessment, Risk Management, and Risk Communication objectives.

FSIS

FY 2008-2013

Update on the Epidemiology of Shiga toxin-producing *E. coli* in the United States

September 14, 2009
Capital Area Food Protection Association Meeting

L. Hannah Gould, MS, PhD
Enteric Diseases Epidemiology Branch
Division of Foodborne, Bacterial, and Mycotic Diseases
Centers for Disease Control and Prevention

Outline

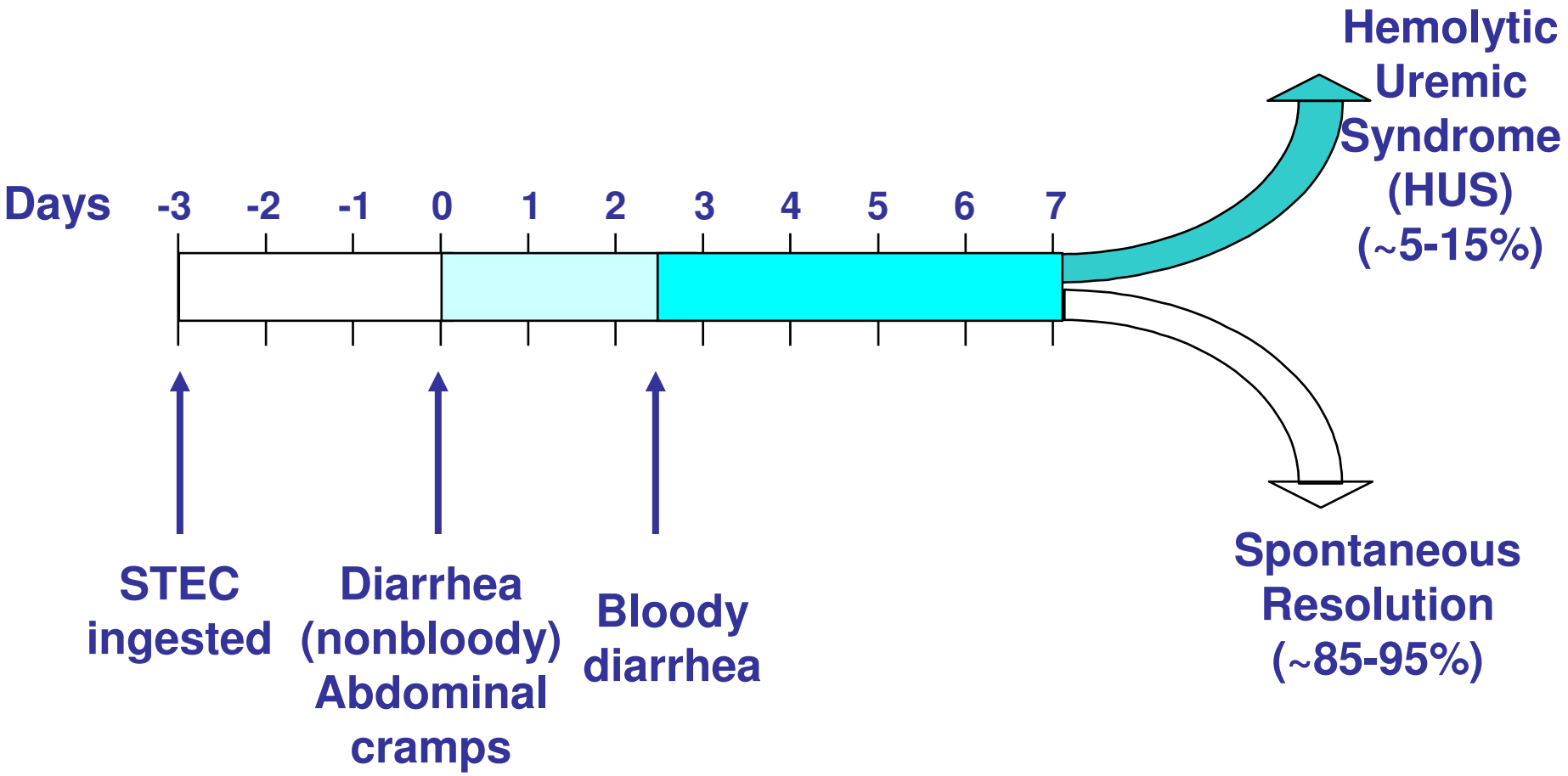
- Background
- STEC Surveillance Data
  - O157
  - Non-O157
- Outbreaks
  - O157
  - Non-O157
- Conclusions

Estimates of annual number of Shiga toxin-producing *E. coli* (STEC) infections, United States

- *E. coli* O157
  - 73,000 illnesses
  - 2200 hospitalizations
  - 61 deaths
- Non-O157 STEC
  - 36,700 illnesses
  - 1100 hospitalizations
  - 30 deaths
- Estimates used as baseline for 2010 targets
  - 50% reduction in specific diseases

Mead et al., 1999

Sequence of events in STEC infection

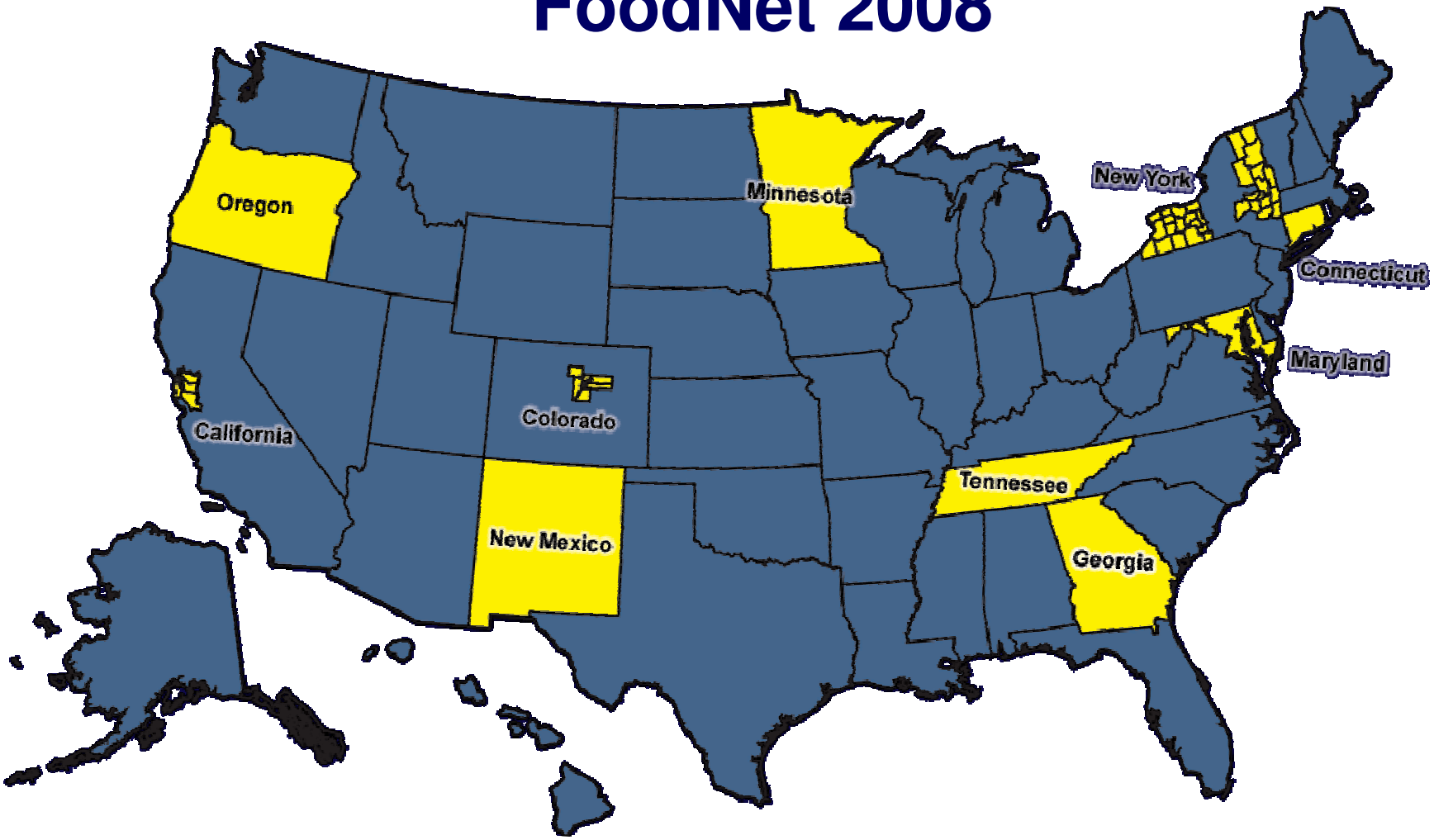


STEC Surveillance

Foodborne Diseases Active Surveillance Network (FoodNet)

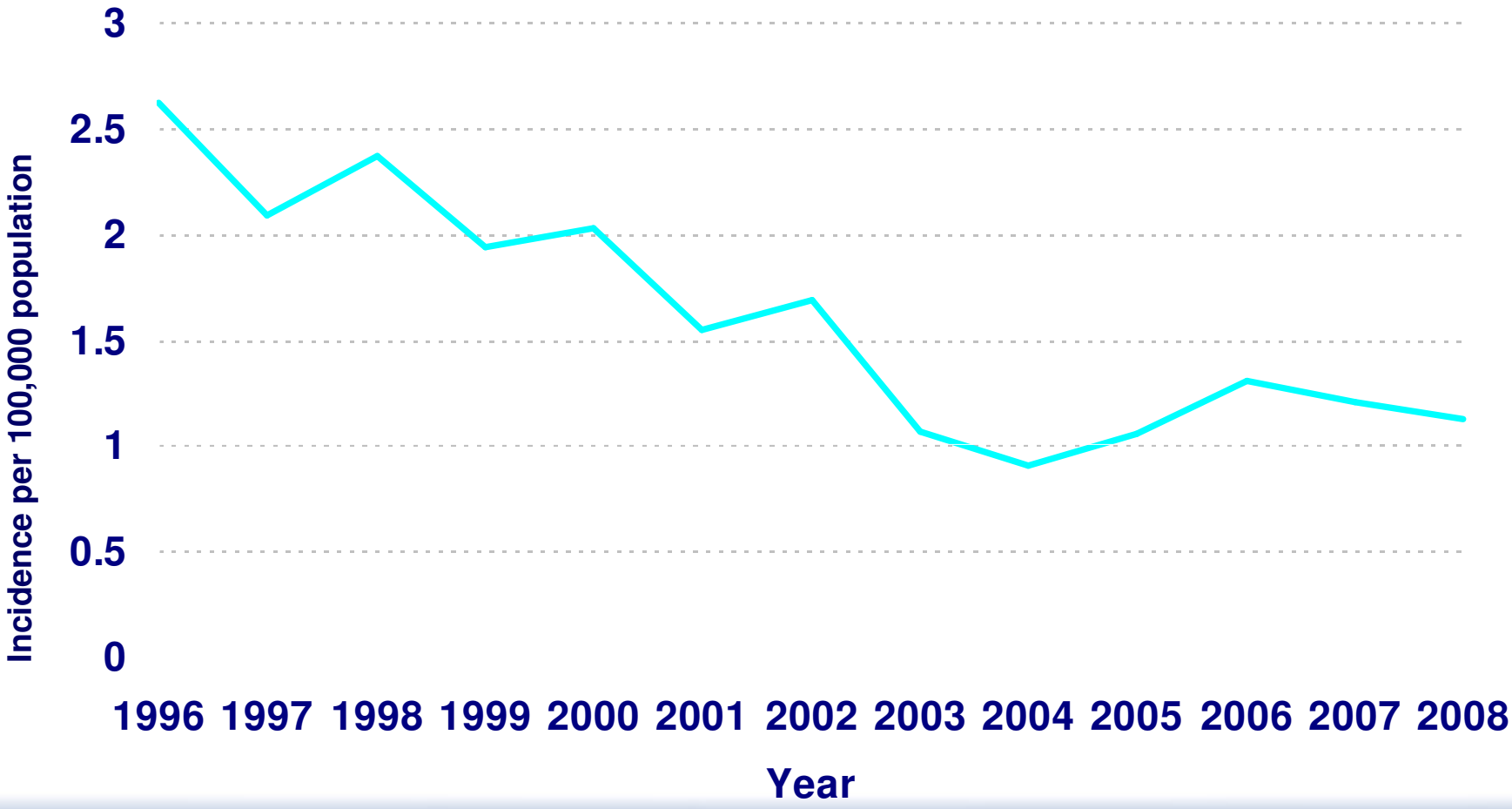
- Sentinel site surveillance system for foodborne diseases
  - Population-based active surveillance
  - 9 bacterial pathogens, hemolytic uremic syndrome
  - Best data on US burden of illness, trends
  - Special studies to determine risk factors
- 10 state health departments, CDC/USDA/FDA
- Annual report card for food safety

FoodNet 2008

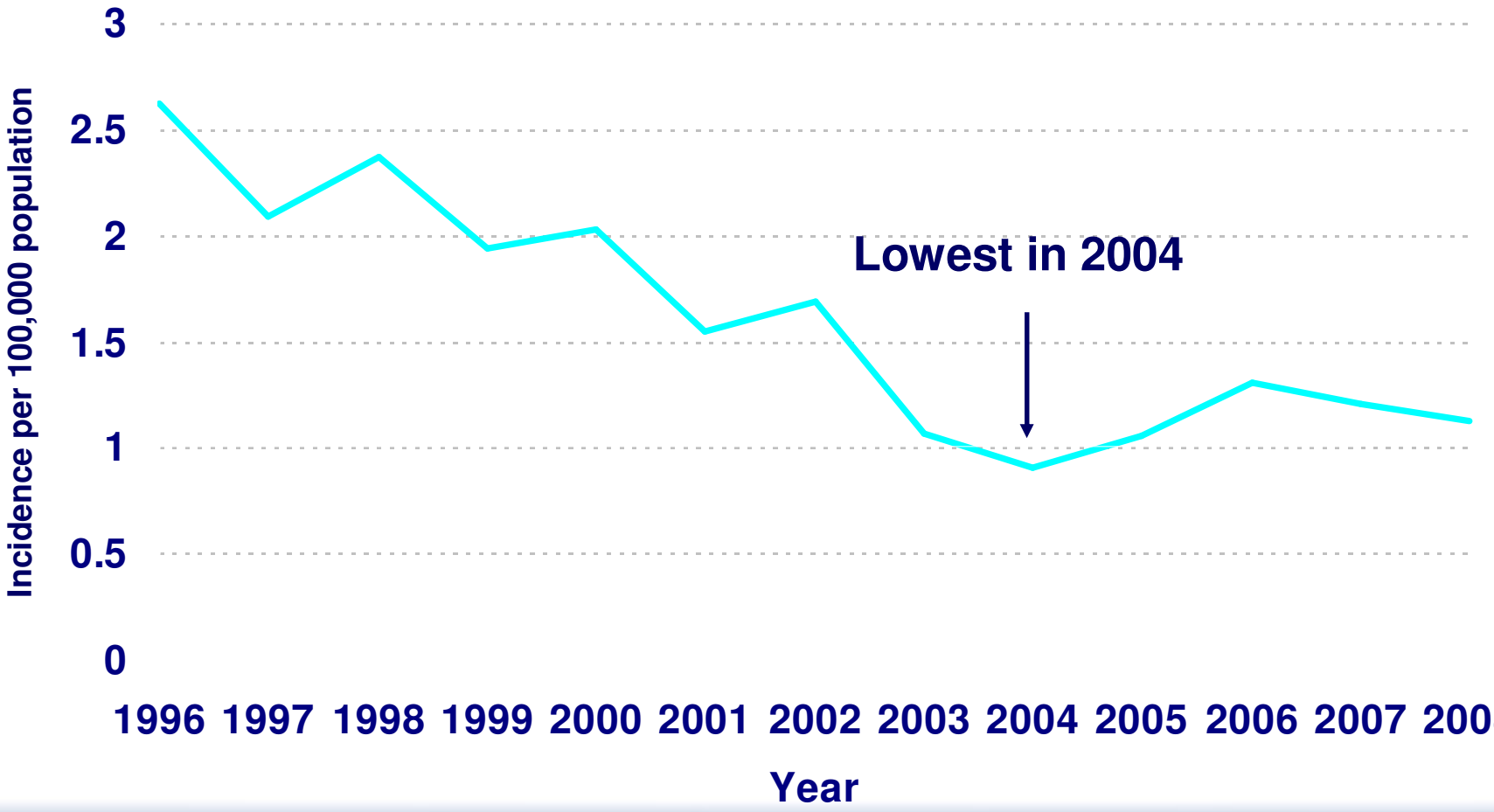


46 million persons—15% of U.S. population

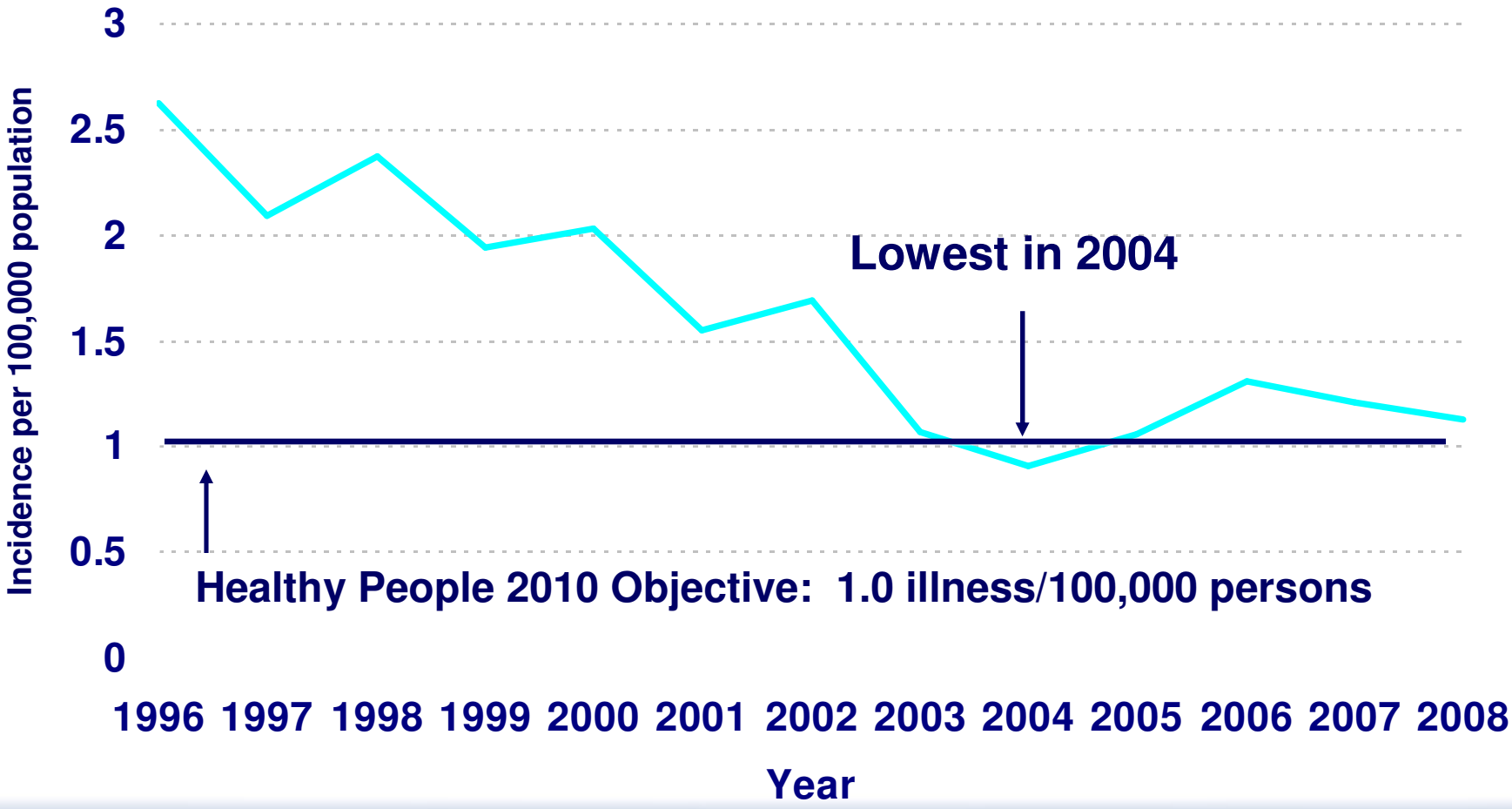
Incidence of O157 STEC infections, FoodNet,1996-2008



Incidence of O157 STEC infections, FoodNet,1996-2008



Incidence of O157 STEC infections, FoodNet,1996-2008

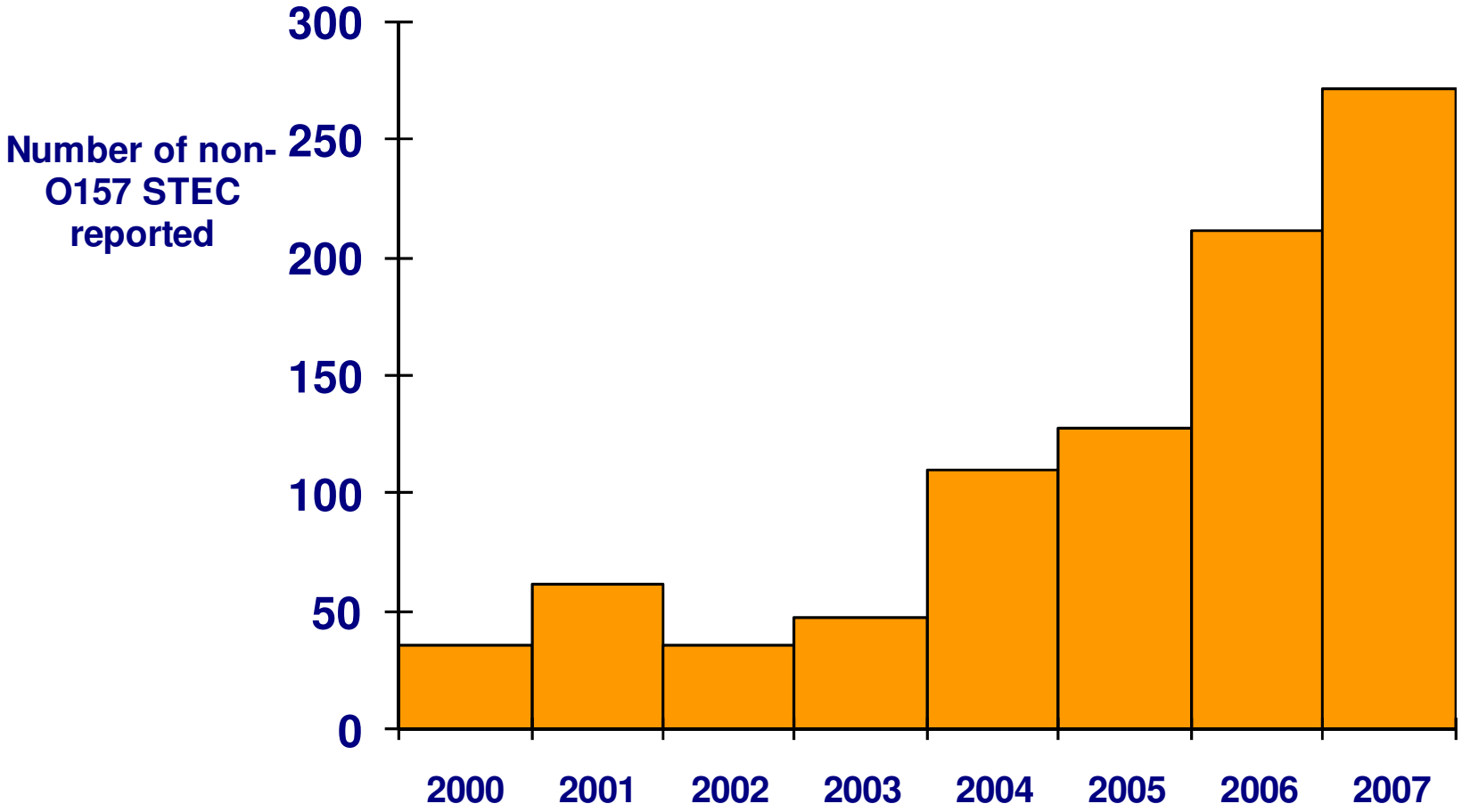


Complications of *E. coli* O157 infection, FoodNet sites, 2000-2006

- 3464 patients with *E. coli* O157 infection in FoodNet surveillance
- 42% hospitalized
  - Highest rate (66%) in ≥60 years old
- 6.3% hemolytic uremic syndrome (HUS)
  - Highest rate (15%) in <5 year olds
- 0.6% died
  - Highest rate (3%) in ≥60 year olds
  - 5% persons with HUS died (33% of ≥60 year olds)

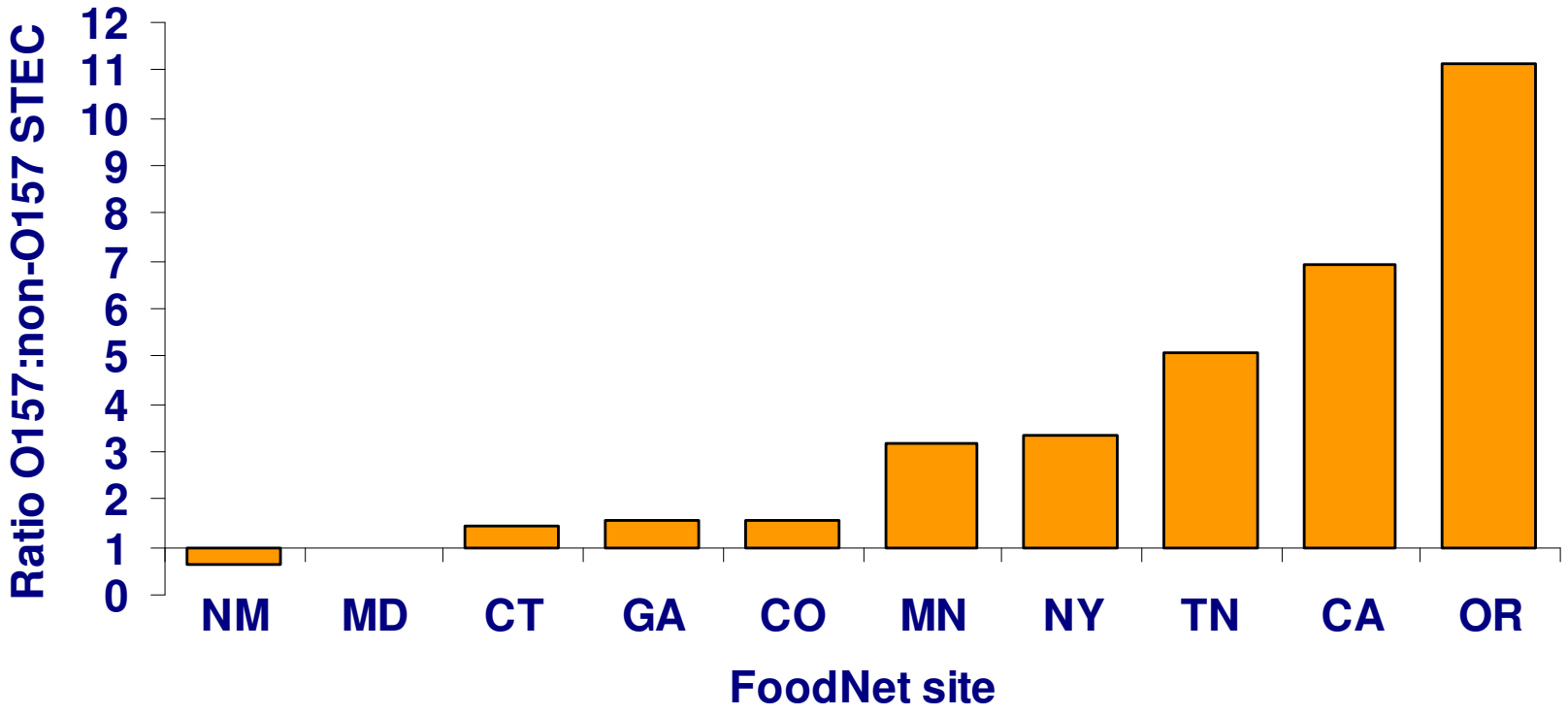
Gould et al., 2009, CID, in press

Number of non-O157 STEC reported in FoodNet sites, 2000-2007



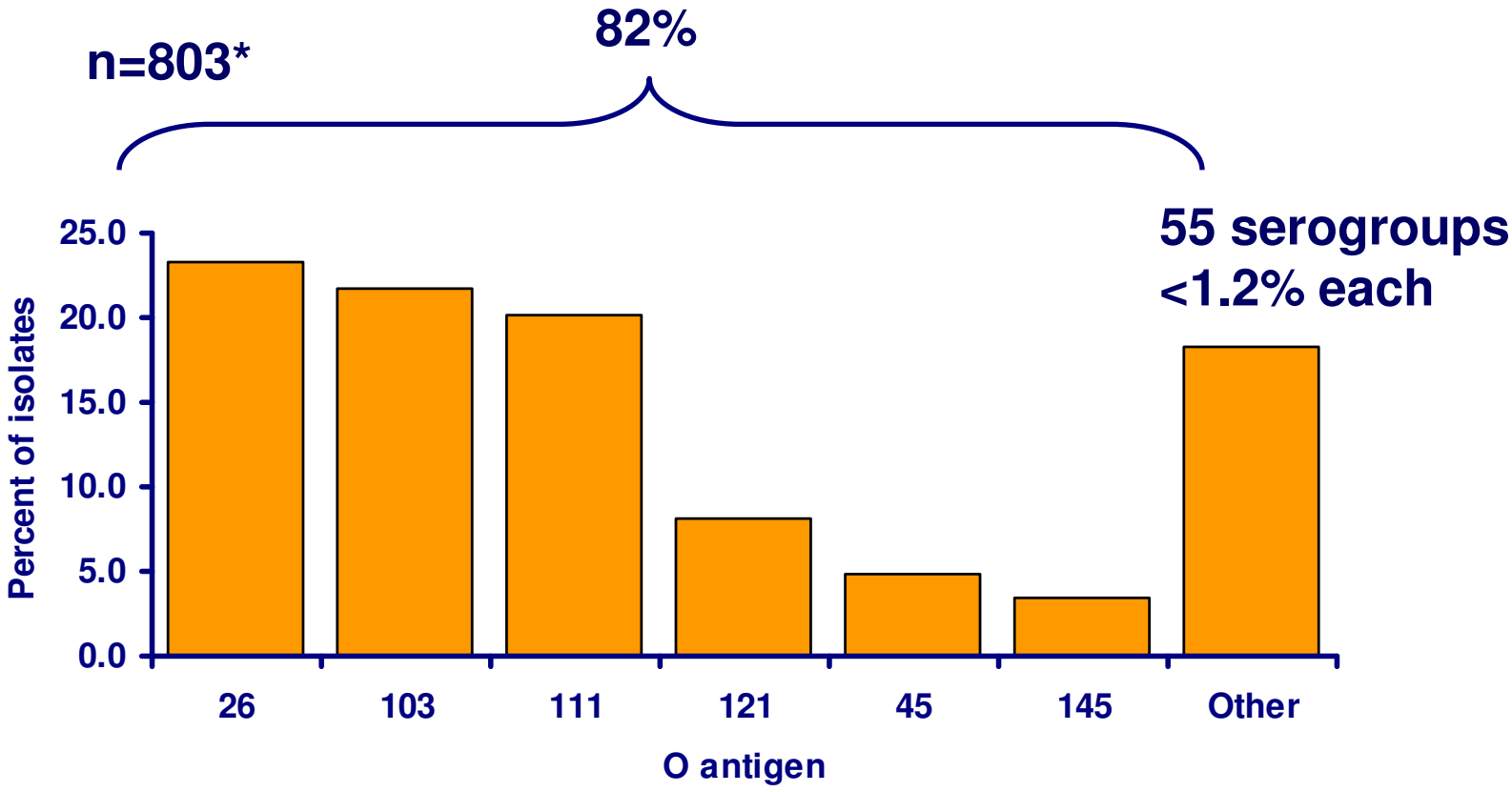
CDC, unpublished preliminary data, 2009

Ratio of O157 to non-O157 STEC, FoodNet sites, 2006-2008



All data are preliminary and subject to change.

Human isolates of non-O157 STEC by O serogroup, FoodNet sites, 2000-2007



*An additional 86 isolates had missing O group information

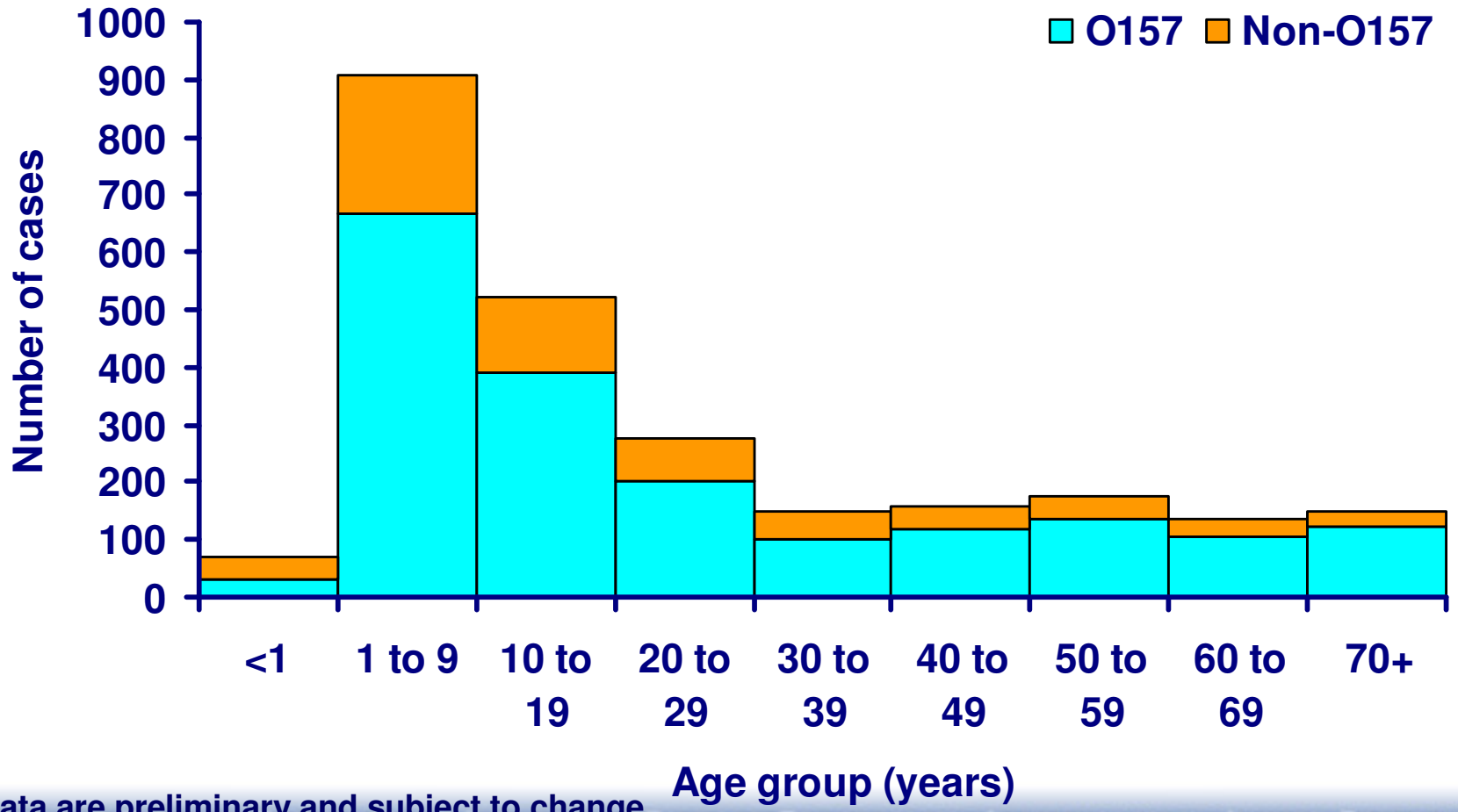
All data are preliminary and subject to change.

Comparison of characteristics of non-O157 and O157 STEC cases, FoodNet, 2000-2008

	Non-O157 (n=1113)	O157 (n=4776)
Similar		
Female	52%	53%
Median age, years	14 (<1-89)	16 (<1-93)
Different		
Part of outbreak	10%	19%
Developed HUS	1.7%	6.3%
Hospitalized	12%	42%
Died	0.1%	0.6%
Traveled internationally	15%	3%

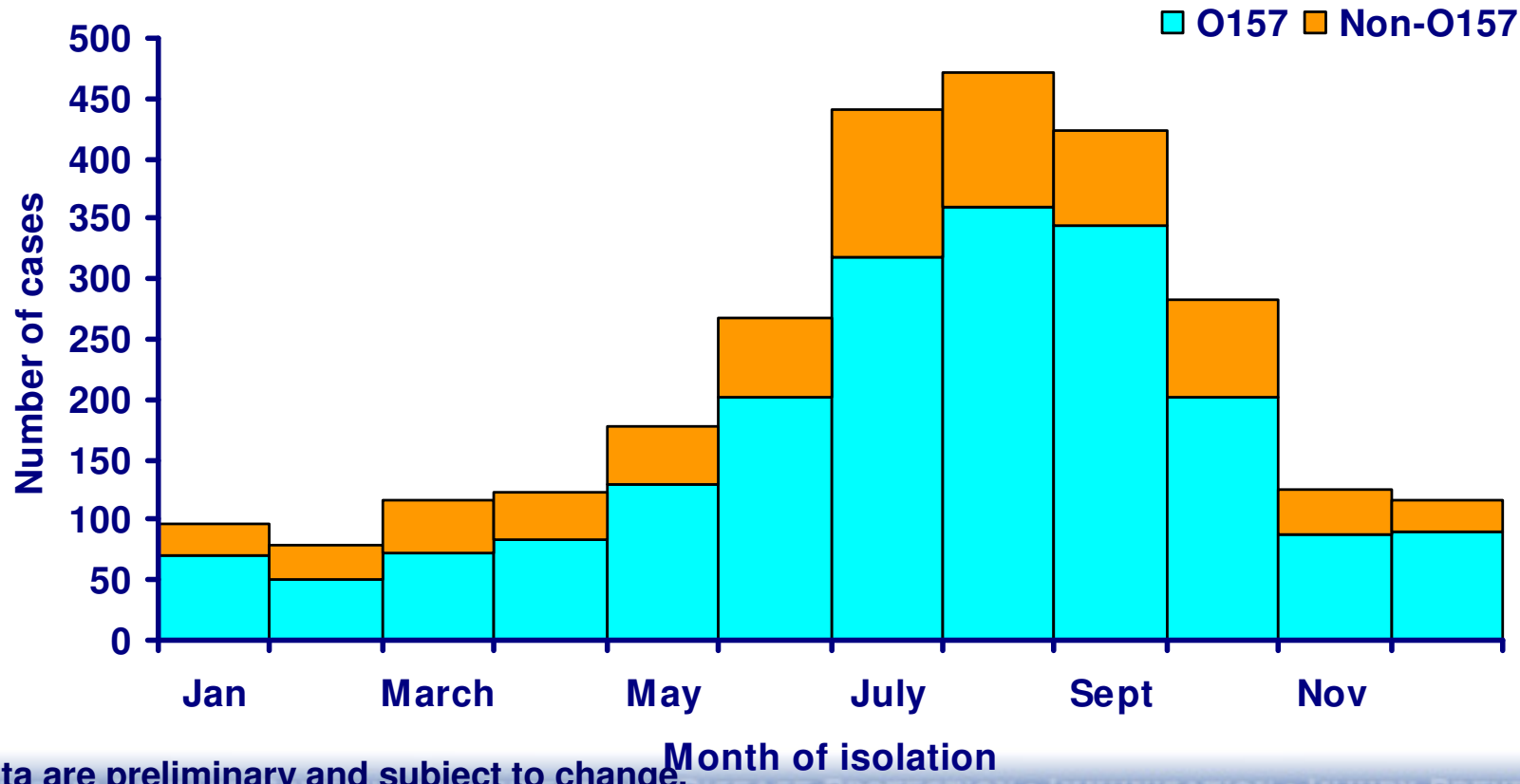
All data are preliminary, HUS data through 2006, travel and outbreak data since 2004

Number of O157 and non-O157 STEC infections by age group, FoodNet, 2004-2007



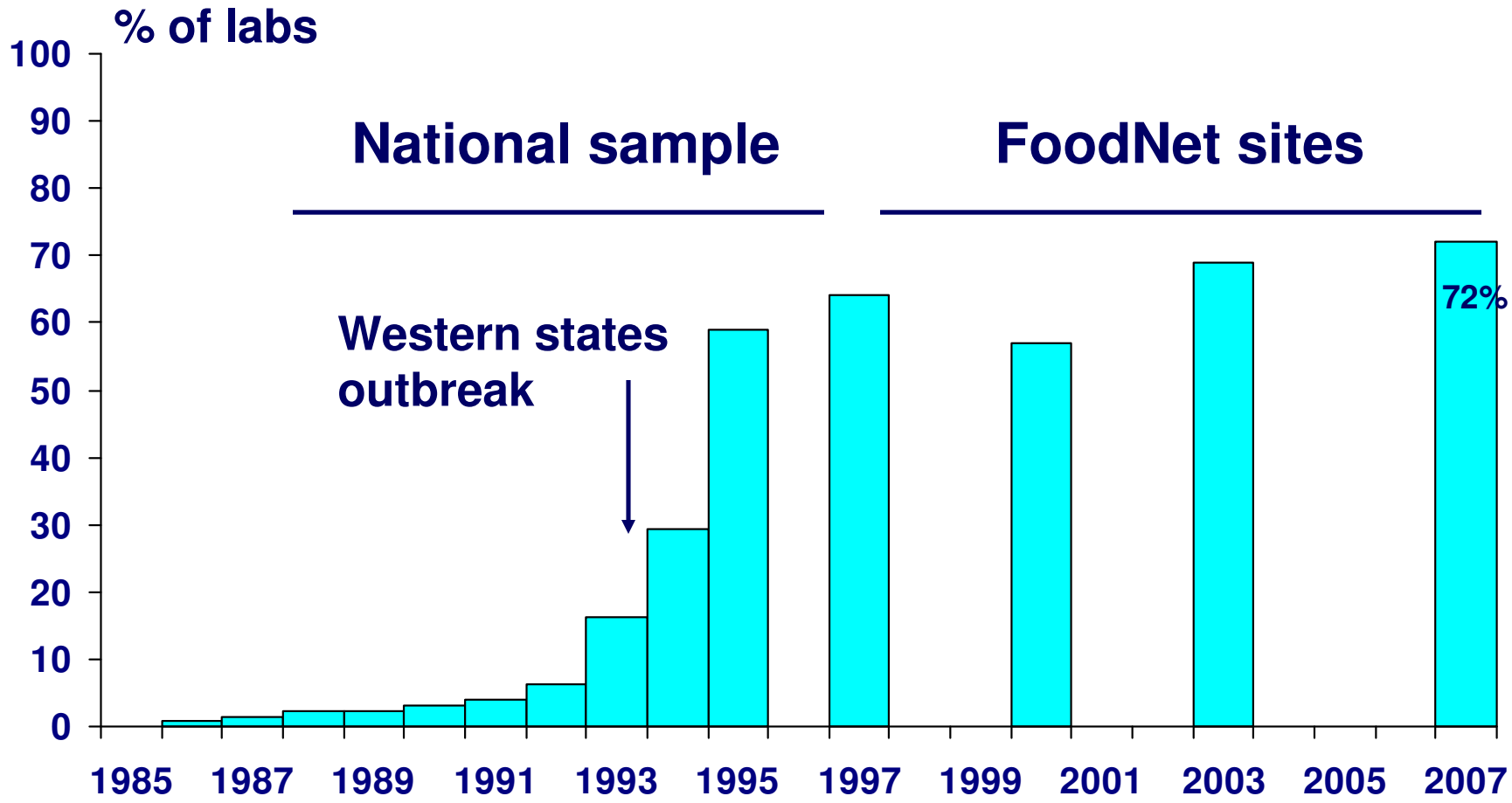
All data are preliminary and subject to change.

Number of non-O157 STEC infections by month of isolation, FoodNet sites, 2004-2007



All data are preliminary and subject to change.

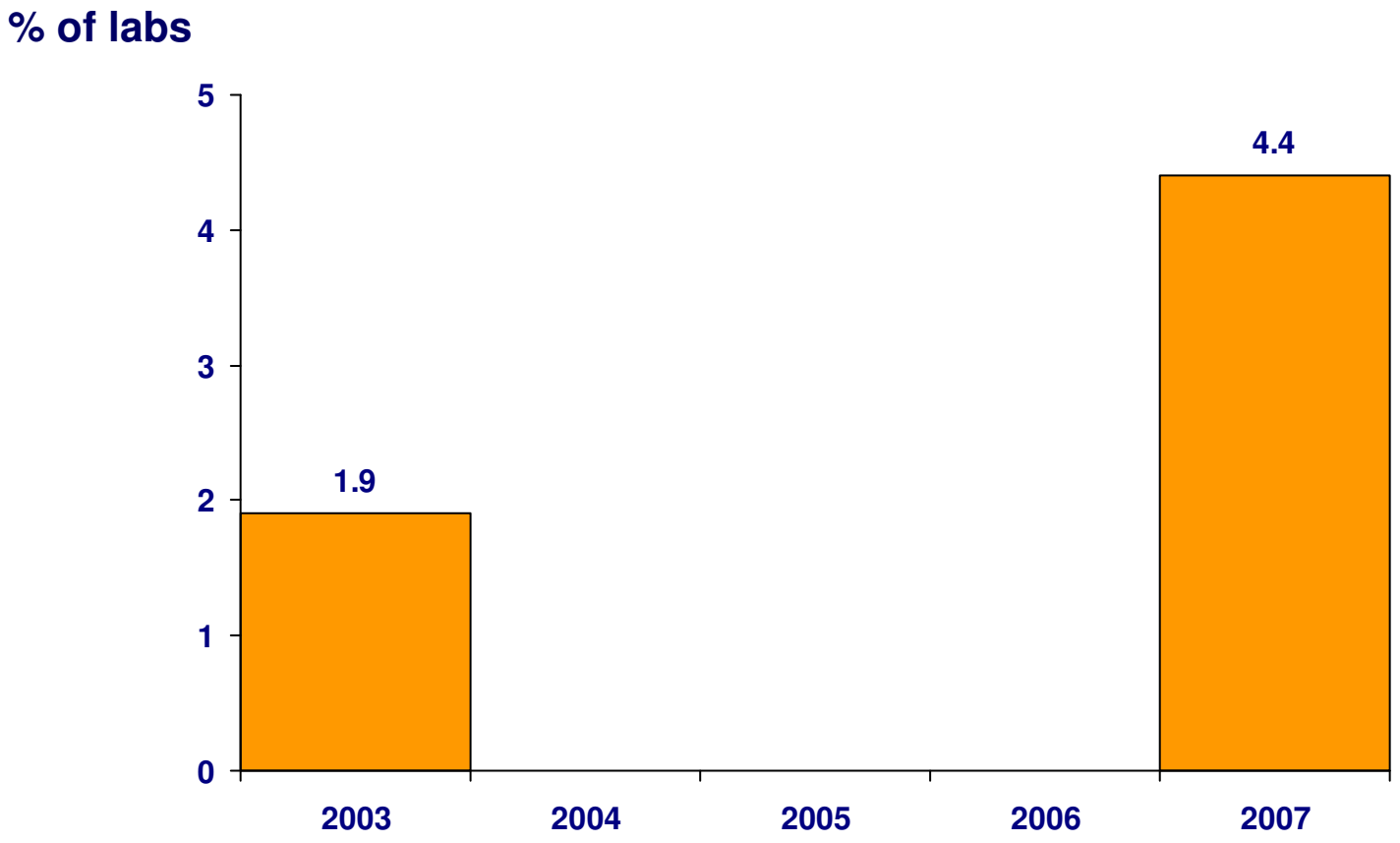
Percent of clinical labs screening all stools for O157 STEC



Boyce, J Clin Micro 1995; Voetsch CID 2004; and unpublished preliminary data

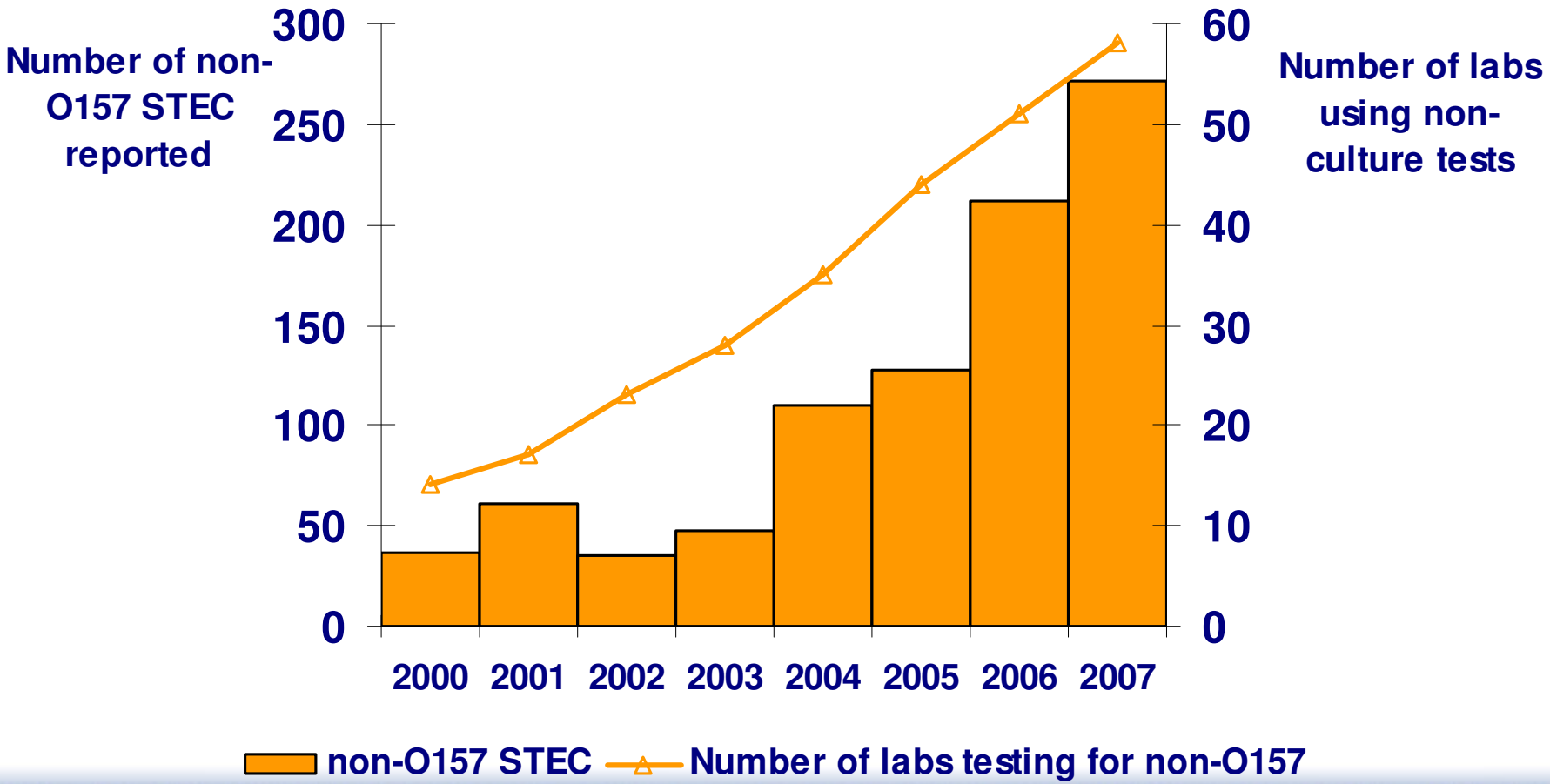
All data are preliminary and subject to change.

Percent of clinical labs that test stools routinely for Shiga toxin using enzyme immunoassay (EIA), FoodNet sites



CDC, unpublished preliminary data, 2009

Number of non-O157 STEC has increased at same time number of labs doing non-culture tests has increased



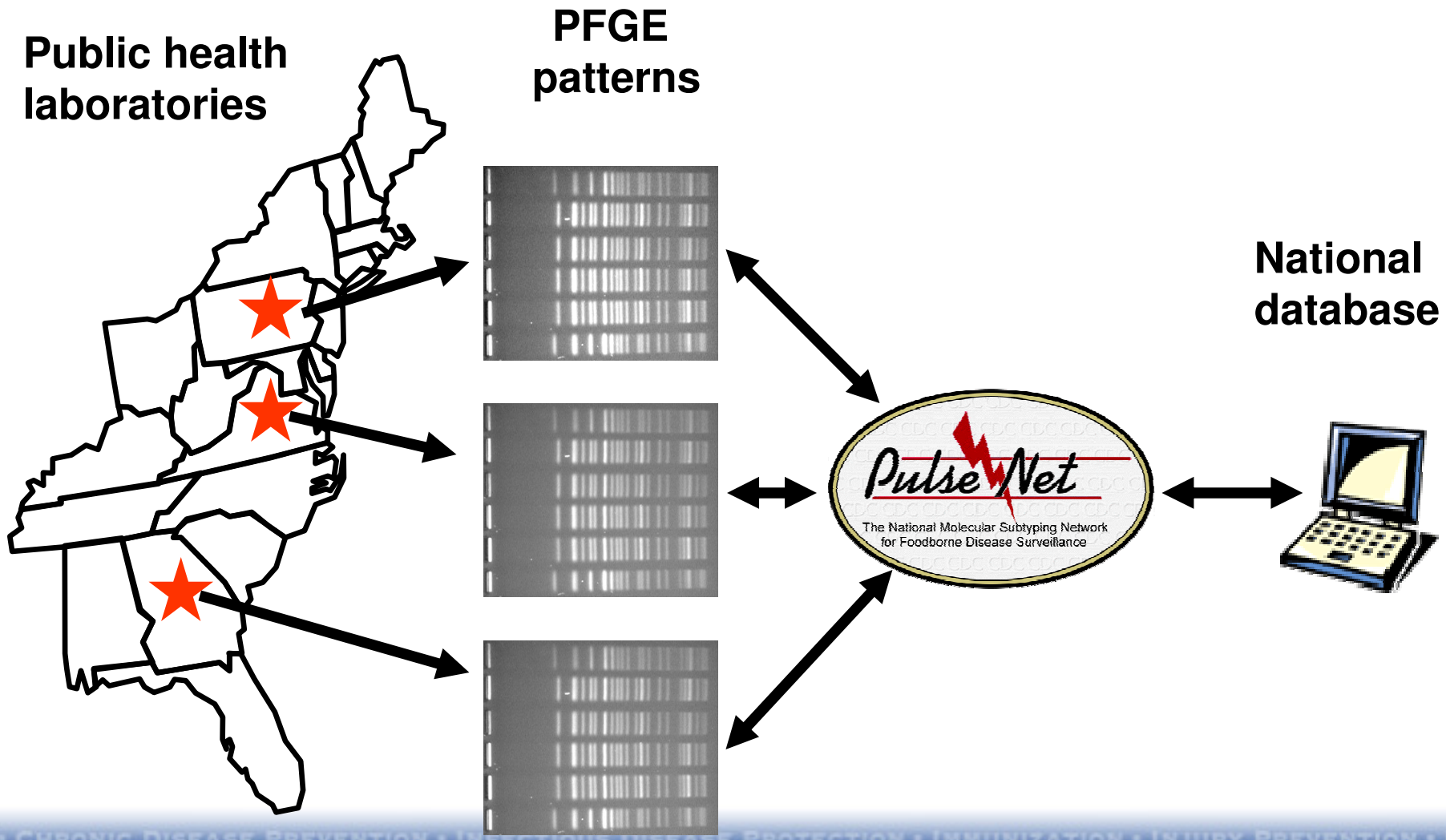
CDC, unpublished preliminary data, 2009

STEC O157 Outbreaks

PulseNet

- Detects clusters of illness with matching DNA "fingerprints"
  - A match suggests that the infections might have a common origin
  - Facilitates early identification of outbreaks
- Assists epidemiologists in investigating outbreaks
  - Persons with the outbreak "fingerprint" are likely to be part of the outbreak
  - A match between an isolate in a suspect food and a patient can help confirm an outbreak

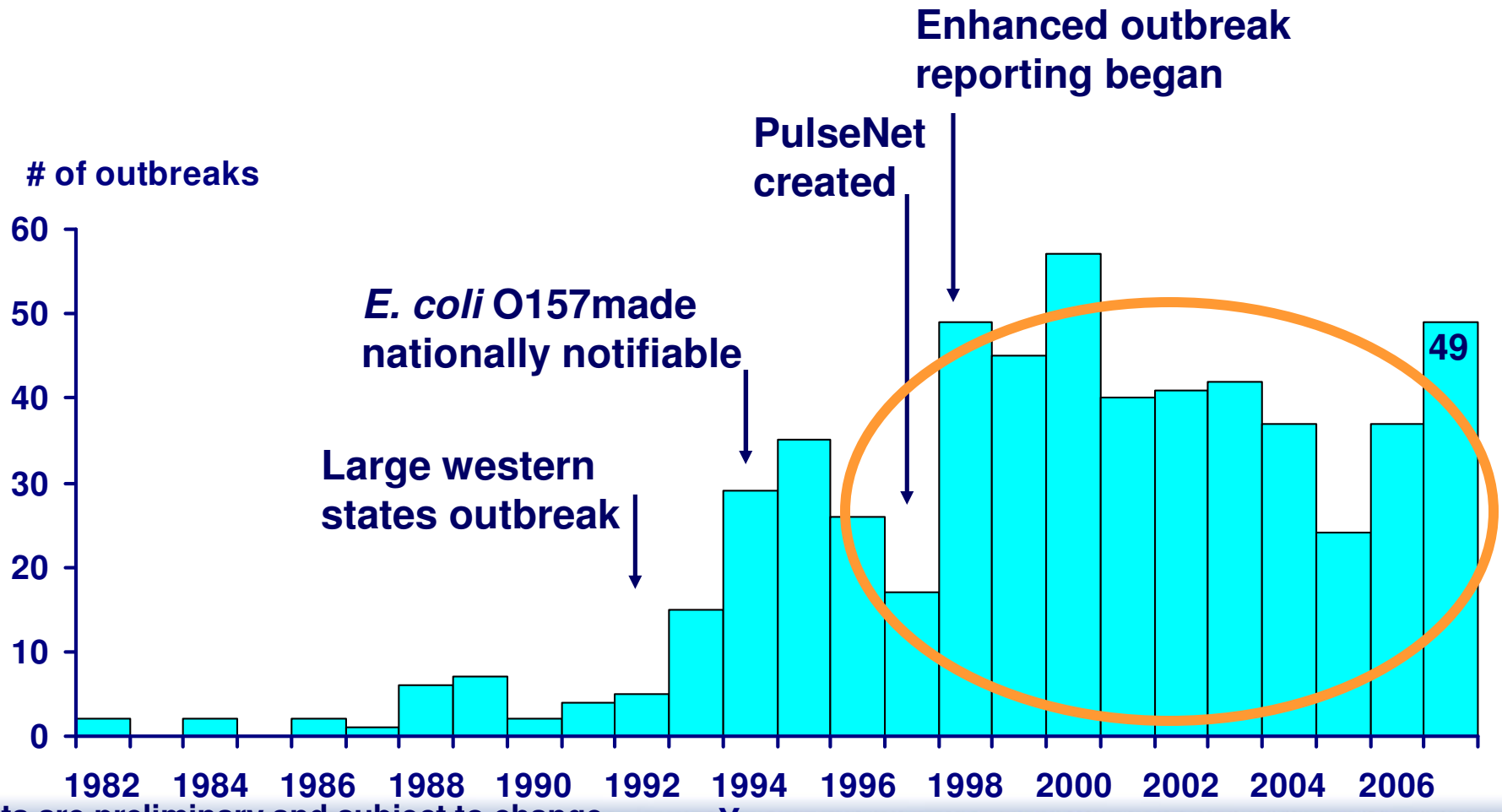
PulseNet Laboratory Communication Network



Coordinating Multi-State Investigations - "OutbreakNet"

- PulseNet detects clusters of possibly linked infections
- Outbreak coordination team at CDC in regular communication with counterparts in every state
- Goal:
  - systematic investigation of cases
  - coordinated investigation of multi-state outbreaks
- Working relationships with FSIS and with FDA, and states
- Systematic collection and review of foodborne outbreaks reported by state health departments (~1200 investigated per year)

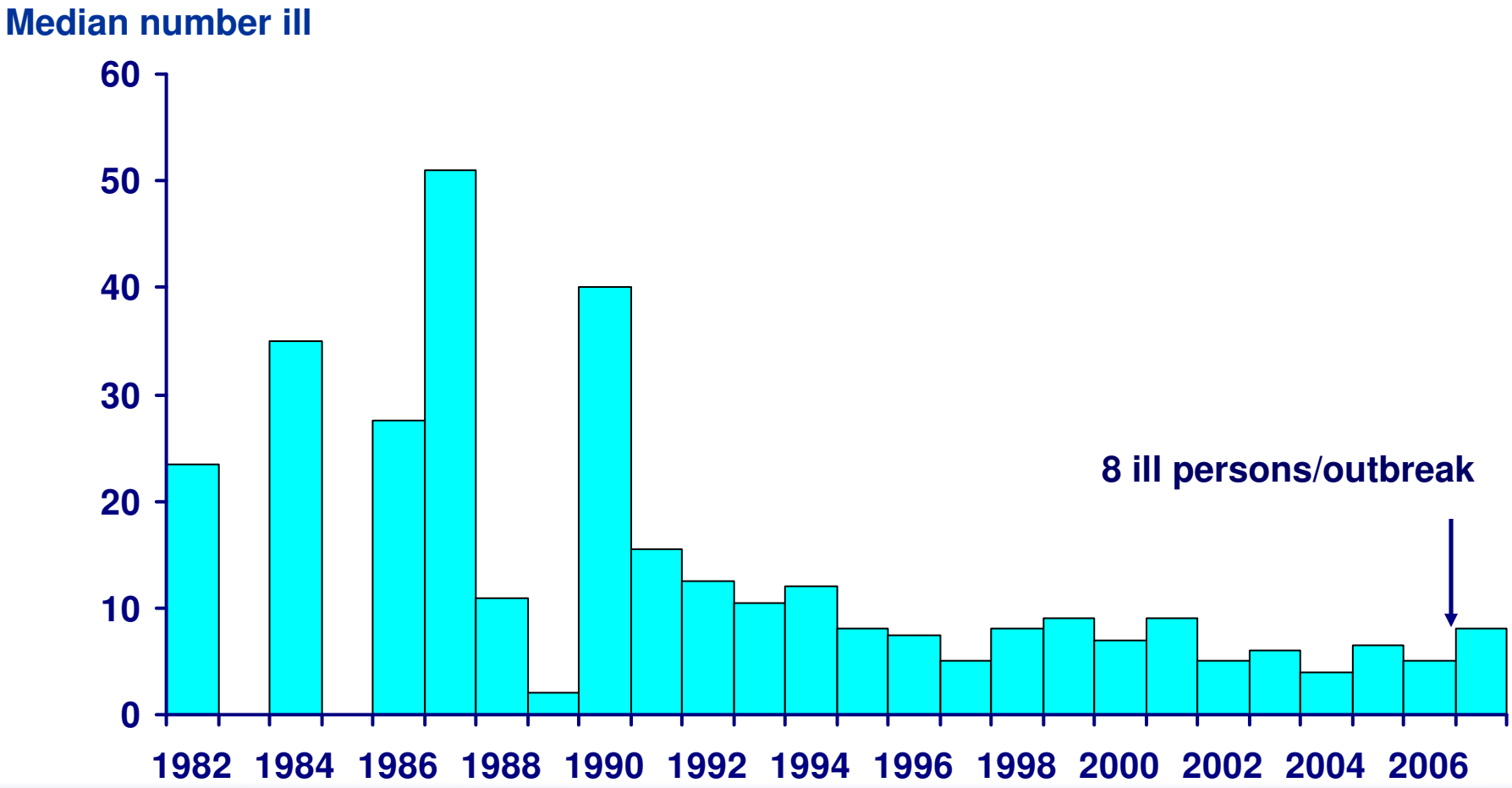
E. coli O157 outbreaks, 1982-2007 (n = 525 outbreaks)



All data are preliminary and subject to change.

Rangel, Emerg Infect Dis, 2005 and unpublished CDC data

Median number of ill persons in *E. coli* O157 outbreaks, 1982-2007



All data are preliminary and subject to change.

Rangel, Emerg Infect Dis, 2005 and unpublished CDC data

Proportion of illnesses due to each mode of transmission in *E. coli* O157 outbreaks, 1998-2007

Mode	% Illness in Outbreaks (n=344 outbreaks, 7864 illnesses)
Foodborne	69
Waterborne	18
Animals or their environment	8
Person-to-person	6
Lab-acquired	<0.1%

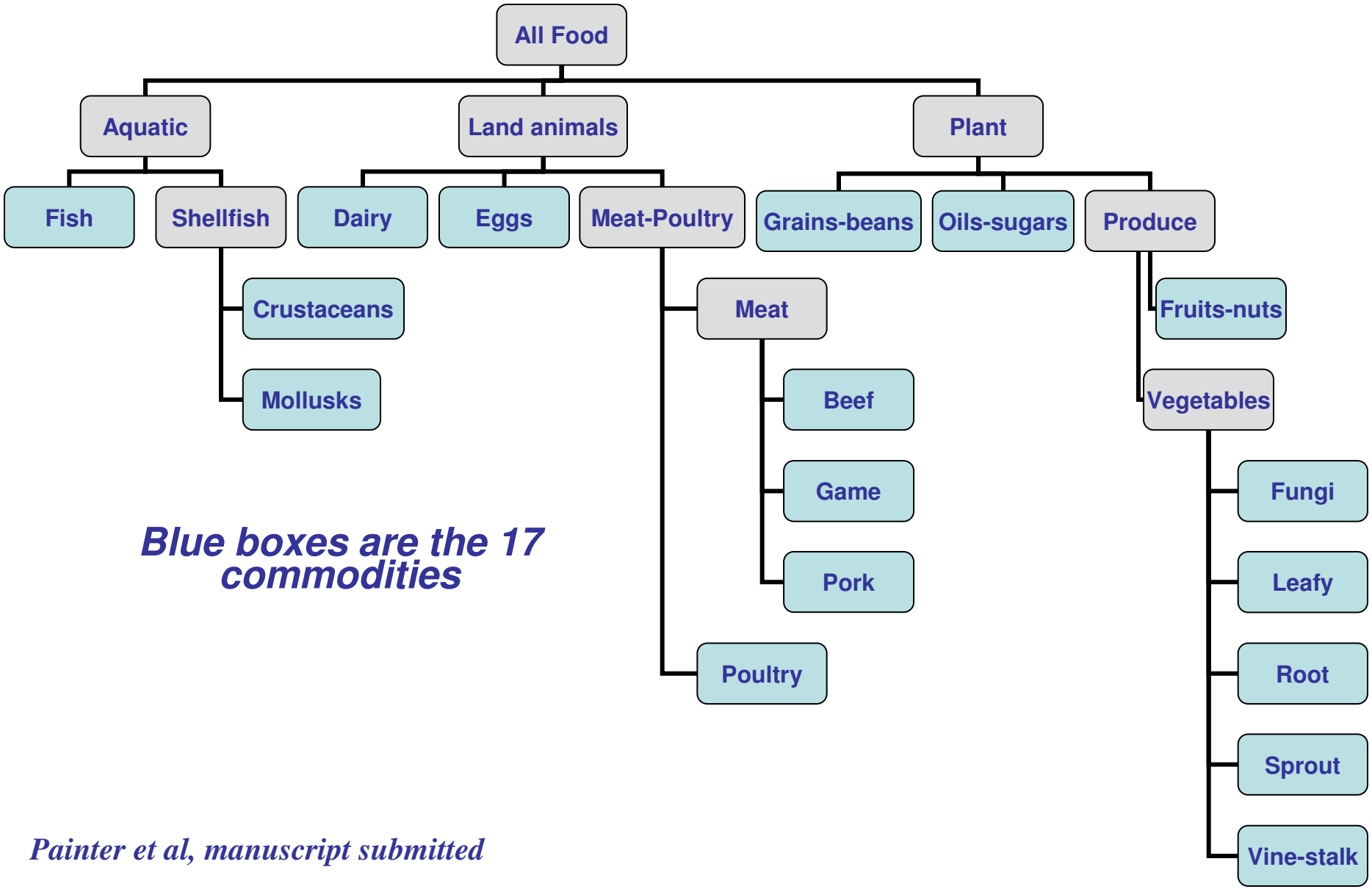
All data are preliminary and subject to change.

Foods causing illness in 245 foodborne outbreaks of *E. coli* O157 infections, 1998-2007

- 5,387 outbreak-associated illnesses
  - 21% due to an unknown food
  - 16% due to a complex food
    - Complex food contains more than one commodity, e.g., a sandwich with beef and lettuce
  - 63% due to a simple food
    - Simple food contains only one commodity
    - Determined the proportion of outbreak-associated illnesses
      - Due to each commodity
      - In outbreaks due to simple foods

All data are preliminary and subject to change.

Hierarchical scheme for categorizing foods into commodities



Blue boxes are the 17 commodities

Painter et al, manuscript submitted

Commodities causing illness in all foodborne outbreaks of *E. coli* O157 infections due to simple foods, 1998-2007

Commodity	1st 5 years (1998-2002) (n=2,053 ill)
Beef	33
Leafy vegetable	11
Dairy	13
Fruits-nuts	41
Sprouts	1
Game	-
Poultry	2

All data are preliminary and subject to change.

Commodities causing illness in all foodborne outbreaks of *E. coli* O157 infections due to simple foods, 1998-2007

Commodity	1st 5 years (1998-2002) (n=2,053 ill)
Beef	33
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Fruits-nuts	41
Sprouts	1
Game	-
Poultry	2

All data are preliminary and subject to change.

Commodities causing illness in all foodborne outbreaks of *E. coli* O157 infections due to simple foods, 1998-2007

Commodity	1st 5 years (1998-2002) (n=2,053 ill)	2nd 5 years (2003-2007) (n=1,312 ill)
Beef	33	42
Leafy vegetable	11	41
Dairy	13	13
Fruits-nuts	41	2
Sprouts	1	2
Game	-	1
Poultry	2	-

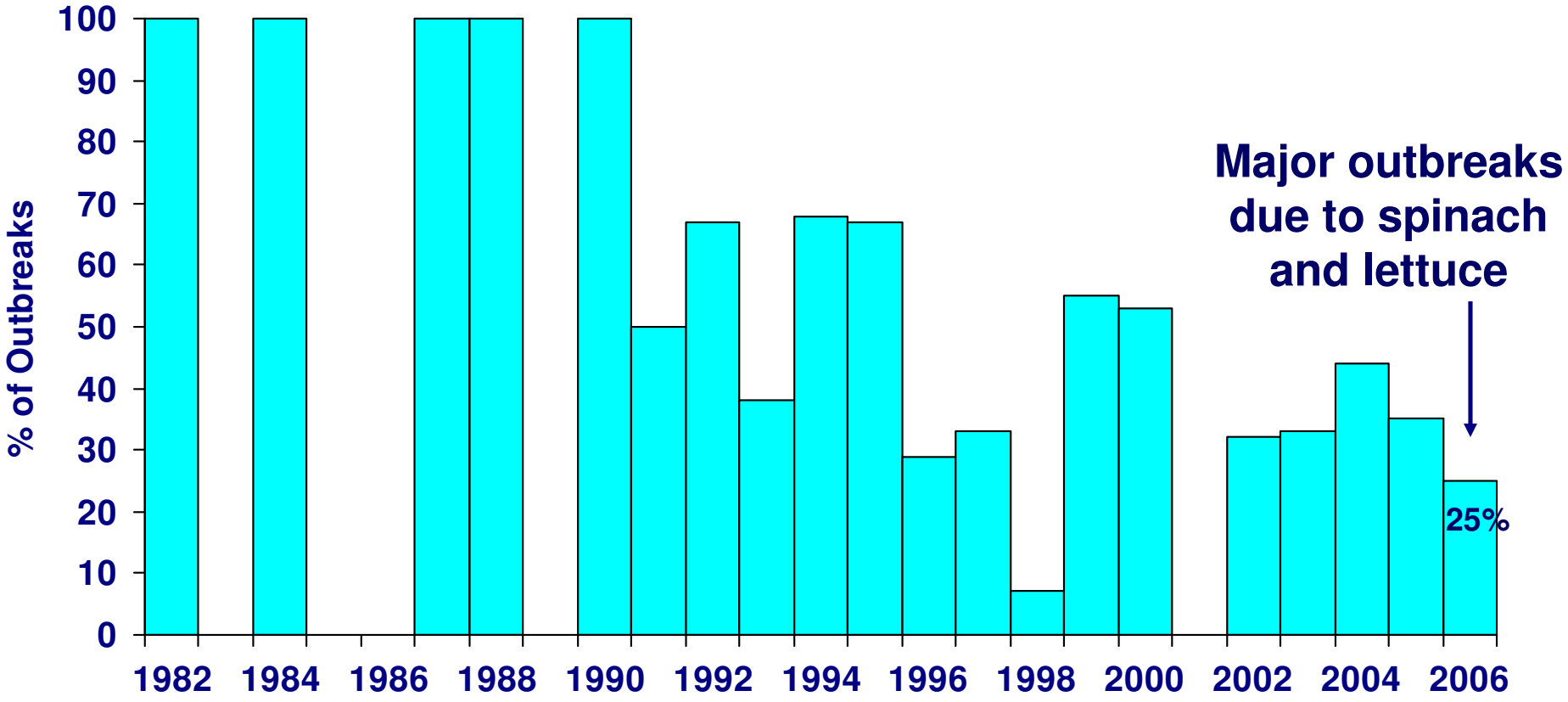
All data are preliminary and subject to change.

Commodities causing illness in all foodborne outbreaks of *E. coli* O157 infections due to simple foods, 1998-2007

Commodity	1st 5 years (1998-2002) (n=2,053 ill)	2nd 5 years (2003-2007) (n=1,312 ill)
Beef	33	42
Leafy vegetable	11	41
Dairy	13	13
Fruits-nuts	41	2
Sprouts	1	2
Game	-	1
Poultry	2	-

All data are preliminary and subject to change.

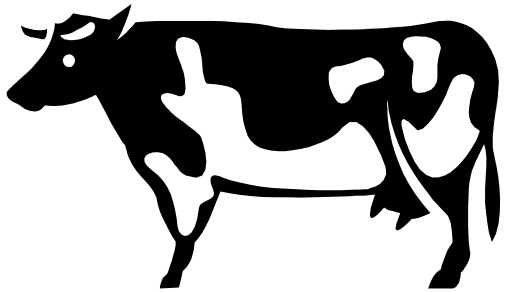
Percent of foodborne STEC O157 outbreaks due to beef, 1982 - 2006



Rangel 2005, and CDC unpublished data

Leafy green vegetables implicated in STEC O157 outbreaks—US, 1982-2005

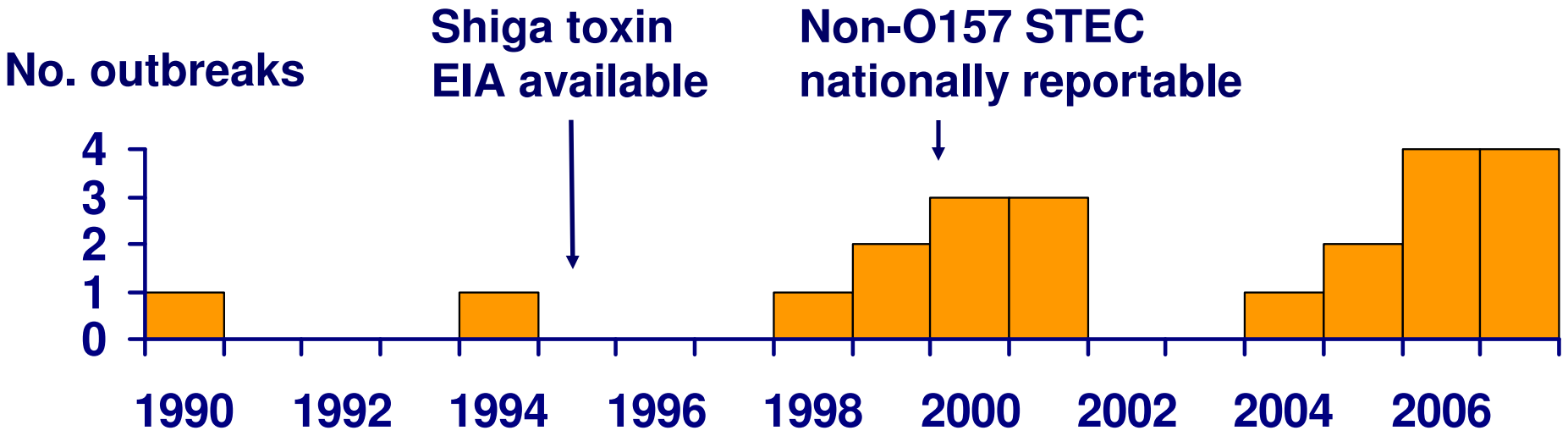
- Before 1995: none implicated
- 1995-2005: 26 outbreaks
  - Lettuce and lettuce salads: 21 outbreaks
  - Cabbage: 3 outbreaks
  - Parsley: 2 outbreaks
  - Spinach: 1 outbreak
- Cattle are part of this picture!



All data are preliminary and subject to change.

Non-O157 STEC Outbreaks

Outbreaks of non-O157 STEC infections (N=22)—United States, 1990-2007



All data are preliminary and subject to change.

Serogroups in non-O157 STEC outbreaks, 1990-2007

Serogroup	Number outbreaks	Number outbreaks with other pathogens
O111	10	5
O121	5	1
O26	3	1
O45	2	
O104	1	
O103	1	
Total	22	7

All data are preliminary and subject to change.

Proportion of illnesses due to each mode of transmission in non-O157 STEC outbreaks, 1990-2007

Mode	Illnesses in outbreaks (n=548 illnesses)*
Foodborne	83%
Waterborne	9%
Animals or their environment	5%
Person-to-person	4%

*Estimated after adjusting for several multi-pathogen outbreaks

All data are preliminary and subject to change.

Food commodities implicated outbreaks of non-O157 STEC infections with known food commodity, 1990-2007

Commodity	Number outbreaks	Food items
Fruit-nuts	3	Juice, apple cider, berries
Dairy	2	Cheese, margarine
Leafy vegetables	1	Lettuce

No outbreaks due to beef

All data are preliminary and subject to change.

Summary of STEC in United States

- **Features of illness**
  - ***E. coli* O157**
    - <5 year olds have highest rate of illness and HUS
    - ≥60 year olds have highest rate of hospitalization and death
  - **Non-O157 STEC compared with O157**
    - Lower proportion part of outbreak, hospitalized, died
    - Higher proportion had international travel

Summary of STEC in United States (continued)

- **Surveillance**
  - ***E. coli* O157**
    - Decline through 2004, reversed in 2005-6
      - Not accounted for by change in lab practices or consumption patterns
  - **Non-O157 STEC**
    - Increase in number of reported cases likely due to changes in clinical laboratory practices
    - Six serogroups comprise >80% of isolates
    - Only ~4% of clinical labs test routinely
      - new recommendations should increase testing

Summary of STEC in United States (continued)

- **Transmission of most STEC in outbreaks is by food**
  - ***E. coli* O157 outbreaks**
    - **Continued at same level, generally smaller**
    - **Beef remains major vehicle**
    - **Leafy vegetables consumed raw are a new major challenge**
      - **complex ecologies result in contamination**
  - **Non-O157 STEC outbreaks**
    - **Beef has not been implicated**

Thank you!

www.cdc.gov/foodnet

www.cdc.gov/ecoli

Acknowledgements

CDC Enteric Diseases
Epidemiology and Laboratory
Branches

FoodNet sites and partners

USDA-FSIS

FDA

The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention

Prevalence and pathogenicity of Shiga toxin-producing Escherichia coli in beef cattle and their products

H. S. Hussein

J Anim Sci 2007.85:E63-E72.
doi: 10.2527/jas.2006-421 originally published online Oct 23, 2006;

Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products[1,2]

H. S. Hussein[3]

Department of Animal Biotechnology, University of Nevada, Reno 89557

ABSTRACT: During the past 23 yr, a large number of human illness outbreaks have been traced worldwide to consumption of undercooked ground beef and other beef products contaminated with Shiga toxin-producing *Escherichia coli* (STEC). Although several routes exist for human infection with STEC, beef remains a main source. Thus, beef cattle are considered reservoirs of O157 and nonO157 STEC. Because of the global nature of the food supply, safety concerns with beef will continue, and the challenges facing the beef industry will increase at the production and processing levels. To be prepared to address these concerns and challenges, it is critical to assess the beef cattle role in human infection with STEC. Because most STEC outbreaks in the United States were traced to beef containing *E. coli* O157:H7, the epidemiological studies have focused on the prevalence of this serotype in beef and beef cattle. Worldwide, however, additional STEC serotypes (e.g., members of the O26, O91, O103, O111, O118, O145, and O166 serogroups) have been isolated from beef and caused human illnesses ranging from bloody diarrhea and hemorrhagic colitis to the life-threatening hemolytic uremic syndrome (HUS). To provide a global assessment of the STEC problem, published reports on beef and beef cattle in the past 3 decades were evaluated. The prevalence rates of *E. coli* O157 ranged from 0.1 to 54.2% in ground beef, from 0.1 to 4.4% in sausage, from 1.1 to 36.0% in various retail cuts, and from 0.01 to 43.4% in whole carcasses. The corresponding prevalence rates of nonO157 STEC were 2.4 to 30.0%, 17.0 to 49.2%, 11.4 to 49.6%, and 1.7 to 58.0%, respectively. Of the 162 STEC serotypes isolated from beef products, 43 were detected in HUS patients and 36 are known to cause other human illnesses. With regard to beef cattle, the prevalence rates of *E. coli* O157 ranged from 0.3 to 19.7% in feedlots and from 0.7 to 27.3% on pasture. The corresponding prevalence rates of nonO157 STEC were 4.6 to 55.9% and 4.7 to 44.8%, respectively. Of the 373 STEC serotypes isolated from cattle feces or hides, 65 were detected in HUS patients and 62 are known to cause other human illnesses. The results indicated the prevalence of a large number of pathogenic STEC in beef and beef cattle at high rates and emphasized the critical need for control measures to assure beef safety.

Key words: beef, beef cattle, *Escherichia coli*, foodborne pathogen, Shiga toxin

 J. Anim. Sci. 2007. 85(E. Suppl.):E63–E72
doi:10.2527/jas.2006-421

INTRODUCTION

The importance of beef safety increased since reporting the first 2 human illness outbreaks caused by consumption of ground beef contaminated with *Escherichia coli* O157:H7 (Riley et al., 1983). Because of tracing a large number of *E. coli* O157:H7 outbreaks to beef in the United States during the past 23 yr (CDC, 1993, 2003; Rodrigue et al., 1995), most US studies focused on this pathogen in beef cattle (Hancock et al., 1994; Galland et al., 2001; Barkocy-Gallagher et al., 2003) or their edible products (Doyle and Schoeni, 1987; Elder et al., 2000; Barkocy-Gallagher et al., 2003). Worldwide, other Shiga toxin-producing *E. coli* (**STEC**) serotypes were isolated from beef cattle (Beutin et al., 1997; Pradel et al., 2000; Leomil et al., 2003) or their products (Sekla et al., 1990; Leung et al., 2001; Khan et al., 2002) and caused human illnesses (WHO, 1998; Blanco et al., 2003; Bettelheim, 2006).

In addition to beef (CDC, 1993; López et al., 1997; CDC, 2003), human infections were traced to vegetables (Cieslak et al., 1993), raw milk (Martin et al., 1986;

[1]Presented at the ADSA-ASAS Joint Annual Meeting, Food Safety Symposium: Ruminants as Reservoirs for Shiga Toxin-Producing *Escherichia coli,* Minneapolis, MN, July 2006.

[2]The author would like to acknowledge the support of the Integrated Research, Education, and Extension Competitive Grants Program—National Integrated Food Safety Initiative (grant No. 2001-51110-11463) of the USDA Cooperative State Research Education, and Extension Service.

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Herriott et al., 1994; Lahti et al., 2002), dairy products (Morgan et al., 1993; Reid, 2001), and drinking water (Yatsuyanagi et al., 2002) containing STEC. Other infection routes included person to person (Reida et al., 1994) and animal to person (Synge et al., 1993; Crump et al., 2002). The infection caused human illnesses (Griffin and Tauxe, 1991; Paton and Paton, 2000) ranging from mild diarrhea to hemolytic uremic syndrome (**HUS**) that could lead to death (Pavia et al., 1990; CDC, 1993; Cowden, 1997). Because beef cattle are considered reservoirs for O157 (Hancock et al., 1994; Chapman et al., 2001; Al-Saigh et al., 2004) and nonO157 STEC (Schurman et al., 2000; Geue et al., 2002; Barkocy-Gallagher et al., 2003), safety concerns with beef, especially in the ground form, will continue to be a major challenge for the beef industry. This is critically important because recent evidence (Hussein and Bollinger, 2005a,b; Hussein and Sakuma, 2005) illustrated a large number of pathogenic STEC serotypes to derive from bovine origin. To be prepared to address current and future safety concerns and challenges, it is critical for the beef industry to develop strategies supporting beef safety. This review is intended to provide a global assessment of the beef cattle role in human infection with STEC.

HUMAN DISEASE OUTBREAKS FROM STEC OF BEEF CATTLE ORIGIN

In a large number of the reported outbreaks and sporadic cases of human illnesses, STEC infection was attributed to consumption of undercooked ground beef or other beef products (i.e., roast or smoked beef, sausage, steak, or tri-tip, and veal) contaminated with O157 (Orr et al., 1994; Cowden, 1997; CDC, 2003) or nonO157 (Caprioli et al., 1994; López et al., 1997; Henning et al., 1998) serotypes. The human illnesses included (Nataro and Kaper, 1998) mild diarrhea, abdominal pain, vomiting, bloody diarrhea, hemorrhagic colitis (**HC**), strokes, and HUS. The HC is characterized by bloody diarrhea, abdominal cramps, fever, and vomiting (Griffin and Tauxe, 1991). The HUS is characterized by thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure due to production of toxins that damage endothelial cells and trigger the clotting mechanism (Donnenberg, 2002). The HUS is more common in infants, children, the elderly, and those with compromised immune function (Paton and Paton, 2000). Although most HUS patients recover, some die and some may develop strokes (Griffin and Tauxe, 1991) or chronic renal failure (Remuzzi, 1987; Fitzpatrick et al., 1991; Siegler et al., 1991). Other symptoms of STEC infection include diabetes mellitus and necrotizing colitis (Paton and Paton, 2000).

Evaluation of published reports in the past 23 yr revealed 146 STEC outbreaks and sporadic cases of human illnesses to be traced to consumption of beef contaminated with various *E. coli* O157 strains (Bollinger, 2004). These strains belonged to *E. coli* O157:H7, O157:H$^-$ (a nonmotile isolate), and others that were not typed for the H antigen. Of these outbreaks and sporadic cases, 88% were traced to ground beef, 89% occurred in the United States, and 11% occurred in the United Kingdom (8), Canada (2), Germany (2), Japan (2), Argentina (1), and Central African Republic (1). The large number of outbreaks and cases in the United States could be explained by the high level of ground beef consumption at fast food restaurants and by availability of *E. coli* O157 diagnostic methods. Some of the outbreaks involved large numbers of affected people (ranging from 303 to 736) as shown in Canada (Orr et al., 1994), the United Kingdom (Cowden, 1997), and the United States (CDC, 1993, 2003). These outbreaks emphasized the role of beef as an important vehicle of *E. coli* O157 transmission (CDC, 1993; USDA-APHIS-VS, 1997; CDC, 2003).

A smaller number of outbreaks (6 total) of human illnesses was attributed to infections with nonO157 STEC strains from contaminated beef (Bollinger, 2004). These infections involved 8 STEC serogroups (O1, O2, O15, O25, O75, O86, O111, and O160) and 3 serotypes (O26:H11, O111:H7, and O111:H$^-$). These outbreaks were reported in Argentina, Australia, Germany, and Italy and were traced to consumption of undercooked ground beef or its sausage. Two of these outbreaks involved large numbers of affected people (161 and 433) as shown in Australia (CDC, 1995) and Argentina (López et al., 1997), respectively. The significance of nonO157 STEC infections through contaminated beef was illustrated in the incidence of HUS cases in 5 of the 6 reported outbreaks (Bollinger, 2004). In these outbreaks, most HUS patients were children or the elderly, reflecting the naivety of the immune system of young children and the declining immune function of the elderly (Paton and Paton, 2000). Although infection with *E. coli* O26:H11 did not lead to HUS in the outbreak reported (Werber et al., 2002), it is known to cause HUS (WHO, 1998; Anonymous, 2001; Blanco et al., 2003).

BEEF CATTLE AS RESERVOIRS FOR STEC

Although various STEC strains have been isolated from different animals (Beutin et al., 1993; 1995), they have been shown to be more prevalent in ruminants than in other animals (Beutin et al., 1993; Caprioli et al., 1993; Beutin et al., 1995). In addition, human illnesses due to STEC infection have been traced in most cases to cattle (Bielaszewska et al., 2000; Crump et al., 2002), their manure (Wilson et al., 1992; Cieslak et al., 1993; Lahti et al., 2002), or their edible products, especially beef (Riley et al., 1983; López et al., 1997; CDC, 2003). A wide distribution of STEC among various beef cattle categories was documented by isolation of different serotypes from bulls (Čižek et al., 1999), cows (Shinagawa et al., 2000; Gannon et al., 2002; Hussein et al., 2003), heifers (Schurman et al., 2000; Thran et al., 2001; Ezawa et al., 2004), steers (Schurman et al.,

2000; Gioffré et al., 2002; Smith et al., 2004), and calves (Gannon et al., 2002; Leomil et al., 2003; Cobbold et al., 2004). Additionally, STEC are commonly detected in cattle in feedlots (Hancock et al., 1997; LeJeune et al., 2004; Padola et al., 2004) and under grazing conditions (Gannon et al., 2002; Cobbold et al., 2004; Pearce et al., 2004).

BEEF CATTLE AS TRANSIENT CARRIERS OF STEC

Beef cattle have not been reported as long-term carriers of STEC. Besser et al. (1997) reported that the duration of detected excretion of *E. coli* O157:H7 by individual US cattle was shorter than 1 mo in 63% of the cattle tested. Similar results were also reported for Japanese cattle (Ohya and Ito, 1999). It is worth noting that the carriage of these pathogens was shown to fluctuate significantly over time in US feedlots (Hancock et al., 1997; Khaitsa et al., 2003; LeJeune et al., 2004). Testing feedlot cattle in 13 states during winter showed a decrease in prevalence rate from 4.6 to 1.3% by increasing the time on feed from 7 to 185 d (Hancock et al., 1997). In a North Dakota study, however, the prevalence rate rose during winter from 1.4% on arrival to 6.9% at 28 d on feed (Khaitsa et al., 2003). Testing cattle in 20 feedlot pens during spring also showed fluctuations in the prevalence rates (15, 28, 22, and 12%) at different times (7, 14, 28, and 42 d, respectively) on feed (LeJeune et al., 2004). Shedding of STEC by beef cattle has been shown to increase during the warm months, which is consistent with the timing of most human illness outbreaks (USDA-APHIS-VS, 1997). In the US, testing beef cattle over 1 yr revealed the highest (9%) and lowest (5%) prevalence rates for the fall and winter, respectively (Cobbold et al., 2004). Similar results were reported in Germany when 2 grazing beef herds were tested over 2 yr (Geue et al., 2002). In our laboratory (Thran et al., 2001), however, fecal testing of grazing beef heifers over 1 yr revealed the highest (15%) prevalence rate to occur in winter and the lowest (4%) to occur in the spring and fall. In another grazing study (Jenkins et al., 2002), the highest (22%) and lowest (6%) prevalence rates occurred in the summer and fall, respectively. Testing cattle at slaughter for *E. coli* O157 revealed the highest and lowest prevalence rates to occur in the warm and cold months, respectively, in Finland (Lahti et al., 2001) and the United Kingdom (Chapman et al., 2001; Paiba et al., 2002). In the United States, testing Midwestern cattle (Barkocy-Gallagher et al., 2003) at slaughter showed *E. coli* O157:H7 to be more prevalent in the summer than in winter (12.9 vs. 0.3%) and nonO157:H7 STEC to be more prevalent in the fall (27.1%) than in the summer or winter (14.0%). In France (Pradel et al., 2000), 58 STEC serotypes were prevalent at very high rates in the summer (85%) and spring (46%), and the cattle were transient in infection.

PREVALENCE OF STEC IN BEEF CATTLE

Hussein and Bollinger (2005a) reviewed published reports in the past 3 decades and summarized the prevalence of STEC in beef cattle feces and hides. In general, the prevalence rates of *E. coli* O157 ranged from 0.3 to 19.7% in feedlot cattle, from 0.7 to 27.3% in cattle on irrigated pasture, and from 0.9 to 6.9% in cattle grazing rangeland forages. These observations suggest a high potential for infection and reinfection of cattle with *E. coli* O157 during grazing of the dense vegetation on pasture. On the range, however, cattle travel in large and less-dense areas seeking edible vegetation. With regard to testing for *E. coli* O157 at slaughter, the prevalence rates ranged from 0.2 to 27.8%. Worldwide, the prevalence rates of nonO157 STEC ranged from 4.6 to 55.9% in feedlot cattle and from 4.7 to 44.8% in grazing cattle. With regard to testing for nonO157 STEC at slaughter, the prevalence rates ranged from 2.1 to 70.1%. These observations indicate that nonO157 STEC are prevalent in all beef production systems at rates as high as 70.1%. The ranges of prevalence rate, however, varied widely and could be explained by the significant impact of environmental factors, by management practices on promoting or decreasing STEC prevalence, or both.

Cattle hides have been identified as an important source of microbial contamination of carcasses (Ridell and Korkeala, 1993; Bell, 1997; McEvoy et al., 2000). It has been shown that O157:H7 and nonO157:H7 STEC can be easily transferred from cattle hides to the carcass (Barkocy-Gallagher et al., 2003). Because of the role that cattle hides can play in carcass contamination with STEC at slaughter, efforts (Bacon et al., 2000; Elder et al., 2000; Barkocy-Gallagher et al., 2003) have been devoted to evaluate its significance. Testing swab samples from cattle hides at 12 US beef processing plants in the fall revealed a 3.6% prevalence rate of *E. coli* O157:H7 (Bacon et al., 2000). A higher prevalence rate (10.7%) of *E. coli* O157 was reported when cattle hides were tested in the summer at 4 Midwestern beef processing plants (Elder et al., 2000). These different prevalence rates could be explained by sampling time (i.e., fall vs. summer). Because a large number of variables (e.g., management practices, diets fed, animal factors, and methods of STEC detection) can influence STEC prevalence, comparisons among studies should be carefully evaluated. Significant seasonal differences in the prevalence rates of O157:H7 and nonO157:H7 STEC were also found (Barkocy-Gallagher et al., 2003) preharvest (i.e., feces and hides) and postharvest (i.e., carcasses). In this study, testing fecal, hide, and carcass swab samples from cattle at 3 Midwestern beef processing plants over 1 yr (Barkocy-Gallagher et al., 2003) revealed the prevalence of O157:H7 and nonO157:H7 STEC at high rates. The prevalence rates for O157:H7 and nonO157:H7 STEC, however, varied among cattle hides (60.6 and 56.6%, respectively), feces (5.9 and 19.4%, respectively), and carcasses (26.7 and 58.0%,

respectively). With regard to cattle hides, prevalence of *E. coli* O157:H7 was highest in the spring, summer, and fall (averaging 71.5%) and lowest in winter (29.4%). Prevalence of nonO157:H7 STEC, however, was lowest in the winter, spring, and summer (averaging 49.2%) and highest in the fall (77.7%). In this study, no attempt was made to serotype the nonO157:H7 isolates.

The concentration at which STEC is shed in feces varies from animal to animal as demonstrated in a US study (Zhao et al., 1995), where a range from 10^2 to 10^5 cfu of *E. coli* O157:H7/g of wet feces was observed. It is important to note that quantitative fecal shedding of STEC is considered (Omisakin et al., 2003; Ogden et al., 2004) a more important factor than prevalence in influencing the risk of human exposure and infection with these foodborne pathogens. For example, prevalence of *E. coli* O157 in Scottish beef cattle at slaughter was found (Ogden et al., 2004) to be greater ($P < 0.05$) during the cooler months (11.2%) than during the warmer months (7.5%). This is the reverse of the known seasonality of human infections with STEC (WHO, 1998). Ogden et al. (2004) reported their high shedding beef cattle (i.e., excreting > 10^4 cfu/g of wet feces) to shed greater concentrations of *E. coli* O157 in the warmer months, which may explain increased human infections at that time. Interestingly, the high shedding cattle (9% of the cattle tested) excreted the largest amount of *E. coli* O157 (96%) produced.

PREVALENCE OF STEC IN BEEF PRODUCTS

Contamination of beef carcasses with STEC usually occurs during removal of the hide or the gastrointestinal tract (Elder et al., 2000; McEvoy et al., 2003). The site and extent of carcass contamination subsequently affect prevalence of STEC in various beef products. Hussein and Bollinger (2005b) evaluated published reports in the past 3 decades on STEC prevalence in beef. With regard to *E. coli* O157, the results showed prevalence rates ranging from 0.01 to 43.4% in packing plants, from 0.1 to 54.2% in supermarkets, and an average of 2.4% in fast food restaurants. In general, the prevalence rates of *E. coli* O157 ranged from 0.1 to 54.2% in ground beef, from 0.1 to 4.4% in sausage, from 1.1 to 36.0% in unspecified retail cuts, and from 0.01 to 43.4% in whole carcasses. In 57% of the *E. coli* O157 studies evaluated by Hussein and Bollinger (2005b), the beef samples were tested only for *E. coli* O157:H7. This was due to the availability of simple methods to detect this serotype (Bettelheim, 2003), which is known for its high virulence (CDC, 2003). It is worth noting that in the remaining 43% of the studies, the *E. coli* O157 isolates were not typed for the H antigen. In general, *E. coli* O157:H7 and O157:H$^-$ were detected on the whole carcass and were isolated from various beef products. These serotypes are known to cause major outbreaks and sporadic cases of human illnesses, including HC and HUS (CDC, 2003). Hussein and Bollinger (2005b) showed increased prevalence rates of *E. coli* O157 in recent years, which could be explained by the development and adaptation of more sensitive methods (e.g., immunomagnetic separation) to detect *E. coli* O157 strains (Chapman et al., 2001). Methods designed to detect only *E. coli* O157 isolates, however, usually underestimate the true prevalence of STEC (Read et al., 1990).

In the same evaluation, Hussein and Bollinger (2005b) found nonO157 STEC to be more prevalent in beef products than *E. coli* O157. The prevalence rates of nonO157 STEC ranged from 1.7 to 58.0% in packing plants, from 3.0 to 62.5% in supermarkets, and an average of 3.0% in fast food restaurants. In general, the prevalence rates of nonO157 STEC ranged from 2.4 to 30.0% in ground beef, from 17.0 to 49.2% in sausage, from 8.6 to 49.6% in unspecified retail cuts, and from 1.7 to 58.0% in whole carcasses. Testing other beef products such as steaks and ground veal revealed prevalence rates of 19.0% (Zhao et al., 2001) and 62.5% (Samadpour et al., 1994), respectively.

PATHOGENICITY OF STEC OF BEEF CATTLE ORIGIN

For 23 yr, *E. coli* O157:H7 has been recognized (Riley et al., 1983) as the cause of major outbreaks of human illnesses in North America. Over 50% of the nonO157 STEC strains are also known for their pathogenicity (WHO, 1998). Some of these strains have caused major outbreaks of human illnesses worldwide (Mariani-Kurkdjian et al., 1993; Karch et al., 1997; Morabito et al., 1998). Examples of these include *E. coli* O26:H11, O26:H$^-$, O91:H10, O91:H21, O103:H2, O103:H$^-$, O111:H2, O111:H8, and O111:H$^-$ (WHO, 1998; Blanco et al., 2003; Bettelheim, 2006). It is important to note that not all STEC strains are harmful to humans and pathogenicity of a STEC strain depends on production of key virulence factors. Pathogenic STEC strains are often referred to as enterohemorrhagic *E. coli* (**EHEC**) and are known to produce 1 or 2 toxins that resemble those of *Shigella dysenteriae* (O'Brien and Holmes, 1987). These are Shiga Toxin 1 (**Stx1**) and Shiga Toxin 2 (**Stx2**). Because of their toxic effects on Vero (African green monkey kidney) cells (Konowalchuk et al., 1977), pathogenic STEC strains are also known as verotoxin-producing *E. coli*.

Although Stx1 and Stx2 are different proteins, encoded by different genes (stx_1 and stx_2, respectively), their biological activities are similar (Acheson and Keusch, 1996; Neill, 1997). These activities involve depurinating specific residues of the host cell's ribosomes, blocking the binding of aminoacyl tRNA to the ribosomes, and inhibiting protein synthesis (Saxena et al., 1989). The toxins also bind to and damage the endothelial cells in the intestine, kidney, and brain (Acheson and Keusch, 1996). This results in formation of tiny clots and other damage in capillary beds within the kidney (Acheson and Keusch, 1996). Various STEC strains are known to produce different toxins (Bettel-

Table 1. Serotypes or serogroups of Shiga toxin-producing *Escherichia coli* (STEC) isolated from beef cattle[1]

Health category and related isolates
Caused hemolytic uremic syndrome[2]
O2:H5, O2:H6, O2:H7, O2:H29, O5:H$^-$,[3] O6:H$^-$, O8:H2, O8:H19, O8:H21, O20:H19, O22:H8, O25:H2, O26:H11, O26:H$^-$, O26:HUT[4] (Kijima-Tanaka et al., 2005), O45:H2, O49:H$^-$, O55:H$^-$, O84:H$^-$, O86:H$^-$ (Zweifel et al., 2005), O91:H10, O91:H21, O91:H$^-$, O98:H$^-$, O103:H2, O103:H$^-$, O105:H18, O105ac:H18,[5] O111:H8, O111:H$^-$, O112ac:H19,[5] O113:H21, O118:H16, O118:H$^-$, O119:H2, O119:H6, O121:H19, O125:H$^-$, O128:H2 (Bollinger et al., 2005), O128ab:H2,[6] O145:H25, O145:H28, O145:H$^-$, O146:H21, O153:H25, O154:H$^-$, O157:H7, O157:H$^-$, O161:H$^-$, O163:H19, O165:H25, O165:H$^-$, O171:H$^-$, O172:H$^-$, O174:H2,[7] O174:H21, O174:H$^-$, O177:H$^-$, OR:H4,[7] OR:H25, OR:H$^-$, OUT:H2,[8] OUT:H11, OUT:H25 (Sheng et al., 2005), and OUT:H$^-$
Caused other illnesses[9]
O1:H2 (Bollinger et al., 2005), O1:H20, O2:H27 (Zweifel et al., 2005), O8:H$^-$, O8:HUT, O15:H$^-$, O20:H7, O22:H16, O22:H$^-$, O26:H2, O26:H21 (Kijima-Tanaka et al., 2005), O26:H32, O28:H$^-$, O39:H8, O45:H$^-$, O70:H11, O74:H$^-$, O75:H8, O76:H7, O77:H18, O82:H8, O84:H2, O88:H$^-$ (Kijima-Tanaka et al., 2005), O91:H14, O91:HUT, O103:H25, O104:H7, O112:H21, O113:H4, O113:H7, O113:H$^-$, O117:H7, O117:H19, O119:H$^-$, O126:H20, O128ab:H8, O128:H12, O128:HUT, O132:H$^-$, O141:H$^-$, O146:H28, O146:H$^-$, O150:H$^-$, O156:H25, O163:H$^-$, O171:H2, OR:H19, OR:HUT, OUT:H1, OUT:H4, OUT:H7, OUT:H8, OUT:H10, OUT:H14 (Zweifel et al., 2005), OUT:H16, OUT:H18, OUT:H19, OUT:H21, OUT:H28, OUT:H33, OUT:H41, and OUT:HUT
Did not cause illnesses
O1:H18, O1:H45 (Kijima-Tanaka et al., 2005), O2 (Shaw et al., 2004; Renter et al., 2005), O2:H4, O2:H8, O2:H21 (Zweifel et al., 2005), O2:H25, O2:H26, O2:H45 (Zweifel et al., 2005), O2:H$^-$, O3:H7, O3:H12, O4:H4, O5 (Renter et al., 2005), O5:H7, O5:H27, O6 (Renter et al., 2005), O6:H10, O6:H34, O6:H49, O7 (Renter et al., 2005), O7:H10, O8 (Shaw et al., 2004; Renter et al., 2005), O8:H5, O8:H8, O8:H16, O8:H20 (Zweifel et al., 2005), O8:H25, O10, O11:H14, O11:H$^-$, O15 (Shaw et al., 2004; Renter et al., 2005), O15:H16 (Zweifel et al., 2005), O15:H21, O15:HUT (Kijima-Tanaka et al., 2005), O16:H2, O16:H21, O20 (Shaw et al., 2004), O20:H16, O20:H41, O20:H44, O20:HUT, O22 (Renter et al., 2005), O22:H7, O22:H25, O22:HUT, O23:H15, O25, O25:H19, O25:H21, O25:HUT, O26, O28ac:H4,[5] O28:H8, O28ac:H21, O28ac:H$^-$, O29 (Renter et al., 2005), O32:H7, O37:H10, O38 (Renter et al., 2005), O38:HUT, O39 (Renter et al., 2005), O39:H49, O39:H$^-$, O40:H21, O42:H25, O43:H2, O44:H15, O45:H8, O46:H11 (Kijima-Tanaka et al., 2005), O46:H38, O46:HUT (Kijima-Tanaka et al., 2005), O51:H$^-$, O54:H2, O55:H2, O68:H$^-$, O69 (Renter et al., 2005), O70:H8, O74 (Renter et al., 2005), O74:H19, O74:H28, O74:H42, O74:H52, O74:HUT, O75, O75:H1, O76:H2, O76:H21, O77:H39, O79:H19, O79:H$^-$, O79:HUT, O81:H31, O82:H40, O83:H7, O84 (Shaw et al., 2004; Renter et al., 2005), O84:H8, O86, O86:H2 (Bollinger et al., 2006), O86:H19 (Bollinger et al., 2005), O87, O87:H8, O87:H16, O87:H31, O88 (Renter et al., 2005), O88:H21, O88:HUT, O90:H24, O91 (Shaw et al., 2004; Renter et al., 2005), O91:H8, O91:H49, O93:H19, O96 (Renter et al., 2005), O96:H19, O98 (Renter et al., 2005), O101:H40, O102:H21, O103, O103:H11 (Kijima-Tanaka et al., 2005), O103:H14, O105:H8, O105:H$^-$, O106 (Renter et al., 2005), O106:H42, O108 (Renter et al., 2005), O108:H2, O108:H7, O109, O109:H16, O109:H$^-$, O110:H2, O110:H40, O111, O111:H11, O111:H16, O111:H21 (Zweifel et al., 2005), O112:H2, O112:H7, O113 (Shaw et al., 2004; Renter et al., 2005), O113:H11, O113:H19, O113:H27, O114:H$^-$, O115 (Renter et al., 2005), O116 (Renter et al., 2005), O116:H11 (Zweifel et al., 2005), O116:H21, O116:H28, O116:H$^-$, O116:HUT, O117 (Renter et al., 2005), O117:H16 (Zweifel et al., 2005), O117:H21, O119:H16, O119:H17, O119:H18, O119:H25, O119:H40, O119:HUT, O120, O121 (Renter et al., 2005), O121:H7, O123:H2, O123:H8, O123:H38, O124:H19, O125:H2, O125:H16 (Bollinger et al., 2006), O125:H19 (Bollinger et al., 2005; 2006), O125:H27 (Bollinger et al., 2005), O125:H28 (Bollinger et al., 2005), O125:H47, O125:HUT (Bollinger et al., 2005), O126 (Renter et al., 2005), O126:H7, O126:H28, O127:H2 (Bollinger et al., 2006), O127:H19 (Bollinger et al., 2006), O127:H28 (Bollinger et al., 2005), O128ab (Shaw et al., 2004), O128:H16 (Bollinger et al., 2006), O128:H20 (Bollinger et al., 2005), O128ab:H21, O130 (Renter et al., 2005), O130:H11, O130:H38, O130:H43, O132 (Renter et al., 2005), O132:H2, O132:H18, O136 (Renter et al., 2005), O136:H1, O136:H2 (Zweifel et al., 2005), O136:H12, O136:H16, O136:H$^-$ (Zweifel et al., 2005), O136:HUT (Bollinger et al., 2006), O138:H$^-$, O140:H32, O141 (Renter et al., 2005), O141:H7, O141:H8, O142 (Renter et al., 2005), O143:H2, O145 (Renter et al., 2005), O146 (Renter et al., 2005), O146:H1, O148:H8 (Zweifel et al., 2005), O149:H19, O149:HUT, O150 (Renter et al., 2005), O150:H8, O152:H7, O152:H$^-$, O153 (Renter et al., 2005), O153:H8, O153:H19, O153:H21, O153:HR,[10] O153:HUT, O156:H1, O156:H4, O156:HUT, O157, O157:H2, O157:H8, O157:H12, O157:H19, O157:H25, O157:H27, O157:H38, O157:H43, O157:H45, O157:HUT, O158:H28 (Bollinger et al., 2005), O158:HUT (Bollinger et al., 2005), O159 (Renter et al., 2005), O159:H12, O159:H28, O159:HUT, O160:H10, O160:H21, O160:H38, O160:H$^-$, O161:H2, O161:H19, O161:HUT, O162 (Shaw et al., 2004), O162:H7, O162:H27, O163 (Renter et al., 2005), O165:H8, O166:H2 (Bollinger et al., 2005), O166:H6 (Bollinger et al., 2006), O166:H20 (Bollinger et al., 2005), O168 (Shaw et al., 2004), O168:H8, O171 (Renter et al., 2005), O171:H38, O172 (Renter et al., 2005), O172:H16, O172:H21, O174:H8, O174:H40, O174:H43, O174:HUT, O175:H8, O178:H19, O182:H21 (Zweifel et al., 2005), O211:H7, OR (Renter et al., 2005), OR:H8, OR:H10, OR:H12, OR:H18, OR:H27, OR:H31, OR:H32, OR:H34, OR:H39, OX3[11] (Renter et al., 2005), OX7[12] (Renter et al., 2005), OX7:H16, OX13[12] (Renter et al., 2005), OX18[12] (Renter et al., 2005), OX25[12] (Renter et al., 2005), E2981:H$^-$,[13] E11362:H11,[13] E11362:H21, E11362:H$^-$, E40874[13] (Shaw et al., 2004), E54071[13] (Shaw et al., 2004), E54071:H19, OUT (Shaw et al., 2004), OUT:H5, OUT:H20 (Bollinger et al., 2005), OUT:H24, OUT:H27, OUT:H29, OUT:H30, OUT:H32, OUT:H34, OUT:H37, OUT:H38, OUT:H40, OUT:H42, OUT:H49, and OUT:HR.

[1]Unless otherwise indicated, the STEC serotypes or serogroups and their origin are listed in Hussein and Bollinger (2005a).

[2]The STEC serotypes were isolated from humans suffering from hemolytic uremic syndrome (WHO, 1998; Blanco et al., 2003; Bettelheim, 2006).

[3]A nonmotile isolate.

[4]An untypeable H antigen.

[5]Within each of the O28, O105, and O112 serogroups, certain antigenic relationships are represented by 'a,' a common factor and 'c,' a specific factor (Lior, 1994).

[6]Within the O128 serogroup, certain antigenic relationships are represented by 'a,' a common factor and 'b,' a specific factor (Lior, 1994).

[7]A rough O antigen.

[8]An untypeable O antigen.

[9] The STEC serotypes were isolated from humans suffering from a wide range of illnesses such as mild diarrhea, bloody diarrhea, abdominal pain, ulcerative colitis, hemorrhagic colitis, and thrombotic thrombocytopenic purpura (WHO, 1998; Blanco et al., 2003; Bettelheim, 2006).

[10]A rough H antigen.

[11]The O174 antigen was formerly designated as OX3.

[12]OX7, OX13, OX18, and OX25 are provisional designations for new O serogroups.

[13]E2981, E11362, E40874, and E54071 are new provisional serogroups.

Table 2. Serotypes or serogroups of Shiga toxin-producing *Escherichia coli* (STEC) isolated from beef products[1]

Health category and related isolates
Caused hemolytic uremic syndrome[2]
O2:H29, O4:H$^-$,[3] O5:H$^-$, O8:H19, O14:H$^-$, O20:H19, O22:H5, O22:H8, O23:H$^-$, O26:H11, O45:H2, O50:H7, O84:H$^-$, O91:H21, O91:H$^-$, O103:H2, O103:H21, O104:H$^-$, O105:H18, O111:H$^-$, O113:H21, O117:H4, O121:H19, O125:H$^-$, O128:H2, O128ab:H2,[4] O128:H7, O137:H41, O145:H$^-$, O146:H21, O153:H25, O157:H7, O157:H$^-$, O163:H19, O165:H25, O165:H$^-$, O172:H$^-$, O174:H2, O174:H21, OR:H$^-$,[5] OUT:H2,[6] OUT:H11, and OUT:H$^-$
Caused other illnesses[7]
O1:H20, O6:H31, O8:H9, O8:H$^-$, O8:HUT,[8] O15:H27, O15:H$^-$, O17:H18, O22:H16, O22:H$^-$, O60:H19, O62:H$^-$, O74:H$^-$, O75:H8, O82:H8, O91:H14, O110:H$^-$, O113:H4, O113:H$^-$, O117:H7, O146:H28, O171:H2, OR:H2, OR:H7, OR:H21, OUT:H4, OUT:H7, OUT:H8, OUT:H12, OUT:H16, OUT:H18, OUT:H19, OUT:H21, OUT:H28, OUT:H47, and OUT:HUT
Did not cause illnesses
O1, O2, O3, O4:H21, O6, O6:H10, O6:H34, O7:H16, O7:H$^-$, O8, O8:H16, O8:H30, O10, O15, O18ac,[9] O20, O20:H12, O21, O22:H4, O22:H54, O22:HUT, O23:H15, O25, O25:H21, O26, O27:H21, O28:H4, O30:H$^-$, O38:H30, O39, O39:H49, O43:H38 (Li et al., 2005), O45, O46:H8, O46:H38, O46:H$^-$, O54:H2, O55, O55:H9, O56:H56, O57:H$^-$, O59, O62:H8, O65:H48, O68, O70, O73:H16, O73:H31, O73:H$^-$, O74, O74:H37, O74:H39, O75, O79:H$^-$, O81, O81:H26, O84, O86 (Hazarika et al., 2004), O87, O87:H16, O88, O88:H21, O88:H25, O88:H49, O91, O98 (Hazarika et al., 2004), O100:H$^-$, O103, O104, O104:H12, O106, O107:H7, O109, O110, O110:HUT, O111, O111:H7, O111:H16, O112:H2, O113, O113:H19, O116:H21, O116:H$^-$, O117, O117:H8, O119, O121, O123, O125ab:H$^-$,[4] O128, O128:H27, O128:H35, O131, O132, O136, O138:H$^-$, O139, O139:H19, O142, O142:H38, O144:H2, O145, O148:H8, O149:H10, O149:H45, O150:H8, O151:H8, O151:H12, O153, O153:H8, O157, O159:H7, O160, O162, O162:H7, O163, O165, O166:H$^-$, O168, O168:H8, O171, O171:H25, O172, O172:H16, O174,[10] O174:H8, OR, OR:H14, OR:H23, OR:H31, OR:H42, OR:H47, OR:H48, OX6,[11] OX25,[11] OC70:H49,[11] OC86:H49,[11] OUT, OUT:H5, OUT:H6, OUT:H9, and OUT:H23

[1]Unless otherwise indicated, the STEC serotypes or serogroups and their origin are listed in Hussein and Bollinger (2005b).
[2]The STEC serotypes were isolated from humans suffering from hemolytic uremic syndrome (WHO, 1998; Blanco et al., 2003; Bettelheim, 2006).
[3]A nonmotile isolate.
[4]Within each of the O125 and O128 serogroups, certain antigenic relationships are represented by "a," a common factor and "b," a specific factor (Lior, 1994).
[5]A rough O antigen.
[6]An untypeable O antigen.
[7]The STEC serotypes were isolated from humans suffering from a wide range of illnesses such as mild diarrhea, bloody diarrhea, abdominal pain, ulcerative colitis, hemorrhagic colitis, and thrombotic thrombocytopenic purpura (WHO, 1998; Blanco et al., 2003; Bettelheim, 2006).
[8]An untypeable H antigen.
[9]Within the O18 serogroup, certain antigenic relationships are represented by "a," a common factor and "c," a specific factor (Lior, 1994).
[10]The O174 antigen was formerly designated as OX3.
[11]OX6, OX25, OC70, and OC86 are provisional designations for new O serogroups.

heim, 2003; Blanco et al., 2003), and the ability of a specific strain to cause human illnesses depends on its toxin production (Karmali et al., 1985; Jacewicz et al., 2000). Human illnesses, however, have been caused by STEC strains producing Stx1, Stx2, or both toxins (Lior, 1994; Willshaw et al., 1997; Bonnet et al., 1998).

Pathogenic STEC strains not only produce Shiga toxins but also can produce other virulence factors that may increase the severity of human illnesses (Paton and Paton, 2000). These factors include intimin and enterohemolysin, which are responsible for the intimate attachment to the intestinal surface and enterocyte damage, respectively (Saunders et al., 1999; Donnenberg, 2002). These virulence factors (i.e., intimin and enterohemolysin) are encoded by the *E. coli* attaching and effacing (***eae***) and enterohemolysin (***ehxA***) genes, respectively). These genes are found in virtually all *E. coli* O157 strains (Neill, 1997) and appear to be more common in pathogenic nonO157 STEC strains (Beutin et al., 1994). Because some STEC strains lacking *ehxA* and *eae* were shown to cause human illnesses (Neill, 1997), these genes do not appear to be absolutely required for pathogenicity. Thus, it was suggested that each STEC strain should be considered a potential EHEC (Bürk et al., 2002). However, many STEC strains still lack association with human illnesses.

Among the STEC strains that have been isolated from humans with illnesses, a subset of EHEC strains has been found (Levine, 1987) to carry common sets of virulence genes that encode factors for attachment to the host cells, elaboration of effector molecules, and productions of either or both toxins (Stx1 and Stx2). The sets of the virulence genes are found in the locus of enterocyte attachment pathogenicity island, lambdoid bacteriophages, and a large virulence associated plasmid (Newland et al., 1985; McDaniel et al., 1995; Schmidt et al., 1997). Population genetic analysis revealed EHEC strains to compose 2 divergent lineages, termed EHEC 1 and EHEC 2, that are only distantly related but apparently experience similar pathways of virulence gene acquisition (Whittam et al., 1993; McGraw et al., 1999; Reid et al., 2000). The EHEC 1 lineage is comprised solely of a geographically disseminated cluster of strains with related genotypes bearing O157:H7 and O157:H$^-$ serotypes, whereas the EHEC 2 lineage is serotypically and genotypically more diverse. New evidence indicated that the *E. coli* O157:H7 lineage of EHEC is a geographically disseminated complex of highly related genotypes that share common ancestry (Kim et al., 2001). Additionally, the DNA sequence analysis of representative polyphyletic markers showed that genome

diversity accrued through random drift and bacteriophage-mediated event (Kim et al., 2001).

Evaluation of published reports on STEC shedding by beef cattle in the past 25 yr (Table 1) revealed the isolation of strains belonging to 121 O serogroups, 4 new E serogroups, and 373 serotypes. Of these STEC serotypes, 65 were isolated from HUS patients and an additional 62 are known to cause human illnesses such as mild or bloody diarrhea, abdominal cramps, and HC (WHO, 1998; Blanco et al., 2003; Bettelheim, 2006). Evaluation of published reports during the same period on STEC contamination of beef (Table 2) revealed isolation of strains belonging to 98 O serogroups, 2 new provisional O serogroups, and 162 serotypes. Of these, 43 were isolated from HUS patients and an additional 36 are known to cause other human illnesses (WHO, 1998; Blanco et al., 2003; Bettelheim, 2006).

CONCLUSIONS

Shiga toxin-producing *E. coli* are known to cause human illnesses ranging from mild diarrhea to the life-threatening HUS. Because a large number of human illness outbreaks were traced to beef consumption, the roles of beef cattle and their edible products in human infection were evaluated. Worldwide testing of beef cattle and their products revealed high prevalence rates for *E. coli* O157 and other nonO157 serotypes known for their high virulence. Thus, beef cattle are considered reservoirs for these foodborne pathogens. These findings emphasized the critical need for long-term strategies to assure beef safety. Current and future strategies should include educational programs to bring awareness of the STEC problem to beef farmers, ranchers, processors, and consumers. Developing and implementing pre- and postharvest control measures to effectively decrease carriage of these pathogens by beef cattle and to eliminate contamination of their products during processing are essential steps toward sustaining a competitive beef industry.

LITERATURE CITED

Acheson, D. W. K., and G. T. Keusch. 1996. Which Shiga toxin-producing types of *E. coli* are important? ASM News 62:302–306.

Al-Saigh, H., C. Zweifel, J. Blanco, J. E. Blanco, M. Blanco, M. A. Usera, and R. Stephan. 2004. Fecal shedding of *Escherichia coli* O157, *Salmonella,* and *Campylobacter* in Swiss cattle at slaughter. J. Food Prot. 67:679–684.

Anonymous. 2001. Non-O157 Shiga toxin-producing *Escherichia coli* (STEC). http://www.nzfsa.govt.nz/science-technology/data-sheets/non-o157-stec.pdf Accessed Nov. 4, 2004.

Bacon, R. T., K. E. Belk, J. N. Sofos, and G. C. Smith. 2000. Incidence of *Escherichia coli* O157:H7 on hide, carcass and beef trimmings samples collected from United States packing plants. http://www.amif.org/pdf/AMIF%20Executive%20Summary%20Carcass%20Sampling%202000.pdf Accessed Dec. 22, 2006.

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli,* including O157:H7 and non-O157:H7 serotypes, and Salmonella in commercial beef processing plants. J. Food Prot. 66:1978–1986.

Bell, R. G. 1997. Distribution and sources of microbial contamination on beef carcasses. J. Appl. Microbiol. 82:292–300.

Besser, T. E., D. D. Hancock, L. C. Pritchett, E. M. McRae, D. H. Rice, and P. I. Tarr. 1997. Duration of detection of fecal excretion of *Escherichia coli* O157:H7 in cattle. J. Infect. Dis. 175:726–729.

Bettelheim, K. A. 2003. Non-O157 verotoxin-producing *Escherichia coli:* A problem, paradox, and paradigm. Exp. Biol. Med. 228:333–344.

Bettelheim, K. A. 2006. Serotypes of VTEC: The VTEC Table. http://www.microbionet.com.au/vtectable.htm Accessed Jan. 9, 2006.

Beutin, L., D. Geier, H. Steinrück, S. Zimmermann, and F. Scheutz. 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. 31:2483–2488.

Beutin, L., S. Aleksic, S. Zimmermann, and K. Gleier. 1994. Virulence factors and phenotypical traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. Med. Microbiol. Immunol. (Berl.) 183:13–21.

Beutin, L., D. Geier, S. Zimmermann, S. Aleksic, H. A. Gillespie, and T. S. Whittam. 1997. Epidemiological relatedness and clonal types of natural populations of *Escherichia coli* strains producing Shiga toxins in separate populations of cattle and sheep. Appl. Environ. Microbiol. 63:2175–2180.

Beutin, L., D. Geier, S. Zimmermann, and H. Karch. 1995. Virulence markers of Shiga-like toxin producing *Escherichia coli* strains originating from healthy domestic animals of different species. J. Clin. Microbiol. 33:631–635.

Bielaszewska, M., S. Herbert, L. Almut, P. Rita, R. Wolfgang, T. Helmut, C. Alois, J. Jan, B. Kveta, and K. Helge. 2000. Cattle can be a reservoir of sorbitol-fermentation Shiga toxin-producing *Escherichia coli* O157:H$^-$ strains and a source of human disease. J. Clin. Microbiol. 38:3470–3473.

Blanco, J., M. Blanco, J. E. Blanco, A. Mora, E. A. González, M. I. Bernárdez, M. P. Alonso, A. Coira, A. Rodriguez, J. Rey, J. M. Alonso, and M. A. Usera. 2003. Verotoxin-producing *Escherichia coli* in Spain: Prevalence, serotypes, and virulence genes of O157:H7 and non-O157 VTEC in ruminants, raw beef products, and humans. Exp. Biol. Med. 228:345–351.

Bollinger, L. M. 2004. Effects of season and animal factors on prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle. MS Thesis Univ. Nevada, Reno.

Bollinger, L. M., H. S. Hussein, M. R. Hall, and E. R. Atwill. 2006. Isolation, characterization, and potential pathogenicity of Shiga toxin-producing *Escherichia coli* from beef cattle in California during winter and spring. Abstract # Z-014 in Abstracts of the 106th Gen. Meet. Am. Soc. Microbiol (CD Edition). Am. Soc. Microbiol., Washington, DC.

Bollinger, L. M., H. S. Hussein, T. Sakuma, M. R. Hall, and E. R. Atwill. 2005. Isolation and characterization of Shiga toxin-producing *Escherichia coli* from beef cattle. Abstract # Z-024 in Abstracts of the 105th Gen. Meet. Am. Soc. Microbiol (CD Edition). Am. Soc. Microbiol., Washington, DC.

Bonnet, R., B. Souweine, G. Gauthier, C. Rich, V. Livrelli, J. Sirot, B. Joly, and C. Forestier. 1998. Non-O157:H7 Stx2-producing *Escherichia coli* strains associated with sporadic cases of hemolytic-uremic syndrome in adults. J. Clin. Microbiol. 36:1777–1780.

Bürk, C., I. G. B. Braumiller, H. Becker, and E. Märtlbauer. 2002. Nuclease fluorescence assay for the detection of verotoxin genes in raw milk. Lett. Appl. Microbiol. 35:153–156.

Caprioli, A., I. Luzzi, F. Rosmini, C. Resti, A. Edefonti, F. Perfumo, C. Farina, A. Goglio, A. Gianviti, and G. Rizzoni. 1994. Community wide outbreak of hemolytic-uremic syndrome associated with non-O157 verocytotoxin-producing *Escherichia coli.* J. Infect. Dis. 169:208–211.

Caprioli, A., A. Nigrelli, R. Gatti, M. Zavanella, A. M. Blando, F. Minelli, and G. Donelli. 1993. Characterization of verocytotoxin-producing *Escherichia coli* from pigs and cattle in northern Italy. Vet. Rec. 133:323–324.

CDC. 1993. Update: Multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers—Western United States, 1992–1993. Morb. Mortal. Wkly. Rep. 42:258–263.

CDC. 1995. *Escherichia coli* O157:H7 linked to commercially distributed dry-cured salami, Washington and California 1994. Morb. Mortal. Wkly. Rep. 44:157–160.

CDC. 2003. *E. coli* O157 outbreak summaries. http://www.cdc.gov/foodborneoutbreaks/ecoli/STEC2002.pdf Accessed Dec. 22, 2006.

Chapman, P. A., M. Ellin, and R. Ashton. 2001. A comparison of immunomagnetic separation and culture, Reveal and VIP for the detection of *E. coli* O157 in enrichment cultures of naturally contaminated raw beef, lamb, and mixed meat products. Lett. Appl. Microbiol. 32:171–175.

Cieslak, P. R., T. J. Barrett, and P. M. Griffin. 1993. *Escherichia coli* O157:H7 infection from a manured garden. Lancet 342:367.

Čižek, A., P. Alexa, I. Literák, J. Hamřík, P. Novák, and J. Smola. 1999. Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and Norwegian rats from a large-scale farm. Lett. Appl. Microbiol. 28:435–439.

Cobbold, R. N., D. H. Rice, M. Szymanski, D. R. Call, and D. D. Hancock. 2004. Comparison of Shiga-toxigenic *Escherichia coli* prevalences among dairy, feedlot, and cow-calf herds in Washington State. Appl. Environ. Microbiol. 70:4375–4378.

Cowden, J. M. 1997. Scottish outbreak of *Escherichia coli* O157, November-December 1996. Euro Surveill. 2:1–2.

Crump, J. A., A. C. Sulka, A. J. Langer, C. Schaben, A. S. Crielly, R. Gage, M. Baysinger, M. Moll, G. Withers, D. M. Toney, S. B. Hunter, R. M. Hoekstra, S. K. Wong, P. M. Griffin, and T. J. Van Gilder. 2002. An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. N. Engl. J. Med. 347:555–560.

Donnenberg, M. S. 2002. VIII. Infections due to *Escherichia coli* and other enteric gram-negative bacilli. WebMD® Scientific American® Medicine. http://online.statref.com/document.aspx?fxid=48&docid=1046 Accessed Jun. 25, 2003.

Doyle, M. P., and J. L. Schoeni. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. 53:2394–2396.

Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. USA 97:2999–3003.

Ezawa, A., F. Gocho, K. Kawata, T. Takahashi, and N. Kikuchi. 2004. High prevalence of enterohemorrhagic *Escherichia coli* (EHEC) O157 from cattle in selected regions of Japan. J. Vet. Med. Sci. 66:585–587.

Fitzpatrick, M. M., V. Shah, R. S. Trompeter, M. H. Dillon, and T. M. Barrett. 1991. Long term renal outcome of childhood haemolytic uraemic syndrome. BMJ 303:489–492.

Galland, J. C., D. R. Hyatt, S. S. Crupper, and D. W. Acheson. 2001. Prevalence, antibiotic susceptibility, and diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. Appl. Environ. Microbiol. 67:1619–1627.

Gannon, V. P. J., T. A. Graham, R. King, P. Michel, S. Read, K. Ziebell, and R. P. Johnson. 2002. *Escherichia coli* O157:H7 infection in cows and calves in a beef cattle herd in Alberta, Canada. Epidemiol. Infect. 129:163–172.

Geue, L., M. Segura-Alvarez, F. J. Conraths, T. Kuczius, J. Bockemuhl, H. Karch, and P. Gallien. 2002. A long-term study on the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) on four German cattle farms. Epidemiol. Infect. 129:173–185.

Gioffré, A., L. Meichtri, E. Miliwebsky, A. Baschkier, G. Chillemi, M. I. Romano, S. Sosa Estani, A. Cataldi, R. Rodríguez, and M. Rivas. 2002. Detection of Shiga toxin-producing *Escherichia coli* by PCR in cattle in Argentina. Evaluation of two procedures. Vet. Microbiol. 87:301–313.

Griffin, P. M., and R. V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli,* and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60–98.

Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice, and M. G. Paros. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington state. Epidemiol. Infect. 113:199–207.

Hancock, D. D., D. H. Rice, L. A. Thomas, D. A. Dargatz, and T. E. Besser. 1997. Epidemiology of *Escherichia coli* O157 in feedlot cattle. J. Food Prot. 60:462–465.

Hazarika, R. A., D. K. Singh, K. N. Kapoor, R. K. Agarwal, A. B. Pandey, and D. N. Rajkumar. 2004. Detection and characterization of verotoxin-producing *Escherichia coli* (VTEC) isolated from buffalo meat. J. Food Safety 24:281–290.

Henning, P. H., E. B. Tham, A. A. Martin, T. H. Beare, and K. F. Jureidini. 1998. Haemolytic-uraemic syndrome outbreak caused by *Escherichia coli* O111:H$^-$: Clinical outcomes. Med. J. Aust. 168:552–555.

Herriott, D. E., D. D. Hancock, W. E. Keene, and D. H. Rice. 1994. Raw milk associated *E. coli* O157:H7 outbreaks in Oregon. Pages FS17–FS21 in Proc. 8th Int. Congr. Anim. Hygiene, Int. Soc. Anim. Hygiene, St. Paul, MN.

Hussein, H. S., and L. M. Bollinger. 2005a. Prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle. J. Food Prot. 68:2224–2241.

Hussein, H. S., and L. M. Bollinger. 2005b. Prevalence of Shiga toxin-producing *Escherichia coli* in beef. Meat Sci. 71:676–689.

Hussein, H. S., and T. Sakuma. 2005. Prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products. J. Dairy Sci. 88:450–465.

Hussein, H. S., B. H. Thran, M. R. Hall, W. G. Kvasnicka, and R. C. Torell. 2003. Verotoxin-producing *Escherichia coli* in culled beef cows grazing rangeland forages. Exp. Biol. Med. 228:352–357.

Jacewicz, M. S., H. Trachtman, D. S. Newberg, and D. W. K. Acheson. 2000. Abstr. 100th Gen. Meet. Am. Soc. Microbiol. B-102:64. (Abstr.)

Jenkins, C., M. C. Pearce, H. Chart, T. Cheasty, G. A. Willshaw, G. J. Gunn, G. Dougan, H. R. Smith, B. A. Synge, and G. Frankel. 2002. An eight-month study of a population of verocytotoxigenic *Escherichia coli* (VTEC) in a Scottish cattle herd. J. Appl. Microbiol. 93:944–953.

Karch, H., C. Geitz, and H. Schmidt. 1997. Increased incidence of infections with EHEC O103:H2. Notiziaro dell'Istituto Superiore di Sanita 10(3):2.

Karmali, M. A., M. Petric, C. Lim, P. C. Fleming, G. S. Arbus, and H. Lior. 1985. The association between idiopathic hemolytic-uremic syndrome and infection by verotoxin-producing *Escherichia coli.* J. Infect. Dis. 151:775–782.

Khaitsa, M. L., M. L. Bauer, P. S. Gibbs, and G. P. Lardy. 2003. Risk assessment of *Escherichia coli* O157:H7 fecal shedding in North Dakota feedlot cattle. Unified Beef Cattle and Range Research Report. North Dakota State Univ., Fargo.

Khan, A., S. Yamasaki, T. Sato, T. Ramamurthy, A. Pal, S. Datta, N. R. Chowdhury, S. C. Das, A. Sikdar, T. Tsukamoto, S. K. Bhattacharya, Y. Takeda, and G. B. Nair. 2002. Prevalence and genetic profiling of virulence determinants of non-O157 Shiga toxin-producing *Escherichia coli* isolated from cattle, beef, and humans, Calcutta, India. Emerg. Infect. Dis. 8:54–62.

Kijima-Tanaka, M., K. Ishihara, A. Kojima, A. Morioka, R. Nagata, M. Kawanishi, M. Nakazawa, Y. Tamura, and T. Takahashi. 2005. A national surveillance of Shiga toxin-producing *Escherichia coli* in food-producing animals in Japan. J. Vet. Med. B52:230–237.

Kim, J., J. Nietfeldt, J. Ju, J. Wise, N. Fegan, P. Desmarchelier, and A. K. Benson. 2001. Ancestral divergence, genome diversification, and phylogeographic variation in subpopulations of sorbitol-negative, β-glucuronidase-negative enterohemorrhagic *Escherichia coli* O157. J. Bacteriol. 183:6885–6897.

Konowalchuk, J., J. I. Speirs, and S. Stavric. 1977. Vero response to a cytotoxin of *Escherichia coli.* Infect. Immun. 18:775–779.

Lahti, E., M. Eklund, P. Ruutu, A. Siitonrn, L. Rantala, P. Nuorti, and T. Honkanen-Buzalski. 2002. Use of phenotyping and genotyping to verify transmission of *Escherichia coli* O157:H7 from dairy farms. Eur. J. Clin. Microbiol. Infect. Dis. 21:189–195.

Lahti, E., M. Keskimäki, L. Rantala, P. Hyvönen, A. Siitonen, and T. Honkanen-Buzalski. 2001. Occurrence of *Escherichia coli* O157 in Finnish cattle. Vet. Microbiol. 79:239–251.

LeJeune, J. T., T. E. Besser, D. H. Rice, J. L. Berg, R. P. Stillborn, and D. D. Hancock. 2004. Longitudinal study of fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle: Predominance and persistence of specific clonal types despite massive cattle population turnover. Appl. Environ. Microbiol. 70:377–384.

Leomil, L., L. Aidar-Ugrinovich, B. E. C. Guth, K. Irino, M. P. Vettorato, D. L. Onuma, and A. F. P. de Castro. 2003. Frequency of Shiga toxin-producing *Escherichia coli* (STEC) isolates among diarrheic and non-diarrheic calves in Brazil. Vet. Microbiol. 97:103–109.

Leung, P. H. M., W. C. Yam, W. W. S. Ng, and J. S. M. Peiris. 2001. The prevalence and characterization of verotoxin-producing *Escherichia coli* isolated from cattle and pigs in an abattoir in Hong Kong. Epidemiol. Infect. 126:173–179.

Levine, M. M. 1987. *Escherichia coli* that cause diarrhea: Enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J. Infect. Dis. 155:377–389.

Li, F., C. Zhao, W. Zhang, S. Cui, J. Meng, J. Wu, and D. Y. Zhang. 2005. Use of ramification amplification assay for detection of *Escherichia coli* O157:H7 and other *E. coli* Shiga toxin-producing strains. J. Clin. Microbiol. 43:6086–6090.

Lior, H. 1994. Classification of *Escherichia coli.* Pages 31–72 in *Escherichia coli* in Domestic Animals and Humans. C. L. Gyles, ed. CAB Int., Wallingford, UK.

López, E. L., M. M. Contrini, M. Sanz, M. R. Viñas, A. Parma, M. F. De Rosa, and T. G. Cleary. 1997. Perspectives in Shiga-like toxin infections in Argentina. J. Food Prot. 60:1458–1462.

Mariani-Kurkdjian, P., E. Denamur, A. Milon, B. Picard, H. Cave, N. Lambert-Zechovsky, C. Loirat, P. Goullet, P. Sansonetti, and J. Elion. 1993. Identification of a clone of *Escherichia coli* O103:H2 as a potential agent of hemolytic uremic syndrome in France. J. Clin. Microbiol. 31:296–301.

Martin, M. L., L. D. Shipman, J. G. Wells, M. E. Potter, K. Hedberg, I. K. Wachsmuth, R. V. Tauxe, J. P. Davis, J. Arnoldi, and J. Tilleli. 1986. Isolation of *Escherichia coli* O157 from dairy cattle associated with two cases of haemolytic uraemic syndrome. Lancet II:1043.

McDaniel, T. K., K. G. Jarvis, M. S. Donnenberg, and J. B. Kaper. 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proc. Natl. Acad. Sci. USA 92:1664–1668.

McEvoy, J. M., A. M. Doherty, M. Finnerty, J. J. Sheridan, L. McGuire, I. S. Blair, D. A. McDowell, and D. Harrington. 2000. The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. Lett. Appl. Microbiol. 30:390–395.

McEvoy, J. M., A. M. Doherty, J. J. Sheridan, F. M. Thomson-Carter, P. Garvey, L. McGuire, I. S. Blair, and D. A. McDowell. 2003. The prevalence and spread of *Escherichia coli* O157:H7 at a commercial beef abattoir. J. Appl. Microbiol. 95:256–266.

McGraw, E. A., J. Li, R. K. Selander, and T. S. Whittam. 1999. Molecular evolution and mosaic structure of alpha, beta, and gamma intimins of pathogenic *Escherichia coli.* Mol. Biol. Evol. 16:12–22.

Morabito, S., H. Karch, P. Mariani-Kurkdjian, H. Schmidt, F. Minelli, E. Bingen, and A. Caprioli. 1998. Enteroaggregative, Shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of hemolytic-uremic syndrome. J. Clin. Microbiol. 36:840–842.

Morgan, D., C. P. Newman, D. N. Hutchinson, A. M. Walker, B. Rowe, and F. Majid. 1993. Verotoxin producing *Escherichia coli* O157 infections associated with the consumption of yoghurt. Epidemiol. Infect. 111:181–187.

Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli.* Clin. Microbiol. Rev. 11:142–201.

Neill, M. A. 1997. Overview of verotoxigenic *Escherichia coli.* J. Food Prot. 60:1444–1446.

Newland, J. W., N. A. Strockbine, F. F. Miller, A. D. O'Brien, and R. K. Holmes. 1985. Cloning of Shiga-like toxin genes from a toxin converting phage of *Escherichia coli.* Science 230:179–181.

O'Brien, A. D., and R. K. Holmes. 1987. Shiga and Shiga-like toxins. Microbiol. Rev. 51:206–220.

Ogden, I. D., M. MacRae, and N. J. C. Strachan. 2004. Is prevalence and shedding of *E. coli* O157 in beef cattle in Scotland seasonal? FEMS Microbiol. Lett. 233:297–300.

Ohya, T., and H. Ito. 1999. Experimental infection of calves with *Escherichia coli* O157:H7. J. Vet. Med. Sci. 61:1187–1189.

Omisakin, F., M. MacRae, I. D. Ogden, and N. J. C. Strachan. 2003. Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. Appl. Environ. Microbiol. 69:2444–2447.

Orr, P., B. Lorencz, R. Brown, R. Kielly, B. Tan, D. Holton, H. Clugstone, L. Lugtig, C. Pim, S. MacDonald, G. Hammond, M. Moffatt, J. Spika, D. Manuel, W. Winther, D. Milley, H. Lior, and N. Sinuff. 1994. An outbreak of diarrhea due to verotoxin-producing *Escherichia coli* in the Canadian Northwest Territories. Scand. J. Infect. Dis. 26:675–684.

Padola, N. L., M. E. Sanz, J. E. Blanco, M. Blanco, J. Blanco, A. I. Etcheverria, G. H. Arroyo, M. A. Usera, and A. E. Parma. 2004. Serotypes and virulence genes of bovine Shigatoxigenic *Escherichia coli* (STEC) isolated from a feedlot in Argentina. Vet. Microbiol. 100:3–9.

Paiba, G. A., J. C. Gibbens, S. J. S. Pascoe, J. W. Wilesmith, S. A. Kidd, C. Byrne, J. B. M. Ryan, R. P. Smith, I. M. McLaren, R. J. Futter, A. C. S. Kay, Y. E. Jones, S. A. Chappell, G. A. Willshaw, and T. Cheasty. 2002. Faecal carriage of verocytotoxin-producing *Escherichia coli* O157 in cattle and sheep at slaughter in Great Britain. Vet. Rec. 150:593–598.

Paton, J. C., and A. W. Paton. 2000. Shiga toxigenic *Escherichia coli* infections. Sci. Med. (Phila.) May/June:28–37.

Pavia, A. T., C. R. Nichols, D. P. Green, R. V. Tauxe, S. Mottice, K. D. Greene, J. G. Wells, R. L. Siegler, E. D. Brewer, D. Hannon, and P. A. Blake. 1990. Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: Clinical and epidemiologic observations. J. Pediatr. 116:544–545.

Pearce, M. C., C. Jenkins, L. Vali, A. W. Smith, H. I. Knight, T. Cheasty, H. R. Smith, G. J. Gunn, M. E. J. Woolhouse, S. G. B. Amyes, and G. Frankel. 2004. Temporal shedding patterns and virulence factors of *Escherichia coli* serogroups O26, O103, O111, O145, and O157 in a cohort of beef calves and their dams. Appl. Environ. Microbiol. 70:1708–1716.

Pradel, N., V. Livrelli, C. De Champs, J. B. Palcoux, A. Reynaud, F. Scheutz, J. Sirot, B. Joly, and C. Forestier. 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. J. Clin. Microbiol. 38:1023–1031.

Read, S. C., C. L. Gyles, R. C. Clarke, H. Lior, and S. McEwen. 1990. Prevalence of verocytotoxigenic *Escherichia coli* in ground beef, pork, and chicken in Southwestern Ontario. Epidemiol. Infect. 105:11–20.

Reid, S. D., C. J. Herbelin, A. C. Brumbaugh, R. K. Selander, and T. S. Whittam. 2000. Parallel evolution of virulence in pathogenic *Escherichia coli.* Nature 406:64–67.

Reid, T. M. S. 2001. A case study of cheese associated *E. coli* O157 outbreaks in Scotland. Pages 201–212 in Verocytotoxigenic *Escherichia coli.* G. Duffy, P. Garvey, and D. McDowell, ed. Food and Nutrition Press Inc., Trumbull, CT.

Reida, P., M. Wolff, H. W. Pohls, W. Kuhlmann, A. Lehmacher, S. Aleksic, H. Karch, and J. Bockemuhl. 1994. An outbreak due to enterohemorrhagic *Escherichia coli* O157:H7 in a children day care center characterized by person-to-person transmission and environmental contamination. Zentralbl. Bakteriol. 281:534–543.

Remuzzi, G. 1987. HUS and TTP: Variable expression of a single entity. Kidney Int. 32:292–308.

Renter, D. G., J. G. Morris, Jr., J. M. Sargeant, L. L. Hungerford, J. Berezowski, T. Ngo, K. Williams, and D. W. K. Acheson. 2005. Prevalence, risk factors, O-serogroups, and virulence profiles of Shiga toxin-producing bacteria from cattle production environments. J. Food Prot. 68:1556–1565.

Ridell, J., and H. Korkeala. 1993. Special treatment during slaughtering in Finland of cattle carrying an excessive load of dung; meat hygienic aspects. Meat Sci. 35:223–228.

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Well, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. 308:681–685.

Rodrigue, D. C., E. E. Mast, K. D. Greene, J. P. Davis, M. A. Hutchinson, J. G. Wells, T. J. Barrett, and P. M. Griffin. 1995. A university outbreak of *Escherichia coli* O157:H7 infections associated with roast beef and an unusually benign clinical course. J. Infect. Dis. 172:1122–1125.

Samadpour, M., J. E. Ongerth, J. Liston, N. Tran, D. Nguyen, T. S. Whittman, R. A. Wilson, and P. I. Tarr. 1994. Occurrence of Shiga-like toxin-producing *Escherichia coli* in retail fresh seafood, beef, lamb, pork, and poultry from grocery stores in Seattle, Washington. Appl. Environ. Microbiol. 60:1038–1040.

Saunders, J. R., M. J. Sergent, A. J. McCarthy, K. J. Mobbs, C. A. Hart, T. S. Marks, and R. J. Sharp. 1999. Genetics and molecular ecology of *Escherichia coli* O157. Pages 1–25 in *Escherichia coli* O157 in Farm Animals. C. S. Stewart and H. J. Flint, ed. CAB International, Wallingford, UK.

Saxena, S. K., A. D. O'Brien, and E. J. Ackerman. 1989. Shiga toxin, Shiga-like toxin II variant, and ricin are all single-site RNA N-glycosidases of 28S RNA when microinjected into Xenopus oocytes. J. Biol. Chem. 264:596–601.

Schmidt, H., B. Henkel, and H. Karch. 1997. A gene cluster related to type II secretion pathway operons of gram-negative bacteria is located on the large plasmid of enterohemorrhagic *Escherichia coli* O157 strains. FEMS Microbiol. Lett. 148:265–272.

Schurman, R. D., H. Hariharan, S. B. Heaney, and K. Rahn. 2000. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* in beef cattle slaughtered on Prince Edward Island. J. Food Prot. 63:1583–1586.

Sekla, L., D. Milley, W. Stackiw, J. Sisler, J. Drew, and D. Sargent. 1990. Verotoxin-producing *Escherichia coli* in ground beef—Manitoba. Can. Dis. Wkly. Rep. 16:103–105.

Shaw, D. J., C. Jenkins, M. C. Pearce, T. Cheasty, G. J. Gunn, G. Dougan, H. R. Smith, M. E. J. Woolhouse, and G. Frankel. 2004. Shedding patterns of verocytotoxin-producing *Escherichia coli* strains in a cohort of calves and their dams on a Scottish beef farm. Appl. Environ. Microbiol. 70:7456–7465.

Sheng, H., M. A. Davis, H. J. Knecht, D. D. Hancock, J. Van Donkersgoed, and C. J. Hovde. 2005. Characterization of a Shiga toxin-, intimin-, and enterotoxin hemolysin-producing *Escherichia coli* ONT:H25 strain commonly isolated from healthy cattle. J. Clin. Microbiol. 43:3213–3220.

Shinagawa, K., M. Kanehira, K. Omoe, I. Matsuda, D. Hu, D. A. Widiasih, and S. Sugii. 2000. Frequency of Shiga toxin-producing *Escherichia coli* in cattle at a breeding farm and at a slaughterhouse in Japan. Vet. Microbiol. 76:305–309.

Siegler, R. L., M. K. Milligan, T. H. Burningham, R. D. Christofferson, S. Y. Changand, and L. B. Jorde. 1991. Long term renal outcome and prognostic indicators in the hemolytic-uremic syndrome. J. Pediatr. 118:195–200.

Smith, D. R., J. T. Gray, R. A. Moxley, S. M. Younts-Dahl, M. P. Blackford, S. Hinkley, L. L. Hungerford, C. T. Milton, and T. J. Klopfenstein. 2004. A diagnostic strategy to determine the Shiga toxin-producing *Escherichia coli* O157 status of pens of feedlot cattle. Epidemiol. Infect. 132:297–302.

Synge, B. A., G. F. Hopkins, W. J. Reilly, and J. C. M. Sharp. 1993. Possible link between cattle and *E. coli* O157 infection in a human. Vet. Rec. 133:507.

Thran, B. H., H. S. Hussein, M. R. Hall, and S. F. Khaiboullina. 2001. Shiga toxin-producing *Escherichia coli* in beef heifers grazing an irrigated pasture. J. Food Prot. 64:1613–1616.

USDA-APHIS-VS. 1997. An update: *Escherichia coli* O157:H7 in humans and cattle. USDA Animal and Plant Health Inspection Service, Veterinary Service, Centers for Epidemiology and Animal Health, Fort Collins, CO.

Werber, D., A. Fruth, A. Liesegang, M. Littmann, U. Buchholz, R. Prager, H. Karch, T. Breuer, H. Tschape, and A. Ammon. 2002. A multistate outbreak of Shiga toxin-producing *Escherichia coli* O26:H11 infections in Germany, detected by molecular subtyping surveillance. J. Infect. Dis. 186:419–422.

Whittam, T. S., M. L. Wolfe, I. K. Wachsmuth, F. Orskov, I. Orskov, and R. A. Wilson. 1993. Clonal relationship among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. Infect. Immun. 61:1619–1629.

WHO. 1998. Zoonotic non-O157 Shiga toxin-producing *Escherichia coli* (STEC). Report of a WHO Scientific Working Group Meeting. World Health Organization, Geneva, Switzerland.

Willshaw, G. A., S. M. Scotland, and B. Rowe. 1997. Vero-cytotoxin-producing *Escherichia coli.* Pages 421–448 in *Escherichia coli:* Mechanisms of Virulence. M. Sussman, ed. Cambridge Univ. Press, Cambridge, UK.

Wilson, J. B., S. A. McEwen, R. C. Clarke, K. E. Leslie, R. A. Wilson, D. Waltner-Toews, and C. L. Gyles. 1992. Distribution and characteristics of verotoxigenic *Escherichia coli* isolated from Ontario dairy cattle. Epidemiol. Infect. 108:423–439.

Yatsuyanagi, J., S. Saito, and I. Ito. 2002. A case of hemolytic-uremic syndrome associated with Shiga toxin 2-producing *Escherichia coli* O121 infection caused by drinking water contaminated with bovine feces. Jpn. J. Infect. Dis. 55:174–176.

Zhao, T., M. P. Doyle, J. Shere, and L. Garber. 1995. Prevalence of *Escherichia coli* O157:H7 in a survey of dairy herds. Appl. Environ. Microbiol. 61:1290–1293.

Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli,* and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC area. Appl. Environ. Microbiol. 67:5431–5436.

Zweifel, C., S. Schumacher, M. Blanco, J. E. Blanco, T. Tasara, J. Blanco, and R. Stephan. 2005. Phenotypic and genotypic characteristics of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) from Swiss cattle. Vet. Microbiol. 105:37–45.

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Invited Review: Prevalence of Shiga Toxin-Producing *Escherichia coli* in Dairy Cattle and Their Products

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ABSTRACT

The main objective of this review was to assess the role of dairy cattle and their products in human infections with Shiga toxin-producing *Escherichia coli* (STEC). A large number of STEC strains (e.g., members of the serogroups O26, O91, O103, O111, O118, O145, and O166) have caused major outbreaks and sporadic cases of human illnesses that have ranged from mild diarrhea to the life-threatening hemolytic uremic syndrome. These illnesses were traced to O157 and non-O157 STEC. In most cases, STEC infection was attributed to consumption of ground beef or dairy products that were contaminated with cattle feces. Thus, dairy cattle are considered reservoirs of STEC and can impose a significant health risk to humans. The global nature of food supply suggests that safety concerns with beef and dairy foods will continue and the challenges facing the dairy industry will increase at the production and processing levels. In this review, published reports on STEC in dairy cattle and their products were evaluated to achieve the following specific objectives: 1) to assemble a database on human infections with STEC from dairy cattle, 2) to assess prevalence of STEC in dairy cattle, and 3) to determine the health risks associated with STEC strains from dairy cattle. The latter objective is critically important, as many dairy STEC isolates are known to be of high virulence. Fecal testing of dairy cattle worldwide showed wide ranges of prevalence rates for O157 (0.2 to 48.8%) and non-O157 STEC (0.4 to 74.0%). Of the 193 STEC serotypes of dairy cattle origin, 24 have been isolated from patients with hemolytic uremic syndrome. Such risks emphasize the importance and the need to develop long-term strategies to assure safety of foods from dairy cattle.
(**Key words:** foodborne pathogen, *Escherichia coli*, dairy cattle, Shiga toxin)

Abbreviation key: ***eae*** = *E. coli* attaching and effacing gene, ***ehxA*** = enterohemolysin gene, **HUS** = hemolytic uremic syndrome, **HUT** = an untypeable H antigen, **H$^-$** = nonmotile, **OR** = a rough O antigen, **OUT** = an untypeable O antigen, **OX3** = the provisional designation for the O174 antigen, **STEC** = Shiga toxin-producing *E. coli*, **Stx1** = Shiga toxin 1, ***stx*$_1$** = Shiga toxin 1 gene, **Stx2** = Shiga toxin 2, ***stx*$_2$** = Shiga toxin 2 gene.

INTRODUCTION

The safety concern about foods of bovine origin emerged 2 decades ago and increased in recent years because of the growing number of human infections with Shiga toxin-producing *Escherichia coli* (**STEC**). These infections result in illnesses (Griffin and Tauxe, 1991) such as mild diarrhea, bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (**HUS**). Hemolytic uremic syndrome can lead to acute or chronic renal failure, especially in children (Siegler et al., 1991). Other illnesses include strokes (Griffin and Tauxe, 1991) and thrombotic thrombocytopenic purpura that is characterized by nervous system abnormalities (Paton and Paton, 2000).

Dairy cattle are considered reservoirs of O157 (Besser and Hancock, 1994; Hancock et al., 1994; Mechie et al., 1997) and non-O157 STEC (Rahn et al., 1997; Conedera et al., 2001; Kobayashi et al., 2001). Contamination of raw milk (Wells et al., 1991; Sandhu et al., 1996; Chiueh et al., 2002), cheese (Clarke et al., 1994; Pradel et al., 2000; Pradel et al., 2001), or ground beef from dairy cattle (Doyle, 1991) poses a significant risk to humans. Raw milk caused a small number of human illness outbreaks (USDA-APHIS-VS, 1997) that were traced to O157 (Martin et al., 1986; Borczyk et al., 1987; Lahti et al., 2002) and non-O157 STEC (CDC, 1995; Bielaszewska et al., 2000; Allerberger et al., 2001). Outbreaks and sporadic cases of illnesses were also traced to consumption of STEC-contaminated cheese (Deschênes et al., 1996; CDC, 2000) and yogurt (Morgan et al., 1993). Culled dairy cows are mainly used for production of ground beef (USDA-APHIS-VS, 1996a) and therefore can impose a significant health risk (Ostroff et al., 1990; Doyle, 1991; Faith et al., 1996). Because of the increased concern with the safety of ground beef and dairy products in recent years, the objective of this review was

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to assess the role of dairy cattle in human infections with STEC.

VIRULENCE FACTORS OF STEC

In addition to *E. coli* O157:H7, >100 STEC serotypes (e.g., members of the O26, O91, O103, O111, O118, O145, and O166 serogroups) are known to cause human illnesses, including HUS (Paton and Paton, 2000). Pathogenic STEC strains are often referred to as enterohemorrhagic *E. coli* and are known to produce 1 or 2 toxins that resemble those of *Shigella dysenteriae* (O'Brien and Holmes, 1987). These are Shiga toxin 1 (**Stx1**) and Shiga toxin 2 (**Stx2**). The STEC strains can also produce other virulence factors such as those responsible for intimate attachment to the intestinal surface (intimin) and for enterocyte damage (enterohemolysin). In addition to *E. coli* O157:H7, members of the O26, O103, and O111 serogroups are considered the most important enterohemorrhagic *E. coli* (Franke et al., 1995). Although Stx1 and Stx2 are different proteins, encoded by the Shiga toxin 1 gene (***stx*$_1$**) and the Shiga toxin 2 gene (***stx*$_2$**), respectively, their biological activities are quite similar (Neill, 1997). It is worth noting that STEC-related illnesses can be caused by an *E. coli* strain that produces Stx1, Stx2, or both toxins (Lior, 1994). Other virulence factors (intimin and enterohemolysin) are encoded by the *E. coli* attaching and effacing (***eae***) and enterohemolysin (***ehxA***) genes. The *eae* and *ehxA* genes are found in virtually all *E. coli* O157 strains (Neill, 1997) and appear to be more common in the non-O157 STEC strains responsible for human illnesses (Beutin et al., 1994). The *eae* and *ehxA* genes, however, are not absolutely required to cause human illnesses because STEC strains lacking these genes were shown to cause human illnesses (Neill, 1997). Therefore, each STEC strain should be considered a potential enterohemorrhagic *E. coli* (Bürk et al., 2002).

HUMAN INFECTIONS WITH STEC FROM DAIRY CATTLE

Most human STEC infections have been traced to consumption of contaminated foods of bovine origin such as ground beef (Griffin and Tauxe, 1991; USDA-APHIS-VS, 1997) and raw milk (Herriott et al., 1994; Lahti et al., 2002). Other infection routes include manure-contaminated vegetables (Cieslak et al., 1993), person to person (Reida et al., 1994), animal to person (Crump et al., 2002), and contaminated water through drinking (Yatsuyanagi et al., 2002) or swimming (Keene et al., 1994). Beef from dairy cattle, raw milk, and some of the dairy products are considered contributing factors to STEC infections (Martin et al., 1986; Steele et al., 1997). Shiga toxin-producing *E. coli* also can spread from the dairy farm environment to humans by touching the animals (Crump et al., 2002). These pathogens were prevalent at high rates on US dairy farms (Zhao et al., 1995) and were widely distributed in the dairy farm environment (Zhao et al., 1995).

Several outbreaks and sporadic cases of human illnesses caused by infection with *E. coli* O157 (O157:H7, O157:**H**$^-$ [nonmotile], or other isolates that were not typed for the H antigen) from dairy cattle (Table 1) emphasized the role of raw milk as an important vehicle of transmission (Reitsma and Henning, 1996). With regard to *E. coli* O157:H7, 2 US outbreaks (Herriott et al., 1994; USDA-APHIS-VS, 1997) were caused by consumption of raw milk in 1992 (9 cases) and 1993 (6 cases) and were traced to 2 dairies that were licensed to sell raw milk (USDA-APHIS-VS, 1994). Drinking raw milk in the US resulted in HUS cases in Wisconsin (Martin et al., 1986), Washington State (Wells et al., 1991), and Oregon (Keene et al., 1997). Raw milk also caused human illnesses in Canada (Borczyk et al., 1987; Wilson et al., 1996) and Finland (Lahti et al., 2002). Fortunately, the number of raw milk drinkers in Western societies is small. It was estimated that 1 to 2% of the US population consume raw milk (USDA-APHIS-VS, 1994). Raw milk consumption, however, has decreased from 429 million kg in 1980 to 230 million kg in 1990 (USDA, 1992). These statistics may explain why the number of STEC outbreaks caused by raw milk consumption has been small.

Outbreaks caused by *E. coli* O157-contaminated dairy products are limited. For example, the only reported yogurt outbreak occurred in the UK in 1991 (Morgan et al., 1993) and involved 16 people, of which 5 had HUS. Only one cheese outbreak was reported in the US (Wisconsin) and involved 55 people. In addition to the 4 cheese outbreaks that occurred in the UK (Table 1), 2 cheese outbreaks were also reported in France (Anonymous, 1994) and the UK (Curnow, 1994). Other contaminated dairy products (butter and unpasteurized cream) were the cause of O157 infection in one (Reid, 2001) and 7 (CDSC, 1998) adults, respectively, in the UK.

Although many human illness outbreaks were traced to ground beef (USDA-APHIS-VS, 1997), the beef source (beef or dairy cattle) was not known in most cases. This is because ground beef is produced mainly from culled dairy and beef cattle and includes low-value cuts from finished steers or heifers. Cows culled because of health, age, or production reasons are a significant component of the hamburger consumed in the US; 6 million culled dairy cows enter the food chain as ground beef annually (Troutt and Osburn, 1997; National Cat-

Table 1. Human infections with *Escherichia coli* O157[1] from dairy cattle.

Country	Year	Cases (no.)	Age	Illness (no.)	Infection route	Reference
US						
WI	1986	2	<13 mo	HUS[2]	Raw milk	Martin et al., 1986
WA	1986	37	Adults	HC[3] (all), HUS (1), and TTP[4] (3)	Ground beef	Ostroff et al., 1990
	1990	2	Adults	HUS	Raw milk	Wells et al., 1991
OR	1993	14	Adults	Diarrhea	Raw milk	Keene et al., 1997
WI	1998	55	15 mo to 90 yr	Abdominal pain/bloody diarrhea	Cheese	CDC, 2000
PA	2000	47	<10 yr	Abdominal pain/bloody diarrhea (all) and HUS (8)	Visiting a dairy farm	Crump et al., 2002
		4	Adults	Abdominal pain/bloody diarrhea		
WA	2000	5	2 to 14 yr	Abdominal pain/bloody diarrhea (all) and HUS (1)	Visiting a dairy farm	Georgia Division of Public Health, 2002
Canada (Ontario)	1986	60	<5 yr	Abdominal pain/diarrhea (all) and HUS (3)	Raw milk	Borczyk et al., 1987
		14	Adults	Abdominal pain/diarrhea		
Canada (Alberta)	1987	15	Elderly	Abdominal pain/bloody diarrhea	Ground beef	Todd et al., 1993
UK (England)	1991	11	<10 yr	Diarrhea or HC (all) and HUS (5)	Yogurt	Morgan et al., 1993
		5	Adults	Diarrhea or HC		
Canada (Ontario)	1992	1	6 mo	No signs of illness	Raw milk or animal/manure contact	Wilson et al., 1996
UK (England)	1993	4	Children	Abdominal pain (all) and HUS (3)	Raw milk	Wall et al., 1996[5]
		3	Adults	Abdominal pain		
UK (Scotland)	1994	3	Children	Abdominal pain/bloody diarrhea (2) and HUS (1)	Cheese	Reid, 2001[5]
		19	Adults	Abdominal pain/bloody diarrhea		
UK (England)	1996	3	Adults	Abdominal pain/diarrhea	Raw milk	CDSC, 1996[5]
Finland	1997	4	<11 yr	Bloody diarrhea (all) and HUS (2)	Raw milk or animal/manure contact	Lahti et al., 2002
		2	<11 yr	Diarrhea		
		1	A child	No signs of illness		
		1	An adult	No signs of illness		
Czech Republic	1998	2	<6 yr	Bloody diarrhea and HUS	Visiting a dairy farm	Bielaszewska et al., 2000[6]
Finland	1998	1	An adult	Bloody diarrhea	Raw milk	Lahti et al., 2002
UK (England)	1998	7	Adults	Abdominal pain/diarrhea (all) and HUS (4)	Cream	CDSC, 1998[5]
UK (Scotland)	1998	1	3 yr	Abdominal pain/diarrhea	Raw milk	Reid, 2001
		1	12 yr	HUS	Cheese	
		1	An adult	Abdominal pain/diarrhea	Butter	
		3	Adults	Abdominal pain/diarrhea (all) and HUS/death (1)	Cheese	
UK (England)	1999	60	Adults	Abdominal pain/diarrhea	Raw milk	CDSC, 1999a[5]
	1999	3	Adults	Abdominal pain/diarrhea	Cheese	CDSC, 1999b[5]
	2000	1	A child	HUS	Raw milk	International Association of Milk, Food, and Environmental Sanitarians, 2000[5]
		3	Adults	Bloody diarrhea		
Austria	2001	1	6 yr	Bloody diarrhea and HUS	Raw milk	Allerberger et al., 2001[6]

[1]All isolates were *E. coli* O157:H7 unless indicated otherwise.

[2]HUS = Hemolytic uremic syndrome.

[3]HC = Hemorrhagic colitis.

[4]TTP = Thrombotic thrombocytopenic purpura.

[5]The *E. coli* O157 isolates were not typed for the H antigen.

[6]A nonmotile *E. coli* O157 isolate.

Table 2. Human infections with non-O157 Shiga toxin-producing *Escherichia coli* from dairy cattle.

Country	Year	Cases (no.)	Age	Illness (no.)	Serotype	Infection route	Reference
US (MT)	1994	4	Adults	Abdominal pain/bloody diarrhea	O104:H21	Raw milk	CDC, 1995
Canada (Ontario)	1992	2	<2 yr	No signs of illness	O5:H^{-1} or O7:H4	Raw milk or animal/manure contact	Wilson et al., 1996
		6	Adults	No signs of illness	O80:H$^-$, O91:H14, O103:H2, O119:H25, O132:H$^-$, or O146:H21		
France	1992-1993	4	<15 mo	HUS[2] (all) and bloody diarrhea (3)	Unknown	Cheese	Deschênes et al., 1996
Germany	1997	1	2 yr	HUS	OUT[3]:H8	Raw milk	Gallien et al., 1997

[1]H$^-$ = A nonmotile *E. coli* isolate.
[2]HUS = Hemolytic uremic syndrome.
[3]OUT = An untypeable O antigen.

tlemen's Beef Association, 2001). Culled dairy cows account for 17% of the ground beef produced in the US (Troutt and Osburn, 1997). In New York State alone, culled dairy cows contribute 4.8 million kg of hamburger annually (Segelken, 1996). In general, it is difficult to trace a specific ground beef outbreak to dairy or beef cattle. The only 2 known dairy beef outbreaks are summarized in Table 1.

Contact with dairy farm environments through touching the cattle or their manure resulted in severe cases of *E. coli* O157:H7-related illnesses (HUS) in the US (Crump et al., 2002; Georgia Division of Public Health, 2002). The illnesses were associated with farm visitors, and no symptoms were reported for farm residents or employees (Crump et al., 2002). Visitors who washed their hands also did not become ill, indicating a protective effect of hand washing. Two severe cases attributable to visiting a dairy farm were also reported in the Czech Republic and were caused by *E. coli* O157:H$^-$ (Bielaszewska et al., 2000). As shown in Table 1, responses to infection varied from no signs of illness (Wilson et al., 1996; Lahti et al., 2002) to bloody diarrhea and HUS (Lahti et al., 2002) and reflected the immunity status of the affected individuals (mostly children). In general, previous exposure to farm animals is known to decrease the risk of infection with STEC (Wilson et al., 1997). In a study of numerous environmental sites on dairy farms (Blanco et al., 2001), evidence of STEC was found in composite samples of calf feeders (19%), calf-barn surfaces (18%), cow feeders (15%), and cow-barn surfaces (11%). Therefore, contaminated farm environments may remain sources of STEC infection for several months.

Infections with non-O157 STEC from dairy cattle are summarized in Table 2. Most of those were traced to consumption of raw milk, as was the case with the US outbreak (CDC, 1995). The remaining raw milk outbreaks occurred in Canada (Wilson et al., 1996) and Germany (Gallien et al., 1997). Although *E. coli* O5:H$^-$ and O103:H2 serotypes were not shown to cause any signs of illness (Table 2), they are known to cause HUS (WHO, 1998). Several outbreaks were also attributed to consumption of cheeses (those made from unpasteurized milk) in Sweden, the UK, the US (Adams and Motarjemi, 1999), and France (Deschênes et al., 1996). Brie cheese was responsible for the outbreaks in Sweden and the US, whereas local cheeses were responsible for the remaining ones. Contact with dairy cattle manure was also the cause of Canadian outbreaks of STEC (O5:H$^-$ and O7:H4) infections (Wilson et al., 1996).

STEC SEROTYPES FROM MILK AND DAIRY PRODUCTS

Studies on contamination of milk or its products with *E. coli*, in general, have been limited. Testing raw milk and cheese samples (500 and 739, respectively) revealed low levels (<20 cfu/g) of *E. coli* contamination (Coia et al., 2001). Detection of STEC in raw milk, milk filters, and cheeses (Table 3) was reported in Canada, Belgium, Germany, the UK, and the US (WHO, 1998). Padhye and Doyle (1991) reported that *E. coli* O157:H7 was detected in raw milk and cheese. Two STEC serotypes (O91:H21 and an untypeable O antigen [**OUT**]:H$^-$) and members of the O91 and O113 serogroups were isolated from raw milk and cheese (Table 3). None of these isolates is known to cause human illnesses attributable to the consumption of dairy foods. However, *E. coli* O91:H21 and OUT:H$^-$ were isolated from patients with HUS and bloody diarrhea, respectively (WHO, 1998).

Table 3. Shiga toxin-producing *Escherichia coli* from raw milk, milk filters, and cheese.

Source	Serotype or serogroup	Reference
Raw milk	O1:H20, O26:H11, O91:H21, O113:H21, O121:H7, O136:H12, O136:H16, O142:H38, O163:H19, OUT[1]:H8, OUT:H21, and OUT:H^{-}[2]	Steele et al., 1997
	O5:H^{-} and O7:H4	Wilson et al., 1996
	O6:H34	Clarke et al., 1994; Sandhu et al., 1996; Wilson et al., 1996
	O21:H21, O30:H8, O114:H4, and OUT:H^{-}	WHO, 1998
	O22:H8	Wells et al., 1991; Sandhu et al., 1996; Wilson et al., 1996
	O91:H21, O156:H25, and OUT:H7	Clarke et al., 1994
	O103:H2	Wells et al., 1991; Clarke et al., 1994; Sandhu et al., 1996; Wilson et al., 1996
	O104:H21	CDC, 1995
	O113:H4	Sandhu et al., 1996; Wilson et al., 1996
	O157	CDSC, 1996; Wall et al., 1996; CDSC, 1999a; Reid, 2001
	O157:H7	Martin et al., 1986; Borczyk et al., 1987; Padhye and Doyle, 1991; Wells et al., 1991; Abdul-Raouf et al., 1996; Wilson et al., 1996; Keene et al., 1997; Lahti et al., 2002
	O157:H^{-}	Klie et al., 1997; Allerberger et al., 2001
	OUT:H2	Clarke et al., 1994; Wilson et al., 1996
	OUT:H8	Gallien et al., 1997
	OUT:HUT[3]	Chiueh et al., 2002
Milk filters	O26:H11	Wells et al., 1991; Sandhu et al., 1996; Wilson et al., 1996
	O43:H2	Clarke et al., 1994
	O44:H^{-} and OUT:H19	WHO, 1998
	O153:H25	Wells et al., 1991; Sandhu et al., 1996
	OUT:H8	Clarke et al., 1994; Wilson et al., 1996
Cheese	O26:H32 and OUT:H^{-}	WHO, 1998
	O91, O113, and OX3[4]	Pradel et al., 2000
	O91:H21	Pradel et al., 2001
	O116:H^{-}	Clarke et al., 1994
	O157	CDSC, 1999b; Reid, 2001
	O157:H7	Padhye and Doyle, 1991; CDC, 2000

[1]OUT = An untypeable O antigen.
[2]H^{-} = A nonmotile *E. coli* isolate.
[3]HUT = An untypeable H antigen.
[4]OX3 = Provisional designation for the O174 antigen.

Interestingly, the *E. coli* OUT:H8 found in milk filters (Table 3) is known to cause HUS (Table 2). Of *E. coli* isolates (O6:H10, O26:H32, O116:H^{-}, and OUT:H^{-}) found in Canadian cheeses (WHO, 1998), 2 serotypes (O26:H32 and OUT:H^{-}) are known to cause HUS (WHO, 1998). Recently, several STEC strains (members of the O8, O103, O156, and O157 serogroups) were isolated from raw milk tanks in the UK (McKee et al., 2003). Although these isolates had 1 (stx_1 in O103 and stx_2 in O8 and O156) or 2 (stx_2 and *eae* in O157) virulence factors, only members of the O103 and O157 serogroups are known to cause human illnesses (WHO, 1998).

DAIRY CATTLE AS RESERVOIRS OF STEC

Although STEC are not host specific, they are more prevalent in ruminants than in other animals (Riemann and Cliver, 1998). In addition, human illnesses caused by STEC infection have been traced mostly to cattle (Dean-Nystrom et al., 1997). Therefore, cattle (Martin et al., 1986; Wilson et al., 1992; Chapman et al., 1993), including dairy cows, heifers, and calves (Hancock et al., 1994; Zhao et al., 1995; Besser et al., 1997), have been considered reservoirs of STEC. Zhao et al. (1995) estimated that 22 to 50% of the US dairy farms are contaminated with *E. coli* O157:H7. Higher estimates should be considered for STEC prevalence when taking into account other serotypes. The wide distribution of STEC on dairy farms was documented (Rahn et al., 1998) in the high prevalence rates reported for cows (58 of 274; 21.2%) and calves (68 of 135; 50.4%). In a recent study of 3 Japanese dairy farms (Ezawa et al., 2004), the prevalence rates of *E. coli* O157 were 33.7, 18.5, and 0% for heifers, cows, and calves, respectively. The high prevalence rate in heifers was attributed to their close contact during grazing. Other studies (Wells et al., 1991, 1992) illustrated the presence of a wide range of STEC serotypes in dairy cattle at different stages of production. Testing 1266 fecal samples from cows, heifers, and calves from 22 farms, a stockyard, and a packing plant (Wells et al., 1991) revealed the presence of *E. coli* O157:H7, O157:H^{-}, and 27 other non-

O157 serotypes (O10:H$^-$, O15:H27, O22:H8, O22:H40, O25:H$^-$, O26:H11, O45:H2, O45:H$^-$, O76:H21, O84:H2, O84:H$^-$, O103:H2, O103:H$^-$, O111:H$^-$, O116:H21, O121:H7, O145:H$^-$, O153:H25, O163:H19, O171:H2, **OX3** [the provisional designation for the O174 antigen]:H21, OX3:H$^-$, **OR** [a rough O antigen]:H2, OR:H8, OR:H$^-$, OUT:H$^-$, and OUT:**HUT** [an untypeable H antigen]). Except for no detection of *E. coli* O157:H7, a similar distribution of non-O157 STEC was found when 1790 fecal samples from dairy cows and calves were tested (Wilson et al., 1992). The STEC strains included O2:HUT, O3:HUT, O4:H7, O4:H16, O4:H$^-$, O6:HUT, O8:H8, O8:H16, O8:H$^-$, O8:HUT, O9:H$^-$, O11:HUT, O15:H$^-$, O22:H1, O22:H8, O26:H$^-$, O32:H7, O32:H16, O32:H$^-$, O32:HUT, O40:HUT, O43:H2, O43:H6, O43:H12, O82:H$^-$, O87:H$^-$, O103:H2, O103:H6, O103:H12, O103:H16, O103:H39, O103:HUT, O106:HUT, O109:H16, O111:H$^-$, O113:H$^-$, O117:H$^-$, O117:HUT, O119:H16, O121:H7, O121:HUT, O146:H$^-$, O153:H7, O153:H32, O153:H$^-$, O153:HUT, O163:HUT, OX3:H7, OX3:H16, OX3:H21, OX3:H39, OX3:H$^-$, OX3:HUT, OX8 [a provisional designation for a new O antigen]:HUT, OUT:H2, OUT:H3, OUT:H6, OUT:H7, OUT:H8, OUT:H12, OUT:H16, OUT:H21, OUT:H40, OUT:H$^-$, and OUT:HUT. Similar trends were also found in Japan (Tada et al., 1992; Furuhata et al., 1999), where members of the O26, O111, O145, and O157 serogroups (Tada et al., 1992) and 84 non-O157 STEC serotypes (Furuhata et al., 1999) were detected in dairy cattle feces. Furuhata et al. (1999) indicated that different serotypes were associated with specific geographical locations or climates and suggested that certain STEC serotypes may have resistance to hot or cold climates. With regard to sex effects on STEC shedding by dairy cattle, only one study was found (Montenegro et al., 1990). Various STEC serotypes (O3:H$^-$, O10:H21, O22:H8, O39:H40, O75:H8, O82:H8, O82:H40, O91:H10, O104:H21, O105:H18, O113:H21, O116:H21, O126:H20, O126:H21, O136:H12, O139:H8, O156:H21, O157:H7, OR:H18, OUT:H16, and OUT:H29) were recovered from 17 and 9% of the 47 cows and 212 bulls tested, respectively. To better define dairy cattle as reservoirs of STEC, published reports on STEC prevalence in dairy cattle were evaluated.

PREVALENCE OF STEC IN DAIRY CATTLE

Prevalence rates of STEC in dairy cattle are summarized in Table 4. With 2 exceptions (Mechie et al., 1997; Bonardi et al., 1999), the prevalence rates of *E. coli* O157:H7 were based on one-time sampling of cattle feces. In these 2 studies, multiple samples (monthly for 15 mo or 1 yr, respectively) were tested. In Canada (Jackson et al., 1998), a very high prevalence rate (59 of 95; 62.1%) was found in a dairy farm that was associated with a child infection with *E. coli* O157:H7. In the US, *E. coli* O157:H7 prevalence rates ranged from 0.2 to 8.4% for cows, from 1.6 to 3.0% for heifers, and from 0.4 to 40.0% for calves. In Canada, Italy, Japan, and the UK, the corresponding ranges of *E. coli* O157:H7 prevalence rates were 0.3 to 16.1%, 10.0 to 14.1%, and 1.7 to 48.8%, respectively. These data emphasized the significant impact of animal age on epidemiology of *E. coli* O157:H7 as illustrated in the higher prevalence rates for younger (2- to 24-mo old) than for older cattle. Testing dairy cattle on 10 Dutch farms (Heuvelink et al., 1998) revealed prevalence rates of *E. coli* O157:H7 ranging from 0.8 to 22%, with calves (4- to 12-mo old) having the highest rate (21%). High prevalence rates were also reported for young beef cattle (Hancock et al., 1997c) and were attributed to the greater susceptibility to colonization for calves and heifers than for cows (Hancock et al., 1998). Prevalence rates of *E. coli* O157:H7 among dairy calves also appear to be affected by age. For example, the prevalence rate increased from 1.4% (6 of 423) before weaning to 4.8% (25 of 518) after weaning (Garber et al., 1995). Testing a large number of calves from 60 dairy herds in Washington State revealed that *E. coli* O157:H7 was more prevalent in weaned (7 of 1083; 0.65%) than in unweaned (0 of 649; 0%) calves (Hancock et al., 1994).

Factors that may affect the prevalence of *E. coli* O157:H7 in dairy cows were evaluated (Hancock et al.1994; Fitzgerald et al., 2003). In the study by Hancock et al. (1994), similar prevalence rates were reported for lactating (2 of 1273; 0.16%) and dry (1 of 477; 0.21%) cows. In contrast, a higher prevalence rate (43% vs. 22%) was reported for lactating cows than for dry cows (Fitzgerald et al., 2003). The time of fecal sampling was shown (Fitzgerald et al., 2003) to affect the number of dry cows shedding *E. coli* O157:H7 (28% for a.m. vs. 17% for p.m.) but not the lactating cows (average, 43%). Parity and DIM did not influence the number of cows shedding *E. coli* O157:H7 (Fitzgerald et al., 2003). The higher prevalence rates reported by Fitzgerald et al. (2003) could be attributed to the hot weather during the time of fecal sampling (August) in New Mexico. In the study by Hancock et al. (1994), however, the reported prevalence rates were average values of fecal testing of different dairy farms over a 1-yr investigation in Washington State.

Prevalence rates of *E. coli* O157:H7 in culled dairy cows have been variable. For example, prevalence rates ranging from 0.5% in Canada (Clarke et al., 1994) to 16.1% in the UK (Chapman et al., 1997) were reported. The 16.1% prevalence rate (Chapman et al., 1997) was much higher than the rates (1 to 2%) reported for younger dairy cows in the UK (Chapman et al., 1993)

or in the US (Hancock et al., 1994). Prevalence rates for culled dairy cows ranged from 0.9 to 3% in New York State (USDA-APHIS-VS, 1996b). Based on data from 91 dairy farms, Garber et al. (1999) showed a higher prevalence rate (2.8% vs. 0.9%) for *E. coli* O157:H7 in culled cows than in those in production. Rice et al. (1997) tested fecal samples from culled dairy cows on the farm (205 cows from 19 herds) and at slaughter (103 cows from 15 herds) in Idaho, Oregon, and Washington State and reported that 3.4 and 3.9% of the cows on the farm and at slaughter, respectively, were positive for *E. coli* O157:H7. Dairy cattle with downer cow syndrome are those suffering from assorted maladies (e.g., mastitis, calving paralysis, and milk fever) and/or injuries (e.g., during transport) that render them immobile to various degrees (Correa et al., 1993). If their condition does not improve, they are culled from the production herd (Faith et al., 1996; Troutt and Osburn, 1997) and enter the food chain as ground beef. As a result, downer dairy cows harboring STEC at slaughter can be a health risk to humans. This potential risk was recently evaluated at 2 packing plants in Wisconsin over a 6-mo (May to October) period (Byrne et al., 2003). The prevalence rate was higher for downer (10 of 203; 4.9%) than for healthy (3 of 201; 1.5%) cows that were harvested at the same time and were from similar geographical locations.

Prevalence rates of non-O157 STEC in dairy cattle are also summarized in Table 4. Multiple sampling (monthly over a 1-yr period) of cattle feces was used in 2 studies (Hancock et al., 1997a; Conedera et al., 2001) but one-time sampling was used in the others. Worldwide, the prevalence rates ranged from 0.4 to 52.0% for cows, from 1.7 to 74.0% for heifers, and from 1.3 to 68.7% for calves. Non-O157 STEC were more prevalent in dairy cattle than *E. coli* O157 (Table 4). In addition to detecting strains that belonged to 39 O serogroups, a total of 77 STEC serotypes were isolated from dairy cattle feces (Table 4). Of these, 15 (O2:H29, O22:H8, O26:H11, O103:H2, O103:H$^-$, O105:H18, O111:H8, O111:H$^-$, O113:H21, O145:H$^-$, O153:H25, O163:H19, OX3:H21, OUT:H2, and OUT:H25) are known to cause HUS and 20 (O7:H4, O15:H27, O22:H40, O22:H$^-$, O25:H$^-$, O45:H2, O45:H$^-$, O70:H11, O84:H2, O91:H$^-$, O113:H2, O113:H4, O119:H$^-$, O125:H$^-$, O146:H21, O171:H2, OR:H$^-$, OUT:H18, OUT:H21, and OUT:H$^-$) are known to cause other illnesses (WHO, 1998).

In contrast to the *E. coli* O157:H7 data, there was no clear age effect on the prevalence of non-O157 STEC (Table 4). Interestingly, Nielsen et al. (2002) reported

Table 4. Prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in dairy cattle.

		Cows		Heifers[1]		Calves[2]		
Country	Year	no.	%	no.	%	no.	%	Reference
US								
WA and WI	Not reported	1/662	0.2	12/394	3.0	5/210	2.3	Wells et al., 1991[3]
WI	Not reported	13/154	8.4	32/168	19.0			Wells et al., 1991[4]
AL, CA, CO, CT, GA, OR, IA, ID, IL, IN, MA, ME, MI, MN, NC, NE, NH, NY, OH, PA, RI, TN, VA, VT, WA, and WI						28/6894	0.4	USDA-APHIS-VS, 1994[3]
CA[5]	1993					7/85	8.2	Zhao et al., 1995[3]
FL[5]						1/12	8.3	
MD[5]						1/56	1.8	
NE[5]						2/21	9.5	
NY[5]						3/80	3.8	
OH[5]						1/27	3.7	
TN[5]						1/43	2.3	
WI[5]						3/32	9.4	
CA[6]						1/50	2.0	
MN[6]						2/5	40.0	
NY[6]						4/54	7.4	
OH[6]						1/8	12.5	
VT[6]						1/28	3.6	
WA[6]						3/42	7.1	
WA[5]	1993	3/1750	0.17			7/1732	0.4	Hancock et al., 1994[3]
WA[6]	1993–1994	20/4762	0.4	58/3483	1.7	13/1040	1.3	Hancock et al., 1997a[7]
ID,[5] OR,[5] and WA[5]	1994	19/4505	0.4	72/4419	1.6	6/1385	0.4	Besser and Hancock, 1994[3]
WA[5]	1995	46/545	8.4			10/116	8.6	Besser et al., 1997[3]
NV	1999			2/23[8]	9.5			Thran et al., 2001

Continued

Table 4 (Continued). Prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in dairy cattle.

		Cows		Heifers[1]		Calves[2]		
Country	Year	no.	%	no.	%	no.	%	Reference
Japan	1991			1/10	10.0	30/92	32.6	Tada et al., 1992[3]
	1992–1994	7/387	1.8					Miyao et al., 1998[3]
		94/387[9]	24.3					Miyao et al., 1998
Canada	1992–1993	4/886	0.45			10/592	1.7	USDA-APHIS-VS, 1997[3]
	1992–1993	3/291	1.0			4/115	3.5	Rahn et al., 1997[10]
		1/291	0.3			2/115	1.7	
						2/115	1.7	
	1993	26/291[11]	8.9			19/115[12]	16.5	Rahn et al., 1997
UK	1993–1994	80/3131	2.6	25/177	14.1	44/336	13.1	Mechie et al., 1997[3]
Canada	1996	133/886	15.0			289/592	48.8	Sandhu et al., 1996[3]
Italy	1996–1997			138/186	74.0	39/186	21.0	Conedera et al., 2001[7]
Spain	1996–1997	5/197[13]	2.5	23/114[14]	20.2	8/101[15]	7.9	Orden et al., 2002
Germany	1996–1998	131/726	18.0					Zschöck et al., 2000
Italy	1997–1998	23/137	16.1					Bonardi et al., 1999[3]
Japan	1997–1998	5/27	18.5	28/83	33.7			Ezawa et al., 2004[7]
	1998	45/183[16]	24.6	28/88[17]	31.8	19/87[18]	21.8	Kobayashi et al., 2001
	1998–1999	21/106[19]	19.8	3/7[20]	42.9	57/83[20]	68.7	Shinagawa et al., 2000
Denmark	1999	23/160	14.4	24/76	31.6	41/176	23.3	Nielsen et al., 2002[7]
Brazil	1999–2000	33/63[21]	52.0	33/58[22]	57.0	53/122[23]	44.0	Moreira et al., 2003
Denmark	2000–2001	11/452	2.4	51/499	10.2	34/572	5.9	Rugbjerg et al., 2003[7]

[1]6 mo to 2 yr old.

[2]<6 mo old.

[3]Studies tested only for *E. coli* O157:H7.

[4]The prevalence rate for the heifers included all animals that were <2 yr old. The cattle shed STEC isolates belonging to various serotypes (O10:H$^-$ [a nonmotile isolate], O15:H27, O22:H8, O22:H40, O25:H$^-$, O26:H11, O45:H2, O45:H$^-$, O76:H21, O84:H2, O84:H$^-$, O103:H2, O103:H$^-$, O111:H$^-$, O116:H21, O121:H7, O145:H$^-$, O153:H25, O157:H$^-$, O163:H19, O171:H2, OX3 [the provisional designation for the O174 antigen]:H21, OX3:H$^-$, OR [a rough O antigen]:H2, OR:H8, OR:H$^-$, OUT [an untypeable O antigen]:H$^-$, and OUT:HUT [an untypeable H antigen]).

[5]Control herds (herds in which *E. coli* O157 was not previously isolated).

[6]Case herds (herds in which *E. coli* O157 was previously isolated).

[7]The *E. coli* O157 isolates were not typed for the H antigen.

[8]One heifer shed STEC isolates belonging to *E. coli* O26:H$^-$, and the other heifer shed an untypeable isolate.

[9]The cows shed STEC isolates belonging to various serotypes (O2:H25, O2:H29, O16:H2, O16:H21, O22:H8, O22:H$^-$, O42:H25, O45:H8, O45:H$^-$, O70:H8, O70:H11, O74:H19, O74:H52, O74:HUT, O84:H8, O84:H$^-$, O87:H8, O105:H18, O109:H$^-$, O113:H21, O113:H$^-$, O119:H16, O132:H2, O136:H1, O136:H16, O145:H25, O145:H$^-$, O146:H21, O153:H19, O153:H25, O153:HUT, O156:H25, OUT:H7, OUT:H8, OUT:H16, OUT:H18, OUT:H19, OUT:H$^-$, and OUT:HUT) with O45:H8, O45:H$^-$, and O145:H$^-$ being the most frequently isolated serotypes.

[10]The prevalence data as illustrated in the 3 consecutive rows were based on 3 fecal samplings (0, 1, and 3 mo, respectively) of the same herd. No animals tested positive at more than one sampling time.

[11]The cows shed STEC isolates belonging to the serotypes O2:H29, O15:H7, O98:H25, O111:H8, O113:H2, O119:H$^-$, O153:H25, O156:H$^-$, OUT:H21, OUT:H25, and OUT:H$^-$.

[12]The calves shed STEC isolates belonging to the serotypes O7:H4, O8:H9, O26:H11, O45:H2, O103:H$^-$, O113:H4, O153:H25, O156:H$^-$, OUT:H2, OUT:H4, OUT21, and OUT:H$^-$.

[13]Each cow shed a STEC isolate that belonged to a different serogroup (O84, O87, O110, O113, and O136).

[14]The serogroups of the STEC isolates and the number of heifers shedding these isolates were O2 (3), O5 (4), O8 (5), O21 (1), O22 (5), O74 (1), O91 (1), O98 (2), O113 (4), O116 (1), O172 (4), O174 (3), O175 (4), and OUT (11).

[15]The serogroups of the STEC isolates and the number of calves shedding these isolates were O4 (2), O8 (2), O23 (3), O103 (3), O111 (5), and O174 (1).

[16]The serogroups of the STEC isolates and the number of cows shedding these isolates were O8 (1), O15 (4), O22 (2), O26 (4), O38 (2), O55 (1), O73 (1), O84 (4), O88 (1), O103 (2), O104 (1), O111 (2), O113 (3), O116 (2), O125 (1), O136 (2), O153 (2), O157 (1), O158 (1), O163 (1), and OUT (7).

[17]The serogroups of the STEC isolates and the number of heifers shedding these isolates were O1 (1), O2 (1), O8 (4), O22 (2), O26 (1), O38 (1), O84 (1), O103 (1), O113 (2), O116 (3), O119 (2), O123 (1), O136 (1), and OUT (7).

[18]The serogroups of the STEC isolates and the number of calves shedding these isolates were O8 (3), O26 (4), O28 (1), O84 (1), O113 (3), O116 (1), O136 (1), O163 (1), and OUT (4).

[19]The serogroups of the STEC isolates were O26, O111, and O157.

[20]The STEC isolates were untypeable.

[21]STEC isolates that belonged to the serotypes O91:H$^-$ and O157:H$^-$.

[22]The heifers (12 to 24 mo old) shed STEC isolates that belonged to the O157:H$^-$ serotype.

[23]The calves (<12 mo old) shed STEC isolates belonged to the serotypes O29:H$^-$, O91:H$^-$, O112:H$^-$, O119:H$^-$, O125:H$^-$, and O157:H$^-$.

variations in prevalence of non-O157 STEC within Danish dairy calves. A higher prevalence rate (8.6% vs. 0.7%) was found for older (2 to 6 mo) than for younger (<2 mo) calves. Rugbjerg et al. (2003) also reported a higher prevalence rate (11.8% vs. 2.1%) in older (3 to 4 mo) than in younger (1 to 2 mo) calves.

PREVALENCE OF STEC IN RAW MILK

Prevalence of STEC in raw milk was determined in a limited number of studies focusing on *E. coli* O157:H7 in bulk tanks (Wells et al., 1991; Murinda et al., 2002). In the US, a low prevalence rate (2 of 268; 0.75%) was reported in Tennessee (Murinda et al., 2002) and a relatively higher rate (1 of 23; 4.3%) was reported in Wisconsin and Washington State (Wells et al., 1991). Similar (2 of 35; 5.7%) and higher (6 of 37; 16.2%) prevalence rates were reported in the UK (Mechie et al., 1997) and Canada (Cardinal, 1993), respectively. A 2-yr study of bulk milk in the UK (McKee et al., 2003) revealed a lower prevalence rate for STEC, in general, than those reported for *E. coli* O157:H7 (Wells et al., 1991; Cardinal, 1993; Mechie et al., 1997). The presumed route of STEC transmission to raw milk is fecal contamination during milking. This could be eliminated by improving sanitation during the milking process, as shown in a Taiwanese study (Chiueh et al., 2002). Raw milk samples from 407 cows tested negative for STEC, although 8 cows in the production herd tested positive (Chiueh et al., 2002).

DAIRY CATTLE AS TRANSIENT CARRIERS OF STEC

Prevalence rates of STEC in cattle have significant fluctuations over time (Hancock et al., 1998). Repeated fecal testing of cattle herds demonstrated that STEC are, at least occasionally, present on most farms (Hancock et al., 1997a, b). In general, cattle have not been reported as long-term carriers of STEC, and fecal shedding has not been associated with any cattle disease (Garber et al., 1995; Hancock et al., 1997b,c). Studies with dairy cattle showed the transient presence of *E. coli* O157:H7 (Hancock et al., 1994; 1997a). In an initial study (Hancock et al., 1994), *E. coli* O157:H7 was found on 5 of the 60 dairy farms that were sampled once. In a following study (Hancock et al., 1997a), 8 of the initially negative farms were sampled monthly for 3 to 12 mo, and *E. coli* O157:H7 was detected in 4 of those farms. It was concluded, therefore, that repeated sampling is needed to establish an accurate prevalence status of STEC.

Fecal shedding of STEC by dairy cattle has been shown to follow a seasonal trend. In the US, the highest prevalence rates of *E. coli* O157:H7 in dairy cattle were in warm weather (Hancock et al., 1997a,b; Garber et al., 1999), which is consistent with the timing of most human illness outbreaks (USDA-APHIS-VS, 1997). Monthly fecal sampling of dairy cattle in Idaho, Oregon, and Washington State (Hancock et al., 1997b) over a 6-mo period (July to December) revealed a sharp decline in *E. coli* O157:H7 prevalence in cold weather (November and December). An investigation of *E. coli* O157 on 9 dairy herds for 13 mo revealed prevalence rates that were several times higher in warm (June to October) than in cold (December to March) weather (Hancock et al., 1997a). The prevalence of *E. coli* O157:H7 peaked at 2.6% in June and was lowest (0%) in December. In another study covering 20 states (CA, FL, ID, IL, IN, IA, KY, MI, MN, MO, NM, NY, OH, OR, PA, TN, TX, VT, WA, and WI), Garber et al. (1999) tested 4361 fecal samples from cows on 91 dairy farms over a 6-mo period (February to July) and reported a higher prevalence rate (52.9% vs. 7.0%) in the summer than in the spring. Herds in the Southern states had a higher prevalence rate (61.9% vs. 12.9%) than those in the Northern states. Hancock et al. (1997b) observed a significant correlation between multiple fecal testing of dairy herds and prevalence of *E. coli* O157:H7 and reported that some herds had tendencies to have higher or lower prevalence rates that were somewhat stable over time.

In a 1-yr study, similar seasonal effects on *E. coli* O157:H7 prevalence in dairy cows, heifers, and calves were detected in Canada, Denmark, Italy, and the UK. In the UK (Mechie et al., 1997), testing of 3593 fecal samples showed the highest prevalence rates (average, 8.3%) to occur from May to July and the lowest rate (0%) to occur from December to April. In Denmark (Rugbjerg et al., 2003), testing of 1706 fecal samples also showed a higher prevalence rate in summer than in winter (9.9% vs. 2.9%). In Northern Italy (Bonardi et al., 1999), testing of 450 fecal samples revealed a higher prevalence rate (17.5% vs. 2.9%) in the warm months (April to October) than in the cold months (November to January). In the same region (Conedera et al., 2001), testing of 650 fecal samples showed higher prevalence rates in August and September (average, 20%) than in January and February (average, 15%). In Canada (Van Donkersgoed et al., 1999), testing of 1247 fecal samples from culled cows at slaughter also showed *E. coli* O157:H7 to be most prevalent from June to August. Except for one investigation (Thran et al., 2001), no studies evaluating prevalence of non-O157 STEC over time were found. Testing fecal samples from 23 heifers over 1 yr resulted in detecting STEC only during winter at a prevalence rate of 9.5% (Thran et al., 2001). Interestingly, the STEC isolates belonged to 2 non-O157 serotypes (O26:H$^-$ and OUT:HUT).

Table 5. Virulence factors[1] in *Escherichia coli* O157[2] from dairy cattle.

Toxin genes		Other genes		
stx$_1$	*stx*$_2$	*eae*	*ehxA*	Reference
+	–	ND[3]	ND	Wells et al., 1991[4]; Faith et al., 1996
		+		Sandhu et al., 1996
			+	Byrne et al., 2003
–	+	ND	ND	Montenegro et al., 1990; Ostroff et al., 1990[4]; Wells et al., 1991; Tada et al., 1992[4]; Faith et al., 1996; Wilson et al., 1996; Cerqueira et al., 1999; Furuhata et al., 1999[4]; Conedera et al., 2001
		+		Sandhu et al., 1996; Wilson et al., 1996; Heuvelink et al., 1998[5]; Bonardi et al., 1999[5]
			+	Allerberger et al., 2001[4,6]; Lahti et al., 2002[6]; Murinda et al., 2002; Byrne et al., 2003
			–	Bielaszewska et al., 2000[6]; Lahti et al., 2002[6]
+	+	ND	ND	Wells et al., 1991[2,6]; Tada et al., 1992; Faith et al., 1996; Conedera et al., 2001
		+		Sandhu et al., 1996; Heuvelink et al., 1998[5]; Jung et al., 2000
			+	Kobayashi et al., 2001[5]; Murinda et al., 2002; Byrne et al., 2003
			–	Byrne et al., 2003

[1]The virulence factors include Shiga toxin 1 (*stx*$_1$), Shiga toxin 2 (*stx*$_2$), *E. coli* attaching and effacing (*eae*), and enterohemolysin (*ehxA*) genes.

[2]All isolates were *E. coli* O157:H7 unless indicated otherwise.

[3]ND = Not determined.

[4]Studies did not test for presence of the toxin genes but examined their expression (e.g., cytotoxicity).

[5]The *E. coli* O157 isolates were not typed for the H antigen.

[6]A nonmotile *E. coli* O157 isolate.

PATHOGENICITY OF STEC FROM DAIRY CATTLE

A few studies evaluated distribution of the virulence genes among STEC isolates from dairy cattle (Sandhu et al., 1996; Wilson et al., 1996; Byrne et al., 2003). For example, Wilson et al. (1996) reported the presence of the *eae* gene in 37% of O157:H7 and non-O157:H7 STEC isolates. Sandhu et al. (1996) also showed that the *eae* gene was more frequently found (42% vs. 18%) in STEC from calves than from cows. In the same study, 35% of *E. coli* O157:H7 strains were positive for the *eae* gene. Of the *eae*-positive isolates, 73% produced only Stx1, suggesting a strong association between certain O serogroups (O5, O26, O69, O84, O103, O111, O145, and O157) and the *eae* gene. It was concluded, therefore, that Stx1 production is more frequently associated with *eae*-positive than with *eae*-negative STEC strains (Sandhu et al., 1996). In another study, 57 *E. coli* O157:H7 isolates were tested for the presence of the *stx*$_1$, *stx*$_2$, *eae*, and *ehxA* genes (Byrne et al., 2003). Results showed 67% of the isolates to have the 4 genes, whereas the remaining isolates had various combinations of 3 genes at different rates (19% had *stx*$_1$, *eae*, and *ehxA*; 9% had *stx*$_2$, *eae*, and *ehxA*; and 5% had *stx*$_1$, *stx*$_2$, and *eae*). The presence of these virulence factors was evaluated for O157 (Table 5) and non-O157 STEC (Table 6) isolates from dairy cattle.

With regard to *E. coli* O157 (Table 5), some isolates had *stx*$_1$, *stx*$_2$, or both genes. The ability of this serotype, as well as other non-O157:H7 STEC strains, to cause human illnesses depends on the production of Stx1, Stx2, or both toxins (Karmali et al., 1985). All *E. coli* O157:H7 isolates that were tested for the presence of the *eae* gene gave positive results (Table 5). Except for one isolate (Byrne et al., 2003), all of the tested ones also were positive for the *ehxA* gene. Beutin et al. (1994) investigated human infections with O157 and non-O157 STEC (O26:H11, O111:H8, and O145:H$^-$) and reported that they had the *eae* and *ehxA* genes at high rates (92 and 88%, respectively). Beutin et al. (1994) also reported that *E. coli* O157:H7 was responsible for the most severe illnesses (hemorrhagic colitis and HUS). The data in Table 5, therefore, suggest a high level of pathogenicity for several *E. coli* O157:H7 isolates of dairy cattle origin (Murinda et al., 2002; Byrne et al., 2003).

Table 6 shows that 44 STEC serotypes and members of 2 O serogroups (O87 and O88) had only *stx*$_1$, whereas 69 serotypes and members of 11 O serogroups (O1, O8, O15, O21, O28, O104, O111, O158, O172, O174, and O175) had only *stx*$_2$. Additionally, 41 serotypes and members of 6 O serogroups (O23, O38, O55, O73, O123, and O125) had both genes. Although isolates from 4 serotypes had only *stx*$_1$ (OUT:H$^-$ and OUT:HUT) or *stx*$_2$ (OUT:H8 and OUT:H21), the isolates from each of the remaining serotypes exhibited different toxin genotypes. For example, isolates from each of the O22:H$^-$, O98:H$^-$, O113:H21, O145:H21, O153:H25, O153:H$^-$, and OUT:H7 serotypes had *stx*$_1$ or *stx*$_2$. Isolates from each of the O105:H18 and O111:H$^-$ serotypes had *stx*$_1$ or both *stx*$_1$ and *stx*$_2$, whereas those from each of the O22:H16, O45:H8, O45:H9, O45:HUT, O82:H8, O116:H21, O153:H19, O153:HUT, O157:H$^-$, OUT:H16,

Table 6. Virulence factors[1] in non-O157 Shiga toxin-producing *Escherchia coli* from dairy cattle.

Toxin genes		Other genes			
stx$_1$	*stx*$_2$	*eae*	*ehxA*	Serotype[2] or serogroup	Reference
+	–	ND[3]	ND	O3:H^{-}[4], O10:H21, **O105:H18**, and O136:H12	Montenegro et al., 1990
				O10:H^{-}, **O26:H11**, O45:H2, O45:H^{-}, O76:H21, O84:H2, **O103:H2**, **O103:H^{-}**, **O111:H^{-}**, O121:H7, **O153:H25**, **OUT[5]:H^{-}**, and OUT:HUT[6]	Wells et al., 1991[7]
				O1:H7, **O5:H^{-}**, **O26:H11**, **O26:H^{-}**, O98:H25, **O98:H^{-}**, **O103:H2**, **O103:H^{-}**, O104:H32, **O111:H^{-}**, **O113:H21**, O116:H16, O145:H8, O145:H11, O145:H16, O153:H^{-}, and OUT:HUT	Tada et al., 1992[7]
				O22:H^{-}, O70:H11, O74:H52, **O113:H21**, O136:H1, **O145:H25**, O156:H25, OUT:H7, and **OUT:H^{-}**	Miyao et al., 1998[7]
				OUT:H^{-}	Cerqueira et al., 1999
				O18:H25, **O26:H11**, **O26:H^{-}**, **O103:H2**, **O103:H^{-}**, O105:H8, **O105:H18**, **O111:H^{-}**, O126:H^{-}, O153:H2, and **OUT:H^{-}**, OUT:HUT	Furuhata et al., 1999[7]
				O26:H^{-}	Thran et al., 2001
		+		**O5:H^{-}**, **O103:H2**, and O119:H25	Wilson et al., 1996
				O5, O84, O87, O98, and O103	Orden et al., 2002
			+	O26 and O103	Kobayashi et al., 2001
				O26	Zschöck et al., 2000
			–	O26	Kobayashi et al., 2001
		–	ND	O91:H41 and O146:H21	Wilson et al., 1996
				O26:H46, **O26:H^{-}**, and **OUT:H^{-}**	Jung et al., 2000
				OUT and O136	Orden et al., 2002
			+	O26, O84, O88, and O136	Kobayashi et al., 2001
				OUT:HUT	Vernozy-Rozand et al., 2002
			–	O26	Kobayashi et al., 2001
				OUT:HUT	Vernozy-Rozand et al., 2002
–	+	ND	ND	**O22:H8**, O39:H40, O75:H8, O82:H8, **O91:H10**, **O113:H21**, O116:H21, O126:H20, O126:H21, O139:H8, OUT:H16, and OUT:H29	Montenegro et al., 1990
				O22:H8, O116:H21, **O145:H^{-}**, **O163:H19**, O171:H2, OX3[8]:H21, OX3:H^{-}, OR[9]:H2, OR:H8, and OR:H^{-}	Wells et al., 1991
				O76:H8, O84:H^{-}, **O98:H^{-}**, O113:H^{-}, O145:H19, **O145:H^{-}**, OUT:H21, and OUT:HUT	Tada et al., 1992
				O2:H25, **O2:H29**, O16:H2, O16:H21, **O22:H8**, O22:H^{-}, O42:H25, O45:H8, O45:H^{-}, O74:H19, O74:HUT, O84:H^{-}, O109:H16, O109:H^{-}, **O113:H21**, O113:H^{-}, O132:H2, O136:H16, **O145:H^{-}**, O146:H21, O153:H19, **O153:H25**, O153:HUT, OUT:H7, OUT:H8, OUT:H16, OUT:H19, **OUT:H^{-}**, and OUT:HUT	Miyao et al., 1998
				O22:H16, OUT:H18, and OUT:H28	Cerqueira et al., 1999
				O2:H^{-}, O2:H12, **O2:H29**, O45:H9, O45:H^{-}, O45:HUT, O82:H8, O82:HUT, O116:H21, O124:H19, O126:H19, O126:H^{-}, O132:H2, O132:H9, O132:H^{-}, O153:H19, O153:H42, O153:H^{-}, O163:H9, OUT:H19, OUT:H21, OUT:H49, **OUT:H^{-}**, and OUT:HUT	Furuhata et al., 1999
				OUT:HUT	Thran et al., 2001
		+		O80:H^{-}	Wilson et al., 1996
				O172	Orden et al., 2002
			+	O111	Kobayashi et al., 2001
			–	O104	Kobayashi et al., 2001
		–	ND	O7:H4 and O132:H^{-}	Wilson et al., 1996
				OUT:H21 and **OUT:H^{-}**	Jung et al., 2000
				O2, O8, O21, O22, O74, O91, O113, O116, O174, O175, and OUT	Orden et al., 2002
			+	O28, O113, O116, O153, and O163	Kobayashi et al., 2001
				OUT:HUT	Vernozy-Rozand et al., 2002
			–	O1, O2, O15, O113, and O158	Kobayashi et al., 2001
				OUT:HUT	Vernozy-Rozand et al., 2002
				OUT:H7 and OUT:H21	Chiueh et al., 2002
+	+	ND	ND	O82:H8, O82:H40, O104:H21, O116:H21, O156:H21, and OR:H18	Montenegro et al., 1990
				O15:H27 and **O22:H8**	Wells et al., 1991
				O111:H^{-}	Tada et al., 1992

Continued

Table 6 (Continued). Virulence factors[1] in non-O157 Shiga toxin-producing *Escherchia coli* from dairy cattle.

Toxin genes		Other genes			
stx$_1$	*stx*$_2$	*eae*	*ehxA*	Serotype[2] or serogroup	Reference
				O45:H8, O45:H$^-$, O70:H8, O84:H8, O87:H8, O105:H18, OUT:H8, OUT:H16, OUT:H18, and **OUT:H$^-$**	Miyao et al., 1998
				O22:H16, O82:H8, OUT:H21, and OR:H19	Cerqueira et al., 1999
				O28ac[10]:H25, O45:H9, O45:H28, O45:HUT, O88:H25, **O91:H21**, **O111:H$^-$**, O126:H$^-$, O153:H19, O158:H16, O163:H9, O163:H49, OUT:H9, OUT:H11, and OUT:HUT	Furuhata et al., 1999
		+		O111	Orden et al., 2002
		–	ND	O111:HUT, O153:HUT, and **OUT:H2**	Jung et al., 2000
				O22 and O23	Orden et al., 2002
			+	O22, O38, O123, O125, and O163	Kobayashi et al., 2001
				OUT:HUT	Vernozy-Rozand et al., 2002
			–	O22, O38, O55, and O73	Kobayashi et al., 2001
				OUT:H51	Chiueh et al., 2002

[1]The virulence factors include Shiga toxin 1 (*stx*$_1$), Shiga toxin 2 (*stx*$_2$), *E. coli* attaching and effacing (*eae*), and enterohemolysin (*ehxA*) genes.

[2]The serotypes in bold are known (WHO, 1998; Blanco et al., 2003) to cause hemolytic uremic syndrome.

[3]ND = Not determined.

[4]H$^-$ = A nonmotile *E. coli* isolate.

[5]OUT = An untypeable O antigen.

[6]HUT = An untypeable H antigen.

[7]Studies did not test for presence of the toxin genes but examined their expression (e.g., cytotoxicity).

[8]OX3 = Provisional designation for the O174 antigen.

[9]OR = A rough O antigen.

[10]Reflecting a certain antigenic relationship within the O28 serogroup in which "a" represents the common factor and "c" represents a specific factor (Lior, 1994).

OUT:H18, OUT:H21, OUT:H$^-$, and OUT:HUT serotypes had *stx*$_2$ or both *stx*$_1$ and *stx*$_2$. Finally, isolates from each of the serotypes O45:H$^-$ and O126:H$^-$ had *stx*$_1$, *stx*$_2$, or both genes. Table 6 shows that 20 STEC serotypes (in bold) commonly isolated from dairy cattle are known to cause HUS (WHO, 1998; Blanco et al., 2003). Several other serotypes (O1:H7, O7:H4, O15:H27, O22:H16, O22:H$^-$, O45:H2, O45:H$^-$, O70:H11, O80:H$^-$, O82:H8, O84:H2, O104:H21, O116:H21, O126:H21, O126:H$^-$, O132:H$^-$, O145:H16, O146:H21, O153:H2, O171:H2, OR:H$^-$, OUT:H18, OUT:H19, and OUT:H21) in Table 6 are also known to cause diarrhea, bloody diarrhea, or hemorrhagic colitis (WHO, 1998).

Based on potential toxicity by having *stx*$_1$ and/or *stx*$_2$ genes, it is clear from Table 6 that it is not necessary for an isolate to have and express both genes to cause human illnesses. With regard to other virulence genes (*eae* and *ehxA*), unfortunately, the 20 STEC serotypes in Table 6 that are known to cause HUS were not evaluated for presence of the *ehxA* gene. Of these serotypes, 5 (O5:H$^-$, O26:H$^-$, O103:H2, OUT:H2, and OUT:H$^-$) were evaluated for presence of the *eae* gene, and only 2 (O5:H$^-$ and O103:H2) tested positive. The ability of these *eae*-positive isolates to produce a functional intimin, however, was not reported. Investigating STEC pathogenicity (HUS cases) as affected by the presence of *eae* and *ehxA* genes (Karch et al., 1997) revealed that most STEC isolates were *eae*-positive. Several STEC strains involved in severe human illnesses (e.g., HUS), however, have lacked this gene or did not express a functional intimin (Keskimäki et al., 1997). This suggests that STEC strains lacking the *eae* gene can colonize the small intestine by expressing additional adherence factors. Studies of STEC in HUS patients showed that both *eae* and *ehxA* genes were found in virtually all *E. coli* O157 strains (Beutin et al., 1994) but were more variably present among the non-O157 strains (Neill, 1997). The data in Tables 5 and 6 support this conclusion. Although the *eae* and *ehxA* genes appear to be more common in the STEC strains associated with human illnesses, they are not absolutely required for pathogenicity, as strains lacking these genes have been shown to cause human illnesses (Neill, 1997).

CONCLUSIONS

The role of dairy cattle and their products in human infections with STEC was evaluated. These pathogens cause human illnesses ranging from mild diarrhea to the life-threatening HUS. Most STEC outbreaks were traced to the consumption of ground beef or dairy products. Contact with dairy farm environments by urban visitors also caused outbreaks of human illnesses. Long-term and short-term testing of dairy cattle feces world-

wide showed high prevalence rates for O157 (ranging from 0.2 to 48.8%) and non-O157 STEC (ranging from 0.4 to 74.0%). Shiga toxin-producing *E. coli* were also isolated from raw milk, milk filters, and dairy products such as yogurt and soft cheeses. Dairy cattle, therefore, are considered reservoirs of STEC. The data summarized in this review showed 193 STEC serotypes to derive from dairy cattle origin. Of these serotypes, 24 have been isolated from HUS patients. Considering the wide distribution of STEC on dairy farms, the high prevalence rates reported, and the isolation of several serotypes of high virulence from dairy cattle or their products, long-term strategies to assure safety of foods from dairy cattle should be developed. These strategies should focus on establishing educational programs to bring awareness of the STEC problem to dairy farmers, processors, and consumers. Developing and implementing pre- and postharvest control methods to effectively decrease STEC carriage by dairy cattle and to eliminate contamination of their products during processing are essential steps toward sustaining a competitive dairy industry.

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REFERENCES

Abdul-Raouf, U. M., M. S. Ammar, and L. R. Beuchat. 1996. Isolation of *Escherichia coli* O157:H7 from some Egyptian foods. Int. J. Food Microbiol. 29:423–426.

Adams, M., and Y. Motarjemi. 1999. Basic Food Safety for Health Workers. World Health Organization, Geneva, Switzerland.

Allerberger, F., M. Wagner, P. Schweiger, H.-P. Rammer, A. Resch, M. P. Dierich, A. W. Friedrich, and H. Karch. 2001. *Escherichia coli* O157 infections and unpasteurised milk. Eurosurveillance 6:147–151.

Anonymous. 1994. Two clusters of haemolytic uraemic syndrome in France. Commun. Dis. Rep. Wkly. 4:1.

Besser, T. E., and D. D. Hancock. 1994. Surveillance for *Escherichia coli* O157:H7: Seasonal variation and duration of infection in cattle. Pages FS13–FS16 in Proc. 8th Int. Congr. Anim. Hygiene. Int. Soc. Anim. Hygiene, St. Paul, MN.

Besser, T. E., D. D. Hancock, L. C. Pritchett, E. M. McRae, D. H. Rice, and P. I. Tarr. 1997. Duration of detection of fecal excretion of *Escherichia coli* O157:H7 in cattle. J. Infect. Dis. 175:726–729.

Beutin, L., S. Aleksic, S. Zimmermann, and K. Gleier. 1994. Virulence factors and phenotypical traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. Med. Microbiol. Immunol. 183:13–21.

Bielaszewska, M., S. Herbert, L. Almut, P. Rita, R. Wolfgang, T. Helmut, C. Alois, J. Jan, B. Kveta, and K. Helge. 2000. Cattle can be a reservoir of sorbitol-fermentation Shiga toxin-producing *Escherichia coli* O157:H$^-$ strains and a source of human disease. J. Clin. Microbiol. 38:3470–3473.

Blanco, J., M. Blanco, J. E. Blanco, A. Mora, M. P. Alonso, E. A. González, and M. I. Bernárdez. 2001. Epidemiology of verocytotoxigenic *Escherichia coli* (VTEC) in ruminants. Pages 113–148 in Verocytotoxigenic *Escherichia coli*. G. Duffy, P. Garvey, and D. McDowell, ed. Food and Nutrition Press Inc., Trumbull, CT.

Blanco, J., M. Blanco, J. E. Blanco, A. Mora, E. A. González, M. I. Bernárdez, M. P. Alonso, A. Coira, A. Rodriguez, J. Rey, J. M. Alonso, and M. A. Usera. 2003. Verotoxin-producing *Escherichia coli* in Spain: Prevalence, serotypes, and virulence genes of O157:H7 and non-O157 VTEC in ruminants, raw beef products, and humans. Exp. Biol. Med. 228:345–351.

Bonardi, S., E. Maggi, A. Bottarelli, M. L. Pacciarini, A. Ansuini, G. Vellini, S. Morabito, and A. Caprioli. 1999. Isolation of Verocytotoxin-producing *Escherichia coli* O157:H7 from cattle at slaughter in Italy. Vet. Microbiol. 67:203–211.

Borczyk, A. A., M. A. Karmali, H. Lior, and L. M. C. Duncan. 1987. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. Lancet i:98.

Bürk, C., I. G. B. Braumiller, H. Becker, and E. Märtlbauer. 2002. Nuclease fluorescence assay for the detection of verotoxin genes in raw milk. Lett. Appl. Microbiol. 35:153–156.

Byrne, C. M., I. Erol, J. E. Call, C. W. Kaspar, D. R. Buege, C. J. Hiemke, P. J. Fedorka-Cray, A. K. Benson, F. M. Wallace, and J. B. Luchansky. 2003. Characterization of *Escherichia coli* O157:H7 from downer and healthy dairy cattle in the upper Midwest region of the United States. Appl. Environ. Microbiol. 69:4683–4688.

Cardinal, P. 1993. Isolation of *Escherichia coli* O157:H7 and detection of verotoxigenic *E. coli* in food and cattle feces in Quebec in 1990–1991. Pages 141–147 in *Escherichia coli* O157:H7 and Other Verotoxigenic *E. coli* in Foods. E. C. D. Todd and J. M. MacKenzie, ed. Food Directorate, Health Protection Branch, Health and Welfare Canada, and the Canadian Meat Council, Ottawa, Canada.

CDC. 1995. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21-Helena, Montana, 1994. Centers for Disease Control and Prevention. Morbid. Mortal. Wkly. Rep. 44:501–503.

CDC. 2000. Outbreak of *Escherichia coli* O157:H7 infection associated with eating fresh cheese curds—Wisconsin, June 1998. Centers for Disease Control and Prevention. Morbid. Mortal. Wkly. Rep. 49:911–913.

CDSC. 1996. VTEC O157 infection in West Yorkshire associated with the consumption of raw milk. Communicable Disease Surveillance Centre. Commun. Dis. Rep. Wkly. 6:181.

CDSC. 1998. *Escherichia coli* O157 infection and unpasteurized cream in England. Communicable Disease Surveillance Centre. Eurosurveillance Wkly. 2:981022.

CDSC. 1999a. VTEC O157 phage type 21/28 infection in North Cumbria. Update. Commun. Dis. Rep. CDR Wkly. 9:105.

CDSC. 1999b. *Escherichia coli* O157 associated with eating unpasteurised cheese – Update. Commun. Dis. Rep. CDR Wkly. 9:131–134.

Cerqueira, A. M. F., B. E. C. Guth, R. M. Joaquim, and J. R. C. Andrade. 1999. High occurrence of Shiga toxin-producing *Escherichia coli* (STEC) in healthy cattle in Rio de Janeiro State, Brazil. Vet. Microbiol. 70:111–121.

Chapman, P. A., C. A. Siddons, A. T. Cerdan Malo, and M. A. Harkin. 1997. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiol. Infect. 119:245–250.

Chapman, P. A., D. J. Wright, P. Norman, J. Fox, and E. Crick. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infection in man. Epidemiol. Infect. 111:439–447.

Chiueh, L., F. Liu, and D. Y. Shih. 2002. Prevalence of Shiga toxin-producing *Escherichia coli* in feed and raw milk of domestic cattle and sheep. J. Food Drug Anal. 10:39–46.

Cieslak, P. R., T. J. Barrett, and P. M. Griffin. 1993. *Escherichia coli* O157:H7 infection from a manured garden. Lancet 342:367.

Clarke, R. C., J. B. Wilson, S. C. Read, S. Renwick, K. Rahn, R. P. Johnson, D. Alves, M. A. Karmali, H. Lior, S. A. McEwen, J. Spika, and C. L. Gyles. 1994. Verocytotoxin-producing *Escherichia coli* (VTEC) in the food chain: Preharvest and processing perspectives. Pages 17–24 in Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections. M. A. Karmali and A. G. Goglio, ed. Elsevier Science, Amsterdam, The Netherlands.

Coia, J. E., Y. Johnston, N. J. Steers, and M. F. Hanson. 2001. A survey of the prevalence of *Escherichia coli* O157 in raw meats,

raw cow's milk and raw-milk cheeses in south-east Scotland. Int. J. Food Microbiol. 66:63–69.
Conedera, G., P. A. Chapman, S. Marangon, E. Tisato, P. Dalvit, and A. Zuin. 2001. A field survey of *Escherichia coli* O157 ecology on a cattle farm in Italy. Int. J. Food Microbiol. 66:85–93.
Correa, M. T., H. N. Erb, and J. M. Scarlett. 1993. Risk factors for downer cow syndrome. J. Dairy Sci. 76:3460–3463.
Crump, J. A., A. J. Langer, R. Gage, M. Baysinger, G. Withers, D. M. Toney, S. B. Hunter, M. Hoekstra, S. K. Wong, P. M. Griffin, and T. J. Van Gilder. 2002. An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. N. Engl. J. Med. 347:555–560.
Curnow, J. 1994. *E. coli* O157:H7 phage type 28 infections in Grampian. Commun. Dis. Environ. Health Scotland Wkly. Rep. 28:1.
Dean-Nystrom, E. A., B. T. Bosworth, W. C. Cray, Jr., and H. W. Moon. 1997. Pathogenecity of *Escherichia coli* O157:H7 in the intestines of neonatal calves. Infect. Immun. 65:1842–1848.
Deschênes, G., C. Casenave, F. Grimont, J. C. Desenclos, S. Benoit, M. Collin, S. Baron, P. Marian, P. A. Grimont, and H. Nivet. 1996. Cluster of cases of haemolytic uraemic syndrome due to unpasteurised cheese. Pediatr. Nephrol. 10:203–205.
Doyle, M. P. 1991. *Escherichia coli* O157:H7 and its significance in foods. Int. J. Food Microbiol. 12:289–302.
Ezawa, A., F. Gocho, K. Kawata, T. Takahashi, and N. Kikuchi. 2004. High prevalence of enterohemorrhagic *Escherichia coli* (EHEC) O157 from cattle in selected regions of Japan. J. Vet. Med. Sci. 66:585–587.
Faith, N. G., J. A. Shere, R. Brosch, K. W. Arnold, S. E. Ansay, M. S. Lee, J. B. Luchansky, and C. W. Kaspar. 1996. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. Appl. Environ. Microbiol. 62:1519–1525.
Fitzgerald, A. C., T. S. Edrington, M. L. Looper, T. R. Callaway, K. J. Genovese, K. M. Bischoff, J. L. McReynolds, J. D. Thomas, R. C. Anderson, and D. J. Nisbet. 2003. Antimicrobial susceptibility and factors affecting the shedding of *E. coli* O157:H7 and Salmonella in dairy cattle. Lett. Appl. Microbiol. 37:392–398.
Franke, S., D. Harmsen, A. Caprioli, D. Pierard, L. H. Wieler, and H. Karch. 1995. Clonal relatedness of Shiga-like toxin-producing *Escherichia coli* O101 strains of human and porcine origin. J. Clin. Microbiol. 33:3174–3178.
Furuhata, K., S. Sakata, T. Okamoto, S. Yamamoto, M. Honda, A. Kai, T. Ito, M. Hara, K. Tabuchi, and M. Fukuyama. 1999. Prevalence and serotypes of verocytotoxin-producing *Escherichia coli* (VTEC) isolates from dairy cattle. Kansenshougaku Zasshi 73:445–450.
Gallien, P., H. Richter, H. Klie, M. Timm, H. Karch, K. W. Perlberg, H. Steinrück, S. Riemer, M. Djuren, and D. Protz. 1997. Detection of STEC and epidemiological investigations of a HUS patient. Berl. Münch. Tierärztl. Wochenschr. 110:342–346.
Garber, L. P., S. J. Wells, D. D. Hancock, M. P. Doyle, J. Tuttle, J. A. Shere, and T. Zhao. 1995. Public veterinary medicine: Food safety and handling—Risk factors for fecal shedding of *Escherichia coli* O157:H7 in dairy calves. JAVMA 207:46–49.
Garber, L., S. Wells, L. Schroeder-Tucker, and K. Ferris. 1999. Factors associated with fecal shedding of verotoxin-producing *Escherichia coli* O157 on dairy farms. J. Food Prot. 62:307–312.
Georgia Division of Public Health. 2002. Outbreaks of *Escherichia coli* O157:H7 infections among children associated with farm visits-Pennsylvania and Washington, 2000. Available: http://www.ph.dhr.state.ga.us/epi/news/apr01/042401.shtml. Accessed Dec. 18, 2003.
Griffin, P. M., and R. V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60–98.
Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice, and M. G. Paros. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol. Infect. 113:199–207.
Hancock, D. D., T. E. Besser, and D. H. Rice. 1998. Ecology of *Escherichia coli* O157:H7 in cattle and impact of management practices. Pages 85–91 in *Escherichia coli* O157:H7 and Other Shiga Toxin-Producing *E. coli* Strains. J. B. Kaper and A. B. O'Brien, ed. ASM Press, Washington, DC.
Hancock, D. D., T. E. Besser, D. H. Rice, D. E. Herriott, and P. I. Tarr. 1997a. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. Epidemiol. Infect. 118:193–195.
Hancock, D. D., D. H. Rice, D. E. Herriott, T. E. Besser, E. D. Ebel, and L. V. Carpenter. 1997b. Effects of farm manure handling practices on *Escherichia coli* O157:H7 prevalence in cattle. J. Food Prot. 60:363–366.
Hancock, D. D., D. H. Rice, L. A. Thomas, D. A. Dargatz, and T. E. Besser. 1997c. Epidemiology of *Escherichia coli* O157 in feedlot cattle. J. Food Prot. 60:462–465.
Herriott, D. E., D. D. Hancock, W. E. Keene, and D. H. Rice. 1994. Raw milk associated *E. coli* O157:H7 outbreaks in Oregon. Pages FS17–FS21 in Proc. 8th Int. Congr. Anim. Hygiene. Int. Soc. Anim. Hygiene, St. Paul, MN.
Heuvelink, A. E., F. L. A. M. Van den Biggelaar, J. T. M. Zwartkruis-Nahuis, R. G. Herbes, R. Huyben, N. Nagelkerke, W. J. G. Melchers, L. A. H. Monnens, and E. de Boer. 1998. Occurrence of verotoxin-producing *Escherichia coli* O157 on Dutch dairy farms. J. Clin. Microibiol. 36:3480–3487.
International Association of Milk, Food, and Environmental Sanitarians. 2000. Outbreaks of VTEC O157 infection linked to consumption of unpasteurized milk. Assoc. Milk Food Environ. San. 20:627.
Jackson, S. G., R. B. Goodbrand, R. P. Johnson, V. G. Odorico, D. Alves, K. Rahn, J. B. Wilson, M. K. Welch, and R. Khakhria. 1998. *Escherichia coli* O157:H7 diarrhoea associated with well water and infected cattle on an Ontario farm. Epidemiol. Infect. 120:17–20.
Jung, B., S. Jung, H. Park, G. Cho, and B. Kim. 2000. Characteristics of verotoxin non-producing *Escherichia coli* O157 and verotoxin-producing *E. coli* isolated from healthy cattle. Korean J. Vet. Res. 40:525–531.
Karch, H., H. I. Huppertz, J. Bockemuhl, H. Schmidt, A. Schwarzkopf, and R. Lissner. 1997. Shiga toxin-producing *Escherichia coli* infections in Germany. J. Food Prot. 60:1454–1457.
Karmali, M. A., M. Petric, C. Lim, P. C. Fleming, G. S. Arbus, and H. Lior. 1985. The association between idiopathic hemolytic-uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J. Infect. Dis. 151:775–782.
Keene, W. E., K. Hedberg, D. E. Herriott, D. D. Hancock, R. W. McKay, T. J. Barrett, and D. W. Fleming. 1997. A prolonged outbreak of *Escherichia coli* O157:H7 infections caused by commercially distributed raw milk. J. Infect. Dis. 176:815–818.
Keene, W. E., J. M. McAnulty, F. C. Hoesly, L. P. Williams, K. Hedberg, G. L. Oxman, T. J. Barrett, M. A. Pfaller, and D. W. Fleming. 1994. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei.* N. Engl. J. Med. 331:579–584.
Keskimäki, M., R. Ikäheimo, P. Kärkkäinen, F. Scheutz, Y. Ratiner, R. Puohiniemi, and A. Siitonen. 1997. Shiga toxin-producing *Escherichia coli* serotype OX3:H21 as a cause of hemolytic-uremic syndrome. Clin. Infect. Dis. 24:1278–1279.
Klie, H., M. Timm, H. Richter, P. Gallien, K. W. Perlberg, and H. Steinruck. 1997. Detection and occurrence of verotoxin-forming and/or Shigatoxin producing *Escherichia coli* (VTEC and/or STEC) in milk. Berl. Munch. Tierärztl. Wochenschr. 110:337–341.
Kobayashi, H., J. Shimada, M. Nakazawa, T. Morozumi, T. Pohjanvirta, S. Pelkonen, and K. Yamamoto. 2001. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* from healthy cattle in Japan. Appl. Environ. Microbiol. 67:484–489.
Lahti, E., M. Eklund, P. Ruutu, A. Siitonrn, L. Rantala, P. Nuorti, and T. Honkanen-Buzalski. 2002. Use of phenotyping and genotyping to verify transmission of *Escherichia coli* O157:H7 from dairy farms. Eur. J. Clin. Microbiol. Infect. Dis. 21:189–195.
Lior, H. 1994. Classification of *Escherichia coli*. Pages 31–72 in *Escherichia coli* in Domestic Animals and Humans. C. L. Gyles, ed. CAB Int., Wallingford, UK.
Martin, M. L., L. D. Shipman, J. G. Wells, M. E. Potter, K. Hedberg, I. K. Wachsmuth, R. V. Tauxe, J. P. Davis, J. Arnoldi, and J. Tilleli. 1986. Isolation of *Escherichia coli* O157 from dairy cattle

associated with two cases of haemolytic uremic syndrome. Lancet ii:1043.
McKee, R., R. H. Madden, and A. Gilmour. 2003. Occurrence of verocytotoxin-producing *Escherichia coli* in dairy and meat processing environments. J. Food Prot. 66:1576–1580.
Mechie, S. C., P. A. Chapman, and C. A. Siddons. 1997. A fifteen-month study of *Escherichia coli* O157:H7 in a dairy herds. Epidemiol. Infect. 118:17–25.
Miyao, Y., T. Kataoka, T. Nomoto, A. Kai, T. Itoh, and K. Itoh. 1998. Prevalence of verotoxin-producing *Escherichia coli* harbored in the intestine of cattle in Japan. Vet. Microbiol. 61:137–143.
Montenegro, M. A., M. Bülte, T. Trumpf, S. Aleksiá, G. Reuter, E. Bulling, and R. Helmuth. 1990. Detection and characterization of fecal verotoxin-producing *Escherichia coli* from healthy cattle. J. Clin. Microbiol. 28:1417–1421.
Moreira, C. N., M. A. Pereira, C. S. Brod, D. P. Rodrigues, J. B. Carvalhal, and J. A. G. Aleixo. 2003. Shiga toxin-producing *Escherichia coli* (STEC) isolated from healthy dairy cattle in southern Brazil. Vet. Microbiol. 93:179–183.
Morgan, D., C. P. Newman, D. N. Hutchinson, A. M. Walker, B. Rowe, and F. Majid. 1993. Verotoxin producing *Escherichia coli* O157 infections associated with the consumption of yoghurt. Epidemiol. Infect. 111:181–187.
Murinda, S. E., L. T. Nguyen, S. J. Ivey, B. E. Gillespie, R. A. Almeida, F. A. Draughon, and S. P. Oliver. 2002. Prevalence and molecular characterization of *Escherichia coli* O157:H7 in bulk tank milk and fecal samples from cull cows: A 12-month survey of dairy farms in East Tennessee. J. Food Prot. 65:752–759.
National Cattlemen's Beef Association. 2001. Cattle and Beef Industry Statistics. National Cattlemen's Beef Association, Centennial, CO.
Neill, M. A. 1997. Overview of Verotoxigenic *Escherichia coli*. J. Food Prot. 60:1444–1446.
Nielsen, E. M., C. Teqtmeier, H. J. Anderson, C. Grønbæk, and J. S. Andersen. 2002. Influence of age, sex and herd characteristics on the occurrence of verocytotoxin-producing *Escherichia coli* O157 in Danish dairy farms. Vet. Microbiol. 88:245–257.
O'Brien, A. D., and R. K. Holmes. 1987. Shiga and Shiga-like toxins. Microbiol. Rev. 51:206–220.
Orden, J. A., D. Cid, J. A. Ruiz-Santa-Quireria, S. García, S. Martínez, and R. de la Fuente. 2002. Verotoxin-producing *Escherichia coli* (VTEC), enteropathogenic *E. coli* (EPEC) and necrotoxigenic *E. coli* (NTEC) isolated from healthy cattle in Spain. J. Appl. Microbiol. 93:29–35.
Ostroff, S. M., P. M. Griffin, R. V. Tauxe, L. D. Shipman, K. D. Greene, J. G. Wells, J. H. Lewis, P. A. Blake, and J. M. Kobayashi. 1990. A statewide outbreak of *Escherichia coli* O157:H7 infections in Washington State. Am. J. Epidemiol. 132:239–247.
Padhye, N. V., and M. P. Doyle. 1991. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* O157:H7 in food. Appl. Environ. Microbiol. 57:2693–2698.
Paton, J. C., and A. W. Paton. 2000. Shiga toxigenic *Escherichia coli* infections. Sci. Med. May/June:28–37.
Pradel, N., K. Boukhors, Y. Bertin, C. Forestier, C. Martin, and V. Livrelli. 2001. Heterogeneity of Shiga toxin-producing *Escherichia coli* strains isolated from hemolytic-uremic syndrome patients, cattle, and food samples in central France. Appl. Environ. Microbiol. 67:2460–2468.
Pradel, N., V. Livrell, C. de Champs, J. Palcoux, A. Reynaud, F. Scheutz, J. Sirot, B. Joly, and C. Forestier. 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. J. Clin. Microbiol. 38:1023–1031.
Rahn, K., S. A. Renwick, R. P. Johnson, J. B. Wilson, R. C. Clarke, D. Alves, S. McEwen, H. Lior, and J. Spika. 1997. Persistence of *Escherichia coli* O157:H7 in dairy cattle and the dairy farm environment. Epidemiol. Infect. 119:251–259.
Rahn, K., S. A. Renwick, R. P. Johnson, J. B. Wilson, R. C. Clarke, D. Alves, S. A. McEwen, H. Lior, and J. Spika. 1998. Follow-up study of verocytotoxigenic *Escherichia coli* infection in dairy farm family. J. Infect. Dis. 177:1138–1139.
Reid, T. M. S. 2001. A case study of cheese associated *E. coli* O157 outbreaks in Scotland. Pages 201–212 in Verocytotoxigenic *Escherichia coli*. G. Duffy, P. Garvey, and D. McDowell, ed. Food and Nutrition Press Inc., Trumbull, CT.
Reida, P., M. Wolff, H. W. Pohls, W. Kuhlmann, A. Lehmacher, S. Aleksic, H. Karch, and J. Bockemuhl. 1994. An outbreak due to enterohemorrhagic *Escherichia coli* O157:H7 in a children day care center characterized by person-to-person transmission and environmental contamination. Zentralbl. Bakteriol. 281:534–543.
Reitsma, C. J., and D. R. Henning. 1996. Survival of enterohemorrhagic *Escherichia coli* O157:H7 during the manufacture and curing of Cheddar cheese. J. Food Prot. 59:460–464.
Rice, D. H., E. D. Ebel, D. D. Hancock, T. E. Besser, D. E. Herriott, and L. V. Carpenter. 1997. *Escherichia coli* O157 in cull dairy cows on farm and at slaughter. J. Food Prot. 60:1386–1387.
Riemann, H. P., and D. O. Cliver. 1998. *Escherichia coli* O157:H7. Pages 41–48 in The Veterinary Clinics of North America—Food Animal Practice: Microbial Food Borne Pathogens. L. Tollefson, ed. W. B. Saunders Co., Philadelphia, PA.
Rugbjerg, H., E. M. Nielsen, and J. S. Andersen. 2003. Risk factors associated with faecal shedding of verocytotoxin-producing *Escherichia coli* O157 in eight known-infected Danish dairy herds. Prev. Vet. Med. 58:101–113.
Sandhu, K. S., R. C. Clarke, K. McFadden, A. Brouwer, M. Louie, J. Wilson, H. Lior, and C. L. Gyles. 1996. Prevalence of the eaeA gene in verotoxigenic *Escherichia coli* strains from dairy cattle in Southwest Ontario. Epidemiol. Infect. 116:1–7.
Segelken, R. 1996. *E. coli* screen aims to keep bacteria out of hamburger. Available: http://www.news.cornell.edu/releases/May96/beefQA.hrs.html. Accessed Jan. 19, 2004.
Shinagawa, K., M. Kanehira, K. Omoe, I. Matsuda, D. Hu, D. A. Widiasih, and S. Sugii. 2000. Frequency of Shiga toxin-producing *Escherichia coli* in cattle at a breeding farm and at a slaughterhouse in Japan. Vet. Microbiol. 76:305–309.
Siegler, R. L., M. K. Milligan, T. H. Burningham, R. D. Christofferson, S. Y. Changand, and L. B. Jorde. 1991. Long term renal outcome and prognostic indicators in the hemolytic-uremic syndrome. J. Pediatr. 118:195–200.
Steele, M. L., W. B. McNab, C. Poppe, M. W. Griffiths, S. Chen, S. A. Degrandis, L. C. Fruhner, C. A. Larkin, J. A. Lynch, and J. A. Odumeru. 1997. Survey of Ontario bulk raw milk for foodborne pathogens. J. Food Prot. 60:1341–1346.
Tada, H., S. Itami, Y. Yamamoto, K. Kobayashi, M. Taguchi, and M. Nakazawa. 1992. Detection of verotoxin-producing *Escherichia coli* using polymerase chain reaction from dairy cattle. Kasnenshougaku Zasshi 66:1383–1389.
Thran, B. H., H. S. Hussein, M. R. Hall, and S. F. Khaiboullina. 2001. Occurrence of verotoxin-producing *Escherichia coli* in dairy heifers grazing an irrigated pasture. Toxicology 159:159–169.
Todd, E., R. Szabo, and J. MacKenzie. 1993. Cultural and enzyme-linked antibody methods to detect *Escherichia coli* O157:H7. Pages 39–52 in *Escherichia coli* O157:H7 and Other Verotoxigenic *E. coli* in Foods. E. C. D. Todd and J. M. MacKenzie, ed. Food Directorate, Health Protection Branch, Health and Welfare Canada, and the Canadian Meat Council, Ottawa, Canada.
Troutt, H. F., and B. I. Osburn. 1997. Meat from dairy cows: Possible microbiological hazards and risks. Rev. Sci. Technol. Off. Int. Epizoot. 16:405–414.
USDA. 1992. Agricultural Statistics 1992. Agricultural Statistics Board, USDA, Washington, DC.
USDA-APHIS-VS. 1994. *Escherichia coli* O157:H7 in US Dairy Calves. January Report, National Animal Health Monitoring System. USDA Animal and Plant Health Inspection Service, Veterinary Service, Centers for Epidemiology and Animal Health, Fort Collins, CO.
USDA-APHIS-VS. 1996a. Economic opportunities for dairy cow culling management options. Info Sheet. USDA Animal and Plant Health Inspection Service, Veterinary Service, Centers for Epidemiology and Animal Health, Fort Collins, CO.
USDA-APHIS-VS. 1996b. New York cull cow study on *Escherichia coli* O157:H7. APHIS Report of FY 1995. Accomplishments in Animal Production Food Safety. USDA Animal and Plant Health

Inspection Service, Veterinary Service, Centers for Epidemiology and Animal Health, Fort Collins, CO.

USDA-APHIS-VS. 1997. An update: *Escherichia coli* O157:H7 in humans and cattle. USDA Animal and Plant Health Inspection Service, Veterinary Service, Centers for Epidemiology and Animal Health, Fort Collins, CO.

Van Donkersgoed, J., T. Graham, and V. Gannon. 1999. The prevalence of verotoxins, *Escherichia coli* O157:H7, and *Salmonella* in the feces and rumen of cattle at processing. Can. Vet. J. 40:332–338.

Vernozy-Rozand, C., M. P. Montet, F. Lequerrec, E. Serillon, B. Tilly, C. Bavai, S. Ary-Gueniot, J. Bouvet, C. Mazuy-Cruchaudet, and Y. Richard. 2002. Prevalence of verotoxin-producing *Escherichia coli* (VTEC) in slurry, farmyard manure and sewage sludge in France. J. Appl. Microbiol. 93:473–478.

Wall, P. G., R. J. McDonnell, G. K. Adak, T. Cheasty, H. S. Smith, and B. Rowe. 1996. General outbreaks of verocytotoxin producing *Escherichia coli* O157 in England and Wales from 1992 to 1994. Commun. Dis. Rep. CDR Rev. 6:R26–R33.

Wells, J. G., L. D. Shipman, K. D. Greene, E. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. E. Potter, R. V. Tauxe, and K. I. Wachsmuth. 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. 29:985–989.

WHO. 1998. Zoonotic non-O157 Shiga toxin-producing *Escherichia coli* (STEC). Report of a WHO Scientific Working Group Meeting. World Health Organization, Geneva, Switzerland.

Wilson, J. B., R. C. Clarke, S. A. Renwick, K. Rahn, R. P. Johnson, M. A. Karmali, H. Lior, D. Alves, C. L. Gyles, K. S. Sandhu, S. A. McEwen, and J. S. Spika. 1996. Vero cytotoxigenic *Escherichia coli* infection in dairy farm families. J. Infect. Dis. 174:1021–1027.

Wilson, J. B., R. P. Johnson, R. C. Clarke, K. Rahn, S. A. Renwick, D. Alves, M. A. Karmali, P. Michel, E. Orrbine, and J. S. Spika. 1997. Canadian perspectives on verotoxin-producing *Escherichia coli* infection. J. Food Prot. 60:1451–1453.

Wilson, J. B., S. A. McEwen, R. C. Clarke, K. E. Leslie, R. A. Wilson, D. Waltner-Toews, and C. L. Gyles. 1992. Distribution and characteristics of verotoxigenic *Escherichia coli* isolated from Ontario dairy cattle. Epidemiol. Infect. 108:423–439.

Yatsuyanagi, J., S. Saito, and I. Ito. 2002. A case of hemolytic-uremic syndrome associated with Shiga toxin 2-producing *Escherichia coli* O121 infection caused by drinking water contaminated with bovine feces. Jpn. J. Infect. Dis. 55:174–176.

Zhao, T., M. P. Doyle, J. Shere, and L. Garber. 1995. Prevalence of *Escherichia coli* O157:H7 in a survey of dairy herds. Appl. Environ. Microbiol. 61:1290–1293.

Zschöck, M., H. P. Hamann, B. Kloppert, and W. Wolter. 2000. Shiga-toxin-producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: Prevalence and virulence properties. Lett. Appl. Microbiol. 31:203–208.

The Emerging Clinical Importance of Non-O157 Shiga Toxin–Producing *Escherichia coli*

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In 1982, hemorrhagic colitis and hemolytic-uremic syndrome were linked to infection with *Escherichia coli* O157:H7, a serotype now classified as Shiga toxin–producing *E. coli* (STEC). Thereafter, hemorrhagic colitis and hemolytic-uremic syndrome associated with non-O157 STEC serogroups were reported, with the frequency of non-O157 STEC illness rivaling that of O157:H7 in certain geographic regions. In the United States, non-O157 *E. coli* may account for up to 20%–50% of all STEC infections. A high index of suspicion, paired with options to test for non-O157 STEC infection, are necessary for early recognition and appropriate treatment of these infections. Supportive care without the use of antibiotics is currently considered to be optimal treatment for all STEC infections. This commentary provides a perspective on the non-O157 STEC as human pathogens, how and when the clinician should approach the diagnosis of these organisms, and the challenges ahead.

In 1982, infection with Shiga toxin–producing *Escherichia coli* (STEC) O157:H7 strains was linked to hemorrhagic colitis and the hemolytic-uremic syndrome (HUS), defining a new foodborne zoonosis. Since then, ~250 different O serogroups of *E. coli* have been shown to produce Shiga toxin, and >100 of these STEC have been associated with sporadic and epidemic human diarrheal disease. Although the virulence of *E. coli* O157:H7 is well described, over the past 20 years, the capacity of the heterogeneous family of non-O157 STEC to induce human disease has spurred considerable, even heated, debate [1–3]. The data, although presented in variable detail, indicate that some non-O157 STEC unequivocally cause human disease and likely account for 20%–50% of STEC infections (an estimated 37,000 cases of illness) annually in the United States [4]. This commentary collates selected data to provide a perspective on the non-O157 STEC as human pathogens, how and when the clinician should approach diagnosis of these organisms, and the challenges ahead. We focused on reports that included ⩾5 patients infected with non-O157 STEC and that provided clinical details of the disease spectrum observed.

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NON-O157 STEC CLINICAL DISEASE: SUBTLE TO DEADLY

Non-O157 STEC pose a substantial dilemma for the clinician, because these bacteria cause illnesses indistinguishable from O157-induced disease and many other foodborne enteric infections. Data defining the overall frequency of non-O157 STEC compared with that of other enteric pathogens are limited. In this review, we analyzed nonoutbreak studies to try to define the community base of non-O157 STEC–associated disease. In 2 studies involving 11 sites in the United States in 1997 and 4 sites during the period 1998–1999, screening of 13,798 stool samples by EIA for STEC yielded a frequency of 0.9% (120 cases of STEC) [5]. Of these 120 cases, isolates were available from 104; 48 (46%) of the isolates were non-O157 STEC, and 56 (54%) were O157 STEC. These isolation rates are similar to rates for *Shigella* species in some locales. Such data suggest that there is a significant burden of non-O157 STEC–associated disease in the United States that is underappreciated. Further, global "hot spots" exist in which non-O157 STEC serogroups dominate over O157 serogroups, including Argentina, Australia, and Germany (table 1). For example, in Germany, non-O157 STEC account for up to 80% of STEC-associated diarrheal illnesses [14].

Clinical manifestations of non-O157 STEC infection range from mild, watery diarrhea to hemorrhagic colitis, HUS, and death; illness due to non-0157 STEC may be equivalent in severity to illnesses induced by *E. coli* O157:H7. The clinical

Table 1. Global identification of non-O157 Shiga toxin–producing *Escherichia coli* (STEC) serogroups.

Country	Common non-O157 STEC serogroups identified[a]	Patients with non-O157 STEC infection among patients with STEC infection,[b] %	No. of cases of non-O157 STEC infection/ no. of cases of STEC infection	Reference(s)
United States	O26, O45, O103, O104, O111, O119, O121, O145, OR	44[c]	62/141	[6–8]
Canada	O2, O26, O91, O103, O111, O113, O145	20	69/341	[9–12]
United Kingdom	O26, O55, O145	28	15/53	[13]
Germany	O26, O55, O76, O91, O103, O111, O113, O118, O128, O145, O146, ONT, OR	44[c]	327/748	[1, 14–16]
Spain	O26, O98, O118, O145, O150	78	87/111	[15, 17]
Italy	O26, O55, O103, O111, O145	34[d]	92/264	[18, 19]
Czech Republic	O1, O5, O26, O55, O111	57	16/28	[20, 21]
Belgium	O26, O91, O111, O113, O118, OR, ONT	63	41/65	[22]
France	O91, O103, O113, OX3	33[e]	39/118	[23–25]
Denmark	O26, O103, O111, O117, O121, O128, O145, O146, O174, OR	75	493/655	[26, 27]
Finland	OR, O15, O26, O103, O145, O174	53[f]	57/107	[28–31]
Sweden	O8, O117, O121, OR, ON	33	20/60	[32]
Australia	O6, O26, O48, O98, O111	69	31/45	[33, 34]
New Zealand	O22, O26, ONT, OR	100	16/16	[35]
Chile	O26, O111	63	5/8	[36]
Argentina	O8, O26, O103, O113, O121, O145, O174, ONT	40	38/96[g]	[37]
Japan	O26, O111, O128, O145, O165	19[h]	10/52	[38, 39]

NOTE. Serogroups were identified via isolation from stool culture and/or serological evidence of STEC infection in the context of clinical illness. Only serogroups reported twice or more across studies evaluated are reported. Only studies evaluating STEC infection due to both O157 and non-O157 serogroups were considered. Data are presented as a percentage of case patients with non-O157 STEC infection of all STEC infections identified in published reports from each country. When identified, domestic strains alone are included in the totals. When reported, number of non-O157 STEC infections includes those with stool culture isolate, serological evidence of infection, or both. Data are limited by missing and unreported information internationally, including reports of STEC infection in which no serogroup identification was performed. Asymptomatic STEC excretors were excluded from the analysis when identified. ONT, O nontypeable; OR, O rough.

[a] Non-O157 infection was defined as (1) absence of O157:H7 on stool culture and/or absence of circulating antibodies to O157 lipopolysaccharide with fecal cytotoxin assays neutralizable by antiserum to Shiga toxin, monoclonal antibody for Shiga toxin 2, or antibodies to Shiga toxin 1 and Shiga toxin 2; or (2) *stx1* and/or *stx2* DNA probe–positive stool isolates; or (3) a ⩾3-fold increase in serum antibody titers to Stx1 or Stx2 without serological evidence of O157:H7 or isolation of O157:H7 on stool culture. If only a subset of total STEC cases reported was identified by serogroup, this subset was used as the denominator for all STEC reported. Serogroups are included if ⩾2 strains of a serogroup were reported.

[b] When identified, cases with dual infection of non-O157 with O157 STEC were not included in the data reported.

[c] Outbreak and single-case reports are excluded from this calculation.

[d] Non-0157 diagnosed by serological testing alone in a subset of cases.

[e] Because of limited information in the studies available, only [25] was used for the calculation.

[f] For calculation, data are from reference [30] only.

[g] Denominator represents the total number of strains isolated after correction for duplicate subjects.

[h] Outbreak cases were not included in calculation of the non-O157 isolation rate.

variability of disease noted with non-O157 STEC may be related to their notable genetic heterogeneity. At least 15 studies help to define the breadth of non-O157 STEC–related illness [1, 6, 9, 13–15, 17, 26, 32, 37, 40–44] (table 2). Although non-O157 serogroups appear to cause watery diarrheal illness more often than bloody diarrheal illnesses [6, 45], it is equally apparent that bloody diarrhea associated with STEC infection cannot be presumed to be due to O157 infection. Conversely, O157 infection does not inevitably cause hemorrhagic colitis. A 2-year prospective study of 5415 cases of sporadic diarrhea in Canada revealed significant differences between the clinical syndromes associated with non-O157 and O157 STEC infection [9]. Patients with non-O157 STEC infection had a longer duration of diarrhea (mean duration, 9.1 days vs. 5.7 days; $P < .001$) and less frequent bloody diarrhea (42% vs. 97%; $P < .001$). In contrast, the rate of abdominal pain, vomiting, and fever of >38°C did not differ between patients with non-O157 infections and those with O157 infections. In a recent study of 343 Danish patients with STEC infections [26], serogroups O157 and O103 were independent risk factors for bloody diarrhea on multivariate analysis with indistinguishable ORs and 95% CIs, confirming that certain non-O157 serogroups can be as virulent as O157:H7 strains. The virulence of non-O157 strains is further illustrated by a study of 394 children with HUS in Germany and Austria, in which 57% of children with non-O157 illness and 71% of those with O157 illness presented with bloody diarrhea. Although statistically significant ($P < .05$), this difference is clinically meaningless [15].

Table 2. Clinical manifestations of non-outbreak disease due to non-O157 Shiga toxin–producing *Escherichia coli* (STEC).

Reference	Year of publication	Design (dates)	Population	Location	Non-O157 STEC infections identified/ no. of patients evaluated[a]	Clinical manifestations, no. of patients with non-O157–associated disease/total no. of patients (%)			Strengths and/or limitations
						Nonbloody diarrhea	Bloody diarrhea	HUS	
Pai et al. [9]	1988	Prospective study (1984–1986)	Children and adults with diarrhea	Canada	29/5415	18/19 (95)	8/19 (42)	2/19 (11)	Clinical comparison of O157:H7-associated cases with non-O157–associated cases
Lopez et al. [42]	1989	Prospective study (1986–1988)	Children with diarrhea and/or HUS	Argentina	62/95	9 (15)	NS[b]	14 (23)	Specific non-O157 serogroups not identified
Kleanthous et al. [13]	1990	Prospective study (1985–1988)	Children with HUS	United Kingdom	15/196	7 (47)	8 (53)	15 (100)	Clinical data provided for each case
Bitzan et al. [40]	1991	Retrospective study (1985–1989)	Children and adults with mucous or bloody diarrhea	Germany	15/155	13 (87)	6 (40)	6 (40)	Clinical data provided for each case
Bockemuhl et al. [41]	1992	Retrospective study (1987–1990)	Children and adults with diarrhea	Germany	49/62	27 (55)	8 (16)	18 (37)	Clinical data limited, retrospective design
Bielaszewska et al. [44]	1994	Prospective study (1988–1991)	Children with diarrhea	Czech Republic	23/1239	6 (26)	6 (26)	10 (43)	Clinical data limited
Huppertz et al. [14]	1996	Prospective study (1994)	Hospitalized children and adolescents <16 years old with diarrhea	Germany	11/468	11 (100)	2 (18)	1 (9)	Well-characterized cohort
Beutin et al. [1]	1998	Retrospective multicenter study (1996)	Non-O157 isolates from adults and children submitted to a central laboratory	Germany	83[c,d,e]	72 (87)	7 (8)	4 (5)	Well-characterized cohort
Gerber et al. [15]	2002	Prospective, multicenter study (1997–2000)	Hospitalized children and adolescents <16 years old with diarrhea	Austria and Germany	186/394[c]	81/87 (93)	47/83 (57)	90 (48)	HUS studied only; clinical comparison of O157:H7-associated with non-O157–associated HUS cases
Jelacic et al. [6]	2003	Prospective study (1988–2000)	Adults and children with clinical indication for stool culture	United States	50/6300	NR	21/37 (57)	NR	Clinical data limited
Wielender-Olsson et al. [32]	2002	Retrospective study (1997–1999)	Adults and children with clinical indication for stool culture	Sweden	20/5617	7 (35)	9 (45)	2 (10)	Well-characterized cohort
Blanco et al. [17]	2004	Prospective study (1992–1999)	Adults and children with clinical indication for stool culture	Spain	87/5054[f]	59 (68)	18 (21)	1 (1)	Clinical comparison of O157:H7-associated with non-O157–associated HUS cases
Ethelberg et al. [26]	2004	Prospective study (1997–2003)	Adults and children with STEC infection	Denmark	262/343[c]	NR	69 (26)	10 (4)	Well-characterized cohort
Brooks et al. [43]	2005	Prospective sample (1993–2004)	Non-O157 isolates from adults and children submitted to CDC	United States	940[d,g]	NR	75/292 (26)	21/292 (7)	Clinical data available from only 31% of source patients
Rivas et al. [37]	2006	Prospective study (2001–2002)	Children with diarrhea and/or HUS	Argentina	38/99	9 (24)	28 (74)	5 (13)	Well-characterized cohort

NOTE. Studies are ordered chronologically by publication year. Non-O157 STEC infection was identified by fecal culture and/or a positive result of any of the following tests: cytotoxin neutralizing assay, *stx* DNA probe assay, or serology. Unless noted, diarrhea is non-bloody. Cases of diarrhea may have been associated with HUS; there is overlap in the cases reported for each clinical category, such that the syndromes of diarrhea (non-bloody vs. bloody) and HUS may have been noted in a single patient. Studies were selected for systematic analysis of STEC strains and reporting of clinical features to demonstrate the spectrum of non-O157 STEC illness. This is intended to provide a global perspective on the diversity of clinical data and serogroup identification. CDC, Centers for Disease Control and Prevention; HUS, hemolytic-uremic syndrome; NR, not reported; NS, not specified.

[a] When HUS was studied prospectively, data reported are total number of HUS cases/total number of non-O157 STEC infections identified. Asymptomatic cases of infection due to non-O157 STEC were eliminated from the analysis when symptom-free data were provided.

[b] Bloody diarrhea was reported only as a percentage of all reported illnesses; thus, we were unable to derive non-O157–associated bloody diarrhea.

[c] Number of STEC strains identified, excluding dual infection with STEC of 2 different serogroups and any asymptomatic cases where applicable.

[d] Study of non-O157–associated disease only.

[e] Total number of non-O157 strains reported is 126. Serogroup identification was reported for 87. Serogroup and clinical information were reported for 83.

[f] Total number of non-0157 STEC strains is 87. Serogroup and clinical information were reported for 85.

[g] Clinical information was available for a limited number of patients with non-O157 STEC infection. Serogroups reported as "other" are considered to be non-O157 STEC.

HUS is a triad of acute renal failure , microangiopathic hemolytic anemia, and thrombocytopenia. In the majority of cases in children, HUS occurs after a diarrheal illness, but bloody diarrhea is not a prerequisite. Worldwide, diarrhea-associated HUS largely accounts for morbidity and mortality due to STEC, resulting in death (in up to 5%) and frequent permanent renal injury (at an estimated rate of 25%) [46]. Because most of the data detailing the risk of progression to HUS, HUS-related mortality, and development of permanent renal injury following HUS have been obtained from O157:H7-associated diarrheal outbreaks, it is unknown whether these data also will be true in diarrheal illness associated with non-O157 strains. However, it is clear that infection with non-O157 strains can result in HUS. In a 4-year study of children and adults with HUS in the United States, 5 (16.7%) of 30 patients had infection due to non-O157 strains [7]. Argentina has the highest global rates of HUS, estimated annually at 12.2 cases per 100,000 [37] among children younger than 5 years, and non-O157 strains account for nearly all of this disease burden [42]. Similar findings are reported from Australia [33]. In Germany and Austria, a prospective evaluation of sporadic cases of HUS in children revealed non-O157 STEC in nearly one-half (43%) of patients studied [47]. Additional studies from Germany, Belgium, Finland, the Czech Republic, and Italy also suggest that the rate of isolation of non-O157 strains exceeds that of O157 strains among patients with diarrhea and HUS in these regions.

Certain non-O157 serotypes, particularly O111, trigger sporadic and outbreak-associated HUS more frequently than other non-O157 STEC. In a recent sampling of 940 non-O157 human isolates sent to the Centers for Disease Control and Prevention (Atlanta, GA) by public health laboratories in 42 states and the District of Columbia from 1983 through 2002, isolates from 21 cases of HUS were identified [43]. Serogroup O111 accounted for 48% of HUS cases, but missing data limited the power of the analysis. In Germany, Austria, and Australia, O111 strains were most frequently associated with HUS [33, 47]. These and other data suggest that infection with serotypes O26, O103, and O145 may also be relatively more likely to precipitate HUS (table 1).

Although it is not clear whether the pathogenesis of HUS following infection with non-O157 strains exactly mirrors that following infection due to O157 strains, non-O157 strains harboring the gene encoding *stx2* (see Disease Pathogenesis: Complex and Evolving) are those most highly associated with onset of HUS, a finding analogous to that for O157 strains. This similarity suggests that some infections due to non-O157 strains trigger the same abnormalities of the clotting cascade that precipitate HUS following infection due to O157 strains [48]. More data are needed to determine whether, for example, peripheral leukocytosis signals a greater probability of HUS, as has been noted in O157-associated disease [49, 50].

DISEASE PATHOGENESIS: COMPLEX AND EVOLVING

The ability to express one or more subtypes of Shiga toxin defines membership in the STEC family. Production of Shiga toxin is central to the pathogenesis of both O157- and non-O157–associated human disease. Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) are the main members of this toxin family. In contrast to STEC that produce Stx1 only, STEC that produce Stx2 alone or both Stx1 and Stx2 are more likely to be associated with HUS [51]. Of note, intravenous administration of purified Stx2 alone in the baboon model of HUS is sufficient to cause the hallmark pathological features of HUS, at doses at which Stx1 alone will not.

Like cholera toxin and pertussis toxin, the Shiga toxins are members of the AB5 toxin family, consisting of 1 enzymatically active A subunit non-covalently associated with a binding pentamer of identical B subunits. The B subunit binds to neutral glycolipids on host cells and mediates cellular uptake of Shiga toxin with trafficking to the endoplasmic reticulum. In the endoplasmic reticulum, the A subunit is translocated across the endoplasmic reticulum membrane to the cytoplasm, thereby gaining access to cytoplasmic ribosomes. The A subunit recognizes and binds to the 28S RNA portion of the 60S ribosomal subunit and cleaves off a specific adenine, preventing aminoacyl t-RNA binding. This results in inhibition of protein synthesis and can trigger host cell signal transduction mechanisms that may favor expression of proinflammatory cytokines. Therefore, Shiga toxins likely have 2 mechanisms by which they cause local and systemic disease: direct (inhibition of protein synthesis) and indirect (proinflammatory cytokine expression).

Because STEC are noninvasive, Shiga toxins must be absorbed across the intestinal epithelium to gain access to the systemic circulation. It is thought that Shiga toxins, once absorbed, act on a variety of host cell subtypes, including endothelial cells in target organs, such as the kidney and the brain, to cause pathological hallmarks of systemic disease: deposition of fibrin-platelet thrombi in affected tissues. Recent data suggest that there may be additional mediators of cell injury and death, including the secreted zinc metalloprotease of C1 esterase inhibitor [52], subtilase cytotoxin (SubAB), and cytolethal distending toxin [53]. Thus, the degree to which different strains of STEC may colonize the gastrointestinal tract, cause disruption of the gastrointestinal barrier, and produce Shiga toxin locally may vary (thereby influencing the likelihood of developing HUS) according to strain-dependent encoded and expressed virulence genes.

Virulence characteristics vary among non-O157 strains and likely influence their pathogenicity. STEC strains may carry the locus of enterocyte effacement (LEE), a 43-kb pathogenicity island containing a cluster of genes whose cooperative protein products contribute to STEC pathogenesis. The presence of LEE

in non-O157 strains correlates with virulence, because strains containing LEE are more frequently associated with HUS and outbreaks than are LEE-negative strains. Consistent with the increased association between STEC carrying LEE and human disease, serogroups O26, O103, O111, O121, and O145 (all associated with outbreaks of severe disease, including HUS [54]) are predominantly LEE-positive [38, 54, 55] and *cdt*-negative strains [56]. These and other data suggest that infection with certain serogroups may be more likely to precipitate HUS (table 3). LEE contains genes whose expression permits formation of the attaching and effacing lesions characteristic of STEC intestinal colonization. LEE also contains genes that encode a type III secretion system that delivers the proteins mediating the attaching and effacing lesions and other virulence proteins to enterocytes. Attaching and effacing lesions form when LEE-encoded intimin (*eae*), an outer membrane protein homologue, binds to the translocated intimin receptor *(tir)* that inserts into the enterocyte membrane—a unique example of a pathogen providing to the host the receptor required for its colonization. In a Finnish study of all human non-O157 strains collected over a decade from symptomatic persons, 70% carried the *eae* gene [29], 55% carried *stx2,* and 75% carried *E-hly. E-hly* encodes enterohemolysin, a pore-forming cytolysin that may be associated with the presence of *eae* in non-O157 versus O157 strains [29]. The precise contribution of enterohemolysin to STEC virulence remains unknown. However, none of these human isolates lacked all 3 of these virulence genes. In other work, the adherence of STEC to enterocytes (dependent, in part, on the expression of *eae*) correlated directly with pathogenicity when strains from sporadic and outbreak-associated HUS were compared with isolates from food and from species other than humans [29, 74]. Genes encoding potential virulence factors are found more frequently in non-O157 STEC serogroups isolated from patients with bloody diarrhea and/or HUS than they are from patients with non-O157 STEC–associated nonbloody diarrhea.

The observations that O157 and non-O157 LEE-negative STEC can lead to significant disease in humans led investigators to look for other virulence traits, resulting in the discovery of new pathogenicity islands. Notably, the high-pathogenicity island is unique to certain non-O157 serogroups, such as O26, one of the most frequently reported serogroups in Europe and the United States [75]. The high-pathogenicity island encodes genes required for synthesis of an iron-recruitment system, called yersiniabactin, that is also found in pathogenic *Yersinia* species and other enterobacteria. Sequencing of the O157 genome identified 8 additional pathogenicity-associated islands that may contribute to strain virulence [76]. Certain non-O157 strains known to cause severe disease in humans carry $\geqslant$1 of these recently recognized pathogenicity islands, but further work is needed to understand the relationship of these islands to both O157- and non-O157–related human disease [54].

Additional investigation into the determinants of virulence of non-O157 STEC will likely yield exciting results, as illustrated by the recent discovery of a novel AB5 toxin, SubAB, in an Australian STEC O113:H21 strain associated with a small outbreak of HUS [77]. Notably, this strain does not possess the *eae* gene.

Intraperitoneal injection of purified SubAB is fatal in a murine model, causing microvascular thrombosis and the subsequent necrosis within multiple organs [77]. SubAB genes have been found in fecal extracts from ~10% of Australian STEC cases and are typically found in STEC that produce only Stx2 [78]. The worldwide distribution of SubAB in both STEC and other *E. coli* is unknown, and its contribution to strain virulence and disease requires further study.

SCREENING AND ISOLATION OF NON-O157: A DIFFICULT SEARCH

Stool samples for cultures must be obtained early in the disease course to reliably isolate O157, and presumably the same is true for non-O157 strains. O157 strains are easier to detect on stool culture, because most strains fail to metabolize sorbitol. This leads to colorless, opaque-appearing colonies on sorbitol-MacConkey agar, rather than the pink colonies (caused by fermentation of sorbitol) typical of most non-O157 STEC, as well as commensal and pathogenic diarrhea-inducing classes of *E. coli* (e.g., enteropathogenic *E. coli* and enterotoxigenic *E. coli,* among others). Thus, given the serogroup diversity of non-O157 STEC, the only practical rapid approach, at present, to identify non-O157 STEC–related disease is direct or indirect detection of Shiga toxin production by these strains. One of 2 EIAs approved by the US Food and Drug Administration to detect Shiga toxin may be performed either directly on stool samples or on supernatants of an overnight enrichment culture of stool [79]. Sensitivity of EIA is enhanced by adding mitomycin C or polymyxin B to stool cultures to stimulate production or liberation of bacterial cell–associated Shiga toxin. Alternatively, a tissue culture cytotoxicity and neutralization assay can detect Shiga toxin, but this reference standard approach is too laborious and costly to be widely adopted for diagnosis. PCR-based methods to detect Shiga toxin genes in diarrheal stools may emerge as a rapid identification method in diagnostic laboratories capable of reliably performing these assays. One limitation of these approaches is that the non-O157 strains are not recovered and characterized. Ultimately, culture remains important for tracking non-O157 disease epidemiology and the development of public health strategies for containment.

Is rapid diagnosis of infection due to non-O157 STEC clinically necessary? Given the severe disease associated with some non-O157 STEC serogroups, rapid diagnostic measures con-

Table 3. Overview of reported non-O157 Shiga toxin–producing *Escherichia coli* (STEC) serogroups and associated clinical syndromes.

STEC serogroup	Nonbloody diarrhea	HUS	Bloody diarrhea	Reference(s)
O1	Yes	Yes	Yes	[14, 44]
O2	No	Yes[a]	Yes	[30, 37, 44]
O5	No	Yes	Yes	[21, 26, 44]
O8	Yes	Yes	Yes	[16, 32, 37]
O9	Yes	No	No	[14]
O15	No	No	Yes	[26, 29, 37]
O18	No	Yes	Yes	[21, 57]
O22	Yes	Yes	No	[32, 41, 58]
O23	No	Yes	Yes	[16, 41]
O25	No	No	Yes	[37]
O26	Yes	Yes	Yes	[14, 33, 34, 36, 37, 41, 59–62]
O28	No	Yes	Yes	[15, 17, 43]
O45	Yes	No	Yes	[6, 41]
O46	Yes	Yes	No	[32, 34]
O48	No	Yes	No	[34]
O50	No	No	Yes	[44]
O55	Yes	Yes	Yes	[16, 20, 26, 41, 43, 63]
O58	No	No	Yes	[37]
O68	No	No	Yes	[64]
O73	Yes	Yes	Yes	[6, 41, 63]
O75[b]	No	Yes	No	[41]
O76	Yes	No	Yes	[1, 32, 58]
O91	Yes	Yes	No	[1, 26, 28, 32, 34, 37, 41]
O92	No	Yes	No	[16]
O98	No	Yes	No	[34]
O100[b]	No	No	Yes	[41]
O101[a]	No	Yes	No	[30]
O103	Yes	Yes	Yes	[1, 11, 19, 37, 39, 62]
O104	Yes	Yes	Yes	[34, 41, 65]
O107[a]	No	Yes	No	[30]
O111	Yes	Yes	Yes	[1, 14, 15, 18–20, 26, 33, 36, 37, 41, 43, 62, 63, 66–71]
O113	Yes	Yes	Yes	[1, 33, 37, 54, 71]
O115	Yes	Yes	No	[38]
O117	No	No	Yes	[26]
O118	Yes	Yes	Yes	[1, 16, 32]
O119	Yes	Yes	Yes	[14–16, 41, 43, 63, 70]
O121	Yes	Yes	Yes	[10, 16, 26, 32, 37, 63, 72]
O126[b]	Yes	No	No	[41]
O128	Yes	Yes	Yes	[1, 20, 39, 41, 43, 63, 72]
O130	No	Yes	No	[33]
O132[b]	Yes	No	No	[41]
O133	Yes	No	No	[14]
O145	Yes	Yes	Yes	[1, 10, 19, 32, 37, 39, 63]
O146	Yes	Yes	Yes	[1, 26, 34]
O165	No	Yes	Yes	[6, 15, 16, 34, 39, 43]
O166	Yes	No	No	[41]
O171	No	No	Yes	[37]
O174	Yes	Yes[a]	Yes	[26, 30, 37]
O177	No	Yes	Yes	[6, 73]
O179	Yes	No	Yes	[73]
O180	Yes	No	No	[73]
O181	Yes	No	Yes	[6, 73]
OR	Yes	Yes	Yes	[41, 6, 32, 33, 16, 30, 39]
ONT	Yes	Yes	Yes	[1, 6, 14, 16, 32, 33, 37–39, 41]

NOTE. Data are derived from case series, outbreak reports, and case reports. Strains reported without clinical information are not included. HUS, hemolytic-uremic syndrome; ONT, O nontypeable; OR, O rough.

[a] Single strain for a patient presenting with either HUS or thrombotic thrombocytopenic purpura; prior clinical syndrome was not specified [30].

[b] Single strains reported for serogroup.

firmed by culture are optimal. Although testing all patients with diarrheal syndromes for non-O157 STEC infection is ideal, costs may well be prohibitive in regions in which incidence is low. For this reason, development and investigations of rapid, cost-effective diagnostic methods are imperative. The leading life-threatening sequela of STEC infection is development of HUS. Because the critical pathophysiological changes of HUS are occurring by the time that the patient presents with the diarrheal prodrome, only early interventions—requiring rapid diagnostics—to interrupt coagulation cascade dysregulation will reduce morbidity and mortality caused by HUS. Further, bloody diarrhea is a serious illness among both children and adults, compelling clinicians to consider early, empirical antibiotic therapy. However, current recommendations and available data (although limited in scope and only formally studied for O157-related infections in children) suggest that antibiotics should be withheld if STEC infection is suspected, given concerns that antibiotics may trigger release of Stx and progression to HUS, resulting in worse clinical outcomes. These considerations support an argument for early diagnosis of STEC infection. The case for rapid specific diagnosis of STEC infection is further supported by a recent prospective study of 29 children with O157-associated bloody diarrhea or HUS, which suggests that early isotonic volume expansion—occurring before culture results are available—may attenuate renal injury and failure [80]. The value and potential complications (e.g., volume overload) of aggressive hydration in adults and the benefit in non-O157 STEC infections are unknown and are likely to remain so, indicating that clinicians may well have to take their management cues from these limited data. Thus, rapid diagnosis of all STEC infections should assist clinical decision-making regarding fluid and antibiotic administration. Because the clinical manifestations of STEC infection may mimic appendicitis, intussusception, inflammatory bowel disease, and *Clostridium*

difficile infection, rapid diagnosis may avert both invasive endoscopy procedures, which are costly and carry potential morbidity, and emergent surgical procedures. Finally, rapid diagnosis may serve to limit outbreaks. Clinician-initiated reports of STEC infection to public health authorities will allow timely outbreak investigations that may permit detection of additional STEC cases, limit spread from persistently contaminated common sources, and/or prevent secondary spread within families.

CONCLUSIONS AND RECOMMENDATIONS

Non-O157 STEC infections may induce a range of illness, from mild gastroenteritis to critical illnesses, including hemorrhagic colitis, HUS, and death, either as sporadic cases or in outbreaks, reported in the United States and internationally. A substantial proportion of HUS is caused by infection due to non-O157 strains worldwide, but the true incidence and burden of illness caused by these *E. coli* serotypes remain unknown. In 2000, only 68% of clinical laboratories in the United States tested routinely for infections due to O157 STEC, with fewer laboratories testing for non-O157 STEC infections [81]. The search for STEC infection should begin, at a minimum, in patients presenting with bloody diarrhea and/or HUS. Ideally, screening for STEC on all stool specimens submitted because of diarrheal illness should be considered, but the cost-effectiveness of this approach, given the geographic variability of both O157 and non-O157 STEC infections, requires assessment. If specific and effective therapies that prevent HUS are developed, the need to diagnose STEC infection will be compelling. Rapid, sensitive, accurate, and inexpensive techniques to enable routine testing for Shiga toxin and non-O157 strains should be developed and incorporated into clinical laboratory protocols. Only through active case investigation will we further understand the distribution and human cost of non-O157–associated disease.

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Potential conflicts of interest. C.M.T. has received financial support in the form of patent royalties from Meridian Diagnostics. K.E.J. and C.L.S.: no conflicts.

References

1. Beutin L, Zimmermann S, Gleier K. Human infections with Shiga toxin–producing *Escherichia coli* other than serogroup O157 in Germany. Emerg Infect Dis **1998**; 4:635–9.
2. Goldwater PN, Bettelheim KA. Role of non-O157:H7 *Escherichia coli* in hemolytic uremic syndrome. Clin Infect Dis **2002**; 35:346–7.
3. Acheson DWK, Keusch GT. Which Shiga toxin–producing types of *E. coli* are important? ASM News **1996**; 62:302–6.
4. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C. Food-related illness and death in the United States. Emerg Infect Dis **1999**;5: 607–25.
5. Andreoli SP, Trachtman H, Acheson DWK, Siegler RL, Obrig TG. Hemolytic uremic syndrome: epidemiology, pathophysiology, and therapy. Pediatr Nephrol **2002**; 17:293–8.
6. Jelacic JK, Damrow T, Chen GS, et al. Shiga toxin–producing *Escherichia coli* in Montana: bacterial genotypes and clinical profiles. J Infect Dis **2003**; 188:719–29.
7. Banatvala N, Griffin PM, Greene KD, et al. The United States National Prospective Hemolytic Uremic Syndrome Study: microbiologic, serologic, clinical, and epidemiologic findings. J Infect Dis **2001**; 183: 1063–70.
8. Fey PD, Wickert RS, Rupp ME, Safranek TJ, Hinrichs SH. Prevalence of non-O157:H7 Shiga toxin–producing *Escherichia coli* in diarrheal stool samples from Nebraska. Emerg Infect Dis **2000**; 6:530–3.
9. Pai CH, Ahmed N, Lior H, Johnson WM, Sims HV, Woods DE. Epidemiology of sporadic diarrhea due to verocytotoxin-producing *Escherichia coli*: a 2-year prospective study. J Infect Dis **1988**; 157:1054–7.
10. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli.* J Infect Dis **1985**; 151: 775–82.
11. Ramotar K, Henderson E, Szumski R, Louie TJ. Impact of free verotoxin testing on epidemiology of diarrhea caused by verotoxin-producing *Escherichia coli.* J Clin Microbiol **1995**; 33:1114–20.
12. Ritchie M, Partington S, Jessop J, Kelly MT. Comparison of a direct fecal Shiga-like toxin assay and sorbitol-MacConkey agar culture for laboratory diagnosis of enterohemorrhagic *Escherichia coli* infection. J Clin Microbiol **1992**; 30:461–4.
13. Kleanthous H, Smith HR, Scotland SM, et al. Haemolytic uraemic syndromes in the British Isles, 1985–8: association with verocytotoxin producing *Escherichia coli.* Arch Dis Child **1990**; 84:722–7.
14. Huppertz HI, Busch D, Schmidt H, Aleksic S, Karch H. Diarrhea in young children associated with *Escherichia coli* non-O157 organisms that produce Shiga-like toxin. J Pediatr **1996**; 128:341–6.
15. Gerber A, Karch H, Allerberger F, Verweyen HM, Zimmerhackl LB. Clinical course and the role of Shiga toxin–producing *Escherichia coli* infection in the hemolytic-uremic syndrome in pediatric patients, 1997–2000, in Germany and Austria: a prospective study. J Infect Dis **2002**; 186:493–500.
16. Friedrich AW BM, Zhang W, Pulz M, Kuczius T, Ammon A, Karch H. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J Infect Dis **2002**; 185:74–84.
17. Blanco JE, Blanco M, Alonso MP, et al. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)—producing *Escherichia coli* isolates from human patients: prevalence in Lugo, Spain, from 1992 through 1999. J Clin Microbiol **2004**; 42:311–9.
18. Caprioli A, Luzzi I, Rosmini F, et al. Communitywide outbreak of hemolytic-uremic syndrome associated with non-O157 verocytotoxin-producing *Escherichia coli.* J Infect Dis **1994**; 169:208–11.
19. Tozzi AE, Caprioli A, Minelli F, et al. Shiga toxin–producing *Escherichia coli* infections associated with hemolytic uremic syndrome, Italy, 1988–2000. Emerg Infect Dis **2003**; 9:106–8.
20. Bielaszewska M, Janda JM, Blahova K, Feber J, Potuznik V, Souckova A. Verocytotoxin-producing *Escherichia coli* in children with hemolytic uremic syndrome in the Czech Republic. Clin Nephrol **1996**; 46:42–4.
21. Sramkova L, Bielaszewska M, Janda J, Blahova K, Hausner O. Vero cytotoxin–producing strains of *Escherichia coli* in children with haemolytic uraemic syndrome and diarrhoea in Czechoslovakia. Infection **1990**; 18:204–9.
22. Pierard D, Stevens D, Moriau L, Lior H, Lauwers S. Three years PCR screening for VTEC in human stools in Brussels. In: Karmali MA, Goglio AG, eds. Recent advances in verocytotoxin-producing *Escherichia coli* infections. Amsterdam: Elsevier Science, **1994**:33–6.
23. Bonnet R, Souweine B, Gauthier G, et al. Non-O157:H7 Stx2-producing *Escherichia coli* strains associated with sporadic cases of hemolytic-uremic syndrome in adults. J Clin Microbiol **1998**; 36:1777–80.

24. Decludt B, Bouvet P, Mariani-Kurkdjian P, et al. Haemolytic uraemic syndrome and Shiga toxin–producing *Escherichia coli* infection in children in France. The Societe de Nephrologie Pediatrique. Epidemiol Infect **2000**; 124:215–20.
25. Pradel N, Boukhors K, Bertin Y, Forestier C, Martin C, Livrelli V. Heterogeneity of Shiga toxin–producing *Escherichia coli* strains isolated from hemolytic-uremic syndrome patients, cattle, and food samples in central France. Appl Environ Microbiol **2001**; 67:2460–8.
26. Ethelberg S, Olsen KE, Scheutz F, et al. Virulence factors for hemolytic uremic syndrome, Denmark. Emerg Infect Dis **2004**; 10:842–7.
27. Nielsen EM, Scheutz F, Torpdahl M. Continuous surveillance of Shiga toxin–producing *Escherichia coli* infections by pulsed-field gel electrophoresis shows that most infections are sporadic. Foodborne Pathog Dis **2006**; 3:81–7.
28. Keskimaki M, Saari M, Heiskanen T, Siitonen A. Shiga toxin–producing *Escherichia coli* in Finland from 1990 through 1997: prevalence and characteristics of isolates. J Clin Microbiol **1998**; 36:3641–6.
29. Eklund M, Scheutz F, Siitonen A. Clinical isolates of non-O157 Shiga toxin–producing *Escherichia coli*: serotypes, virulence characteristics, and molecular profiles of strains of the same serotype. J Clin Microbiol **2001**; 39:2829–34.
30. Eklund M. Enterohemorrhagic *Escherichia coli* (EHEC) findings from humans in Finland [academic dissertation]. Helsinki: National Public Health Institute, **2005**.
31. Eklund M, Nuorti JP, Ruutu P, Siitonen A. Shigatoxigenic *Escherichia coli* (STEC) infections in Finland during 1998–2002: a population-based surveillance study. Epidemiol Infect **2005**; 133:845–52.
32. Welinder-Olsson C, Badenfors M, Cheasty T, Kjellin E, Kaijser B. Genetic profiling of enterohemorrhagic *Escherichia coli* strains in relation to clonality and clinical signs of infection. J Clin Microbiol **2002**; 40: 959–64.
33. Elliott EJ, Robins-Browne RM, O'Loughlin EV, et al. Nationwide study of haemolytic uraemic syndrome: clinical, microbiological, and epidemiological features. Arch Dis Child **2001**; 85:125–31.
34. Goldwater PN, Bettelheim KA. The role of enterohaemorrhagic *E. coli* serotypes other than O157:H7 as causes of disease. In: Karmali MA, Goglio AG, eds. Recent advances in verocytotoxin-producing *Escherichia coli* infections. Amsterdam: Elsevier Science, **1994**:57–60.
35. Brooks HJ, Bettelheim KA, Todd B, Holdaway MD. Non-O157 Vero cytotoxin producing *Escherichia coli*: aetiological agents of diarrhoea in children in Dunedin, New Zealand. Comp Immunol Microbiol Infect Dis **1997**; 20:163–70.
36. Cordovez A, Prado V, Maggi L, et al. Enterohemorrhagic *Escherichia coli* associated with hemolytic-uremic syndrome in Chilean children. J Clin Microbiol **1992**; 30:2153–7.
37. Rivas M, Miliwebsky E, Chinen I, et al. Characterization and epidemiologic subtyping of Shiga toxin–producing *Escherichia coli* strains isolated from hemolytic uremic syndrome and diarrhea cases in Argentina. Foodborne Pathog Dis **2006**; 3:88–96.
38. Kudoh Y, Kai A, Obata H, et al. Epidemiological surveys on verocytotoxin-producing *Escherichia coli* infections in Japan. In: Karmali MA, Goglio AG, eds. Recent advances in verocytotoxin-producing *Escherichia coli* infections. Amsterdam: Elsevier Science, **1994**:53–6.
39. Uchida H, Kanegane H, Yoshiya K, Kitamura K, Ihara T, Kamiya H. Four cases of hemolytic uremic syndrome (HUS) associated with serotype O165 verotoxin producing *Escherichia coli* [in Japanese]. Kansenshogaku Zasshi **1995**; 69:678–83.
40. Bitzan M, Karch H, Maas MG, et al. Clinical and genetic aspects of Shiga-like toxin production in traditional enteropathogenic *Escherichia coli.* Zentralbl Bakteriol **1991**; 274:496–506.
41. Bockemuhl J, Aleksic S, Karch H. Serological and biochemical properties of Shiga-like toxin (verocytotoxin)–producing strains of *Escherichia coli,* other than O-group 157, from patients in Germany. Zentralbl Bakteriol **1992**; 276:189–95.
42. Lopez EL, Diaz M, Grinstein S, et al. Hemolytic uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. J Infect Dis **1989**; 160:469–75.
43. Brooks JT, Sowers EG, Wells JG, et al. Non-O157 Shiga toxin–producing *Escherichia coli* infections in the United States, 1983–2002. J Infect Dis **2005**; 192:1422–9.
44. Bielaszewska M, Janda JM, Blahova K, Sramkova L, Havlik J, Potuznik V. Verocytotoxin-producing *Escherichia coli* in children with hemolytic uremic syndrome and diarrhea in the Czech Republic. In: Karmali MA, Goglio AG, eds. Recent advances in verocytotoxin-producing *Escherichia coli* infections. Amsterdam: Elsevier Science, **1994**:37–40.
45. Werber D, Fruth A, Heissenhuber A, et al. Shiga toxin–producing *Escherichia coli* O157 more frequently cause bloody diarrhea than do non-O157 strains. J Infect Dis **2004**; 189:1335–6.
46. Garg AX, Suri RS, Barrowman N, et al. Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome. JAMA **2003**; 290: 1360–70.
47. Gerber A, Karch H, Allerberger F, Verweyen HM, Zimmerhackl LB. Clinical course and the role of Shiga toxin–producing *Escherichia coli* infection in the hemolytic-uremic syndrome in pediatric patients, 1997–2000, in Germany and Austria: a prospective study. J Infect Dis **2002**; 186:493–500.
48. Chandler WL, Jelacic S, Boster DR, et al. Prothrombotic coagulation abnormalities preceding the hemolytic-uremic syndrome. N Engl J Med **2002**; 346:23–32.
49. Dundas S, Todd WTA, Stewart AI, Murdoch PS, Chaudhuri AKR, Hutchinson SJ. The central Scotland *Escherichia coli* O157:H7 outbreak: risk factors for the hemolytic uremic syndrome and death among hospitalized patients. Clin Infect Dis **2001**; 33:923–31.
50. Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. N Engl J Med **2000**; 342:1930–6.
51. Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J Infect Dis **1989**; 160: 994–8.
52. Lathem WW, Grys TE, Witowski SE, et al. StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. Mol Microbiol **2002**; 45:277–88.
53. Karch H, Friedrich AW, Gerber A, Zimmerhackl LB, Schmidt MA, Bielaszewska M. New aspects in the pathogenesis of enteropathic hemolytic uremic syndrome. Semin Thromb Hemost **2006**; 32:105–12.
54. Karmali MA, Mascarenhas M, Shen S, et al. Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic or serious disease. J Clin Microbiol **2003**; 41:4930–40.
55. Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of Shiga toxin–producing *Escherichia coli* and disease in humans. J Clin Microbiol **1999**; 37: 497–503.
56. Bielaszewska M, Fell M, Greune L, et al. Characterization of cytolethal distending toxin genes and expression in Shiga toxin–producing *Escherichia coli* strains of non-O157 serogroups. Infect Immun **2004**; 72: 1812–6.
57. Bielaszewska M, Sramkova L, Janda J, Blahova K, Ambrozova H. Verotoxigenic (enterohaemorrhagic) *Escherichia coli* in infants and toddlers in Czechoslovakia. Infection **1990**; 18:352–6.
58. Welinder-Olsson C, Eriksson E, Kaijser B. Virulence genes in verocytotoxigenic *Escherichia coli* strains isolated from humans and cattle. APMIS **2005**; 113:577–85.
59. Tozzi AE, Caprioli A, Minelli F, et al. Shiga toxin–producing *Escherichia coli* infections associated with hemolytic uremic syndrome, Italy, 1988–2000. Emerg Infect Dis **2003**; 9:106–8.
60. Werber D, Fruth A, Liesegang A, et al. A multistate outbreak of Shiga toxin–producing *Escherichia coli* O26:H11 infections in Germany, detected by molecular subtyping surveillance. J Infect Dis **2002**; 186: 419–22.
61. Allerberger F, Friedrich AW, Grif K, et al. Hemolytic-uremic syndrome associated with enterohemorrhagic *Escherichia coli* O26:H infection and

consumption of unpasteurized cow's milk. Int J Infect Dis **2003**; 7: 42–5.
62. Schmidt H, Geitz C, Tarr PI, Frosch M, Karch H. Non-O157:H7 pathogenic Shiga toxin–producing *Escherichia coli:* phenotypic and genetic profiling of virulence traits and evidence for clonality. J Infect Dis **1999**; 179:115–23.
63. Bitzan M, Karch H, Maas MG, et al. Clinical and genetic aspects of Shiga-like toxin production in traditional enteropathogenic *Escherichia coli.* Zentralbl Bakteriol **1991**; 274:496–506.
64. Bokete TN, O'Callahan CM, Clausen CR, et al. Shiga-like toxin–producing *Escherichia coli* in Seattle children: a prospective study. Gastroenterology **1993**; 105:1724–31.
65. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21—Helena, Montana, 1994. MMWR CDC Surveill Summ **1994**; 44:501–3.
66. Community outbreak of hemolytic uremic syndrome attributable to *Escherichia coli* O111:NM—South Australia, 1995. MMWR CDC Surveill Summ **1995**; 44:550–1.
67. Bitzan M, Karch H, Altrogge H, Strehlau J, Blaker F. Hemolytic-uremic syndrome associated with a variant Shiga-like cytotoxin of *Escherichia coli* O111. Pediatr Infect Dis J **1988**; 7:128–32.
68. Gunzburg S, Gracey M, Forbes D, Hewitt I, Bettelheim K. Haemolytic-uraemic syndrome and verocytotoxigenic *Escherichia coli.* Med J Aust **1988**; 149:54–5.
69. Guth BEC, Ramos SRTS, Cerqueira AMF, Andrade JRC, Gomes TAT. Phenotypic and genotypic characteristics of Shiga toxin–producing *Escherichia coli* strains isolated from children in São Paulo, Brazil. Mem Inst Oswaldo Cruz **2002**; 97:1085–9.
70. Brooks JT, Bergmire-Sweat D, Kennedy M, et al. Outbreak of Shiga toxin–producing *Escherichia coli* O111:H8 infections among attendees of a high school cheerleading camp. Clin Infect Dis **2004**; 38:190–8.
71. Banatvala N, Debeukelaer MM, Griffin PM, et al. Shiga-like toxin–producing *Escherichia coli* O111 and associated hemolytic-uremic syndrome: a family outbreak. Pediatr Infect Dis J **1996**; 15: 1008–11.
72. McCarthy TA, Barrett NL, Hadler JL, et al. Hemolytic-uremic syndrome and *Escherichia coli* O121 at a lake in Connecticut, 1999. Pediatrics **2001**; 108:1–7.
73. Scheutz F, Cheasty T, Woodward D, Smith HR. Designation of O174 and O175 to temporary O groups OX3 and OX7, and six new *Escherichia coli* O groups that include verocytotoxin-producing *E. coli* (VTEC): O176, O177, O178, O179, O180, and O181. APMIS **2004**; 112:569–84.
74. Paton AW, Voss E, Manning PA, Paton JC. Shiga toxin–producing *Escherichia coli* isolates from cases of human disease show enhanced adherence to intestinal epithelial (Henle 407) cells. Infect Immun **1997**; 65:3799–805.
75. Karch H, Schubert S, Zhang D, et al. A genomic island, termed high-pathogenicity island, is present in certain non-O157 Shiga toxin–producing *Escherichia coli* clonal lineages. Infection and Immunity **1999**; 67:5994–6001.
76. Perna NT, Plunkett G III, Burland V, et al. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature **2001**; 409:529–33.
77. Paton AW, Srimanote P, Talbot UM, Wang H, Paton JC. A new family of potent AB5 cytotoxins produced by Shiga toxigenic *Escherichia coli.* J Exp Med **2004**; 200:35–46.
78. Paton AW, Paton JC. Multiplex PCR for direct detection of Shiga toxigenic *Escherichia coli* strains producing the novel subtilase cytotoxin. J Clin Microbiol **2005**; 43:2944–7.
79. Carroll KC. Immunoassay detection of Shiga toxin–producing *Escherichia coli.* In: Isenberg H, ed. Clinical microbiology procedures handbook. Washington, DC: American Society for Microbiology Press (in press).
80. Ake JA, Jelacic S, Clark C, et al. Relative nephroprotection during *Escherichia coli* O157:H7 infections: association with intravenous volume expansion. Pediatrics **2005**; 115:673–80.
81. Voetsch AC, Angulo FJ, Rabatsky-Ehr T, et al. Laboratory practices for stool-specimen culture for bacterial pathogens, including *Escherichia coli* O157:H7, in the FoodNet sites, 1995–2000. Clin Infect Dis **2004**; 38(Suppl 3):S190–7.

LEXSEE 265 WIS.2D 476

Estate of Brianna L. Kriefall, deceased, by her Special Administrator, Douglas A. Kriefall, Douglas A. Kriefall, Connie J. Kriefall, and Chad Kriefall, a minor, by his Guardian ad Litem, William M. Cannon, Plaintiffs-Appellants, Humana Employers Health Insurance Co., a Wisconsin insurance corporation, Involuntary-Plaintiff, v. Sizzler USA Franchise, Inc., a foreign corporation, Defendant-Third-Party Plaintiff-Co-Appellant, Federal Insurance Co., a foreign insurance corporation, E&B Management Co. Waukesha, Inc., a Wisconsin corporation, Secura Insurance, a Wisconsin insurance corporation, Sysco Services of Eastern Wisconsin, a wholly owned subsidiary of Sysco Corporation, a foreign corporation, Fidelity and Guaranty Insurance Co., a foreign insurance corporation, Lee M. Eschenbach, Steven C. Boysa, AAA Insurance Co., and BBB Insurance Co., Defendants, Excel Corporation, a foreign corporation, and American Home Assurance Co., a foreign insurance corporation, Defendants-Respondents, v. Lee M. Eschenbach, Steven C. Boysa, AAA Insurance Co., BBB Insurance Co., Cargill, Inc., a foreign corporation, and Excel Food Distribution, Inc., a foreign corporation, Third-Party Defendants. Ervin J. Lesak and Florence Lesak, Plaintiffs-Co-Appellants, v. E&B Management Co. Waukesha, Inc., Defendant, Excel Corporation, Defendant-Respondent. Jeffrey Fortier, Judith Fortier, Tristan Fortier, and Carly Fortier, Plaintiffs-Co-Appellants, Great West Life & Annuity Insurance, Involuntary-Plaintiff, v. Excel Corporation, Defendant-Respondent. Kevin McCormick, Sandy McCormick, and Kelsea McCormick, Plaintiffs-Co-Appellants, Blue Cross Blue Shield United, Involuntary-Plaintiff, v. Excel Corporation, Defendant-Respondent.

Appeal No. 02-1939

COURT OF APPEALS OF WISCONSIN, DISTRICT ONE

2003 WI App 119*; *265 Wis. 2d 476*; *665 N.W.2d 417*; *2003 Wisc. App. LEXIS 482

May 13, 2003, Decided
May 13, 2003, Opinion Filed

SUBSEQUENT HISTORY: Released for Publication June 25, 2003.
Review denied by *Estate of Kriefall v. Sizzler USA, 2003 WI 140, 266 Wis. 2d 63, 671 N.W.2d 849, 2003 Wisc. LEXIS 885 (2003)*
US Supreme Court certiorari denied by, Motion granted by *Excel Corp. v. Estate of Brianna L. Kriefall, 158 L. Ed. 2d 392, 124 S. Ct. 1656, 2004 U.S. LEXIS 2333 (U.S., Mar. 22, 2004)*

PRIOR HISTORY: APPEAL from judgments of the circuit court for Milwaukee County: MICHAEL P. SULLIVAN, Judge. Cir. Ct. Nos. 00 CV 6463, 00 CV 6360, 00 CV 8756, & 00 CV 8793.

DISPOSITION: Reversed.

COUNSEL: On behalf of the plaintiffs-appellants, the cause was submitted on the briefs of William M. Cannon and Edward E. Robinson of Cannon & Dunphy, S.C., Milwaukee. There was oral argument by Edward E. Robinson.

On behalf of the plaintiffs-co-appellants, Ervin J. Lesak and Florence Lesak, the cause was submitted on the briefs of Denis W. Stearns of Marler Clark, LLP, PS, Seattle, Washington and Michael J. Hanrahan of Fox, O'Neill & Shannon, S.C., Milwaukee. There was oral argument by Denis W. Stearns.

On behalf of the defendant-third-party plaintiff-co-appellant, the cause was submitted on the briefs of Russell A. Klingaman of Hinshaw & Culbertson, Milwaukee; J. Ric Gass, Mark M. Leitner, and Thomas Gonzalez of Kravit, Gass, Hovel & Leitner, S.C., Milwaukee; and Frederic L. Holmes of Gordon & Holmes, San Diego, California. There was oral argument by James R. Gass.

On behalf of the defendant-respondent, Excel Corporation, the cause was submitted on the brief of Ralph A. Weber, Amelia L. McCarthy, and Shawn K. Stevens of Reinhart Boerner Van Deuren, s.c., Milwaukee. There was oral argument by Ralph A. Weber.

An amicus curiae brief was filed by Timothy A. Bascom of Bascom, Budish & Ceman, S.C., Wauwatosa; Gary Jay Kushner of Hogan & Hartson, L.L.P., Washington, D.C.; Mark D. Dopp of American Meat Institute, Arlington, Virginia; Brett T. Schwemer, Dennis R. Johnson, and Philip C. Olsson of Olsson, Frank and Weeda, P.C., Washington, D.C.

JUDGES: Before Fine, Schudson and Hoover, JJ.

OPINION BY: FINE

OPINION

[**482] [***420] [*P1] FINE, J. This is a consolidated appeal from the trial court's grant of summary judgment to Excel Corporation, a meat processor, dismissing claims against Excel for damages allegedly caused by Excel's sale to a Milwaukee area Sizzler restaurant of beef contaminated with the bacterium E. coli O157:H7. The plaintiffs involved in this appeal contend that E. coli bacteria from the meat sold by Excel to the Sizzler restaurant contaminated other food that was eaten by either them or those through whom they derive their claims. [1] Sizzler [***421] USA Franchise, Inc., the franchisor of [**483] the Milwaukee Sizzler restaurant, is a defendant in some of the actions and also appeals from the trial court's grant of summary judgment dismissing Sizzler USA's claims against Excel. The trial court ruled that the claims against Excel were barred by the federal-preemption doctrine. We disagree and reverse.

1 The plaintiffs involved in this appeal are: The Estate of Brianna L. Kriefall, Douglas A. Kriefall, Connie J. Kriefall, and Chad Kriefall in Milwaukee County Circuit Court Case No. 00-CV-006463; Ervin J. Lesak and Florence Lesak in Milwaukee County Circuit Court Case No. 00-CV-006360; Jeffrey Fortier, Judith Fortier, Tristan Fortier, and Carly Fortier in Milwaukee County Circuit Court Case No. 00-CV-008756; and Kevin McCormick, Sandy McCormick, and Kelsea McCormick in Milwaukee County Circuit Court Case No. 00-CV-008793. Only the Kriefalls, the Lesaks, and Sizzler USA have filed appellate briefs. A joint *amici curiam* brief has been filed by The American Meat Institute, the National Chicken Council, the National Meat Association, the National Turkey Federation, the North American Meat Processors Association, and the Southwest Meat Association. All submissions to this court provided helpful analyses.

I.

[*P2] In July of 2000, a number of persons were injured and three-year-old Brianna Kriefall died from eating food that everyone party to this appeal, the plaintiffs, Sizzler USA, and Excel, recognize was cross-contaminated by E. coli O157:H7 bacteria from meat sold by Excel. Although some of the parties' arguments on appeal focus on both to what extent the E. coli contamination of the Excel beef was a cause of Brianna's death and the other injuries, and whether Excel was either negligent or sold a dangerously defective product, the only issue we need decide on this appeal is whether the claims against Excel are preempted by federal law. We conclude that federal preemption does not close the doors of Wisconsin's courts to the claims against Excel; the merits of those claims still have to be determined.

[**484] [*P3] Federal preemption is based on Article VI of the United States Constitution, which makes federal law "the supreme Law of the Land." ***Cipollone v. Liggett Group, Inc.***, *505 U.S. 504, 516, 120 L. Ed. 2d 407, 112 S. Ct. 2608 (1992)*. As material here, there are two steps to an analysis of whether federal regulation preempts state common-law claims: (1) whether the controlling federal statute "expressly pre-empts common-law claims," and, if not, (2) whether "the potential conflict between diverse state rules and the federal interest in a uniform system of regulation impliedly pre-empts such claims." ***Sprietsma v. Mercury Marine***, *537 U.S. 51, 154 L. Ed. 2d 466, 123 S. Ct. 518, 522-523 (2002)*; *see also* ***Geier v. American Honda Motor Co.***, *529 U.S. 861, 869, 146 L. Ed. 2d 914, 120 S. Ct. 1913 (2000)* ("ordinary" preemption principles may bar state claims even though those claims are not expressly preempted by the applicable federal statute). [2]

2 ***Sprietsma*** also identified a third element, which does not apply here: whether a decision by the agency vested by Congress with regulatory responsibility to not prescribe a safety standard preempts claims seeking to impose liability on a manufacturer in a regulated industry for the man-

ufacturer's failure to adopt that safety standard. *123 S. Ct. at 522-523*.

[*P4] The interstate sale of beef and other meat products intended for human consumption is regulated by the Federal Meat Inspection Act, *21 U.S.C. §§ 601-695*. The Act has a preemption clause, which provides, as applicable here:

> Requirements within the scope of this chapter with respect to premises, facilities and operations of any establishment at which inspection is provided under subchapter I of this chapter *[§§ 601-624*], which are in addition to, or different than those made under this chapter may not be imposed by any State ... This chapter shall not preclude any [***422] State ... from making [**485] requirement [*sic*] or taking other action, consistent with this chapter, with respect to any other matters regulated under this chapter.

21 U.S.C. § 678. [3] This section thus: (1) [***423] prevents states from imposing "requirements ... with respect to premises, [**486] facilities and operations of any establishment at [**487] which inspection is provided under" *21 U.S.C. §§ 601-624* that "are in addition to, or different than those made under" the Act, and (2) permits states to impose "requirements" and to take "other action" that is "consistent" with the Act "with respect to any other matters regulated under" the Act.

3 *21 U.S.C. § 678* reads in full:

> Requirements within the scope of this chapter with respect to premises, facilities and operations of any establishment at which inspection is provided under subchapter I of this chapter, which are in addition to, or different than those made under this chapter may not be imposed by any State or Territory or the District of Columbia, except that any such jurisdiction may impose recordkeeping and other requirements within the scope of section 642 of this title, if consistent therewith, with respect to any such establishment.
>
> Marking, labeling, packaging, or ingredient requirements in addition to, or different than, those made under this chapter may not be imposed by any State or Territory or the District of Columbia with respect to articles prepared at any establishment under inspection in accordance with the requirements under subchapter I of this chapter, but any State or Territory or the District of Columbia may, consistent with the requirements under this chapter, exercise concurrent jurisdiction with the Secretary over articles required to be inspected under said subchapter I, for the purpose of preventing the distribution for human food purposes of any such articles which are adulterated or misbranded and are outside of such an establishment, or, in the case of imported articles which are

> not at such an establishment, after their entry into the United States. This chapter shall not preclude any State or Territory or the District of Columbia from making requirement [*sic*] or taking other action, consistent with this chapter, with respect to any other matters regulated under this chapter.

We assume, without deciding, that the word "requirements" encompasses state common-law claims, although the law on this is not yet entirely settled. Thus, in ***Medtronic, Inc. v. Lohr***, *518 U.S. 470, 135 L. Ed. 2d 700, 116 S. Ct. 2240 (1996)*, the preemption clause provided that, as material here:

> "No State ... may establish or continue in effect with respect to a device intended for human use any requirement-
>
> (1) which is different from, or in addition to, any requirement applicable under this chapter to the device, and
>
> (2) which relates to the safety or effectiveness of the device or to any other matter included in a requirement applicable to the device under this chapter."

Id., *518 U.S. at 481-482*. Justice John Paul Stevens, writing only for himself and Justices Anthony M. Kennedy, David H. Souter, and Ruth Bader Ginsburg (and not "the" Court, as some of the appellants represent), rejected the view "that any common-law cause of action is a 'requirement' which alters incentives and imposes duties 'different from, or in addition to,' the generic federal standards that the [Food and Drug Administration] has promulgated in response to mandates under the [Medical Device Amendments of 1976]." ***Id.***, *518 U.S. at 486-487*. The same plurality, however, specifically declined to hold "that common-law duties are never 'requirements' within the meaning of the [Medical Device act's preemption clause] and that the statute therefore never pre-empts common-law actions." ***Id.***, *518 U.S. at 502*. Four years earlier, however, Justice Stevens, this time writing for himself, Chief Justice William H. Rehnquist, and Justices Byron R. White and Sandra Day O'Connor, opined in ***Cipollone v. Liggett Group, Inc.***, *505 U.S. 504, 120 L. Ed. 2d 407, 112 S. Ct. 2608 (1992)*, that a preemption phrase in that case, which sought damages for the cigarette-related death of the plaintiff's decedent, encompassed state-court claims. ***Id.***, *505 U.S. at 520-523*. The phrase provided: "No requirement or prohibition based on smoking and health shall be imposed under State law with respect to the advertising or promotion of any cigarettes the packages of which are labeled in conformity with the provisions of this Act." ***Id.***, *505 U.S. at 515*. Justice Stevens wrote:

> The phrase "no requirement or prohibition" sweeps broadly and suggests no distinction between positive enactments and common law; to the contrary, those words easily en-

> compass obligations that take the form of common-law rules. As we noted in another context, "[state] regulation can be as effectively exerted through an award of damages as through some form of preventive relief. The obligation to pay compensation can be, indeed is designed to be, a potent method of governing conduct and controlling policy."
>
> ....
>
> Moreover, common-law damages actions of the sort raised by petitioner are premised on the existence of a legal duty, and it is difficult to say that such actions do not impose "requirements or prohibitions."

Id., *505 U.S. at 521* (quoted source omitted; bracketing by Justice Stevens). *See also* ***Lynnbrook Farms v. Smithkline Beecham Corp.***, *79 F.3d 620, 627 (7th Cir. 1996)* ("State tort actions can therefore be as much of a threat to national uniformity as affirmative state regulation.") (relying on Justice Stevens's plurality opinion in ***Cipollone***, but mistakenly referring to it as the view of "the Supreme Court"). We also assume, without deciding, that the claims asserted here against Excel would, if successful, affect Excel's "operations" by encouraging or even compelling Excel to change those "operations" in order to avoid future liability caused by E. coli contaminated meat.

[*P5] For the purpose of this appeal, we assume that all the facts asserted by Excel are true. *See* ***City of Milwaukee v. Burnette***, *2001 WI App 258, P8, 248 Wis. 2d 820, 834, 637 N.W.2d 447, 454* (court reviewing grant or denial of summary judgment ignores disputed facts unless those facts are material to the legal issue to be decided). We analyze whether either *21 U.S.C. § 678* expressly preempts the tort claims asserted here or whether those claims are impliedly preempted by federal law because they present "an actual conflict with a [**488] federal objective." ***Geier***, *529 U.S. at 871*. Whether state tort claims are preempted by federal law is a legal issue that we review *de novo*. ***International Ass'n of Machinists & Aerospace Workers v. United States Can Co.***, *150 Wis. 2d 479, 487, 441 N.W.2d 710, 713 (1989), cert. denied, 493 U.S. 1019, 107 L. Ed. 2d 738, 110 S. Ct. 718*.

II.

[*P6] Congressional intent concerning the interstate sale of meat is set out in *21 U.S.C. § 602*, which we reprint in full:

> Meat and meat food products are an important source of the Nation's total supply of food. They are consumed throughout the Nation and the major portion thereof moves in interstate or foreign commerce. It is essential in the public interest that the health and welfare of consumers be protected by assuring that meat and meat food products distributed to them are wholesome, not adulterated, and properly marked, labeled, and packaged. Unwholesome, adulterated, or misbranded meat or meat food products impair the effective regulation of meat and meat food products in interstate or foreign commerce, are injurious to the public welfare, destroy markets for wholesome, not adulterated, and properly labeled and packaged meat and meat food products, and result in sundry losses to livestock producers and processors of meat and meat food products, as well as injury to consumers. The unwholesome, adulterated, mislabeled, or deceptively packaged articles can be sold at lower prices and compete unfairly with the wholesome, not adulterated, and properly labeled and packaged articles, to the detriment of consumers and the public generally. It is hereby found that all articles and animals which are regulated under this [***424] chapter are either in interstate or foreign

commerce or substantially affect such commerce, [**489] and that regulation by the Secretary and cooperation by the States and other jurisdictions as contemplated by this chapter are appropriate to prevent and eliminate burdens upon such commerce, to effectively regulate such commerce, and to protect the health and welfare of consumers. [4]

4 "Meat food product" is defined by the *Meat Inspection Act* as:

> Any product capable of use as human food which is made wholly or in part from any meat or other portion of the carcass of any cattle, sheep, swine, or goats, excepting products which contain meat or other portions of such carcasses only in a relatively small proportion or historically have not been considered by consumers as products of the meat food industry, and which are exempted from definition as a meat food product by the Secretary under such conditions as he may prescribe to assure that the meat or other portions of such carcasses contained in such product are not adulterated and that such products are not represented as meat food products. This term as applied to food products of equines shall have a meaning comparable to that provided in this paragraph with respect to cattle, sheep, swine, and goats.
>
> *21 U.S.C. § 601(j).*

(Footnote added.) Thus, as expressed in *§ 602*, Congress wanted to: (1) protect consumers "by assuring that meat and meat food products distributed to them are wholesome, not adulterated, and properly marked, labeled, and packaged"; (2) protect those in the meat-production chain from unscrupulous competitors; and (3) "prevent and eliminate burdens upon [interstate or foreign] commerce." ***Ibid.*** The overriding congressional purpose is, however, public safety-as evidenced by not only the section's direct statements to that effect but also by one of the stated rationales underlying the concurrent congressional desire to preserve fair competition for those who sell wholesome and properly packaged [**490] and labeled meat. Accordingly, congressional focus is on ensuring that only meat that is "not adulterated" makes it into the interstate-commerce market. ***Ibid.***

[*P7] The Excel beef that was shipped to the Sizzler restaurant, and according to the plaintiffs caused their illnesses and Brianna's death, were wrapped "intact" (not ground or chopped or minced or shredded) cuts of sirloin muscle when they left the Excel plant. Excel's preemption argument is based on two main contentions. First, it asserts that the sale of intact meat contaminated with E. coli O157:H7 is not "adulterated" under federal law. Accordingly, under its view, permitting the claims to proceed would prohibit or punish that which federal law allows, thereby running afoul of the preemption clause. Second, it argues that the meat left its plant after it was inspected and approved by government inspectors, and thus, again, permitting these claims against it would, in effect, prohibit or punish that which federal law allows. An evaluation of these contentions requires an analysis of the *Meat Inspection Act* and the applicable governing regulations.

[*P8] Most if not all congressional enactments delegate their implementation to one or more administrative agencies. Some statutes have an all-encompassing clause and state "simply that the agency may 'make ...

such rules and regulations as may be necessary to carry out the provisions of this Act.'" *See* ***Mourning v. Family Publ'ns Serv., Inc.****, 411 U.S. 356, 369, 36 L. Ed. 2d 318, 93 S. Ct. 1652 (1973)* (quoting, as an example, § 8 of the United States Housing Act of 1937, as amended, 42 U.S.C. § 1408). When that is the situation, the administrative agency's regulations are followed as long as they are [***425] "'reasonably related to the purposes of the enabling legislation.'" ***Ibid.*** (quoted source omitted). Other delegations of regulatory authority [**491] are more limited, and the agency is authorized to promulgate regulations only within narrow confines. In such a situation, the statute, not a regulation that may conflict with the statute, governs, ***Touche Ross & Co. v. Redington****, 442 U.S. 560, 577 n.18, 61 L. Ed. 2d 82, 99 S. Ct. 2479 (1979)* ("The language of the statute and not the rules must control."); ***Koshland v. Helvering****, 298 U.S. 441, 447, 80 L. Ed. 1268, 56 S. Ct. 767 (1936)* (where "provisions of the act are unambiguous, and its directions specific, there is no power to amend it by regulation"), because "it is axiomatic that an administrative agency's power to promulgate legislative regulations is limited to the authority delegated by Congress," ***Bowen v. Georgetown Univ. Hosp.****, 488 U.S. 204, 208, 102 L. Ed. 2d 493, 109 S. Ct. 468 (1988)*.

[*P9] Congress delegated enforcement of the *Meat Inspection Act* to the Secretary of the United States Department of Agriculture or designee. *21 U.S.C. §§ 602, 601(a)*. The Secretary has, in turn, delegated authority under the *Meat Inspection Act*, with exceptions that are not material here, to the Under Secretary for Food Safety, Agriculture, *7 C.F.R. § 2.18(a)(1)(ii)(B)*, who, in turn, subdelegated that authority to the Administrator of the Food Safety and Inspection Service, Agriculture, *7 C.F.R. § 2.53(a)(2)(ii)*.

[*P10] In contrast to some other delegations of authority by Congress to administrative agencies, Congress's delegation here is focused. Thus, as we will see, although the Secretary has a wide berth in implementing the congressional mandate to inspect meat-processing plants, the Secretary has only limited authority to affect the congressional definition of "adulterated," other than in the area of labeling (*21 U.S.C. § 601(m)(5), (7)-(9)*). And that limitation, as we will explain, is critical in this case because of Excel's [**492] argument that the Secretary views intact meat contaminated with E. coli O157:H7 as not "adulterated."

III.

[*P11] Whether the claims against Excel are expressly preempted by *21 U.S.C. § 678* because they would impose requirements "with respect to premises, facilities and operations ... which are in addition to, or different than those made under" the *Meat Inspection Act* turns on: (1) what is "adulterated" under the Act, and (2) the nature of federal inspection of meat-processing plants under the Act.

A. *The Act's Definition of "Adulterated."*

[*P12] The word "adulterated" has a special meaning under the *Meat Inspection Act*, and, significantly, the Secretary is given authority to affect the statute's definition in only one limited instance:

> The term "adulterated" shall apply to any carcass, part thereof, meat or meat food product under one or more of the following circumstances:
>
> (1) if it bears or contains any poisonous or deleterious substance which may render it injurious to health; but in case the substance is not an added substance, such article shall not be considered adulterated under this clause if the quantity of such substance in or on such article does not ordinarily render it injurious to health;
>
> (2)(A) if it bears or contains (by reason of administration of any substance to the live animal or otherwise) any added poisonous or added deleterious substance [**493] ... which may, in the judgment of the Secretary, make such article unfit for human food;
>
> ....
>
> [***426] (3) if it consists in whole or in part of any filthy, putrid, or decomposed substance or is for any other reason unsound, unhealthful, unwholesome, or otherwise unfit for human food; [or]
>
> (4) if it has been prepared, packed, or held under insanitary conditions where [***427] by it may have become contaminated with filth, or whereby it may have been rendered injurious to health.

21 U.S.C. § 601(m). We look at these subsections in turn.

[*P13] *Subsection (1)* The E. coli strain that killed Brianna and made the others sick is a "deleterious substance which *may* render [meat] injurious to health." There is no dispute about this. Thus, under the first part of *21 U.S.C. § 601(m)(1)*, meat that either "bears or con-

tains" E. coli O157:H7 (the "deleterious substance") is "adulterated." That E. coli O157:H7 contamination can be rendered non-"injurious to health" by cooking thoroughly, as discussed below, does not negate this; Congress used the phrase "may render," not "in every circumstance renders." Moreover, if the E. coli bacteria is not considered to be "an added substance," because it comes from some of the animals themselves and is not either applied or supplied during the slaughtering process (although we do not decide this), it cannot be said that the E. coli strain "does not ordinarily render [the meat on or in which it appears] injurious to health." Accordingly, meat contaminated by E. coli O157:H7 is also "adulterated" under the second part of *§ 601(m)(1)*.

[**494] [*P14] *Subsection (2)(A)*. This section defers to the Secretary (and thus, derivatively, to the Food Safety and Inspection Service) to determine whether the contaminating substance that is alleged to make the affected meat "adulterated" does, in fact, "make such article unfit for human food." As we will see, the Food Safety and Inspection Service determined that E. coli-infected meat is not unfit as human food as long as it is: (1) an intact cut, and (2) treated by cooking or otherwise to kill the surface E. coli contamination.

[*P15] *Subsection (3)*. Meat contaminated by the E. coli strain that was on the Sizzler meat falls within this definition of "adulterated" because the E. coli made the infected meat at least "in part ... unsound, unhealthful, unwholesome, or otherwise unfit for human food" within the meaning of the all-encompassing phrase "for any other reason."

[*P16] *Subsection (4)*. Meat contaminated by E. coli O157:H7 also falls within this definition of "adulterated" because it is "prepared" in such a way "whereby it may have been rendered injurious to health."

[*P17] The only regulations defining "adulterated" in the context here are found in Animals and Animal Products, *9 C.F.R. § 301.2*:

> *Adulterated.* This term applies to any carcass, part thereof, meat or meat food product under one or more of the following circumstances:
>
> (1) If it bears or contains any such poisonous or deleterious substance which may render it injurious to health; but in case the substance is not an added substance, such article shall not be considered adulterated under this clause if the quantity of such substance in or on such article does not ordinarily render it injurious to health;
>
> [**495] ....
>
> (3) If it consists in whole or in part of any filthy, putrid, or decomposed substance or is for any other reason unsound, unhealthful, unwholesome, or otherwise unfit for human food; [or]
>
> (4) If it has been prepared, packed, or held under unsanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health.

Excel contends that the meat it produced that ultimately went to the Sizzler restaurant was not "adulterated" because the Food Safety and Inspection Service has determined that E. coli adulterates only non-intact meat-that is, meat that is ground or otherwise processed so that the E. coli contamination is not restricted to meat surfaces that are seared, broiled, or otherwise heated to kill the bacteria. To put Excel's argument in context, we examine in detail the Food Safety and Inspection Service's determinations.

[*P18] In a policy statement published in the Federal Register on January 19, 1999, the Food Safety and Inspection Service opined that it:

> believes that in evaluating beef products contaminated with *E. coli* O157:H7, intact cuts of muscle that are to be distributed for consumption as intact cuts should be distinguished from non-intact products, as well as from intact cuts of muscle that are to be further processed into non-intact product prior to distribution for consumption.

Beef Products Contaminated With Escherichia Coli O157:H7, *64 Fed. Reg. 2803, 2804 (Jan. 19, 1999)*. The Inspection Service further explained:

> [**496] Non-intact beef products include beef that has been injected with solutions, mechanically tenderized by needling, cubing, Frenching, or pounding devices, or reconstructed into formed entrees (*e.g.*, beef that has been scored to incorporate a marinade, beef that has a solution of proteolytic enzymes applied to or injected into the cut of meat, or a

formed and shaped product such as beef gyros). Pathogens may be introduced below the surface of these products as a result of the processes by which they are made. In addition, non-intact beef products include those beef products in which pathogens may be introduced below the surface by a comminution process such as chopping, grinding, flaking, or mincing (*e.g.*, fresh veal sausage and fabricated beef steak).

Intact cuts of beef that are to be further processed into non-intact cuts prior to distribution for consumption must be treated in the same manner as non-intact cuts of beef, since pathogens may be introduced below the surface of these products when they are further processed into non-intact products. Manufacturing trimmings (*i.e.*, pieces of meat remaining after steaks, roasts, and other intact cuts are removed) are an example of this type of product. Although manufacturing trimmings may be intact, they are generally further processed into non-intact products.

The Agency believes that with the exception of beef products that are intact cuts of muscle that are to be distributed for consumption as intact cuts, an *E. coli* O157:H7-contaminated beef product must not be distributed until it has been processed into a ready-to-eat product-*i.e.*, a food product that may be consumed safely without any further cooking or other preparation. Otherwise, such products (*i.e.*, non-intact products and intact cuts of muscle that are to be further processed into non-intact products prior to distribution for consumption) must be deemed adulterated. Intact steaks and roasts and other intact cuts of muscle with [**497] surface contamination are customarily cooked in a manner that ensures that these products are not contaminated with *E. coli* O157:H7 when consumed. Consequently, such intact products that are to be distributed for consumption as intact cuts are not deemed adulterated.

[***428] **Ibid.** (footnote omitted). The meat sold by Excel arrived at the Sizzler restaurant as wrapped intact cuts of beef that bore labels warning the Sizzler employees to cook the meat thoroughly, to keep the raw meat away from other foods, and to wash working surfaces, tools, utensils, and hands after their contact with raw meat. Excel contends that this, together with the fact that the meat left its plant approved by the federal inspection process, lets it off the hook. There are two problems with this contention.

[*P19] First, when Excel shipped the meat that was sold to the Sizzler restaurant, meat processors like Excel were required by regulations governing the processors' assessment of food-safety hazards in their plants to consider "the intended use or consumers of the finished product." Animals and Animal Products, *9 C.F.R. § 417.2(a)(2)*. This regulation was effective on January 26, 1998, for establishments employing more than 500 persons. *Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems, 61 Fed. Reg. 38806, 38869 (July 25, 1996)*. According to evidence in the record, significantly more than 500 persons worked at the Excel plant when it produced the meat that was sold to the Sizzler restaurant. The scope of this direction to consider the consumers' "intended use" was underscored by the Food Safety and Inspection Service in a policy statement published in the Federal Register on October 7, 2002:

> [**498] Even establishments that produce intact product will need to reassess their [hazard-analysis] plans based on the new *E. coli* O157:H7 data [indicating that the bacterium "is more prevalent than was previously thought" "and that this pathogen may he a hazard that is reasonably likely to occur at all stages of handling raw beef products"]. These establishments are required to reassess their [hazard-analysis] plans because much intact beef product may be used to make non-intact product such as ground beef. According to [*9 C.F.R.] § 417.2(a)(2)*, establishments are required to identify the intended use or consumers of the finished product. Therefore, to be able to determine the adequacy of their [hazard-analysis] plans, establishments that produce intact beef products need to determine whether their products will be used to produce raw, non-intact product.

E. coli O157:H7 *Contamination of Beef Products, 67 Fed. Reg. 62325, 62329 (Oct. 7, 2002)*. Thus, Excel's hazard-analysis plan recognized that its intact cuts of beef were "intended to be sold raw ... for further processing at retail."

[*P20] Second, although Congress has in *21 U.S.C. § 601(m)(2)(A)* delegated to the Secretary the responsibility to make a "judgment" whether "any ... added deleterious substance" makes the meat to which the substance is added "unfit for human food," Congress has itself, in *21 U.S.C. § 601(m)(1), (3), & (4)*, defined "adulterated" without seeking the Secretary's input. As discussed earlier, insofar as the statutory definitions of "adulterated" conflict with the gloss put on them by the Food Safety and Inspection Service, the statutory definition controls. ***Touche Ross**, 442 U.S. at 577 n.18* ("The language of the statute and not the rules must control."). The focus of subsections *(1), (3),* and *(4) of § 601(m)* is on people's health and safety. None of the [**499] definitions of "adulterated" in the Act makes a distinction between intact or non-intact meat, and the Food Safety and Inspection Service was powerless to add that distinction to Congress's definitions of "adulterated" in *subsections (1), (3),* and *(4)*. *See* ***Koshland**, 298 U.S. at 447* (agency has no power to contravene a statute where its "provisions ... [***429] are unambiguous, and its directions specific"). Moreover, as noted, even the Department's own regulations defining the word "adulterated," as opposed to its less-formal pronouncements, make no distinction between contaminated intact meat and contaminated non-intact meat.

[*P21] Based on the foregoing, we reject Excel's contention that the claims against it are barred because holding it liable for shipping in interstate commerce intact meat contaminated with E. coli O157:H7 would contravene the Act's express "premises, facilities and operations" preemption clause. We now turn to whether the Act's meat-inspection provisions and the regulations promulgated thereunder expressly preempt those claims.

B. *Federal Inspection Under the Act.*

[*P22] As we have seen, the *Meat Inspection Act*'s preemption clause prohibits states from imposing requirements "with respect to premises, facilities and operations of any establishment," which, like Excel, are regulated by the Act, that "are in addition to, or different than those made under" the Act. *21 U.S.C. § 678*. Again, we assume, without deciding, that the claims asserted here against Excel would, if successful, affect Excel's "operations."

[*P23] As material to this appeal, *21 U.S.C. § 603(a)* governs the inspection of meat and meat food products:

> [**500] For the purpose of preventing the use in commerce of meat and meat food products which are adulterated, the Secretary shall cause to be made, by inspectors appointed for that purpose, an examination and inspection of all cattle ... before they shall be allowed to enter into any slaughtering, packing, meat-canning, rendering, or similar establishment, in which they are to be slaughtered and the meat and meat food products thereof are to be used in commerce ... all as provided by the rules and regulations to be prescribed by the Secretary, as provided for in this subchapter. [5]

5 *21 U.S.C. § 603(a)* provides in full:

> For the purpose of preventing the use in commerce of meat and meat food products which are adulterated, the Secretary shall cause to be made, by inspectors appointed for that purpose, an examination and inspection of all cattle, sheep, swine, goats, horses, mules, and other equines before they shall be allowed to enter into any slaughtering, packing, meat-canning, rendering, or similar establishment, in which they are to be slaughtered and the meat and meat food products thereof are to be used in commerce; and all cattle, sheep, swine, goats, horses, mules, and other equines found on such inspection to show symptoms of disease shall be set apart and

> slaughtered separately from all other cattle, sheep, swine, goats, horses, mules, or other equines, and when so slaughtered the carcasses of said cattle, sheep, swine, goats, horses, mules, or other equines shall be subject to a careful examination and inspection, all as provided by the rules and regulations to be prescribed by the Secretary, as provided for in this subchapter.

(Footnote added.) Effective January 26, 1998, for meat processors with more than 500 employees, the Food Safety and Inspection Service delegated to the meat processors themselves the responsibility of coming up with procedures, designated as a Hazard Analysis and Critical Control Point system, adapted to the processors' own circumstances, to safeguard the wholesomeness of the meat they produce:

> [**501] Every official establishment shall conduct, or have conducted for it, a hazard analysis to determine the food safety hazards reasonably likely to occur in the production process and identify the preventive measures the establishment can [***430] apply to control those hazards. The hazard analysis shall include food safety hazards that can occur before, during, and after entry into the establishment. A food safety hazard that is reasonably likely to occur is one for which a prudent establishment would establish controls because it historically has occurred, or because there is a reasonable possibility that it will occur in the particular type of product being processed, in the absence of those controls.

9 C.F.R. § 417.2(a)(1); *Pathogen Reduction, 61 Fed. Reg. at 38869.* As further summarized by the Department in a June 2000 report issued by its Office of Inspector General, the new program was designed to "reverse[]" the arrangement under which "the production of meat and poultry products was monitored at every stage by Government employees" to a system that "allowed a plant to monitor itself." U.S.D.A. Rep. No. 24001-3-At, at 1 (2000). Thus, the new plan, as phrased by the report, "gave industry, not Government, the primary responsibility for ensuring the safety of meat and poultry products." ***Ibid.***

[*P24] In a report dated July 25, 1996, the Food Safety and Inspection Service detailed the nature of the extensive inspection overhaul:

> The Food Safety and Inspection Service is establishing requirements applicable to meat and poultry establishments designed to reduce the occurrence and numbers of pathogenic microorganisms on meat and poultry products, reduce the incidence of foodborne illness associated with the consumption of those products and provide a new framework for modernization of the [**502] current system of meat and poultry inspection. The new regulations (1) require that each establishment develop and implement written sanitation standard operating procedures; (2) require regular microbial testing by slaughter establishments to verify the adequacy of the establishments' process controls for the prevention and removal of fecal contamination and associated bacteria; (3) establish pathogen reduction performance standards for Salmonella that slaughter establishments and establishments producing raw ground products must meet; and (4) require that all meat and poultry establishments develop and implement a system of preventive controls designed to improve the safety of their products, known as [Hazard Analysis and Critical Control Point systems].

Pathogen Reduction, 61 Fed. Reg. at 38806 (acronyms omitted). E. coli O157:H7 is a bacterium associated with "fecal contamination." ***Id.*** *at 38837*. The report noted that the overhaul was triggered by "recent outbreaks of foodborne illness and studies conducted over the past decade ... [that] have established the need for fundamental change in the [Food Safety and Inspec-

tion Service] meat and poultry inspection program to improve food safety, reduce the risk of foodborne illness in the United States, and make better use of the Agency's resources." ***Id.*** *at 38807* (acronyms omitted).

[*P25] The Service also explained that the Hazard Analysis and Critical Control Point systems "focus on attributes affecting product safety, not those affecting economic adulteration or quality" and that it was "a conceptually simple system whereby meat and poultry establishments can identify and evaluate the food safety hazards that can affect the safety of their products, institute controls necessary to prevent those hazards from occurring or keeping them within acceptable [**503] limits, monitor the performance of controls, and maintain records routinely." ***Id.*** *at 38814*. Among the matters that meat processors had to consider [***431] in establishing an applicable Hazard Analysis and Critical Control Point plan for their facilities were:

> (1) What potential hazards may be present in the animals to be slaughtered or the raw materials to be processed? (2) What are the avenues that might lead to contamination of finished product with pathogenic microorganisms, hazardous chemicals, or other potentially hazardous contaminants? (3) What is the likelihood of such contamination and what are the means for preventing it? (4) Does the food contain any ingredient historically associated with a known microbiological hazard? (5) Does the food permit survival or multiplication of pathogens or toxin formation during processing? (6) Does the process include a controllable processing step that destroys pathogens? (7) Is it likely that the food will contain pathogens and are they likely to increase during the times and conditions under which the food is normally stored before being consumed? (8) What product safety devices are used to enhance consumer safety (e.g., metal detectors, filters, thermocouples)? (9) Does the method of packaging affect the multiplication of pathogenic microorganisms and/or the formation of toxins? (10) Is the product epidemiologically linked to a foodborne disease?

Id. *at 38815*. The Service also focused specifically on E. coli contamination:

> In slaughter establishments, fecal contamination of carcasses is the primary avenue for contamination by pathogens. Pathogens may reside in fecal material and ingesta, both within the gastrointestinal tract and on the exterior surfaces of animals going to slaughter. Therefore, without care being taken in handling and dressing procedures during slaughter and processing, [**504] the edible portions of the carcass can become contaminated with bacteria capable of causing illness in humans. Additionally, once introduced into the establishment environment, the organisms may be spread from carcass to carcass.
>
> Because the microbial pathogens associated with fecal contamination are the single most likely source of potential food safety hazard in slaughter establishments, preventing and removing fecal contamination and associated bacteria are vital responsibilities of slaughter establishments. Further, because such contamination is largely preventable, controls to address it will be a critical part of any slaughter establishment's [Hazard Analysis and Critical Control Point] plan. Most slaughter establishments already have in place procedures designed to prevent and remove visible fecal contamination.
>
> There is general agreement within the scientific community that generic E. coli is the best single microbial indicator for fecal contamination. [The Food Safety and Inspection Service], therefore, is requiring that establishments slaughtering livestock or poultry begin testing for E. coli.

Id. *at 38837*. The upshot of all this is that Excel and the rest of the meat-processing industry were well aware of both the danger to health posed by E. coli contamination and the need for their Hazard Analysis and Critical Control Point plans to address eliminating that contamination in their respective facilities. Indeed, the dangers were deemed to be so significant that the Food Safety and Inspection Service saw as the goal of a facility's Hazard Analysis and Critical Control Point System the *prevention* of fecal contamination:

> [**505] Establishments that slaughter livestock and poultry currently have an

> obligation to control the slaughter and sanitary dressing process so that contamination [***432] with fecal material and other intestinal contents is prevented. This means that establishments must maintain sanitary conditions and use good manufacturing practices to avoid contamination with visible feces and ingesta and associated bacteria.

Id. *at 38838*. The Service recognized, however, that mere visible inspection was insufficient:

> [Food Safety and Inspection Service] inspectors apply a zero tolerance performance standard for visible feces and ingesta on dressed carcasses. As a practical matter, however, *additional measures must be taken if inspectors are to assess the extent to which the invisible bacteria associated with feces and ingesta may be present on the carcass.*
>
> [The Food Safety and Inspection Service] has concluded, based on its proposal and the comments received, that the current practice of organoleptic examination by inspectors and the physical removal of visible contamination by establishments needs to be supplemented with an establishment-conducted microbial verification activity. This microbial testing is designed to verify, for the establishment and [the Food Safety and Inspection Service], that the establishment has controlled its slaughter process with respect to *prevention and removal* of fecal material and ingesta *and* associated bacteria.

Ibid. (emphasis added). [6] The Food Safety and Inspection Service characterized the goal as requiring "a [**506] slaughter establishment's adherence to zero tolerance for fecal contamination." ***Id.*** *at 38850*. Indeed, in a report published in the Federal Register on October 7, 2002, the Service repeated that it "considers an acceptable reduction for *E. coli* O157:H7 to be a reduction to an undetectable level." E. coli O157:H7, *67 Fed. Reg. at 62329*.

6 "Organoleptic" is defined as "affecting or making an impression upon one or more of the organs of special sense." Webster's Third New International Dictionary 1590 (1993).

[*P26] Significantly, in light of Excel's focus on the distinction between intact cuts of beef, which were sold to the Milwaukee Sizzler, and non-intact beef, the Service's statement that E. coli contamination must be reduced to "an undetectable level" appears on the same page of the Federal Register reiterating the already in-place requirement in *9 C.F.R. § 417.2(a)(2)* that meat processors identify and consider "the intended use or consumers of the finished product." E. coli O157:H7, *67 Fed. Reg. at 62329*. Even in 1996, however, the Food Safety and Inspection Service recognized that "food-borne illness" was "a substantial and intolerable public health problem" and explained why it was so important for processors to consider what would happen to even intact meat after it left the processors' plants:

> The health effects of enteric pathogens are relatively well documented. If the pathogens enter the food supply, they do, under certain conditions, cause foodborne illness. If their presence can be prevented, no amount of temperature abuse, mishandling or undercooking can lead to foodborne illness.

Pathogen Reduction, 61 Fed. Reg. at 38962. Given the realities of what it saw as consumers' food-handling patterns, the Food Safety and Inspection Service bored in on the only effective way to reduce or eliminate food-borne illness:

> [**507] Occurrence of foodborne disease is a multi-step process. The first, and critical, step is the introduction of a pathogen into or onto the raw product. If a pathogen is present, then subsequent temperature abuse or mishandling may [***433] permit bacterial counts to increase to levels which increase the likelihood that illness will occur; mishandling may result in cross-contamination of other foods which are not cooked before being eaten; or improper cooking may not kill all pathogenic bacteria present in the product. In these instances, it may be said that the illness was "caused" by improper handling. However, *disease would not have occurred if the pathogen had not*

> *been present on the raw product in the first place.*

Id. at 38966 (emphasis added). We now turn to how all this affects whether the claims against Excel are barred by the express-preemption clause.

IV.

[*P27] We start our analysis, as we must, with the words of the statute. In discerning whether Congress intended the *Meat Inspection Act* to preempt state claims we must give to unambiguous statutory language the meaning it denotes. ***Sprietsma**, 123 S. Ct. at 526* (statute's "'plain wording'" of preemption clause "'necessarily contains the best evidence of Congress' pre-emptive intent'") (quoted source omitted). This is also the general rule of statutory construction in Wisconsin. ***Anderson v. City of Milwaukee**, 208 Wis. 2d 18, 25, 559 N.W.2d 563, 566 (1997)* (legislative intent discerned from statute's plain language); ***Jungbluth v. Hometown, Inc.**, 201 Wis. 2d 320, 327, 548 N.W.2d 519, 522 (1996)* (plain statutory language is applied as it is written).

[**508] [*P28] Although our analysis is governed by the statute's words, interpretation and application of preemption "language does not occur in a contextual vacuum," but must be "informed by two presumptions about the nature of pre-emption." ***Medtronic, Inc. v. Lohr**, 518 U.S. 470, 485, 135 L. Ed. 2d 700, 116 S. Ct. 2240 (1996)*. First, the United States Supreme Court has "long presumed that Congress does not cavalierly pre-empt state-law causes of action," and thus the Court "'starts with the assumption that the historic police powers of the States were not to be superseded by the Federal Act unless that was the clear and manifest purpose of Congress.'" ***Ibid.*** (quoted source omitted). Further, this presumption against preemption applies to not only "whether Congress intended any pre-emption at all," but also to the scope of any preemption that Congress may have intended. ***Ibid.***

[*P29] Second, the "'purpose of Congress is the ultimate touchstone' in every pre-emption case." ***Ibid.*** (quoted source omitted). As we have seen, Congressional intent "primarily is discerned from the language of the pre-emption statute and the 'statutory framework' surrounding it," and, also, "the 'structure and purpose of the statute as a whole' as revealed not only in the text but through the reviewing court's reasoned understanding of the way in which Congress intended the statute and its surrounding regulatory scheme to affect business, consumers, and the law." ***Id.**, 518 U.S. at 485-486* (quoted source and internal citations omitted).

[**509] **A.**

[*P30] The express preemption clause here prohibits any state from imposing on producers regulated by the *Meat Inspection Act* any "requirements within the scope of [the Act] with respect to premises, facilities and operations ... which are in addition to, or different than those made under" the Act. *21 U.S.C. § 678*. There is, however, a savings clause that, as we have also seen, permits states to "make[] requirements or take other [***434] action, consistent with [the Act], with respect to any other matters regulated under this [Act.]" ***Ibid.***

[*P31] On its surface, the phrase "with respect to premises, facilities and operations" applies only to the physical plant, the type and quantity of various categories of equipment, and the method of running the business. Thus, without deciding this, it seems to us that a state law that required a meat processor to employ a certain number of quality-control personnel or to irradiate its meat would fall within the express-preemption provision. But it is one thing to view the scope of the preemption clause to encompass meat inspection, meat treatment, or, indeed, the entire reach of the Hazard Analysis and Critical Control Point program, and entirely different to hold that state claims based on the sale of meat contaminated with E. coli O157:H7 are preempted by the clause. ***Medtronic*** makes this clear.

[*P32] ***Medtronic*** concerned a suit for damages sustained by a person injured by an allegedly negligently manufactured and defective pacemaker part. ***Id.**, 518 U.S. at 480-481*. The preemption clause there was similar to the one here, and provided, as material:

> [**510] "No State ... may establish or continue in effect with respect to a device intended for human use any requirement-
>
> (1) which is different from, or in addition to, any requirement applicable under this chapter to the device, and
>
> (2) which relates to the safety or effectiveness of the device or to any other matter included in a requirement applicable to the device under this chapter."

Id.**, 518 U.S. at 481-482*. ***Medtronic held that the claims in that case were not preempted because the "violations of common-law duties" for which the damages were sought were either "parallel" to the requirements set out in the federal statute, or more narrow than those requirements. ***Id.**, 518 U.S. at 495*. ***Medtronic*** explained:

> Nothing in [the preemption clause] denies Florida the right to provide a traditional damages remedy for violations of common-law duties when those duties parallel federal requirements. Even if it may be necessary as a matter of Florida law to prove that these violations were the result of negligent conduct, or that they created an unreasonable hazard for users of the product, such additional elements of the state-law cause of action would make the state requirements narrower, not broader, than the federal requirement. While such a narrower requirement might be "different from" the federal rules in a literal sense, such a difference would surely provide a strange reason for finding pre-emption of a state rule insofar as it duplicates the federal rule. The presence of a damages remedy does not amount to the additional or different "requirement" that is necessary under the statute; rather, it merely provides another reason for manufacturers to comply with identical existing "requirements" under federal law.

[**511] ***Ibid.*** A similar analysis governs this case.

[*P33] No one disputes but that the major goal of the *Meat Inspection Act* is to prevent the sale of "adulterated" meat products. As we have explained in Part III.A. of this opinion, the Excel meat that was sold to the Sizzler restaurant was "adulterated" as Congress defined that word, even though the meat left the Excel facility as intact cuts. Thus, a claim premised on damages resulting from the sale of "adulterated" meat, in the words of ***Medtronic***, "merely provides another reason for manufacturers to comply with identical existing 'requirements' under federal law." ***Ibid.***

[***435] [*P34] By the same token, and as seen in Part III.B. of this opinion, a goal of the Food Safety and Inspection Service and the Hazard Analysis and Critical Control Point plans it implements is to "prevent" fecal and E. coli contamination-what the agency called "zero tolerance" for fecal contamination and the concomitant reduction of the E. coli bacterium to an "undetectable level." *Pathogen Reduction, 61 Fed. Reg. at 38850*; E. Coli O157:H7, *67 Fed. Reg. at 62329*. Here also, in the words of ***Medtronic***, claims based on allegations that Excel meat sold to the Sizzler restaurant had detectable levels of E. coli contamination "merely provide[] another reason for manufacturers to comply with identical existing 'requirements' under federal law." ***Medtronic***, *518 U.S. at 495*. Indeed, the Hazard Analysis and Critical Control Point program leaves it largely to the processors themselves to determine how best to achieve the goals of zero tolerance for bacteria-laden fecal matter and undetectable levels of E. coli O157:H7. Thus, a processor's responsibility to produce wholesome, non-pathogenic meat is, under the Act's inspection-mandate and the interpretation of the Food [**512] Safety and Inspection Service of that mandate, parallel to and not divergent from the processor's goal of avoiding tort-claims liability.

[*P35] Furthermore, insofar as the preemption doctrine implicates a federal need for uniformity of regulation, *see* ***Sprietsma***, *123 S. Ct. at 523*, the federal inspection scheme here *eschews* uniformity in favor of non-uniform plant-by-plant Hazard Analysis and Critical Control Point plans developed by the plant operators themselves. Simply put, rather than a nation-wide uniform, one-size-fits-all approach present in so many preemption cases, the Food Safety and Inspection Service now lets meat processing plants monitor themselves with only comparatively minimal federal oversight. *Pathogen Reduction, 61 Fed. Reg. at 38852* ("The Agency is responsible for establishing and enforcing reasonable standards; it intends to give the industry the maximum flexibility to decide how best to meet such standards. It does not intend to regulate or prescribe how the standards are to be met.").

[*P36] Thus, the potential success of the claims asserted here against Excel because of its alleged failure to reach the goal of non-detectable E. coli contamination set by the Food Safety and Inspection Service is not a "requirement" either "in addition to, or different than" requirements established under the *Meat Inspection Act* "with respect to premises, facilities and operations"; rather, it is wholly consistent with the predominant intent of the Act, which is to keep health-threatening meat out of the commerce stream. Therefore, the state claims are adjunct to, rather than a displacement of, the standards enacted by Congress and the conforming regulations promulgated by the Secretary. Permitting assertion of those claims supplements, in a way consistent [**513] with the "touchstone" of Congressional intent, the federal goal to remove pathogen-laden meat from the food supply.

[*P37] The record here demonstrates in a concrete way how the claims asserted against Excel supplement protection afforded by the meat-inspection program and what the Food Safety and Inspection Service has recognized are the significant limitations of the "organoleptic examination by inspectors." ***Id.*** *at 38838*. Only two federal inspectors oversee a meat fabrication area in Excel's plant where several hundred workers daily cut the approximately seven-foot-long, 350-pound split carcasses

into some 8,000 intact cuts of beef weighing approximately two to four or three to five pounds each. Federal inspectors do not inspect each one of these [***436] smaller cuts of beef. Moreover, the seven-foot-long carcasses arrive at the fabrication area after whizzing by the Service inspection station at the rate of one side every six seconds.

[*P38] In sum, since, the "'purpose of Congress is the ultimate touchstone' in every pre-emption case," ***Medtronic**, 518 U.S. at 485* (quoted source omitted), and since the claims asserted against Excel are wholly congruent with the overarching purpose of the *Meat Inspection Act*, and in light of the savings clause, which, as we have seen, permits states to "make[] requirements or take other action, *consistent* with [the Act], with respect to any other matters regulated under this [Act]" (emphasis added), we hold that those claims are not preempted by the express-preemption clause in *21 U.S.C. § 678.* [7]

> 7 During oral argument, Excel forcefully contended that a failure to apply federal preemption will subject the meat-processing industry to intractable dilemmas. But all manufacturers confront difficult cost/benefit choices when balancing expense and methods of production on the one hand, against, on the other hand, potential liability for injuries that may be caused by their products; we see no special burden on Excel or other meat processors beyond that faced by anyone who puts potentially dangerous products into the stream of commerce.

[**514] **B.**

[*P39] As noted, "ordinary" preemption principles may bar state claims even though those claims are not expressly preempted by the applicable federal statute. ***Geier**, 529 U.S. at 869*. The prerequisite to finding an implied preemption, however, is "an actual conflict with a federal objective." ***Id.**, 529 U.S. at 871*. As already explained at some length, there is no "actual conflict" here; permitting claims against Excel for putting into the stream of commerce meat that was contaminated with E. coli O157:H7 is consistent with the objective of the *Meat Inspection Act*-the sale in interstate commerce of safe, wholesome meat. Accordingly, there is no implied preemption. *See* ***Sprietsma**, 123 S. Ct. at 522-523*.

V.

[*P40] There are two final matters we must discuss.

A.

[*P41] Excel's briefs rely heavily on ***Boulahanis v. Prevo's Family Market, Inc.**, 230 Mich. App. 131, 583 N.W.2d 509 (Mich. Ct. App. 1998), cert. denied, 530 U.S. 1203, 147 L. Ed. 2d 232, 120 S. Ct. 2194*, which held that the *Meat Inspection Act* preempted state claims for a death and injuries caused by the sale of ground beef [**515] contaminated with E. coli O157:H7. The trial court also relied heavily on ***Boulahanis*** in deciding to grant summary judgment to Excel. Accordingly, we now turn to that case, which, in our view, is irrelevant to our decision.

[*P42] In ***Boulahanis***, the contaminated meat was purchased in 1993, before the United States Department of Agriculture determined that the E. coli bacterium was dangerous. ***Id.**, 583 N.W.2d at 512*. The plaintiffs contended that their claims could not be preempted because the Department had not promulgated any regulations concerning the sale of E. coli-infected meat when the meat was purchased, and thus there could be no "conflict with then-existing federal law." ***Ibid. Boulahanis*** rejected that argument, holding that the Department's "intentional decision not to regulate the presence of E. Coli because it was not considered an adulterant carries the force of positive enactment." ***Ibid.*** Thus, under ***Boulahanis***'s view, holding the "defendants [***437] liable for failing to detect the presence of E. Coli bacteria in the meat purchased by plaintiffs in 1993 would run contrary to the [Department]'s then-existing determination that inspection for E. Coli bacteria was not required." ***Ibid.***

[*P43] Aside from its other infirmities, including its paucity of analysis, ***Boulahanis***'s rationale for finding preemption-agency *in*action-is, without a deeper analysis than ***Boulahanis*** made, questionable in light of ***Sprietsma***, which held that although agency inaction could be evidence of a federal mandate to leave an area unregulated, it also could be, and was held in ***Sprietsma*** to be, "fully consistent with an intent to preserve state regulatory authority pending the adoption of specific federal standards." ***Sprietsma**, 123 S. Ct. at 527-528*. Moreover, contrary to the situation in ***Boulahanis***, the [**516] Food Safety and Inspection Service is now not only concerned with E. coli O157:H7, but, indeed, is striving to reduce its presence in meat sold as human food to, as we have seen, "undetectable levels." ***Boulahanis*** gives us no guidance.

B.

[*P44] Although it is hardly dispositive, for the reasons we explain below, the Food Safety and Inspection Service has assumed that tort claims against meat processors are *not* preempted by the *Meat Inspection Act*.

[*P45] As we have seen, the Service issued a lengthy report dated July 25, 1996, which explained its

proposed Hazard Analysis and Critical Control Point plan and set out proposed implementing rules. The report also noted concerns expressed by some of those with whom it had consulted. Responding to a concern raised by "[a] few commenters ... about product liability due to product recalls stemming from test results," the Service explained: "Establishments' liability to civil lawsuits should not be adversely affected by this rule precisely because it is an establishment's process, not individual lots of product, that is being assessed, for inspection purposes, on the basis of this testing." *Pathogen Reduction, 61 Fed. Reg. at 38854*. Moreover, the Service opined that if meat producers "did not suffer legal consequences" as a result of selling pathogen-infested meat, it was because "consumers often cannot trace a transitory illness [caused by pathogenic microorganisms] to any particular food or even be certain it was caused by food." ***Ibid.***

[**517] [*P46] Nowhere in the July 25, 1996, report did the Service even hint that it considered tort claims against meat processors to be preempted by the *Meat Inspection Act*, and we are aware of no other place where it has so opined. We mention all this in passing, however, because it is far from settled that an agency's view of the preemptive effect of a statute is given *any* deference. Indeed, the United States Supreme Court has assumed that the agency's view is entitled to *no* deference. ***Smiley v. Citibank (South Dakota), N.A.***, *517 U.S. 735, 744, 135 L. Ed. 2d 25, 116 S. Ct. 1730 (1996)* (assuming, but not deciding, that "whether a statute is pre-emptive ... must always be decided *de novo* by the courts"). *See also* ***Commonwealth of Massachusetts v. United States Dep't of Transp.***, *320 U.S. App. D.C. 227, 93 F.3d 890, 892-897 (D.C. Cir. 1996)* (recognizing ***Smiley***, discussing deference to the agency and holding contrary to the agency's view).

VI.

[*P47] We reverse the summary judgments granted to Excel dismissing the claims of the Kriefalls, the Lesaks, the [***438] Fortiers, the McCormicks, and Sizzler USA. [8]

8 In light of our holding that the claims asserted against Excel are not preempted by federal law, we do not address the additional argument that Excel waived by contract its right to rely on the preemption doctrine. *See* ***Gross v. Hoffman***, *227 Wis. 296, 300, 277 N.W. 663, 665 (1938)* (only dispositive issue need be addressed); ***State v. Blalock***, *150 Wis. 2d 688, 703, 442 N.W.2d 514, 520 (Ct. App. 1989)* (cases should be decided on the "narrowest possible ground").

By the Court.-Judgments reversed.

Risk of Hemolytic Uremic Syndrome After Antibiotic Treatment of Escherichia coli O157:H7 Enteritis: A Meta-analysis

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Risk of Hemolytic Uremic Syndrome After Antibiotic Treatment of *Escherichia coli* O157:H7 Enteritis

A Meta-analysis

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Context The use of antibiotics for treatment of *Escherichia coli* O157:H7 infection has become controversial since a recent small study found that it may increase the risk of hemolytic uremic syndrome (HUS). However, other larger studies have reported a protective effect or no association.

Objective To determine whether antibiotic therapy for *E coli* O157:H7 enteritis increases the risk of HUS.

Data Sources PubMed and MEDLINE computer searches were performed for studies published from January 1983 to February 2001 using the key words *hemolytic uremic syndrome, risk factor, antibiotics,* and *Escherichia coli* O157:H7. Reference lists of relevant publications were reviewed, and 12 experts in the field were contacted to identify additional reports. No language restrictions were applied to the search.

Study Selection Studies were included if they reported a series of patients with documented *E coli* O157:H7 enteritis, some of whom developed HUS; had clear definitions of HUS; and had adequate data delineating the relationship between antibiotic therapy and the occurrence of HUS. Nine of the 26 identified studies fulfilled these criteria.

Data Extraction Two authors (N.S. and A.S.) independently reviewed each report identified by the searches and recorded predetermined information relevant to the inclusion criteria. A pooled odds ratio was calculated using a fixed-effects model, with assessment of heterogeneity among the studies.

Data Synthesis The pooled odds ratio was 1.15 (95% confidence interval, 0.79-1.68), indicating that there does not appear to be an increased risk of HUS with antibiotic treatment of *E coli* O157:H7 enteritis. Incomplete reporting of data in individual studies precluded adjustment for severity of illness.

Conclusion Our meta-analysis did not show a higher risk of HUS associated with antibiotic administration. A randomized trial of adequate power, with multiple distinct strains of *E coli* O157:H7 represented, is needed to conclusively determine whether antibiotic treatment of *E coli* O157:H7 enteritis increases the risk of HUS.

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HEMOLYTIC UREMIC SYNDROME (HUS) is a complex multisystem disorder characterized by hemolytic anemia, thrombocytopenia, and acute renal failure.[1,2] The classic form of this syndrome often follows a prodrome of acute enteritis, most often caused by Shiga toxin–producing strains of *Escherichia coli*.[3,4] Since the first report by Karmali et al[5] causally linking toxin-producing *E coli* to HUS, *E coli* serotype O157:H7 enteric infection has been recognized as the most common cause of HUS in the United States, with 6% of patients developing HUS within 2 to 14 days of onset of diarrhea.[6] Occurring primarily in children, this serious complication carries a mortality rate of 3% to 5%[1,2,7] and is currently the most common cause of acute renal failure in children in the United States.[8] Approximately half of children with HUS require dialysis,[9] and 5% of patients who survive have long-term renal impairment.[9] No cure exists for HUS, and although management beyond intensive support with needed renal replacement therapy remains controversial, there appears to be a growing consensus that plasma exchange therapy brings benefit and should be used.[2,10]

For editorial comment see p 1014.

Numerous studies[11-19] have examined risk factors that may predispose patients to development of HUS. Of those risk factors that have been shown in epidemiological studies to predis-

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pose patients to development of HUS following *E coli* O157:H7 infection, the most controversial to date is antibiotic therapy for acute *E coli* O157:H7 enteritis.[20] Studies[11-19] that have addressed this issue have been limited by small sample sizes and use of varying antibiotic regimens for varying periods and have given conflicting results. Although there is no clear consensus on whether antibiotics should be administered as treatment of *E coli* O157:H7, since the report by Wong et al,[14] avoidance of antibiotic therapy for any presumably infectious enteritis appears to be a growing practice.

We report a meta-analysis of published studies undertaken to better understand the association between antibiotic therapy for *E coli* O157:H7 enteritis and the risk of HUS. We critically examine the heterogeneity of the study results, especially the methods used to adjust for severity of illness. Controlling for severity of illness is desirable, since sicker patients are more likely to both develop HUS and receive antibiotics, thus confounding the relationship between HUS and antibiotic use.

METHODS

Using MEDLINE and PubMed database searches, we identified published studies using the key words *hemolytic uremic syndrome, antibiotic, risk factor,* and *Escherichia coli O157:H7*. No language restrictions were applied to the search. The search was limited to reports on human infections published between January 1983 (the year when Shiga toxin–producing *E coli* was first found to be associated with HUS) and February 2002. Reference lists of recent publications, the Cochrane Network, and the National Institutes of Health Web site listings of ongoing trials were reviewed and 12 authorities in the field were solicited to identify unpublished studies.

The following criteria for the inclusion of studies were defined before reviewing specific reports: the study must report on a series of patients with documented *E coli* O157:H7 enteritis, including patients who developed HUS; clear definitions of HUS must be given; and adequate data delineating the relationship between antibiotic therapy and the occurrence of HUS must be reported. Two authors (N.S. and A.S.) independently reviewed each report identified by these searches. Both investigators recorded predetermined information relevant to the inclusion criteria.

Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated, using data provided in the studies. Data on both antibiotic use and HUS were abstracted as dichotomous variables. If primary data were not reported, we used the OR and 95% CI reported in the study. Pooled estimates of the OR and 95% CI were obtained using the fixed-effects model of Mantel and Haenszel,[21] and testing for heterogeneity was performed with the Breslow-Day test[22] using SAS statistical software version 8.0 (SAS Institute Inc, Cary, NC). Publication bias was assessed by funnel plot.[23] We did not attempt to calculate a pooled adjusted OR since 3 of the 9 studies did not adjust for severity of illness[13,17,19] and there were substantial differences in the methods used to adjust for confounding among the remaining 6 studies.

RESULTS

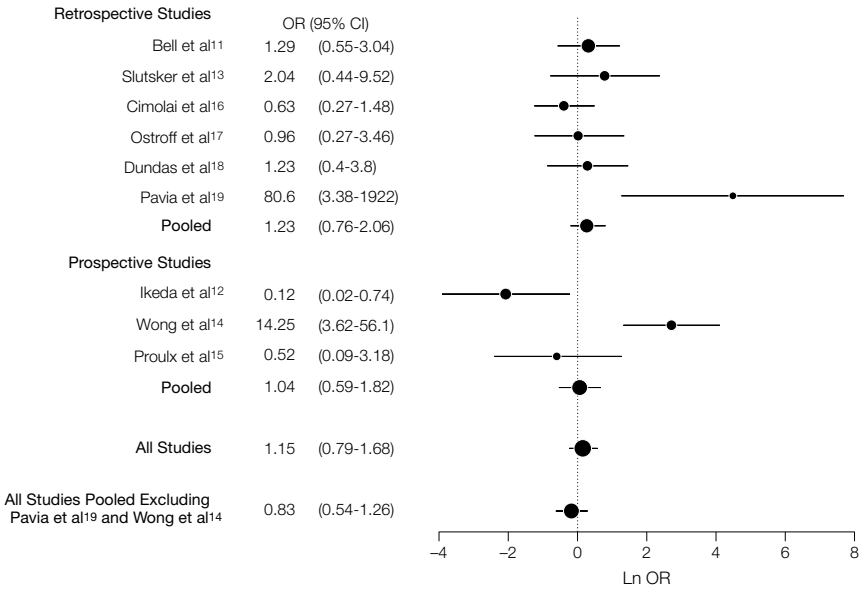
Our search yielded 26 reports. Seven studies[7,24-29] were excluded because of lack of a control group, 2 studies[30,31] because of failure to define clear diagnostic criteria for HUS, 4 studies[32-35] for failure to evaluate risk factors for HUS, and 3 studies[36-38] for lack of data on antibiotic use as a risk factor for HUS. One study[39] was excluded because infections with *E coli* serotypes other than O157:H7 were included.

Table. Studies Evaluating the Association Between Antibiotic Therapy of *Escherichia coli* O157:H7 Enteritis and Risk of Hemolytic Uremic Syndrome (HUS)

Source	Type of Study	Age Range of Patients, y	No. of Patients With *E coli* O157:H7 Enteritis	No. of Patients Developing HUS	Antibiotics Used for Treatment	Interval Between Onset of Acute Diarrhea and Introduction of Antibiotic Therapy, d
Bell et al,[11] 1997	Retrospective cohort	<16	278	36	Trimethoprim, ampicillin, cephalosporins, metronidazole	≤3
Ikeda et al,[12] 1999	Prospective cohort	6-11	292	36	Fosfomycin	≤5
Slutsker et al,[13] 1998	Retrospective case-control	<1-82	93	7	Sulfamethoxazole	≤3
Wong et al,[14] 2000	Prospective cohort	<10	71	10	Trimethoprim-sulfamethoxazole, amoxicillin, cephalosporins	≤3
Proulx et al,[15] 1992	Prospective randomized	<1-17	47	6	Trimethoprim-sulfamethoxazole	7.4*
Cimolai et al,[16] 1994	Retrospective case-control	5 (Mean)	128	27	Not stated	Not stated
Ostroff et al,[17] 1989	Retrospective case-control	<1-78	69	11	Trimethoprim-sulfamethoxazole, erythromycin, ampicillin, gentamicin sulfate, tetracycline	4.3 (1-10)*
Dundas et al,[18] 2001	Retrospective case-control	<1-94	120	34	Ciprofloxacin	≤4
Pavia et al,[19] 1990	Retrospective case-control and randomized trial	6-39	23	8	Sulfonamides, trimethoprim-sulfamethoxazole	≤3

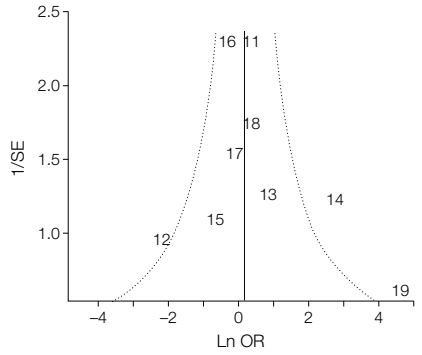
*Data are mean (range).

Figure 1. Relationship Between Antibiotic Therapy for *Escherichia coli* O157:H7 Enteritis and Risk of Hemolytic Uremic Syndrome



An odds ratio (OR) of greater than 1.0 (ln OR >0) suggests that antibiotic therapy is associated with an increased risk of hemolytic uremic syndrome, and an OR of less than 1.0 (ln OR <0) suggests a decreased risk. CI indicates confidence interval. Sizes of data markers are proportional to sample sizes of the studies.

Figure 2. Funnel Plot of Estimated Ln ORs From 9 Studies



Solid vertical line represents pooled estimated log odds ratio (OR) across the studies. Dotted lines represent 2 SE bounds around the pooled estimate. Studies are represented by their reference numbers.

Nine studies, 6 retrospective and 3 prospective, met the inclusion criteria. Their characteristics are summarized in the TABLE. Only 1 of the retrospective studies showed a statistically significant deleterious effect of antibiotic use and HUS.[19] Four retrospective studies[11,13,17,18] showed no association, and 1 study[16] showed a protective effect, but only in the univariate analysis.

Of the 3 prospective studies, only 1 study[14] reported a statistically significant increased risk of HUS with antibiotic use. One prospective study[12] reported a protective effect of fosfomycin for treatment of *E coli* O157:H7 infection, and 1 prospective randomized study[15] showed no association between antibiotic use and HUS.

The pooled OR was 1.15 (95% CI, 0.79-1.68), indicating a lack of association between HUS and antibiotic use (FIGURE 1). The test for heterogeneity was highly significant ($P<.001$). We examined potential explanations for this heterogeneity. Substantial heterogeneity was observed among studies in the populations studied (adults vs children) and in the types of antibiotics used, the timing and length of therapy, and methods used to control for severity of illness.

Two studies, 1 retrospective[19] and 1 prospective,[14] were found to account for most of the heterogeneity, and when these were removed from the analysis, the test for heterogeneity was no longer significant. Pavia et al[19] did not control for severity of illness in their analysis, which may have led to an inflated OR. Wong et al[14] did not use physiological measures to control for severity of illness, which may have contributed to an increased magnitude of the association found.

The funnel plot (FIGURE 2) did not show a substantial publication bias.

COMMENT

Antibiotic treatment for *E coli* O157:H7 enteritis has become controversial because a recent epidemiological study[14] suggested that it may increase the risk of HUS. In vitro and animal studies suggest varying effects of antibiotic exposure on toxin production by *E coli* O157:H7.[40-43] Subinhibitory concentrations of trimethoprim-sulfamethoxazole in vitro have been shown to enhance toxin production by *E coli*.[44]

One in vitro study[40] of the effect of 13 different antibiotics on the release of Shiga toxin by 3 different *E coli* O157:H7 strains showed that the response of *E coli* O157:H7 isolates to subinhibitory concentrations of antibiotics seems to be highly dependent on the individual strain involved. This may explain in part the conflicting findings of earlier in vitro studies,[41-43] where in some instances antibiotics were found to decrease toxin production and in others to increase it. Kurioka et al[43] recently reported that norfloxacin, fosfomycin, kanamycin sulfate, ampicillin, and clarithromycin reduced the risk of complications in a murine model of enteritis with a Shiga toxin–producing *E coli* O157:H7; antibiotic therapy shortened the length of *E coli* O157:H7 excretion in stool samples and decreased the amount of toxin in both feces and blood. However, trimethoprim-sulfamethoxazole was associated with increased mortality when given to some of the mice 3 days after initiation of infection.

Similar conflicting results have been found in clinical studies. In a large outbreak of *E coli* O157:H7 enteritis in adult patients, a retrospective case-

control study found that coincidental antibiotic use, defined as antibiotic use in the 4 weeks before development of *E coli* infection, appeared to be associated with an increased risk of HUS (OR, 4.7).[18] However, when patients who received antibiotic treatment for *E coli* O157:H7 enteritis with ciprofloxacin were compared with those who did not, there was no statistically significant difference in risk.

A widely promulgated, recent cohort study of 71 children with *E coli* O157:H7 enteritis, 10 of whom developed HUS, reported a 14-fold increased risk of HUS when various antibiotics were given for treatment of *E coli* O157:H7 enteritis.[14] The relative risk was adjusted for initial white blood cell count and the day the stool sample was collected as markers of disease severity. The authors examined the risk of HUS by class of antibiotic used, and trimethoprim-sulfamethoxazole and β-lactams appeared to be associated with increased risk. An accompanying editorial strongly endorsed the study findings.[45] As a consequence, many US clinicians have become reluctant to give antibiotic therapy to children or adults with acute enteritis, even if they have dysentery and are severely ill. In the study by Wong et al,[14] the reported CI for the adjusted relative risk was very wide (2.2-137). If 2 fewer children who developed HUS had received antibiotics, the association would no longer be statistically significant.

Antibiotic therapy has been shown to be highly beneficial for *Campylobacter jejuni* enteritis,[46] traveler's diarrhea caused by enterotoxigenic *E coli*,[47] and shigellosis.[48] These ubiquitous enteric infections have clinical manifestations indistinguishable from *E coli* O157:H7 enteritis, and withholding antibiotic therapy for these infections until *E coli* O157:H7 infection can be ruled out could be deleterious for many patients.

The paucity of randomized trials does not allow a definitive conclusion to be reached regarding this matter. A prospective study[12] in children with *E coli* O157:H7 enteritis found that administration of fosfomycin within the first 2 days of illness was associated with a significantly reduced risk of HUS.The significant protective effect remained in a multivariable analysis that controlled for severity of illness using the presence of fever as an indicator (adjusted OR, 0.09; 95% CI, 0.01-0.79).

In a case-control study, Slutsker et al[13] reported no association between antibiotic use within 3 days of onset of *E coli* O157:H7 infection and HUS. However, in a subgroup analysis, children younger than 13 years who developed HUS were more likely to have received an antibiotic, primarily sulfamethoxazole (relative risk, 11.5; $P=.02$), than those who did not. However, no adjustment for severity of illness was performed.

In a retrospective cohort study of 278 children with culture-confirmed *E coli* infection, 50 of whom received antibiotic therapy, Bell et al[11] did not find an association between prior antibiotic use and HUS in a multivariate analysis.

A retrospective study by Cimolai et al,[16] using multivariable techniques of data analysis, also did not find an increased likelihood of progression to HUS after antibiotic treatment of *E coli* enteritis. Antibiotic use was defined in this study as being either appropriate or inappropriate. Appropriate antibiotics were arbitrarily defined as those effective against shigellosis, such as ampicillin or trimethoprim-sulfamethoxazole, or the isolate was shown to be susceptible in vitro. Inappropriate antibiotics were those that have not been shown to have therapeutic value for treatment of shigellosis, or the isolate was resistant in vitro. Univariate analysis showed a trend toward a reduced risk of HUS with appropriate antibiotic use, but this variable was not significant in a multivariable analysis.

In a randomized trial of trimethoprim-sulfamethoxazole treatment of *E coli* O157:H7 enteritis in 47 children, no association was found between antibiotic treatment and subsequent development of HUS (2/22 vs 4/25; $P=.67$).[15] Trimethoprim-sulfamethoxazole had no effect on the course of symptoms, duration of excretion of the organism, or likelihood of progression to HUS. However, antibiotic therapy was initiated a mean of 7 days after the onset of diarrhea.

Pavia et al[19] reported an outbreak of *E coli* O157:H7 infection among the residents and staff of an institution for mentally handicapped persons. Eight persons developed HUS and half died. Antibiotics, mainly trimethoprim-sulfamethoxazole, were administered to 5 of the 8 with HUS compared with 0 of the 7 without HUS; however, no adjustment for severity of illness was performed, and it is likely that antibiotics were given to the more severely ill patients, many of whom may have been biologically destined to develop HUS.

A retrospective study performed by Ostroff et al[17] found no association between antibiotic use and HUS. These authors examined differences between patients who received antibiotics for *E coli* O157:H7 infection and those who did not and found similar durations of overall illness in both groups.

In our analysis, we did not use adjusted ORs from the included studies to obtain a summary OR because too few of the 9 studies evaluated possible confounding in their analysis. Three studies[13,17,19] did not assess differences in patients' severity of illness, 3 studies[11,16,18] used multivariate analysis but did not provide sufficient data regarding which factors were adjusted for, 1 study[12] adjusted for severity of illness using the presence of fever, 1 study[14] adjusted for white blood cell count and day of illness on which stool sample was obtained as markers of disease severity, and only 1 study[15] was randomized.

Our analysis does not show an increased risk of HUS after antibiotic treatment of *E coli* O157:H7 infection (Figure 1). Although the included studies differed substantially in the duration and types of therapeutic antibiotics used, we believe our results highlight the ongoing controversy surrounding the use of antibiotics for *E coli* O157:H7 enteritis and the development of HUS. We have focused our analysis on the effect of antibiotic therapy in general on the risk of HUS and have made no

attempt to examine the influence of different antibiotic classes because of wide variation in the type, timing, and duration of antibiotic treatment.

In vitro and animal studies indicate that the timing and duration of antibiotic therapy may have great relevance for the risk of developing HUS. Early in the onset of the enteritis, antibiotic therapy appears, in some studies, to have a protective effect.[49] Of the 9 studies used in our meta-analysis, antibiotics were administered within 3 days of the onset of diarrhea in 3 of the studies.[11,13,14] Of these 3 studies, 2 studies[11,13] did not have a statistically significant increased risk of HUS with antibiotic therapy and 1 study[14] showed an increased risk. In the remainder of the studies, antibiotics were given within 5 days, except in the randomized trial conducted by Proulx et al,[15] in which trimethoprim-sulfamethoxazole was given a mean of 7 days after the onset of acute illness.

The class of antibiotics used for treatment of *E coli* O157:H7 enteritis is also important when assessing the risk of HUS with antibiotic treatment. It is not clear which particular category of antibiotics is most likely to be associated with increased toxin production and which with decreased toxin production by Shiga toxin–producing *E coli*.[42,50-53] Trimethoprim-sulfamethoxazole has been the antibiotic most frequently reported to have a detrimental effect in animal studies.[41,44,54] In their clinical trial, Proulx et al[15] did not show a harmful effect of trimethoprim-sulfamethoxazole. In Japanese studies of antibiotic use for *E coli* O157:H7 infection, fosfomycin is the most frequently used antibiotic and appears to have a protective effect.[30,43]

The major limitation of our meta-analysis is our inability to adjust for severity of illness and to analyze the risk of HUS with various classes and duration of antibiotics and the timing of therapy. Publication bias may also play a role, in that studies showing no effect of antibiotic use on HUS may be less likely to be published. Our assessment of this possibility does not, however, suggest that a substantial publication bias exists.

In summary, we believe that better data are needed—ideally, an adequately powered, nationwide randomized trial in which multiple distinct strains of *E coli* O157:H7 are represented and rapid diagnostic methods for identification of *E coli* O157:H7 infection are used to permit early randomization—before it can be concluded unequivocally that administration of antibiotic therapy to critically ill children or adults with severe, presumably infectious enteritis, especially dysentery that might represent *E coli* O157:H7 infection, is deleterious.

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Drafting of the manuscript: Safdar, Said, Maki.
Critical revision of the manuscript for important intellectual content: Said, Gangnon, Maki.
Statistical expertise: Safdar, Said, Gangnon.
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REFERENCES

1. Boyce TG, Swerdlow DL, Griffin PM. *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. *N Engl J Med.* 1995;333:364-368.
2. Elliott MA, Nichols WL. Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *Mayo Clin Proc.* 2001;76:1154-1162.
3. Siegler R. The hemolytic uremic syndrome. *Pediatr Clin North Am.* 1995;42:1505-1529.
4. Griffin PM, Ostroff SM, Tauxe RV, et al. Illnesses associated with *Escherichia coli* O157:H7 infections: a broad clinical spectrum. *Ann Intern Med.* 1988;109:705-712.
5. Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases of hemolytic-uremic syndrome associated with fecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet.* 1983;1:619-620.
6. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J Infect Dis.* 1985;151:775-782.
7. Martin DL, MacDonald KL, White KE, Soler JT, Osterholm MT. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. *N Engl J Med.* 1990;323:1161-1167.
8. Andreoli SP. The pathophysiology of the hemolytic uremic syndrome. *Curr Opin Nephrol Hypertens.* 1999;8:459-464.
9. Siegler R, Pavia AT, Christofferson RD, Milligan MK. A 20-year population-based study of postdiarrheal hemolytic uremic syndrome in Utah. *Pediatrics.* 1994;94:35-40.
10. Dundas S, Murphy J, Soutar RL, Jones GA, Hutchinson SJ, Todd WT. Effectiveness of therapeutic plasma exchange in the 1996 Lanarkshire *Escherichia coli* O157:H7 outbreak. *Lancet.* 1999;354:1327-1330.
11. Bell BP, Griffin PM, Lozano P, Christie DL, Kobayashi JM, Tarr PI. Predictors of hemolytic uremic syndrome in children during a large outbreak of *Escherichia coli* O157:H7 infections. *Pediatrics.* 1997;100:E12.
12. Ikeda K, Ida O, Kimoto K, Takatorige T, Nakanishi N, Tatara K. Effect of early fosfomycin treatment on prevention of hemolytic uremic syndrome accompanying *Escherichia coli* O157:H7 infection. *Clin Nephrol.* 1999;52:357-362.
13. Slutsker L, Ries AA, Maloney K, Wells JG, Greene KD, Griffin PM. A nationwide case-control study of *Escherichia coli* O157:H7 infection in the United States. *J Infect Dis.* 1998;177:962-966.
14. Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med.* 2000;342:1930-1936.
15. Proulx F, Turgeon JP, Delage G, Lafleur L, Chicoine L. Randomized controlled trial of antibiotic therapy for *Escherichia coli* O157:H7 enteritis. *J Pediatr.* 1992;121:299-303.
16. Cimolai N, Basalyga S, Mah DG, Morrison BJ, Carter JE. A continuing assessment of risk factors for the development of *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Clin Nephrol.* 1994;42:85-89.
17. Ostroff SM, Kobayashi JM, Lewis JH. Infections with *Escherichia coli* O157:H7 in Washington State: the first year of statewide disease surveillance. *JAMA.* 1989;262:355-359.
18. Dundas S, Todd WT, Stewart AI, Murdoch PS, Chaudhuri AK, Hutchinson SJ. The central Scotland *Escherichia coli* O157:H7 outbreak: risk factors for the hemolytic uremic syndrome and death among hospitalized patients. *Clin Infect Dis.* 2001;33:923-931.
19. Pavia A, Nichols CR, Green DP, et al. Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. *J Pediatr.* 1990;116:544-551.
20. Proulx F, Seidman E. Is antibiotic therapy of mice and humans useful in *Escherichia coli* O157:H7 enteritis? *Eur J Clin Microbiol Infect Dis.* 1999;18:533-534.
21. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst.* 1959;29:719-748.
22. Breslow NE, Day NE. Statistical methods in cancer research. In: *Analysis of Case-Control Studies.* Vol 1. Lyon, France: International Agency for Research on Cancer; 1980:142-146.
23. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315:629-634.
24. Shiomi M, Togawa M, Fujita K, Murata R. Effect of early oral fluoroquinolones in hemorrhagic colitis due to *Escherichia coli* O157:H7. *Pediatr Int.* 1999;41:228-232.
25. Kawamura N, Yamazaki T, Tamai H. Risk factors for the development of *Escherichia coli* O157:H7 associated with hemolytic uremic syndrome. *Pediatr Int.* 1999;41:218-222.
26. Fukushima H, Hashizume T, Morita Y, et al. Clinical experiences in Sakai City Hospital during the massive outbreak of enterohemorrhagic *Escherichia coli* O157 infections in Sakai City, 1996. *Pediatr Int.* 1999;41:213-217.
27. Higami S, Nishimoto K, Kawamura T, Tsuruhara T, Isshiki G, Ookita A. Retrospective analysis of the relationship between HUS incidence and antibiotics among patients with *Escherichia coli* O157 enterocolitis in the Sakai outbreak. *Kansenshogaku Zasshi.* 1998;72:266-272.
28. Kohsaka T, Tagawa M, Suzuki T, Ito H. The old and new problems of the antibiotics treatment in hemorrhagic enterocolitis and hemolytic uremic syndrome, according to Japanese epidemiological studies. *Nippon Rinsho.* 1997;55:706-714.
29. Oshima T. Predictive factors for development of

hemolytic uremic syndrome (HUS) and early intensive treatments for prevention of HUS enterohemorrhagic *Escherichia coli* infection. *Jpn J Antibiot.* 1997; 50:855-861.
30. Takeda T, Yoshino K, Uchida H, Ikeda N, Tanimura M. Early use of fosfomycin for Shiga toxin-producing *Escherichia coli* O157 infection reduces the risk of hemolytic-uremic syndrome. In: Kaper JB, O'Brien A, eds. *Escherichia coli O157:H7 and Other Shiga Toxin–Producing E coli*. Washington, DC: American Society of Microbiology; 1998:385-387.
31. Cimolai N, Carter JE, Morrison BJ, Anderson JD. Risk factors for the progression of *Escherichia coli* O157:H7 enteritis to hemolytic-uremic syndrome. *J Pediatr.* 1990;116:589-592.
32. Besser RE, Lett SM, Weber JT, et al. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA.* 1993;269:2217-2220.
33. Friedman MS, Roels T, Koehler JE, Feldman L, Bibb WF, Blake P. *Escherichia coli* O157:H7 outbreak associated with an improperly chlorinated swimming pool. *Clin Infect Dis.* 1999;29:298-303.
34. Carter AO, Borczyk AA, Carlson JA, et al. A severe outbreak of *Escherichia coli* O157:H7 associated hemorrhagic colitis in a nursing home. *N Engl J Med.* 1987;317:1496-1500.
35. Ryan CA, Tauxe RV, Hosek GW, et al. *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical, epidemiological and pathological findings. *J Infect Dis.* 1986;154:631-638.
36. Rowe PC, Orrbine E, Lior H, et al, for the Investigators of the Canadian Pediatric Kidney Disease Research Center. Risk of hemolytic uremic syndrome after sporadic *Escherichia coli* O157:H7 infection: results of a Canadian collaborative study. *J Pediatr.* 1998; 132:777-782.
37. Buteau C, Proulx F, Chaibou M, et al. Leukocytosis in children with *Escherichia coli* O157:H7 enteritis developing the hemolytic-uremic syndrome. *Pediatr Infect Dis J.* 2000;19:642-647.
38. Spika JS, Parsons JE, Nordenberg D, Wells JG, Gunn RA, Blake PA. Hemolytic uremic syndrome and diarrhea associated with *Escherichia coli* 0157:H7 in a day care center. *J Pediatr.* 1986;109:287-291.
39. Lopez EL, Contrini MM, Devoto S, et al. Incomplete hemolytic-uremic syndrome in Argentinean children with bloody diarrhea. *J Pediatr.* 1995;127:364-367.
40. Grif K, Dierich MP, Karch H, Allerberger F. Strain-specific differences in the amount of Shiga toxin released from enterohemorrhagic *Escherichia coli* O157 following exposure to subinhibitory concentrations of antimicrobial agents. *Eur J Clin Microbiol Infect Dis.* 1998;17:761-766.
41. Walterspiel JN, Ashkenazi S, Morrow AL, Cleary TG. Effect of subinhibitory concentrations of antibiotics on extracellular Shiga-like toxin I. *Infection.* 1992; 20:25-29.
42. Yoh M, Frimpong EK, Honda T. Effect of antimicrobial agents, especially fosfomycin, on the production and release of Vero toxin by enterohaemorrhagic *Escherichia coli* O157:H7. *FEMS Immunol Med Microbiol.* 1997;19:57-64.
43. Kurioka T, Yunou Y, Harada H, Kita E. Efficacy of antibiotic therapy for infection with Shiga-like toxin-producing *Escherichia coli* O157:H7 in mice with protein-calorie malnutrition. *Eur J Clin Microbiol Infect Dis.* 1999;18:561-571.
44. Karch H, Strockbine NA, O'Brien AD. Growth of *Escherichia coli* in the presence of trimethoprim-sulfamethoxazole facilitates detection of Shiga-like toxin producing strains by colony blot assay. *FEMS Microbiol Lett.* 1986;35:141-145.
45. Zimmerhackl LB. *E. coli*, antibiotics, and the hemolytic-uremic syndrome. *N Engl J Med.* 2000;342: 1990-1991.
46. Salazar-Lindo E, Sack RB, Chea-Woo E, et al. Early treatment with erythromycin of *Campylobacter jejuni*–associated dysentery in children. *J Pediatr.* 1986;109: 355-360.
47. DuPont HL, Galindo E, Evans DG, Cabada FJ, Sullivan P, Evans DJ Jr. Prevention of travelers' diarrhea with trimethoprim-sulfamethoxazole and trimethoprim alone. *Gastroenterology.* 1983;84:75-80.
48. Haltalin KC, Nelson JD, Ring R III, Sladoje M, Hinton LV. Double-blind treatment study of shigellosis comparing ampicillin, sulfadiazine, and placebo. *J Pediatr.* 1967;70:970-981.
49. Sawamura S, Tanaka K, Koga Y. Therapeutic effects of antibiotics against enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 (O157) infection: in vivo analysis using germfree mice. *Kansenshogaku Zasshi.* 1999;73:1054-1063.
50. Isogai E, Isogai H, Hayashi S, et al. Effect of antibiotics, levofloxacin and fosfomycin, on a mouse model with *Escherichia coli* O157 infection. *Microbiol Immunol.* 2000;44:89-95.
51. Murakami J, Kishi K, Hirai K, Hiramatsu K, Yamasaki T, Nasu M. Macrolides and clindamycin suppress the release of Shiga-like toxins from *Escherichia coli* O157:H7 in vitro. *Int J Antimicrob Agents.* 2000;15:103-109.
52. Yoshimura K, Fujii J, Taniguchi H, Yoshida S. Chemotherapy for enterohemorrhagic *Escherichia coli* O157:H infection in a mouse model. *FEMS Immunol Med Microbiol.* 1999;26:101-108.
53. Izumikawa K, Hirakata Y, Yamaguchi T, et al. *Escherichia coli* O157 interactions with human intestinal Caco-2 cells and the influence of fosfomycin. *J Antimicrob Chemother.* 1998;42:341-347.
54. Wolf LE, Acheson DW, Lincicome LL, Keusch GT. Subinhibitory concentrations of antibiotics increase the release of Shiga-like toxin from *Escherichia coli* O157:H7 in vitro. Paper presented at: Third International Symposium and Workshop on Shiga Toxin (Verotoxin) Producing *Escherichia coli* Infections; June 22-26, 1997; Baltimore, Md.

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The Epidemiology of Infections Caused by *Escherichia coli* O157:H7, Other Enterohemorrhagic *E. coli*, and the Associated Hemolytic Uremic Syndrome

Patricia M. Griffin and Robert V. Tauxe

INTRODUCTION

In 1982, investigation of two outbreaks of a distinctive bloody diarrheal syndrome led to the identification of a new bacterial pathogen, *Escherichia coli* O157:H7 (1). In the decade since, this organism has emerged as an important public health concern on the North American and European continents, and epidemiologic, clinical, and laboratory investigations have rapidly expanded our knowledge about it and the diseases it causes. It was soon recognized that other *E. coli* serotypes shared a similar pathogenic potential, and the group was called the enterohemorrhagic *E. coli* (2). *E. coli* O157:H7 is the most common and most studied member of this group. Infections caused by this organism are being recognized more frequently, which in part reflects increased interest in the organism and the availability of commercial reagents for its detection, but also a real increase in its incidence and geographic scope. The distinctive colitis it causes appears to have been uncommon until recently; outbreaks of this disease would have attracted close scrutiny whenever they occurred, and the organism was first identified using classic microbiologic techniques. The hemolytic uremic syndrome was first described in 1955 (3). Thus, *E. coli* O157:H7 and the diseases it causes have only recently become prominent. This organism is a prototype both for the enterohemorrhagic *E. coli* group and for new and emerging bacterial pathogens in general.

The frequent appearance of *E. coli* O157:H7 in the stools of patients with hemorrhagic colitis, its general absence in stools of healthy persons, and the demonstration of the pathogenic nature of both the organism and its toxins in animal models fulfill the three Koch-Henle criteria for causation (4). These are buttressed by the identification of the organism in implicated food vehicles and in the animal sources of those foods, and by the specific antibody responses detected in infected humans. Much of our knowledge has come from careful epidemiologic field investigation and clinical observation, since the gravity of the infection and lack of effective therapy have precluded volunteer studies. Excellent reviews have summarized the early research into the organism (5), the microbiologic (6) and clinical (7) aspects of the infection, and the biochemistry of its toxins (8). This review will summarize the epidemiologic data on *E. coli* O157:H7 and similar *E. coli* that have accumulated in the first decade of research.

The nomenclature of this group of *E. coli* is confusing and lacks consensus. We will use the term Shiga-like toxin for the toxins they produce, known also as verotoxins. This term emphasizes the link between these toxins and the body of knowledge on Shiga

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toxin. We use the term Shiga-like toxin-producing *E. coli* for strains of *E. coli* that elaborate Shiga-like toxin (verotoxin) (6), without reference to their association with a clinical syndrome. This term is equivalent to verotoxigenic *E. coli*. Shiga-like toxin-producing *E. coli* are generally characterized by tissue culture assays of the supernatant for the presence of toxin, or by hybridization of bacterial colony DNA with toxin gene probes. We use the term enterohemorrhagic *E. coli* to refer to those serotypes of *E. coli* that cause a clinical illness similar to that caused by *E. coli* O157:H7, produce one or more phage-encoded Shiga-like toxins, possess a 60-megadalton virulence plasmid, and produce attaching-effacing lesions in an animal model (2, 9). Thus, enterohemorrhagic *E. coli* are a defined subset of Shiga-like toxin-producing *E. coli*. Levine et al. (9) defined *E. coli* serotypes O157:H7 and O26:H11 as enterohemorrhagic *E. coli*; Tzipori (10) proposed the addition of serotypes O4:NM, O45:H2, O111:NM, and O145:NM (NM denotes nonmotile strain) on the basis of a piglet model. A much larger number have been characterized as Shiga-like toxin-producing *E. coli*, and future investigations are likely to enlarge both lists. (Organisms that make only low levels of a cell-associated toxin neutralized by anti-Shiga toxin are not considered Shiga-like toxin-producing *E. coli* (8).)

MICROBIOLOGY

The enterohemorrhagic *E. coli* prototype, *E. coli* O157:H7, like all *E. coli*, is a gram-negative bacillus (the O refers to the somatic, the H to the flagellar, antigen). Unlike most *E. coli*, it does not ferment sorbitol rapidly (11) and does not produce β-glucuronidase (12). In addition, it does not grow well above 41°C, so it may not be detected by standard procedures for enumerating fecal coliforms in food or water (13). Almost all *E. coli* O157:H7 are uniformly susceptible to antimicrobial agents effective against gram-negative organisms (14), although the *E. coli* O157:H7 strain isolated in a 1990 waterborne outbreak was resistant to streptomycin, sulfamethoxazole, and tetracycline (15).

The pathogenesis of infections with *E. coli* O157:H7 and other enterohemorrhagic *E. coli* is incompletely understood. The virulence properties involved are distinct from those of the enteropathogenic, enterotoxigenic, and enteroinvasive classes of *E. coli* (2). It is not invasive in standard in vitro assays (16). Studies in gnotobiotic piglets and in cell cultures have shown no evidence of the extensive invasion and intracellular multiplication seen with invasive bacteria such as *Shigella* (17, 18). Adherence to intestinal mucosal cells and production of Shiga-like toxin appear to be the major virulence properties.

Adherence and attachment

The mechanism of bacterial attachment to the intestinal mucosal cell remains controversial, but may include an initial contact step followed by more intimate attachment, such as has been described for enterpathogenic *E. coli* (19). The initial attachment may be mediated by the 60-megadalton plasmid while the intimate attachment may be chromosomally mediated (18). In the colon of the gnotobiotic piglet, *E. coli* O157:H7 and other enterohemorrhagic *E. coli* strains produce a distinctive microscopic lesion characterized by intimate attachment of the bacteria to the apical intestinal mucosal cell and localized destruction of the microvilli (10, 20). These bacteria also exhibit localized adherence to cells in culture, with dense concentrations of actin microfilaments in a cup-like structure in the cytoplasm beneath the attached bacterium (19). This attaching-effacing lesion resembles that produced by enteropathogenic *E. coli* strains in piglets and cell culture (19, 20). However, this lesion has not been demonstrated in pathologic studies of human colonic specimens (21–27).

Toxins

By definition, all Shiga-like toxin-producing *E. coli* produce one or more

Shiga-like toxins (6). In structure and activity, they resemble Shiga toxin produced by *Shigella dysenteriae* type 1, which was described in 1903 (8). Konowalchuk (28) identified a Shiga-like toxin produced by *E. coli* strains in 1977, before its pathogenic significance was established, and called it a verotoxin because of its direct cytotoxic activity to cultured Vero cells (African green monkey kidney cells). After *E. coli* O157:H7 was recognized as a cause of hemorrhagic colitis, Shiga-like toxin was detected in culture supernatants and lysates (29, 30), and was neutralized by antiserum raised against Shiga toxin (30). Subsequent efforts demonstrated that two toxins were elaborated: the toxin neutralized by anti-Shiga toxin was named Shiga-like toxin I, and the other was named Shiga-like toxin II (31). Other investigators, following the original Konowalchuk nomenclature, named these toxins verotoxin 1 and verotoxin 2 (32). The parallel nomenclature has persisted; as noted above, we chose to use the term Shiga-like toxin.

Both Shiga-like toxins I and II are cytotoxins which, like Shiga toxin, kill Vero and HeLa cells, cause fluid accumulation in the ligated rabbit ileal loop, and are paralytic-lethal to the mouse and rabbit (8). Both Shiga and Shiga-like toxins share the same receptor, and have similar structure and modes of action (33–35). The gene coding for Shiga-like toxin I is very similar to that of Shiga toxin, while the gene for Shiga-like toxin II shows 58 percent overall homology with that for Shiga-like toxin I (36). The two Shiga-like toxins are antigenically distinct and differ in their biologic effects: Shiga-like toxin II is less toxic to Vero cells, but more toxic for mice, and causes hemorrhagic colitis in the adult rabbit, while Shiga-like toxin I does not (8). Genes for both toxins are phage-encoded, which suggests that *E. coli* O157:H7 may have acquired these toxins through phage-mediated transfer (31). There is further variety in the toxins; antigenic differences of unclear biologic significance were demonstrated by cross-neutralization studies of Shiga-like toxin II purified from two different strains of *E. coli* (37), and Shiga-like toxin II genes with two different sequences have been described within a single strain of *E. coli* (38). Shiga-like toxin-producing *E. coli* associated with edema disease of pigs produce a variant of Shiga-like toxin II that probably binds to a different cellular receptor than do other Shiga-like toxins (39).

The Shiga-like toxins are proteins with one large A subunit, the active portion, and five smaller B subunits, which bind to the cell surface receptor (8). Following binding and internalization, the active subunit catalyzes the destructive cleavage of ribosomal RNA, thereby halting protein synthesis (40, 41). The chief determinant of cellular sensitivity appears to be the surface receptor, globotriosyl ceramide (34, 35). This receptor is present on Vero cells and other cell lines sensitive to Shiga-like toxin, and in human renal tissue (6, 34, 42).

Subtyping

Efforts to subtype *E. coli* O157:H7 strains have demonstrated a remarkable homogeneity consistent with the appearance and spread of a new, clonally-derived pathogen. Strain differences in antimicrobial susceptibility patterns have been rare. Isoenzyme typing of *E. coli* O157:H7 strains from North America revealed only slight differences, although they differed substantially from the heterogeneous group of other Shiga-like toxin-producing *E. coli*, suggesting that the O157:H7 strains represent a recent clonal expansion from a common progenitor strain (43). Plasmid profile determination has been of some utility in epidemiologic investigations, but the range of different profiles is limited (14). Toxin typing is of some epidemiologic use: human isolates of *E. coli* O157:H7 that produce both Shiga-like toxin I and Shiga-like toxin II, and those producing only Shiga-like toxin II, are common; those that produce only Shiga-like toxin I are uncommon (44, 45). The greatest strain diversity is shown by phage typing; 62 phage types have been described in one system (46).

Diagnosis

Because *E. coli* O157:H7 does not ferment sorbitol rapidly, sorbitol MacConkey agar can be used to quickly screen clinical stool specimens (11, 47). About 10–15 percent of human diarrheal stool specimens contain non-sorbitol-fermenting organisms (47, 48). Coliform colonies that are colorless at 24 hours on sorbitol MacConkey agar can be identified using direct agglutination with commercially available latex-conjugated O157 antisera (49, 50). Agglutination with O157 antisera alone does not provide certain identification, since sorbitol-negative *E. coli* of serogroup O157 have been reported that are neither *E. coli* O157:H7 nor Shiga-like toxin-producing *E. coli*, and another sorbitol-negative species, *Escherichia hermanii*, has been reported to be agglutinated by O157 antisera (51, 52). Both would be excluded by routine determination of H type and other markers (11, 52). *E. coli* O157:H7 is easily isolated from feces in the first week after symptoms begin, after which it becomes difficult to isolate (16).

Other methods of clinical detection have been described that may be more sensitive, and that can identify other Shiga-like toxin-producing *E. coli* (which do ferment sorbitol and do not agglutinate in O157 antisera). Methods of detecting free fecal Shiga-like toxin have the advantage of detecting toxins in the absence of living organisms and toxins produced by any Shiga-like toxin-producing *E. coli* (53, 54). Several enzyme-linked immunosorbent assays for detection of Shiga-like toxins in culture or stool extracts have been described (55, 56). DNA probes to the Shiga-like toxin genes, used to screen hundreds of *E. coli* colonies per isolation plate, provide a sensitive way to detect the small number of organisms present late in infection (57). A probe to the 60-megadalton plasmid genes (termed the enterohemorrhagic *E. coli* probe) is very sensitive in detecting *E. coli* O157:H7 and less sensitive for other Shiga-like toxin-producing *E. coli* (9), but it also hybridizes with some *E. coli* strains that do not produce Shiga-like toxin (58, 59). Refinement of the polymerase chain reaction for Shiga-like toxin may enable Shiga-like toxin-producing *E. coli* infection to be diagnosed when there are few or no viable organisms (60, 61). Further development of these techniques and commercial marketing of reagents may make these reference and research laboratory techniques more widely available.

Serologic response

Antibodies to O157 antigen have been detected in the sera of patients with recent *E. coli* O157:H7 infection, and may be especially useful in determining the cause of hemolytic uremic syndrome (62, 63). Antibodies to H7 were not detected in one study (62). In the epidemic setting, an immunoglobulin G enzyme-linked immunosorbent assay for anti-O157 antigen was over 90 percent sensitive and specific for patients with recent culture-confirmed infection (64). Although measurement of a fourfold rise in Shiga-like toxin neutralizing activity has been reported in patients with hemolytic uremic syndrome, the sensitivity and specificity of this cell culture assay have not been reported (53). Detection of a specific antibody response to Shiga-like toxin II has not been reported; this has been hampered by finding nonspecific Shiga-like toxin II neutralizing activity in all normal sera examined (64). The full pattern of the serologic response to infection with *E. coli* O157:H7 and other Shiga-like toxin-producing *E. coli* remains to be characterized, and the duration and laboratory markers of natural immunity are unknown.

CLINICAL MANIFESTATIONS

Hemorrhagic colitis caused by *E. coli* O157:H7

Hemorrhagic colitis caused by *E. coli* O157:H7 is characterized by severe abdominal cramps, bloody stools, little or no fever, and evidence of colonic mucosal edema, erosion, or hemorrhage in the absence of conventional enteric pathogens in the stools (1). Typical illness begins with nonbloody diarrhea and severe abdominal cramps (figure 1). The stools usually become bloody on

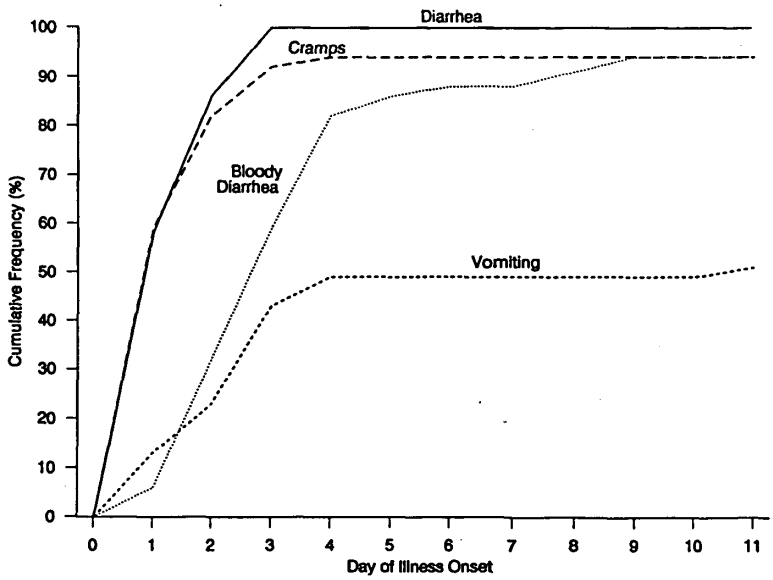

FIGURE 1. Day of illness on which symptoms first appeared during infection with *Escherichia coli* O157:H7, among 88 patients (95 percent of whom developed bloody diarrhea) reported in the first year of laboratory-based surveillance, Washington State, 1987. (From Ostroff, S.M., Kobayashi, J.M., Lewis, J.H. Infections with *Escherichia coli* O157:47 in Washington State: the first year of statewide surveillance. JAMA 1989;262:355–9. Reproduced with permission.)

the second or third day of illness, with the amount of blood ranging from streaks to stools that are essentially all blood (7, 65, 66). The bloody stools continue for 2 to 4 days, and the illness resolves 6 to 8 days after onset (7, 65, 66). Vomiting occurs in about half of patients (7, 66). Fever occurs in fewer than one-third of patients; it is usually not high, and occurs more commonly in more severely ill persons (7). The illness is often severe enough to result in hospitalization. Patients whose stools do not become bloody have less severe illness: in one study, the duration of diarrhea, number of stools per day, and proportion of patients with abdominal cramps, vomiting, and fever were all lower in patients with nonbloody diarrhea (67). Asymptomatic infection has also been reported (65, 68–71).

Nonbloody diarrhea caused by *E. coli* O157:H7

In large series of sporadic cases, over 95 percent of patients with *E. coli* O157:H7 infection had bloody diarrhea (65, 66, 72). Although bloody diarrhea is the most frequently reported manifestation of illness, this may reflect a tendency of patients with nonbloody diarrhea not to seek medical care (65) and physicians' tendencies to perform stool cultures on more severely ill patients (73). Similarly, in community outbreaks, investigators have usually focused case-finding on persons with bloody diarrhea, so the proportion of cases with bloody diarrhea is usually over 90 percent (7). Some outbreaks have occurred in settings in which data on most exposed persons can be obtained. In these settings, the proportion of ill persons (not all of whom were culture-confirmed) with bloody diarrhea is often lower: rates of 65 percent, 56 percent, and 75 percent were reported among residents of three nursing homes (68, 71, 74), 28 percent among staff in a nursing home (74), and 31 percent and 75 percent in two day care center outbreaks (67, 70). In a community outbreak in which a household survey was performed, only 35 percent of 243 persons with diarrhea reported bloody stools (Swerdlow, D., Centers for Disease Control, personal communication, 1991). When illness occurs in family members of patients with bloody diarrhea

due to *E. coli* O157:H7, it is often nonbloody (65, 75).

Extraintestinal isolations of *E. coli* O157:H7

Isolations of *E. coli* O157:H7 from extraintestinal sites are extremely rare and have only been reported from patients who also had diarrhea. The organism was isolated from the glans penis of a 10-month old boy 28 days after he developed bloody diarrhea (76). Urine isolates were reported from two patients who also had intestinal infections. One patient was a girl with hemolytic uremic syndrome who had *E. coli* O157:H7 in the stool. Her initial urine culture was sterile, but she developed hematuria after insertion of a urinary catheter (76). The other was a patient with mild diarrhea and no urinary symptoms (77). An elderly patient with hemorrhagic colitis had *E. coli* O157:H7 isolated from a blood culture 2 days before death (78).

Risk factors for diarrhea caused by *E. coli* O157:H7

Reported risk factors for diarrhea caused by *E. coli* O157:H7, other than ingesting contaminated food or water and person-to-person spread (see section "Outbreak investigations and studies of transmission" below), include very young or very old age, recent antimicrobial use, and previous gastrectomy. In outbreaks in two day-care centers and a kindergarten, the youngest children had the highest rate of illness (67, 69, 70). In a nursing home outbreak, the highest rate of diarrhea was in the very old, and antimicrobial therapy before the onset of illness and previous gastrectomy were both associated with diarrhea (74). In population-based sporadic case series, the highest attack rates have been in children under 5 years of age, with the attack rate then decreasing with increasing age up to 45–64 years, then increasing in elderly persons (65, 66). Three large studies of sporadic cases reported no difference in incidence by sex in general, but, in one study, infections were more common in females than males over the age of 15 years (65, 66, 79). Persons with occupational exposure to cattle, ground beef, and clinical stool specimens may also be at increased risk of illness (66).

Hemolytic uremic syndrome and thrombotic thrombocytopenic purpura caused by *E. coli* O157:H7

Illness caused by *E. coli* O157:H7 resolves in most patients with no sequelae. However, some develop hemolytic uremic syndrome or thrombotic thrombocytopenic purpura. In the outbreak setting, the proportion of patients who develop these complications has varied with the population affected, with the lowest rates in communities and generally higher rates in children and the elderly (7, 80). In four series of sporadic cases in which almost all patients had bloody diarrhea, a total of 15 (9 percent) of the 166 patients developed hemolytic uremic syndrome (66, 72, 81, 82). In a retrospective study of the 72 children (over 90 percent of whom had bloody diarrhea) with *E. coli* O157:H7 infection diagnosed by one laboratory over 4 years, six (8 percent) developed hemolytic uremic syndrome (83). In Canada in 1987, the number of childhood cases of hemolytic uremic syndrome (most of which were preceded by diarrhea and about half of which were culture confirmed as caused by *E. coli* O157:H7) was 86 (84), and the number of culture-confirmed cases of *E. coli* O157:H7 infection reported was 1,342 (85), giving a crude case-complication rate of 6 percent for hemolytic uremic syndrome. The addition of culture-negative cases of hemorrhagic colitis likely caused by *E. coli* O157:H7 to the denominator would decrease this rate, and the addition of cases of hemolytic uremic syndrome in adults would increase the rate. If only 30 to 75 percent of infected persons develop bloody diarrhea (see subsection "Nonbloody diarrhea caused by *E. coli* O157:H7" above), then the true rate of development of hemolytic uremic syndrome is probably 2 to 7 percent, depending on the population.

Some patients with *E. coli* O157:H7 infection develop abnormal laboratory values suggestive of an incomplete form of hemolytic uremic syndrome (86). In a retrospective study of children with *E. coli* O157:H7 infection without hemolytic uremic syndrome, three of 72 had hemolytic anemia with red cell fragmentation (83). In a similar study, four of eight patients had erythrocyte fragments, three of 11 had decreased platelets, and six of 11 had hematuria or proteinurea (87). In another study, four of 16 patients had hematuria or proteinuria, and two of 23 had an elevated lactate dehydrogenase level (7).

Other complications of infection with *E. coli* O157:H7

Other complications of *E. coli* O157:H7 infection include intussusception (83, 88), gross anal dilatation (89), and death. Probably unnecessary diagnostic laparotomies (83, 90) and bowel resections (7) have also been performed. Deaths caused by *E. coli* O157:H7 occur mostly in the elderly and in persons with hemolytic uremic syndrome or thrombotic thrombocytopenic purpura (7). In ill residents of four nursing homes, the combined case-fatality rate was 18 percent (25 of 136) (68, 71, 74, 91). In a community outbreak, the two elderly patients who died had thrombotic thrombocytopenic purpura (7). Reported causes of death in the elderly have included hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, colitis, pulmonary edema, pulmonary effusions, pneumonia, myocardial infarction, congestive heart failure, and bacteremia (71, 74, 78, 91). In an outbreak in institutions for mentally retarded persons, the four ill persons who died had hemolytic uremic syndrome (92). In series of children with hemolytic uremic syndrome, the acute mortality rate in those with a diarrheal prodrome (that is, with disease likely caused by *E. coli* O157:H7) was three (3 percent) of 101 in Minnesota between 1979 and 1988 (93), and 14 (5 percent) of 273 in the United Kingdom in 1985–1988 (94).

Risk factors for hemolytic uremic syndrome caused by *E. coli* O157:H7

A single strain of *E. coli* O157:H7 can produce the entire spectrum of illness, including nonbloody diarrhea, bloody diarrhea, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (7). However, the likelihood of complications may be determined by host factors, dose, or by characteristics of the infecting strain. Reported risk factors for developing hemolytic uremic syndrome or thrombotic thrombocytopenic purpura among patients with *E. coli* O157:H7 infection include very young or old age, female sex, mental retardation, P antigen expression by red blood cells, bloody diarrhea, fever, elevated leukocyte count early in the diarrheal illness, toxin type of the infecting strain, and use of antimotility agents and antimicrobial therapy for the diarrhea (table 1). Young age, especially less than 5 years, has been a risk factor in many studies (7, 66, 82, 92, 95). The elderly are also at increased risk (7). One study suggested that female sex could be a risk factor for hemolytic uremic syndrome (95), but

TABLE 1. Major reported risk factors for developing hemolytic uremic syndrome or thrombotic thrombocytopenic purpura among patients with *Escherichia coli* O157:H7 infection

Factor	References supporting association	References not supporting association
Very young age	7, 66, 82, 92, 95	
Very old age	7	
Female sex	83, 95	96
Mental retardation	84, 92	
P antigen expression by red blood cells	98	
Bloody diarrhea	74	99
Fever	92, 99	
Elevated leukocyte count by third day of illness	92	95
Toxin type of *E. coli* O157:H7	44, 45	
Antimotility agents	95	101
Antimicrobial therapy for diarrhea	66, 92	95

additional data from the same group did not confirm this finding (96). Another study found that females had a significantly greater risk of developing hemolytic anemia after *E. coli* O157:H7 infection than did males (83). In an institutional outbreak of *E. coli* O157:H7 infections, eight (40 percent) of 20 ill retarded persons, but none of the 31 ill employees, developed hemolytic uremic syndrome, suggesting that retarded persons may be at increased risk, perhaps because of exposures specific to the institutionalized retarded (92). Among the 226 Canadian children with hemolytic uremic syndrome reported between 1986 and 1988, 95 percent of whom had preceding diarrhea, 14 (6 percent) had cerebral palsy, developmental delay, or Down's syndrome (84).

The P1 and Pk antigens of red blood cells possess the sugar sequence gal 1–4 alpha gal that is required for binding of Shiga-like toxin to its receptor (97). In a retrospective study comparing patients who had had hemolytic uremic syndrome (over half of whom had Shiga-like toxin-producing *E. coli* infection) and controls with other renal disorders, the proportion of each group whose red blood cells expressed the P1 antigen was similar (98). However, the trend for degree of P1 antigen expression was significantly weaker among hemolytic uremic syndrome patients, and the hemolytic uremic syndrome patients with the worst outcome had the weakest P1 expression. The authors (98) speculated that patients with low expression of the P1 antigen on red cells may be at increased risk for hemolytic uremic syndrome because less toxin is absorbed by red cells, leaving more to damage other tissues. Another possibility is that these patients have more P1 in secretions to bind to toxin.

Clinical characteristics of the initial infection may predict hemolytic uremic syndrome. In a nursing home outbreak, 12 (29 percent) of 41 patients with bloody diarrhea, but none of 14 with nonbloody diarrhea, developed hemolytic uremic syndrome ($p = 0.02$) (74). In another study, the proportion with bloody diarrhea was the same among patients with *E. coli* O157:H7 infection with and without hemolytic uremic syndrome, but the two groups were obtained by different methods (99). In this study, patients with hemolytic uremic syndrome were more likely to have fever, but the timing of the fever was not reported. In another outbreak, patients who developed hemolytic uremic syndrome had higher temperatures and leukocyte counts by the third day of illness than did other patients (92).

Characteristics of the infecting strain may affect the incidence of hemolytic uremic syndrome. In a series of *E. coli* O157 strains from patients in the United Kingdom, 10 (91 percent) of 11 strains that produced only Shiga-like toxin II came from patients with hemolytic uremic syndrome, compared with five (50 percent) of 10 strains that produced both Shinga-like toxin I and II or only Shinga-toxin I ($p = 0.06$) (44). In another series from the United Kingdom, 34 (89 percent) of the 38 hemolytic uremic syndrome patients with *E. coli* O157 had an organism that produced only Shiga-like toxin II (100). In a study of 88 strains of *E. coli* O157:H7 from Washington State, seven (39 percent) of 18 strains that had only the Shiga-like toxin II gene were from patients with hemolytic uremic syndrome or thrombotic thrombocytopenic purpura, compared with four (6 percent) of 70 strains that had both Shiga-like toxins I and II or only Shiga-like toxin I ($p < 0.001$) (45). This association was independent of age and antimicrobial use. Further studies are needed to confirm this association and to determine possible reasons for it.

Receiving an antimotility agent for more than 24 hours was a risk factor in one study (95), but was not demonstrated in another (101). Further studies of these agents are warranted because in one study, 29 percent of children with *E. coli* O157:H7 infection received an antimotility agent (usually for <24 hours) (102).

In one case series, treatment with trimethoprim-sulfamethoxazole or gentamicin was possibly associated with an increased risk of hemolytic uremic syndrome or thrombotic thrombocytopenic purpura (66). In an outbreak, all five patients who received

trimethoprim-sulfamethoxazole, but only two of seven who received no antimicrobial drug for bloody diarrhea, developed hemolytic uremic syndrome ($p = 0.03$). Possible explanations for an association of trimethoprim-sulfamethoxazole therapy with development of hemolytic uremic syndrome include that *E. coli* O157:H7 is resistant to trimethoprim-sulfamethoxazole, that trimethoprim-sulfamethoxazole kills *E. coli* O157:H7 releasing Shiga-like toxin and/or endotoxin, that trimethoprim-sulfamethoxazole increases the production of Shiga-like toxin, and that the association is not causal. Treatment with an antimicrobial agent to which the infecting organism is resistant is likely to accelerate progression to hemolytic uremic syndrome, as has been reported for *Shigella dysenteriae* type 1 (103). However, almost all strains of *E. coli* O157:H7 are susceptible to ampicillin, trimethoprim-sulfamethoxazole, tetracycline, and quinolones, although they are resistant to erythromycin, metronidazole, and vancomycin (14, 82, 104, 105). If trimethoprim-sulfamethoxazole increases the risk of progression to hemolytic uremic syndrome by killing *E. coli* O157:H7, thus releasing Shiga-like toxin or endotoxin, then other cytolytic antimicrobial agents should also accelerate progression to hemolytic uremic syndrome.

One group of investigators has reported that subinhibitory concentrations of trimethoprim-sulfamethoxazole markedly increase production of Shiga-like toxin, and that other antimicrobial agents increase or decrease toxin production (106, 107). However, it is not known whether therapeutic doses of antimicrobial agents influence toxin production. It is possible that the association is not causal, but that more severely ill patients are more likely both to receive trimethoprim-sulfamethoxazole and to progress to hemolytic uremic syndrome. Indeed, in the outbreak in which this association was reported, those patients who received trimethoprim-sulfamethoxazole had higher initial temperatures and leukocyte counts than those who did not receive trimethoprim-sulfamethoxazole (92). In another study, patients with hemolytic uremic syndrome were less likely than patients with only diarrhea to have received an "appropriate" antimicrobial for >24 hours (95). It is possible that antimicrobial agents could have both beneficial and harmful effects, ameliorating the intestinal illness, and increasing the risk of hemolytic uremic syndrome. However, in retrospective analyses, these drugs have not been shown to decrease the duration of diarrhea (7, 66, 108). Treating patients who have *E. coli* O157:H7 infection with erythromycin for presumptive *Campylobacter* infection, or with metronidazole for presumptive *Clostridium difficile* colitis, may worsen the disease by killing normal competing bowel flora. Prospective studies are needed to determine the risk and benefits of antimicrobial drug therapy for diarrhea caused by *E. coli* O157:H7 (104).

Diarrhea associated with non-O157 Shiga-like toxin-producing *E. coli*

Unlike *E. coli* O157:H7, non-O157 Shiga-like toxin-producing *E. coli* do not appear to be a common cause of bloody diarrhea (see also subsections "Frequency of diarrhea caused by non-O157 Shiga-like toxin-producing *E. coli* in North America" and "Frequency of diarrhea caused by non-O157 Shiga-like toxin-producing *E. coli* outside North America" in the "Incidence" section below). No outbreaks of bloody diarrhea caused by non-O157 Shiga-like toxin-producing *E. coli* have been reported from the United States, Canada, or the United Kingdom. Reports of sporadic cases are uncommon, although the absence of a biochemical marker (such as slow sorbitol fermentation for *E. coli* O157:H7) likely leads to underrecognition (59, 65). The non-O157 Shiga-like toxin-producing *E. coli* appear less likely than *E. coli* O157:H7 to be associated with bloody stools. In one study of persons with diarrhea, 110 (99 percent) of 111 persons with *E. coli* O157:H7 infection, but only eight (44 percent) of 18 with non-O157 Shiga-like toxin-producing *E. coli* had bloody stools ($p < 0.001$) (65). In another study, of 51 patients with hemolytic uremic syndrome (with a diarrheal prodrome and

from whom a single Shiga-like toxin-producing *E. coli* strain was isolated), 31 (84 percent) of 37 with *E. coli* O157, but only eight (57 percent) of 14 with other Shiga-like toxin-producing *E. coli*, had a prodrome of bloody stools ($p = 0.05$) (100).

Over 25 non-O157 Shiga-like toxin-producing *E. coli* serotypes have been isolated from humans with diarrhea, of which *E. coli* O26:H11 (9, 65, 109–113), O103:H2 (65, 114), O111:NM (53, 109, 115–118), and O113:H21 (58, 119) have been reported most frequently. Among these, serotypes O26:H11 and O111:NM are also among the most common serotypes of classic enteropathogenic *E. coli* (2, 120). The O26:H11 serotype, for one, appears to be heterogeneous; all strains in one study demonstrated the fluorescent actin staining that correlates with the ability to cause attaching-effacing lesions (19), but not all produced Shiga-like toxin (121). Not all Shiga-like toxin-producing *E. coli* isolated from persons with diarrhea contain the enterohemorrhagic *E. coli* plasmid, which may be important in virulence (9, 58). Identification of the presence or absence of virulence determinants in other Shiga-like toxin-producing *E. coli* may help to determine which are pathogens. Large studies of patients with diarrhea and healthy controls should help to determine the role of these organisms in causing diarrhea.

OUTBREAK INVESTIGATIONS AND STUDIES OF TRANSMISSION

From 1982 through 1990, 12 outbreaks of *E. coli* O157:H7 infections were investigated in the United States (table 2). Outbreaks have also been documented in the United Kingdom and Canada, some of which are also listed on table 2. No outbreak caused by other enterohemorrhagic *E. coli* serotypes has been recognized in North America or Europe; one outbreak has been reported from Japan that was caused by *E. coli* O145:NM, which may have produced Shiga-like toxin (122). In the United States, for reasons that remain to be clarified, outbreaks have tended to occur in northern states (seven of the 12 outbreaks occurred in states bordering Canada), in the last two-thirds of the year (11 of 12 outbreaks), and in even-numbered years (10 of 12 outbreaks). Outbreaks have occurred in schools, custodial and chronic-care institutions, day-care centers, family clusters, and the community at large; the latter are usually associated with a restaurant exposure. Among the U.S. outbreaks, the mean number of cases was 56 (median 39); among these cases, 18 percent of persons were hospitalized, 3.6 percent developed hemolytic uremic syndrome or thrombotic thrombocytopenic purpura, and 1.9 percent died. In outbreaks for which a common source vehicle was determined, mean incubation periods range from 3.1 to 8 days. As shown in table 2, attack rates calculated on the denominator of persons exposed to a specific implicated vehicle ranged from 0.1 percent (outbreak 1) to 71 percent (outbreak 15).

Outbreaks are usually detected because of a cluster of hemolytic uremic syndrome or thrombotic thrombocytopenic purpura cases, or because a large number of persons are hospitalized simultaneously with a severe diarrheal illnesses. The detection of smaller outbreaks not associated with hemolytic uremic syndrome, or of geographically diffuse outbreaks, is unlikely in the absence of routine laboratory-based surveillance for *E. coli* O157:H7 infection. Even a single case of hemolytic uremic syndrome or thrombotic thrombocytopenic purpura associated with a day-care center, school, or nursing home may indicate an unrecognized outbreak. In one outbreak (outbreak 5, table 2) associated with a restaurant, investigation identified a wider outbreak affecting a nursing home in another town, and demonstrated a probable link to a surge of sporadic cases around the state.

The lack of reported outbreaks caused by other Shiga-like toxin-producing *E. coli* is not due to lack of exposure to these organisms. The non-O157 Shiga-like toxin-producing *E. coli* are isolated much more frequently than is *E. coli* O157:H7 from dairy cattle and foods (see section "Animal reservoirs and food vehicles" below).

TABLE 2. Outbreaks of *Escherichia coli* O157:H7 infection in the United States, and selected outbreaks in Canada and the United Kingdom

Outbreak no.	Reference(s)	Month and year	State, province or location	Setting	No. affected	No. hospitalized	No. with HUS*/TTP*	No. who died	Incubation period (days)	Likely vehicle	Vehicle-specific attack rate (%)
					United States						
1	1	February 1982	OR	Community	26	19	0	0	3.9	Ground beef	0.1
2	1	May 1982	MI	Community	21	14	0	0	3.8	Ground beef	NR*
3	71	September 1984	NE	Nursing home	34	14	1	4	8	Ground beef	43
4	67	September 1984	NC	Day-care center	36	3	3	0	NR	Person-to-person	
5	216	October 1986	WA	Community	37	17	4	2	3.1	Ground beef/ranch dressing	NR
6	92	June 1987	UT	Custodial institutions	51	8	8	4	6	Ground beef/person-to-person	NR
7	CDC†	May 1988	WI	School	61	2	0	0	NR	Roast beef	NR
8	222	October 1988	MN	School	54	4	0	0	5.3	Precooked ground beef	8
9	70	August 1988	MN	Day-care center	16	NR	3	0	NR	Person-to-person	NR
10	CDC†	December 1989	MO	Community	243	32	2	4	NR	Water	6.7
11	223	July 1990	ND	Community	65	16	2	0	3.1	Roast beef	13
12	CDC†	November 1990	MT	School	10	2	1	0	3.5	School lunch	1.3

Canada											
13	80	September 1985	Ontario	Home	5	5	5	0	NR	Unknown	NR
14	74	September 1985	Ontario	Nursing home	73	NR	12	19	5.7	Cold sandwiches/ person-to-person	NR
15	69, 196	April 1986	Ontario	School	46	NR	3	0	6.8	Raw milk	71
United Kingdom											
16	224	July 1985	E. Anglia	Community	24	10	NR	0	NR	Raw potatoes	NR
17	211	June 1987	Hereford	Home	26	6	1	0	4.5	Turkey roll	71

* HUS, hemolytic uremic syndrome; TTP, thrombotic thrombocytopenic purpura; NR, not reported.
† CDC, Centers for Disease Control, Atlanta, GA, unpublished data.

Food-borne transmission

The majority of outbreaks have been the result of transmission via foods of bovine origin. Among the eight outbreaks with an identified food vehicle in the United States, six were traced to ground beef and two to roast beef. A Canadian outbreak (outbreak 15, table 2) was associated with drinking raw milk (123). Food handling practices that contributed to these outbreaks were typically not gross food handling errors but more subtle deficits in cooking times and/or temperatures, such that not all coliform bacteria in the meat were killed. In the two 1982 outbreaks (outbreaks 1 and 2, table 2), cooler spots were shown to develop on the restaurant griddle during times of peak use; in outbreak 7 (table 2) a new slow-cook procedure that was applied to frozen cryovac-packaged roasts may have been insufficient; in outbreak 8 (table 2), commercial frozen precooked meat patties were shown to still have active catalase, a marker for insufficient cooking; and in outbreak 11 (table 2), illness was associated with roast beef eaten rare or medium rare, and no illness occurred among those who ate their beef well-done.

Non-bovine food vehicles have also been identified. In a large nursing home outbreak in Ontario, Canada (outbreak 14, table 2), illness in the first wave was associated with eating cold sandwiches held without refrigeration. In the second British outbreak (outbreak 17, table 2), it was suspected that a turkey roll was cross-contaminated, possibly from raw beef in the private kitchen where the meal was prepared, or earlier in processing. In the first British outbreak (outbreak 16, table 2), illness was associated with preparing new potatoes, which were packed in peat that could have been contaminated with calf manure (Palmer, S. R., Public Health Laboratory Service, United Kingdom, personal communication, 1991). This outbreak was also noteworthy because it affected a group of food handlers who became infected by contact with the raw vegetables but did not transmit the infection to those for whom they prepared food. Indeed, in none of the outbreaks reported was there

evidence that an infected food handler was the source of contamination.

Other modes of transmission

Person-to-person transmission, presumably by a direct fecal-oral route, has been documented on a small scale in several outbreaks, and has been the dominant mode of transmission in outbreaks in day-care centers. In the outbreak at an institution for the severely mentally retarded (outbreak 6, table 2), and in the large Canadian nursing home outbreak (outbreak 14, table 2), both foodborne transmission and person-to-person transmission from ill residents of the institution to their caretakers were important. In a number of other outbreaks, person-to-person transmission was either suggested or documented, usually with milder nonbloody illness in the secondary cases: 10 of 56 family members of affected day-care attenders reported a diarrheal illness in outbreak 4 (table 2); two cases of secondary transmission in families were documented in outbreak 5 (table 2), and one episode of intrafamilial transmission was reported in outbreak 9 (table 2). As with *Shigella*, this may be most likely to occur when young children are ill.

In a large waterborne outbreak (outbreak 11, table 2), a community water supply was contaminated following freeze-damage and repairs to the city mains; leaks in the aging sewerage and water distribution systems and lack of chlorination before, during, and after the repairs may have contributed to the contamination. The original source of the contamination was unknown.

Infectious dose

The general patterns of transmission in these outbreaks suggest that the infectious dose is low. The implicated meat vehicles were usually only slightly undercooked and were not subsequently held for many hours at warm temperatures that would have permitted bacterial growth. This suggests that fewer bacteria are needed to cause illness than for outbreaks of salmonellosis, which are often associated with more egregious food handling errors. The apparent ease of person-to-person transmission in settings with limited hygiene is reminiscent of *Shigella*, an organism that can be transmitted by exposure to extremely few organisms. Cold bulk liquid vehicles, such as raw milk or municipal water, are likely to dilute any organisms present without permitting rapid bacterial growth, yet these vehicles were associated with appreciable attack rates. However, the infectious dose is most likely greater than a single organism, since in the large Canadian nursing home outbreak (outbreak 14, table 2), gastrectomy and preceding antimicrobial therapy were identified as risk cofactors, suggesting that gastric acidity and normal bowel flora offer some degree of protection against illness, presumably by killing or inhibiting a substantial proportion of ingested organisms. No clear correlation between attack rate and incubation period emerges from these data; however, incubation periods tended to be long in outbreaks in nursing homes and custodial institutions, where some cases were likely the result of person-to-person spread of small inocula.

Control measures

Control measures applied in ongoing outbreaks of *E. coli* O157:H7 infections need to be thorough to be successful. Identification of flaws in cooking has often required careful review of procedures, and even duplication of the cooking process under observation. In the waterborne outbreak (outbreak 10, table 2), an initial order to boil water did not stop the outbreak, and cases continued to appear until the water supply was chlorinated. Transmission among children at a day-care center continued despite increased hand washing and exclusion of ill children (outbreak 9, table 2); it was concluded that closing the center, while monitoring the children's care at home to prevent their appearance in other day-care centers, was necessary to stop the outbreak. In the institution for the mentally retarded (outbreak 6, table 2), person-to-person transmission stopped coincident with the imposition of rigorous isolation and infection control procedures. Nonetheless, the rarity of nosocomial transmission in acute-care hospitals,

and the lack of documented laboratory-acquired infections, suggest that routine hospital and laboratory infection control procedures are adequate to prevent transmission in most clinical circumstances.

Sources of sporadic infections

Less is known about the sources of sporadic infections with *E. coli* O157:H7, although these appear to be much more common than outbreaks. Two case-control investigations of sporadic cases have been reported (72, 79). Among 25 cases identified at a Seattle health maintenance organization, none were linked to recognized outbreaks (72). Illness was associated with eating rare ground beef (21 percent of cases, $p = 0.054$), and two patients had drunk raw milk shortly before onset of their illness. Among 49 cases and matched healthy controls in a 2-year study in Alberta, Canada, exposure to beef steak, roast beef, hamburger patties, chicken, and barbecued food yielded elevated odds ratios, and exposure to raw or undercooked meat was significantly associated with illness (79). In another study, two sporadic cases of *E. coli* O157:H7-associated hemolytic uremic syndrome occurred in children who drank raw milk (123). *E. coli* O157:H7 has been isolated from untreated surface water (124), and exposure to untreated and potentially sewage-contaminated surface water was suspected as the source of two cases of hemolytic uremic syndrome associated with *E. coli* O157:H7 infections (90). One case has been reported of nosocomial transmission from a child with hemolytic uremic syndrome to a nurse caring for the child (125), and one case of possible laboratory-acquired infection has been reported (66). Thus, the range of sources implicated or suggested by studies of sporadic cases is similar to that for outbreaks: undercooked meat (particularly ground beef), raw milk, untreated water, and person-to-person spread.

INCIDENCE

The syndrome of hemorrhagic colitis was recognized by the early 1970s and was reported under several names, including evanescent colitis (126), transient ischemic colitis, and transient hemorrhagic colitis (127). After *E. coli* O157:H7 was identified as a cause of hemorrhagic colitis, national laboratories in the United States, Canada, and the United Kingdom reviewed their records to look for this serotype. Review of over 3,000 *E. coli* strains serotyped by the Centers for Disease Control between 1973 and 1983 revealed only one O157:H7, isolated in 1975 from a California woman with abdominal cramps and grossly bloody diarrhea (1). However, many of these strains were not from human stool specimens, and the proportion of strains from persons with bloody diarrhea is unknown. The Laboratory Centre for Disease Control in Canada reviewed over 2,000 *E. coli* strains isolated from patients with diarrhea between 1978 and 1982. *E. coli* O157:H7 was isolated from six patients, two of whom had hemorrhagic colitis; information on the other four could not be obtained (29). The Public Health Laboratory in the United Kingdom found a single O157:H7 strain among over 15,000 *E. coli* that were serotyped between 1978 and 1982; the clinical story was unknown (128).

The evidence suggests that infections with *E. coli* O157:H7 are increasing, although the data are limited and are difficult to interpret because the number of laboratories screening for this organism has increased. In Canada, the number of isolates has at least doubled most years from 1980 to 1987 (85). There are no data on incidence over time from the United States. However, the Centers for Disease Control has no record of outbreaks of bloody diarrhea of unknown origin before 1982, suggesting that this organism is not likely to have been a frequent cause of outbreaks in the United States in the past. In the United Kingdom, no O157 strain was isolated from 161 outbreaks of diarrhea between 1973 and 1983 (128). In addition, data from several countries indicate that the incidence of hemolytic uremic syndrome is increasing, and this suggests that infections with *E. coli* O157:H7 are increasing (see section "Hemolytic uremic syndrome" below).

Seasonality and geographic distribution of *E. coli* O157:H7

The number of sporadic *E. coli* O157:H7 infections peaks in the summer. In Washington State in 1987, the peak incidence of cases occurred from June through August (figure 2) (66). In Alberta, Canada, in 1985, the number of cases peaked in July and August (65).

Many isolations of this organism have been reported from the United States, Canada, and the United Kingdom, and the organism appears to be most frequently isolated in the more developed countries. *E. coli* O157:H7 strains were isolated from travelers returning from Spain (105). Other countries that have reported isolates from humans include Ireland (129), Belgium (130, 131), Germany (132, 133), Italy (134), Czechoslovakia (135, 136), Australia (117), Argentina (137, 138), Japan (139), China (140), and South Africa (140a).

Like outbreaks, sporadic cases of *E. coli* O157:H7 infection appear to be more common in Canada than in the United States, and are more common in the northwestern United States than in the south. In Canada, *E. coli* O157:H7 infection and hemolytic uremic syndrome have been reported more frequently from western than eastern provinces (103, 109, 141). Whether these differences reflect biases due to culturing and reporting or are real is unknown. The high isolation rate for *E. coli* O157:H7 in a small study of ground beef in Alberta (142) suggests that these differences may relate to different levels of animal carriage or differences in processing methods for meat.

Frequency of diarrhea caused by *E. coli* O157:H7 in North America

A number of studies in the United States and Canada have examined the frequency of isolation of *E. coli* O157:H7. In each of the five major studies (table 3) that compared the isolation rate of this pathogen with the isolation rate of other bacterial pathogens, all of which involved over 1,000 specimens, *E. coli* O157:H7 was the second or third most frequently isolated known pathogen, and was more common than *Shigella* (65, 72, 99, 141, 143). *E. coli* O157:H7 is a major cause of bloody diarrhea. Three Canadian studies reported similar proportions (15 percent, 18 percent, and 15 percent) of cultures with *E. coli* O157:H7 from patients with bloody diarrhea (table 3) (47, 82, 144). Higher rates were found in a U.S. study of

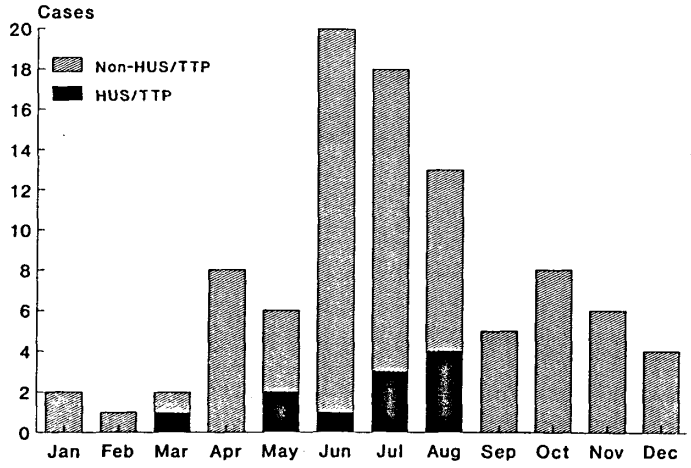

FIGURE 2. Number of cases of infection with *Escherichia coli* O157:H7 by month, Washington, State, 1987. HUS, hemolytic uremic syndrome; TTP, thrombotic thrombocytopenic purpura; solid bars, hemolytic uremic syndrome or thrombotic thrombocytopenic purpura; shaded bars, non-hemolytic uremic syndrome or thrombotic thrombocytopenic purpura. (From Ostroff, S.M., Kobayashi, J.M., Lewis, J.H. Infections with *Escherichia coli* O157:H7 in Washington State: the first year of statewide disease surveillance. JAMA 1989;262:355–9. Reproduced with permission.)

patients with typical symptoms of hemorrhagic colitis and negative routine cultures (37 percent positive) (81), and in a Canadian study conducted during the summer (39 percent positive) (79). In the latter study, the isolation rates for *Campylobacter*, *Salmonella*, and *Shigella* among patients with bloody diarrhea were much lower than for *E. coli* O157:H7.

Few population-based studies have been performed. In 1985–1986, in a health maintenance organization which screened all stool specimens for this organism, the incidence rate for laboratory-confirmed infection with *E. coli* O157:H7 was eight per 100,000 persons per year (72). In a statewide study in Washington State in 1987, the rate was only 2.1 per 100,000 persons, but many laboratories were not screening for the organism (66). In Canada, where many, but not all, laboratories screen for *E. coli* O157:H7, 1,342 isolates were identified in 1987, corresponding to 5.2 per 100,000 persons (85).

Frequency of diarrhea caused by *E. coli* O157:H7 outside North America

A few studies outside North America have determined the proportion of stool specimens with *E. coli* O157:H7 (table 4). Three studies of bloody stools from the United Kingdom reported high rates of isolation of *E. coli* O157:H7, 29 percent of 89 specimens (105), 29 percent of 65 specimens (145), and 75 percent of 40 specimens (146). In the latter study, *E. coli* O157:H7 was also isolated from two (0.9 percent) of 229 age- and sex-matched patients with nonbloody diarrhea. Two other U.K. studies of bloody stools reported low isolation rates, 6 percent of 50 specimens and 5 percent of 80 specimens (48, 100). In one of these, only 33 percent of the specimens had any pathogen (48); in the other, specimens may have been collected late (100). In the latter study (100), *E. coli* O157:H7 was also isolated from one of 62 patients with nonbloody diarrhea. In a study of over 3,000 stool specimens in Belgium in 1987–1988 (130), *E. coli* O157:H7 was isolated from only 0.2 percent of specimens, and was less common than *Shigella*.

No *E. coli* O157:H7 were detected in many studies from less developed areas. In central Australia, none were detected in diarrheal stools from 1,443 aboriginal patients living in poor hygienic conditions (147). In Thailand, no *E. coli* O157:H7 were isolated from stools of 54 children with bloody diarrhea from which no other pathogens were identified (59), from 115 infants with diarrhea (59), from 393 children with diarrhea (148), or from 458 adults with diarrhea (58). In Korea, no *E. coli* O157:H7 were isolated from 231 children with diarrhea (110). In Sao Paulo, Brazil, no *E. coli* O157:H7 were isolated from stools of 95 children under 1 year of age with diarrhea (149). In China, 15 (7 percent) of 221 children with diarrhea, but also six (6 percent) of 108 controls, had stool blots that hybridized with the enterohemorrhagic *E. coli* probe (9); one strain from a case was *E. coli* O157:H7, the others were not isolated (140). In India, no *E. coli* hybridizing with the enterohemorrhagic *E. coli* probe were detected in stool samples from 240 children under 3 years of age (150).

Frequency of diarrhea caused by non-O157 Shiga-like toxin-producing *E. coli*

Few studies of the incidence of diarrheal illness caused by non-O157 Shiga-like toxin-producing *E. coli* have been performed. No single serotype of non-O157 Shiga-like toxin-producing *E. coli* is a common cause of diarrhea, although *E. coli* O26:H11 may be the most frequently reported. A 2-year survey in Canada reported that 36 (0.7 percent) of 5,415 stools had non-O157 Shiga-like toxin-producing *E. coli*, a higher isolation rate than for *Shigella* (table 3) (65). The serotypes isolated included O26:H11 (11 isolates), O103:H2 (five isolates), O?:H21 (three isolates), O91:H- (H- denotes a nonmotile strain, as does abbreviation NM) (two isolates), and isolate one each of O5:H-, O6:H-, O38:H21, O111:H-, and O145:H-; 10 isolates were not serotyped. However, in seven of these 36 patients *E. coli* O157:H7

TABLE 3. Frequency of isolation of *Escherichia coli* O157:H7 and other bacterial pathogens from stools submitted to microbiology laboratories, United States and Canada, 1984–1988*

Location	Reference	Population	Year	Stools					Bloody stools		Comments
				No.	*Campylobacter* (%)	*Salmonella* (%)	*E. coli* O157:H7 (%)	*Shigella* (%)	No.	*E. coli* O157:H7 (%)	
United States	81	All ages	August 1982–April 1984						76†	37	Centers for Disease Control; criteria: gross blood, abdominal pain, no or low fever, routine culture negative
Alberta	82	All ages	June–December 1983						125	15	Three hospitals; criteria: history of blood or bloody specimen
Newfoundland and Labrador	47	?	1983–1984? (20 months)	1,043			1.7		99	18	Public health laboratory
Illinois	77	?	1984? (12 months)	2,552			0.08				Hospital; did not use sorbitol-MacConkey medium
Newfoundland and Labrador	144	All ages	April–December 1984						47†	15	Three hospitals
Alberta	65	All ages	July 1984–June 1986	5,415†	2.0	2.7	2.5	0.5			Three hospitals; 36 (0.7%) had other Shiga-like toxin-producing *E. coli*, but seven of these also had *E. coli* O157:H7
British Columbia	99	Children	August 1984–September 1985	1,425†	2.3	1.7	1.9	0.3			Tertiary care hospital; solicited stools from children with hemolytic uremic syndrome
Washington	72	All ages	May 1985–April 1986	4,539†	3.6	1.5	0.6	0.5			Health maintenance organization; excluded duplicates and adjusted number of cultures to represent number of persons screened

Ontario	141	All ages	April–September 1986	7,252	4.1	4.0	0.7	0.1		Two public health laboratories; the 49 *E. coli* O157:H7 isolates were from 19 patients, five of whom had nonbloody diarrhea
Alberta	79	All ages	1986–1987, (summer only)						266† 39	Three hospitals; *Campylobacter* 11.3%, *Salmonella* 7.5%, *Shigella* 2.3%
Minnesota	143	All ages	April–September 1987	2,164	1.1	1.0	0.5	0.05		One hospital, inpatients and outpatients
Washington	225	All ages	1988	799			2.0			Two hospitals plus physicians' offices

*Sorbitol-MacConkey medium was used to screen for *E. coli* O157:H7 in all these studies except the one in Alberta in 1983, and the one in Illinois; in these two studies, 10 colonies resembling *E. coli* were picked from routine media and further identified.
† Number represents number of patients.

was also isolated, suggesting that the other Shiga-like toxin-producing *E. coli* may not have been responsible for the diarrhea. A limited 7-month survey in Canada did not yield *E. coli* O26, O11, O113, or O115 (99). In Korea, Shiga-like toxin-producing *E. coli* were isolated from two (0.9 percent) of 231 children with diarrhea, but from none of 104 controls; both strains were O26:H11 and both children had nonbloody stools (110). In Thailand, none of 40 children with bloody diarrhea in one study (151), and none of 393 children with diarrhea in another study, had Shiga-like toxin-producing *E. coli* in their stools (148). However, five (1.1 percent) of 458 Thai adults with diarrhea had non-O157 Shiga-like toxin-producing *E. coli*; all five had nonbloody stools (58). In Chile, one (0.6 percent) of 154 children with diarrhea, but none of 66 controls, had an *E. coli* that hybridized with the enterohemorrhagic *E. coli* probe and produced Shiga-like toxin; the serotype from the one child was O26:H11, and the clinical syndrome was not reported (9). In Argentina, only one (2.3 percent) of 44 children with diarrhea had a non-O157 *E. coli* isolate that hybridized with a Shiga-like toxin probe (137). However, 11 (25 percent) of the children with diarrhea, but none of 19 controls, had free fecal toxin or seroconversion to Shiga-like toxin, and the authors suggested that non-O157 Shiga-like toxin-producing *E. coli* may be an important cause of diarrhea in that area. Non-O157 Shiga-like toxin-producing *E. coli* have also been isolated from patients with ulcerative colitis, but whether they have a role in this illness or its exacerbations is unknown (152, 153).

HEMOLYTIC UREMIC SYNDROME

Hemolytic uremic syndrome, first described in 1955 in Switzerland (3), is characterized by microangiopathic hemolytic anemia, thrombocytopenia, renal failure, and central nervous system symptoms (154). It can be categorized into hemolytic uremic syndrome with and without a prodrome of diarrhea. Diarrhea-associated hemolytic uremic syndrome includes that associated

TABLE 4. Frequency of isolation of *Escherichia coli* O157:H7 and other bacterial pathogens from stools submitted to microbiology laboratories outside North America, including studies in which at least one *E. coli* O157:H7 strain was isolated, 1985–1988*

Location	Reference	Population	Year	Stools					Bloody stools		Comments
				No.	*Campylobacter* (%)	*Salmonella* (%)	*E. coli* O157:H7 (%)	*Shigella* (%)	No.†	*E. coli* O157:H7 (%)	
United Kingdom	100	Children	April 1985–March 1988						50‡ (48)	6	Public health laboratory
England and Wales§	105	All ages	October 1985–October 1986						89‡ (83)	29	Public health laboratory; also isolated four O157:H-, one O91:H-, and 1 O?:H8 Shiga-like toxin-producing *E. coli*; criteria: frank blood, routine culture negative, no recent travel or antibiotic
United Kingdom§	145	All ages	April 1986–April 1987						65‡ (63)	29	Public health laboratory; also isolated two O157:H-, one O91:H-, and one O?:H8 Shiga-like toxin-producing *E. coli*; criteria same as above
England	48	All ages	June–September 1986	1,319			0.5		80	5	Public health laboratory; did not report H type; bloody stools included those with red cells by microscopy; of the bloody stools, *Campylobacter* 18.8%, *Salmonella* 7.5%, *Shigella* 1.3%
England	146	All ages	1986–1987						40‡	75	Public health laboratory; also, isolated O128 Shiga-like toxin-producing *E. coli*; criterion: bloody diarrhea
Ireland	226	Children	?	894‡			0.1				Did not plate specimens directly onto sorbitol-MacConkey agar
Belgium	130	All ages	April 1987–September 1988	3,940‡	3.4	3.5	0.2	0.3			One laboratory; also isolated three *E. coli* O157:H-
Argentina	137	Children	September 1986–April 1988	44‡	0	0	4.5	6.8			One hospital

*Sorbitol-MacConkey medium, usually in addition to other tests, was used to screen for *E. coli* O157:H7 in all these studies.
† Number in parentheses indicates number with coliform growth.
‡ Number represents number of patients.
§ These studies appear to contain some duplicate data.

with *Shigella dysenteriae* type 1 infection (103, 155) and with Shiga-like toxin-producing *E. coli* infection. Nondiarrheal hemolytic uremic syndrome includes that attributed to *Streptococcus pneumoniae* (156), defects in serum complement (154), chemotherapy (157, 158), and other poorly understood forms of hemolytic uremic syndrome (154, 159–163). Diarrhea-associated hemolytic uremic syndrome is the most common form; in recent large series of cases, the proportion with a diarrheal prodrome was 86 percent in the United States (93), 95 percent in the United Kingdom (94), 95 percent in Canada (84), and 91 percent in Argentina (137).

The occurrence of most cases of hemolytic uremic syndrome after a diarrheal prodrome, and the tendency for cases to occur in clusters in communities and families, led many researchers to postulate an infectious etiology (154, 164, 165). Among the etiologic agents proposed in developed countries were Coxsackie B viruses (166), multiple different viruses (167), and *Campylobacter* sp. (168, 169); however, none of these were evaluated in prospective studies. *E. coli* O157:H7 was suspected as an important cause of hemolytic uremic syndrome after hemolytic uremic syndrome complicated sporadic (81, 82) and outbreak-associated cases of *E. coli* O157:H7 infection (7, 92).

Prospective studies of the association between Shiga-like toxin-producing *E. coli* and hemolytic uremic syndrome

Karmali et al. (115) in Canada became interested in the role of Shiga-like toxin-producing *E. coli* in hemolytic uremic syndrome after isolating a Shiga-like toxin-producing *E. coli* from the bowel of a patient with fatal hemolytic uremic syndrome in 1980 (115). This group conducted the first prospective study examining the association between Shiga-like toxin-producing *E. coli* infection and hemolytic uremic syndrome (53). Among children with hemolytic uremic syndrome following acute diarrhea, 30 (75 percent) had evidence of infection with Shiga-like toxin-producing *E. coli* by culture, free fecal toxin, or a rise in antibody titer to Shiga-like toxin (table 5). Of these 30 children, 12 were culture positive, but *E. coli* O157:H7 was isolated from only three. Six patients were identified only by a four-fold rise in antibody titers to toxin. None of 40 controls had evidence of Shiga-like toxin-producing *E. coli* infection. In a study of 66 children with hemolytic uremic syndrome in the United Kingdom, 22 (33 percent) had evidence of Shiga-like toxin-producing *E. coli* infection by culture or free fecal toxin. *E. coli* O157 was isolated from 15 (23 percent) children, other Shiga-like toxin-producing *E. coli* from four (6 percent) children (57).

In a study of 52 persons with hemolytic uremic syndrome following diarrhea in Seattle, Washington, *E. coli* O157:H7 was isolated from the stools of 33 (63 percent); other Shiga-like toxin-producing *E. coli* were not sought (170). Twenty-four (96 percent) of 25 persons whose stool culture was obtained within 6 days of onset of diarrhea had *E. coli* O157:H7. The most significant difference between patients from whom *E. coli* O157:H7 was isolated and culture-negative patients was that for the former there were fewer mean days between the onset of diarrhea and the stool culture. Other differences were that patients with *E. coli* O157:H7 isolated were more likely to have bloody diarrhea (85 vs. 80 percent), fecal leukocytes (42 vs. 15 percent), or transfusion (85 vs. 53 percent). The largest study, involving 196 children, was conducted in the United Kingdom (100). Only 58 (30 percent) children had stools positive for Shiga-like toxin-producing *E. coli* by culture or free fecal toxin. Thirty-eight (19 percent) children had *E. coli* O157, and 15 (8 percent) children had Shiga-like toxin-producing *E. coli* of other serotypes. In this study, only two of 51 healthy controls had evidence of Shiga-like toxin-producing *E. coli* infection, and neither had *E. coli* O157:H7. However, the proportion of controls who had other Shiga-like toxin-producing *E. coli* isolated (4 percent) was similar to that for hemolytic uremic syndrome patients.

The differences between these prospective

TABLE 5. Major prospective studies of the association between hemolytic uremic syndrome (HUS) and infection with Shiga-like toxin-producing *E. coli* (SLTEC) in North America and Europe

Locatino	Reference	Years	Syndrome	No. of patients*	Total with evidence of SLTEC infection		SLTEC isolated				Free fecal toxin detected			
							E. coli O157:H7		Other SLTEC		Patients from whom SLTEC isolated		Patients from whom SLTEC not isolated	
					No.	%	No.	%	No.	%	No.	%	No.	%
Canada	53	1980–1983	HUS	40†	30‡	75	3	8	9§	23	9 of 9	100	12 of 28	43
			Controls	40¶	0	0	0	0	0	0	0	0	0 of 40	0
United Kingdom	57	1983–1985	HUS	66 (59)	22	33	15‖	23	4	6	10 of 14	71	3 of 42	7
United States	170	1985–1987	HUS	52†	33	63	33	63	ND**		ND		ND	
United Kingdom	100	1985–1988	HUS	196†† (185)	58	30	38‡‡	19	15§§	8	31 of 52	60	6 of 132	5
			Bloody diarrhea	50 (48)	4	8	3	6	0		0 of 48		1 of 48	
			Nonbloody diarrhea	62 (54)	3	5	1	2	0		0 of 54		2 of 54	
			Healthy	51 (46)	2	4	0	0	2	4	0 of 46		0 of 46	

* Number in parentheses indicates number of patients whose specimens had coliform growth.
† By definition, all patients had a prodrome of diarrhea.
‡ Includes six patients with a fourfold rise in antibody titer to toxin as the only evidence of infection.
§ Includes two nontypable strains.
¶ Controls were age-, sex-, and season-matched; 17 had *Campylobacter* enteritis and 23 were healthy.
‖ One was nonmotile.
** ND, not done.
†† Fifty-three of these patients, plus seven others with hemolytic uremic syndrome (nine with *Escherichia coli* O157:H7 isolated) had sera tested for antibodies to the lipopolysaccharide of *E. coli* O157; 44 (73%) had positive titers (63).
‡‡ Three were O157:H-.
§§ One patient had *E. coli* O157:H7 and another Shiga-like toxin-producing *E. coli* cultured.

studies in the proportion of hemolytic uremic syndrome cases associated with *E. coli* O157:H7 can be explained in several ways. It is possible that there are regional and temporal differences in the causes of hemolytic uremic syndrome between Canada, the United Kingdom, and the United States, but differences in specimen collection and laboratory methodology may be more important. The low isolation rate of *E. coli* O157:H7 in the initial Canadian study may be partially attributed to the fact that neither sorbitol-MacConkey medium nor DNA probes for toxin (171) were in use at that time. The data from these investigators from later years suggest that some of their culture-negative patients had *E. coli* O157:H7 (116). The results of the other three major studies suggest that *E. coli* O157:H7 is a more common cause of hemolytic uremic syndrome than are all the other Shiga-like toxin-producing *E. coli* combined.

Two retrospective studies reported high rates of isolation of *E. coli* O157:H7—13 (46 percent) of 28 hemolytic uremic syndrome patients in Minnesota (93) and 87 (51 percent) of 169 patients in Canada (84). In these studies, like the prospective Seattle study, stool cultures were performed locally, which may have decreased the opportunity for compromise of the specimen in transit. The Seattle study suggests that, in that area and during that time period, *E. coli* O157:H7 caused all or almost all hemolytic uremic syndrome that was preceded by diarrhea. The low isolation rate for Shiga-like toxin-producing *E. coli* in the U.K. studies may be because of delay in specimen collection. In the second major U.K study (94), 27 (49 percent) of the 55 patients whose stool specimens were obtained within the first week of diarrhea had Shiga-like toxin-producing *E. coli* isolated, a much higher isolation rate than for the total. Serologic data from this study also suggest that *E. coli* O157:H7 caused many of the culture-negative cases: 53 of these patients, plus seven others with hemolytic uremic syndrome, had sera tested for antibodies to the lipopolysaccharide of *E. coli* O157. Although only nine (15 percent) patients had *E. coli* O157:H7 isolated from stool, 44 (73 percent) had positive antibody titers (63). The low rate of detection of free fecal toxin in culture-negative specimens in the U.K. studies compared with the Canadian study is unexplained.

Role of the non-O157 Shiga-like toxin-producing *E. coli* in hemolytic uremic syndrome

The data suggest that non-O157 Shiga-like toxin-producing *E. coli* are a much less common cause of hemolytic uremic syndrome than *E. coli* O157:H7 in North America and Europe. The low rate of isolation of non-O157 Shiga-like toxin-producing *E. coli* in the U.K. studies compared with the Canadian study is unexplained. The overall isolation rate of Shiga-like toxin-producing *E. coli* was low in the U.K. studies, but the relative proportion of non-O157 Shiga-like toxin-producing *E. coli* was also very low. Certain non-O157 Shiga-like toxin-producing *E. coli* strains, particularly O26:H11 (53, 57, 100, 135), O111:NM (53, 115–118), and O113:H21 (53, 115, 116, 119) appear to be isolated more frequently than others from patients with hemolytic uremic syndrome. This, along with evidence from other studies that they are enteric pathogens (see subsection "Diarrhea associated with non-O157 Shiga-like toxin-producing *E. coli*" above), suggests that they may be important causes of hemolytic uremic syndrome. However, the isolation of both *E. coli* O157:H7 and another Shiga-like toxin-producing *E. coli* (100, 135) from some patients with hemolytic uremic syndrome, and the demonstration of immunoglobulin M antibodies to *E. coli* O157 in a child with a non-O157 Shiga-like toxin-producing *E. coli* in the stool (63), suggest that, in some cases, the isolation of a non-O157 Shiga-like toxin-producing *E. coli* may be a marker for exposure to *E. coli* O157:H7 acquired from the same food. Further studies to determine which Shiga-like toxin-producing *E. coli* are diarrheal pathogens will help to elucidate their role in hemolytic uremic syndrome. Further research on children with hemolytic uremic syndrome in Argentina—on whom

initial studies showed that only one (2 percent) of 51 children had *E. coli* O157:H7, only 8 percent had colonies that hybridized with Shiga-like toxin probes, but 48 percent had free fecal toxin (137)—may provide helpful information. Studies on risk factors for infection with non-O157 Shiga-like toxin-producing *E. coli* are needed.

Other evidence that Shiga-like toxin-producing *E. coli* cause hemolytic uremic syndrome

The summer peak in incidence of cases of hemolytic uremic syndrome is explainable by the parallel summer peak in *E. coli* O157:H7 infections (figure 2). In the United Kingdom in 1983–1984, the 77 reported cases of hemolytic uremic syndrome peaked in July and August (172, 173), and in Canada in 1986–1988, the 226 cases of hemolytic uremic syndrome likewise peaked in these months (figure 3) (84).

The isolation of Shiga-like toxin-producing *E. coli*, along with another pathogen from some stool specimens, dictates caution in interpreting studies in which evidence of Shiga-like toxin-producing *E. coli* infection was not sought. *Blastocystis hominis* and adenovirus were each found in the stool of a patient with hemolytic uremic syndrome from which *E. coli* O157:H7 was also isolated (99). Another patient had varicella infection, *Salmonella* bacteremia, group A beta streptococcal bacteremia, and *E. coli* O157:H7 isolated from the stool, all just before or when hemolytic uremic syndrome developed (Griffin, P. M., Centers for Disease Control, unpublished data). In another study, Shiga-like toxin-producing *E. coli* (serotypes not stated) were isolated concurrently with *Campylobacter jejuni* from one hemolytic uremic syndrome patient, and enteropathogenic *E. coli* from another (94).

Some epidemiologic features of the diarrheal form of hemolytic uremic syndrome that have not been previously explained are likely related to the epidemiologic features of Shiga-like toxin-producing *E. coli* infection. In Canada in 1986–1988, the annual incidence of hemolytic uremic syndrome in Alberta was 2.78 per 100,000 population, almost three times the incidence of 0.97 per 100,000 population in the province of Ontario (84). It is of interest that high rates of contamination of meat by *E. coli* O157:H7 were also found in Alberta (142). Regional

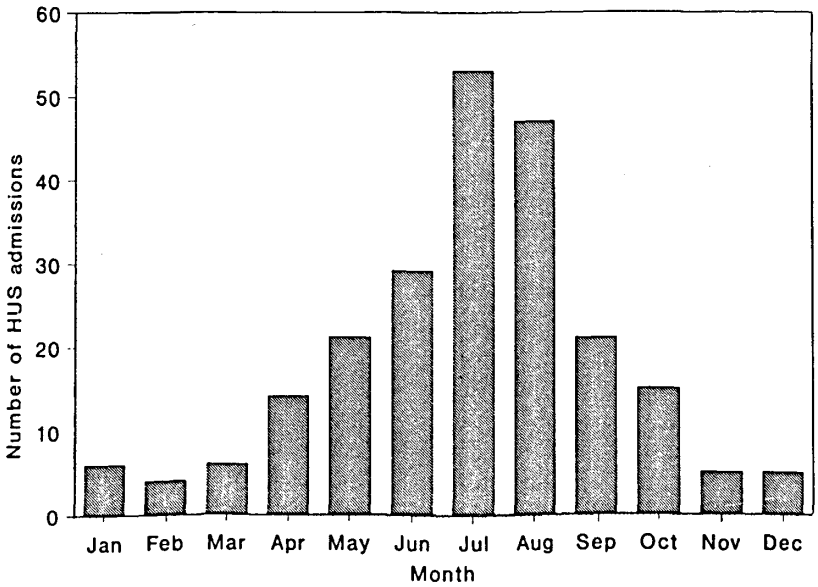

FIGURE 3. The seasonal pattern of hospital admissions for childhood hemolytic uremic syndrome, Canada, 1986–1988. (Courtesy of Dr. P. N. McLaine, Director, Canadian Pediatric Kidney Disease Reference Center.)

variations in incidence (84) of hemolytic uremic syndrome could be due to differences in carriage rates of *E. coli* O157:H7 by cattle, in use of dairy versus beef cattle for ground beef, in slaughter practices, in processing practices for beef, in food preparation practices, or in consumption of undercooked beef. Human population differences in P1 antigen expression by red blood cells could also affect the incidence of hemolytic uremic syndrome (97). Regional differences in the mean age of hemolytic uremic syndrome patients have not been explained but may reflect differences in hygiene or in susceptible age groups. For example, in large series (30 or more cases) of children with hemolytic uremic syndrome before 1970, the mean age was under 2 years in the Netherlands (174), Argentina (164), and southern Africa (175). By the 1970s and 1980s, the mean age had increased to 3.5 years or more for children in the United States (93, 176, 177), Canada (53), and the United Kingdom (178, 179), but was only 1.4 years in Argentina (137). Follow-up studies in the Netherlands and southern Africa, and among populations with different socioeconomic conditions, may help to determine the reasons for the age differences. The increased incidence of hemolytic uremic syndrome in higher socioeconomic groups (84, 164, 175) could be because of greater exposure to Shiga-like toxin-producing *E. coli* among children in lower socioeconomic groups and consequent development of immunity, to a preference for rare meat in higher socioeconomic classes, as documented in Canada (84), or to other factors.

Role of Shiga-like toxin-producing *E. coli* in hemolytic uremic syndrome without a diarrheal prodrome

Hemolytic uremic syndrome not preceded by diarrhea or abdominal pain is unlikely to be caused by Shiga-like toxin-producing *E. coli*. In a study in the United Kingdom, none of seven children with hemolytic uremic syndrome without preceding diarrhea had Shiga-like toxin-producing *E. coli* isolated or free fecal toxin detected in their stools; however, stools were cultured 8 to 21 days after the diagnosis of hemolytic uremic syndrome (94). In another study, *E. coli* O157:H7 was not isolated from stools of two hemolytic uremic syndrome patients without a diarrheal prodrome (75). However, two patients developed hemolytic uremic syndrome or thrombotic thrombocytopenic purpura associated with Shiga-like toxin-producing *E. coli* without a diarrheal prodrome. One man had a prodrome of severe abdominal cramps and hemorrhagic gastroduodenitis; his stool culture grew *E. coli* O157:H7 (180). The other had acquired immunodeficiency syndrome; the prodrome for this patient was vomiting and abdominal pain, and his stool sample showed *E. coli* O26 and free toxin (181). It is not known whether Shiga-like toxin-producing *E. coli* can colonize the bowel and cause hemolytic uremic syndrome without causing any gastrointestinal symptoms.

Evidence that the incidence of hemolytic uremic syndrome is increasing

Several studies suggest that the incidence of hemolytic uremic syndrome increased markedly after it was first reported in the 1950s, and that it is continuing to increase. A global review of all reported pediatric cases of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura up to 1965 concluded that a real increase in the incidence of this disease had occurred in the previous decade (96). A clinic in the Netherlands saw only four cases from 1959 to 1964, but 50 cases from 1965 to 1970 (174). The number of cases of hemolytic uremic syndrome in all the Netherlands increased from seven in 1965 to 82 in 1982 (174); in King County, Washington, the incidence in children less than 15 years of age increased from 0.69 per 100,000 children between 1971 and 1975 to 1.77 per 100,000 between 1976 and 1980 and remained stable (1.74 per 100,000) between 1981 and 1986 (182). This increase was unlikely to be due to ascertainment bias because the severity of patients' illnesses on admission was similar in all periods (182). In Minnesota, the inci-

dence of hemolytic uremic syndrome in children less than 18 years of age increased from 0.5 per 100,000 in 1979 to 2.0 per 100,000 in 1988 (93). The increase was most marked for children less than 5 years of age, from 1.6 to 5.8 cases per 100,000. In the United Kingdom, reports of cases of hemolytic uremic syndrome in children increased from 54 in 1983 to 188 in 1989. Although part, but not all, of this increase was likely due to increased reporting, the rate for the United Kingdom in 1989 was thus at least 1.6 per 100,000 children under 16 years of age (173, 179).

Evidence that hemolytic uremic syndrome was caused by Shiga-like toxin-producing *E. coli* before 1980

Most hemolytic uremic syndrome cases reported before the 1980s, including some of the original cases, had features typical of *E. coli* O157:H7-associated hemolytic uremic syndrome, suggesting that Shiga-like toxin-producing *E. coli* caused hemolytic uremic syndrome in the past. Most patients with this disease had a prodrome of diarrhea, often bloody, in series of cases before 1980 in California (183, 184), London (178), the Netherlands (174, 185), Australia (186), Argentina (164), and southern Africa (175). Most cases occurred in the warmer months in series of cases that occurred before 1980 in Seattle (166), London (178), the Netherlands (174, 185), and southern Africa (175). In one series of cases from Argentina in 1957–1963, the lowest incidence of cases was in the summer (164), but later analysis, including patients up to 1972, demonstrated no clear seasonal incidence (187). In an analysis of hemolytic uremic syndrome cases at one hospital in England, the authors concluded that a new form emerged in 1982. The proportion of patients with bloody diarrhea increased from 37 percent in 1970–1981 to 71 percent in 1982–1987, and predictors for a poor outcome were different for the two periods. However, the proportion of their patients in the earlier period who had bloody diarrhea was much lower than in other early series, suggesting that their patients may not have been typical (164, 174, 184, 185).

Lack of identification of Shiga-like toxin-producing *E. coli* pathogens in early hemolytic uremic syndrome cases most likely had several causes. Hemolytic uremic syndrome was rarer before the 1980s, so there were fewer opportunities to identify pathogens. Although outbreaks of hemolytic uremic syndrome occurred in which patients had symptoms typical of Shiga-like toxin-producing *E. coli* infection, pathogens were not identified in stool cultures, probably mainly because searches for unknown pathogens are very difficult (165, 188). *E. coli* was not generally suspected as the etiologic agent, and proper serotyping, which requires technical expertise and high quality antisera, was not widely available. It is of interest that in 1968 Kibel and Barnard (175) in Rhodesia postulated that hemolytic uremic syndrome is caused by an *E. coli* that acquired a bacteriophage; they noted similarities between hemolytic uremic syndrome and edema disease of pigs, and raised concerns about antimicrobial agents contributing to the pathogenesis of illness.

Perhaps the best evidence that *E. coli* O157:H7 caused hemolytic uremic syndrome before 1980 comes from the Netherlands: sera from six of 16 hemolytic uremic syndrome cases hospitalized at one medical center between 1974 and 1977 contained antibody to the O157 antigen (189).

THROMBOTIC THROMBOCYTOPENIC PURPURA

Thrombotic thrombocytopenic purpura is characterized by microangiopathic hemolytic anemia, thrombocytopenia, neurologic disease, renal disease, and fever (190). The major proposed distinctions between hemolytic uremic syndrome and thrombotic thrombocytopenic purpura include more frequent and severe renal failure in hemolytic uremic syndrome and more frequent and severe neurologic involvement in thrombotic thrombocytopenic purpura, but these do not clearly separate these syndromes (191). However, although most patients with hemolytic uremic syndrome have

preceding diarrhea, diarrhea is not even mentioned as a symptom in major reviews of thrombotic thrombocytopenic purpura cases, and abdominal pain occurred in only 14 percent of patients (190, 192).

E. coli O157:H7 was isolated from stools in five reported sporadic cases of thrombotic thrombocytopenic purpura (26, 180, 193, 194), and three additional cases were reported in association with an outbreak of *E. coli* O157:H7 infections (7). Seven persons had bloody diarrhea preceding thrombotic thrombocytopenic purpura, the eighth had hemorrhagic gastroduodenitis. All were adults, and all had microangiopathic hemolytic anemia and thrombocytopenia. Renal failure was mild, with reported serum creatinine levels of 110–300 μmol/l (1.2–3.4 mg/100 ml); none of the reports stated that the patients underwent dialysis. All except one had fever, usually low-grade. All had neurologic involvement: five had generalized seizures, another had myoconic jerking, aphasia, and confusion, another had dysarthria and dysesthesias, and another had only confusion. Five died. The autopsy of one patient showed diffuse fibrin microthrombi in multiple organs (193), another showed features of thrombotic thrombocytopenic purpura (194), and two others showed no fibrin microthrombi (7).

The patients with thrombotic thrombocytopenic purpura following diarrhea had a disease that was indistinguishable from severe hemolytic uremic syndrome. Further studies are needed to determine the spectrum of *E. coli* O157:H7-associated hemolytic uremic syndrome/thrombotic thrombocytopenic purpura in adults, to determine whether the renal disease is truly milder and the neurologic disease more severe than in children, or whether this represents an artifact of reporting. Further studies of thrombotic thrombocytopenic purpura patients with prodromal diarrhea are needed to determine the proportion with Shiga-like toxin-producing *E. coli* infection. Patients with thrombotic thrombocytopenic purpura following diarrhea should be considered to have the same disease as the diarrheal form of hemolytic uremic syndrome.

ANIMAL RESERVOIRS AND FOOD VEHICLES

Isolations of *E. coli* O157:H7 from cattle

After the first outbreaks of *E. coli* O157:H7 infection were traced to ground beef, a bovine reservoir for this organism was suspected. However, the U.S. Department of Agriculture Animal Laboratories at Ames, Iowa, and the Pennsylvania State University Veterinary Research Laboratory reported no previous identification of *E. coli* O157:H7 from animal sources in the United States (16). Investigators, therefore, cultured specimens from cattle on farms implicated in human infections. Two dairy farm surveys were performed as part of investigations of human infections. In Wisconsin, *E. coli* O157:H7 was isolated from five (5.9 percent) fecal samples from 85 heifers and calves, and from none of 141 adult cows on two dairy farms associated with hemolytic uremic syndrome cases (table 6) (123, 195). A strain from one heifer was indistinguishable from the strain isolated from a child with hemolytic uremic syndrome who had drunk raw milk from that farm. In Canada, *E. coli* O157:H7 was isolated from two calves on a farm where cows supplied raw milk implicated in an outbreak among kindergarten children (69, 196). In Washington State, *E. coli* O157:H7 was isolated from fecal samples from seven (2.2 percent) of 315 heifers and calves, and from none of 224 adult cows on nine dairy farms (195).

Culture surveys of fecal specimens from healthy cattle not linked to outbreaks have also been conducted (table 6). Many of these surveys targeted dairy cattle because much ground beef comes from dairy cows. *E. coli* O157:H7 has been isolated from cattle not associated with human illness in the United States, Canada, England, and Germany (146, 195, 197, 198). The rate of isolation has been 1 percent or less in most surveys, with generally higher rates, up to 5.3 percent, in heifers and calves. The organism was not isolated from healthy cattle in Sri Lanka or Thailand (199, 200). *E. coli* O157:H7 has also been sought in cattle with diarrhea. In 1977, it was isolated from a calf with coli

TABLE 6. Results of testing of fecal samples from cattle for Shiga-like toxin-producing *Escherichia coli*

Location	Reference	Years	No. of animal tested	*E. coli* O157:H7 isolated		Non-O157 Shiga-like toxin-producing *E. coli* isolated		Description of cattle and comments
				No.	%	No.	%	
Cattle possibly associated with human illness								
Wisconsin farms	195	1986	85	5	5.9	ND*		Heifers and calves on two dairy farms associated with two cases of hemolytic uremic syndrome, one case with *E. coli* O157:H7 infection confirmed
			141	0		ND		Adult cows on two dairy farms associated with two cases of hemolytic uremic syndrome, one case with *E. coli* O157:H7 infection confirmed
Washington farms	195	1987	315	7†	2.2	ND		Heifers and calves on nine dairy farms possibly associated with human *E. coli* O157:H7 infections
			224	0		ND		Adult cows on nine dairy farms possibly associated with human *E. coli* O157:H7 infections
Presumably healthy cattle not known to be associated with human illness								
Wisconsin farms	195‡	1986	149	3	2.0	25	16.8	Dairy heifers and calves
			242	1	0.4			Dairy adult cows
			135			12	8.9	Dairy adult cows
Wisconsin stockyard	195‡	1986	19	1	5.3	7	36.8	Dairy heifers and calves
			27	0				Dairy adult cows
			19			1	5.3	Dairy adult cows
Washington packing house	195	1987	27	0		ND		Dairy heifers and calves
Ontario slaughter plant	197	?	200	0		7	3.5	Veal calves
			200	1	0.5	38	19.0	Culled dairy cows
			200	3	1.5	18	9.0	Beef cattle

English abbatoir	146	1987	207	2	1.0	ND		Did not determine H type
Germany	198	?	47	0		8	17.0	Dairy cows
			212	2	0.9	18	8.4	Bulls
Sri Lanka	199	?	54	0		3	5.5	Calves (buffalo excluded)
			40	0		4	10.0	Asymptomatic calves (buffalo excluded) from herds with diarrhea
Thai farms	200	?	66	0		10	15.1	Beef cattle
	200	?	20	0		3	15.0	Dairy cows
Thai holding pens	200	?	10	0		4	40.0	Beef cattle
					Cattle with diarrhea			
United States	203	?	46	0		31	67.4	Calves with attaching and effacing *E. coli* infections. Of 17 isolates serotyped, eight were O111:NM§
Scotland and England	204	?	306	0		9	2.9	Calves; five had O26 or O111
Spain	202	?	78	1	1.3	16	20.5	Calves
Sri Lanka	199	?	78	0		21	26.9	Calves (buffalo excluded)

* ND, not done.
† One strain isolated was nonmotile.
‡ Some data from Wells J, Centers for Disease Control, personal communication, 1991.
§ NM denotes nonmotile strain.

bacillosis in Argentina (201). It was also isolated from one of 78 calves with diarrhea in Spain (202), but not from calves with diarrhea in the United States (203), Scotland and England (204), and Sri Lanka (199). It is not known whether *E. coli* O157:H7 is an animal pathogen.

Isolations of non-O157 Shiga-like toxin-producing *E. coli* from cattle

Non-O157 Shiga-like toxin-producing *E. coli* have been isolated from fecal samples of 3.5 to 40 percent of healthy cattle (table 6). Similar to *E. coli* O157:H7, the isolation rates are generally higher in heifers and calves (195). In Sri Lanka, non-O157 Shiga-like toxin-producing *E. coli* were isolated from 26.9 percent of calves with diarrhea, 10 percent of asymptomatic calves from herds with diarrhea, and 5.5 percent of healthy calves (199). However, the organisms included many serotypes, suggesting that one Shiga-like toxin-producing *E. coli* pathogen alone was not responsible for the diarrhea (205). Non-O157 Shiga-like toxin-producing *E. coli* serotypes that cause diarrhea in humans, including O26:H11 and O111:NM, have been isolated from cattle with diarrhea (121, 204, 206, 207). Attaching-effacing lesions were seen in the colon of an ill calf from which *E. coli* O26:H11 was isolated (208).

Recent studies have suggested that infection with attaching-effacing *E. coli* is an emerging problem in cattle in the United States, and that it is most common in dairy herds (203). In one study, Shiga-like toxin-producing *E. coli* were isolated from 31 (67 percent) of 46 calves with diarrhea whose intestinal specimens showed attaching-effacing *E. coli* (203); eight of the 17 isolates serotyped were *E. coli* O111:NM. Disease characterized by attaching-effacing *E. coli* appears to be more common in dairy than in beef calves, and it has been postulated that this could be due to immunodeficiency caused by inadequate colostral intake, which is more likely to occur in dairy operations (203). A large study of *E. coli* O26:H11 strains found that strains from humans and animals with diarrhea were similar, supporting the probability that animals are the source of human infection (121).

Ecology of Shiga-like toxin-producing *E. coli* in animals

The isolation of *E. coli* O157:H7 and other Shiga-like toxin-producing *E. coli* from many different foods suggests that Shiga-like toxin-producing *E. coli* can colonize many animal species. Other data support this: *E. coli* O157:H7 has been shown to colonize chicken ceca (209), non-O157 Shiga-like toxin-producing *E. coli* have been isolated from buffalo in Sri Lanka (199) and from pigs (210), and attaching-effacing lesions (characteristic of infection with Shiga-like toxin-producing *E. coli* or enteropathogenic *E. coli*) are being increasingly described in animals (210). The fact that human outbreaks have not been traced to foods made from animals other than cattle, with the possible exception of turkeys (74, 211), suggests that colonization of other food animals with Shiga-like toxin-producing *E. coli* pathogens is now low, or that slaughter or processing techniques limit contamination of foods produced from those animals.

It is not known why Shiga-like toxin-producing *E. coli* are more readily isolated from feces of young than adult cattle, yet human illness is associated with meat and dairy products derived from adult cows. This may reflect the ecology of all *E. coli* in cattle, which were isolated more frequently in fecal samples from calves than from adult cows in one study (212). Coliforms were also excreted intermittently and in lower numbers in adult cattle than in calves (212). If *E. coli* are, in fact, relatively uncommon in adult cattle, then isolation of *E. coli* in meat would suggest gross fecal contamination.

The duration of excretion, routes of transmission, farm ecology, pathogenicity, and other aspects of *E. coli* O157:H7 and other Shiga-like toxin-producing *E. coli* in cattle are mostly unknown. In one study, nine cattle fecal cultures obtained 8 to 50 days after an initial culture grew *E. coli* O157:H7 revealed that three of the cattle still had the

same strain (195). Herds in which cattle have been colonized with one strain of *E. coli* O157:H7 have later had other cattle with a different strain of the same organism (195). The isolation of *E. coli* O157:H7 from veal kidneys (see subsection "Isolation of *E. coli* O157:H7 from foods" below) raises the possibility that the organism may colonize or infect sites other than the intestine.

Reports of human *E. coli* O157:H7 infections are more common in Canada and the northern and northwestern United States than in the southern United States (65, 213); if these differences are not due to reporting bias, then regional differences in animal carriage rates or in sources or processing of ground beef could be responsible. Serologic cross-reaction occurs between *Brucella abortus* antigens and the O157 lipopolysaccharide (214). Most cattle herds with laboratory-confirmed brucellosis in the United States are now in the south and south central states, a pattern opposite to that of human *E. coli* O157:H7 infection. There have been many changes in the *Brucella* eradication program that could have affected titers of cross-reacting antibodies in cattle. *Brucella* vaccination rates decreased markedly in the late 1960s, and this contributed to an increase in cattle brucellosis. Therefore, vaccination efforts were increased, and vaccination rates peaked in the late 1980s. Now, with eradication of bovine brucellosis in many northern states, vaccination rates are slowly declining in cattle herds in those states (Gilsdorf, M. J., U. S. Department of Agriculture, personal communication, 1991). Although these opposite patterns in human and cattle illness may be coincidental, it would be interesting to learn if animals vaccinated against *Brucella* are less likely to be colonized with *E. coli* O157.

Isolation of *E. coli* O157:H7 from foods

E. coli O157:H7 has been isolated from samples of foods linked to human illness. The same strain was isolated from ground beef linked to the implicated beef and from humans in the first outbreak of these infections (16); the same strain was also isolated from humans and beef in another outbreak (215). In still another outbreak, ground beef obtained from the implicated supplier contained *E. coli* O157:H7, although the organism was different from the outbreak strain (216). *E. coli* O157:H7 was also isolated from veal chops associated with a family outbreak (85). A raw milk sample from a farm where a child drank raw milk shortly before developing hemolytic uremic syndrome contained an *E. coli* O157:H7 strain different from the patient's isolate (123, 195).

Surveys have confirmed that meat likely to be of dairy farm origin, and other meats, can be contaminated with *E. coli* O157:H7. In 1985 and 1986, *E. coli* O157:H7 was isolated from six (3.7 percent) of 164 ground beef samples, four (2.0 percent) of 205 lamb samples, four (1.5 percent) of 263 chicken and turkey samples, and four (1.5 percent) of 264 pork samples obtained from grocery stores in Wisconsin and Alberta (142). The proportion of positive ground beef samples from Alberta, an area with a high incidence of *E. coli* O157:H7 infections (65) and hemolytic uremic syndrome (84), was particularly high (five of 17, 29 percent). In a national survey of raw meats obtained in the United States between 1987 and 1989, *E. coli* O157 was isolated from seven (0.5 percent) of 1,478 bob veal kidneys, but from only two (0.06 percent) of 3,475 fancy veal kidneys (Okrend, A., U. S. Department of Agriculture, personal communication, 1991). Bob veal usually refers to male calves from dairy herds, whereas fancy veal refers to milk-fed calves raised for veal meat. In this study, two (0.12 percent) of 1,668 raw beef samples, and none of 3,977 chicken backs and necks contained *E. coli* O157. In 1989, *E. coli* O157:H7 was isolated from four (2.4 percent) of 165 ground beef samples from stores and restaurants in Manitoba (217). No *E. coli* O157:H7 was detected in ground beef, ground pork, and chicken samples from a processing plant in Ontario (218), in samples of retail ground meat in Newfoundland, Canada (144), or in beef, chicken, pork, or vegetable samples in Thailand (200).

Isolation of non-O157 Shiga-like toxin-producing *E. coli* from foods

Some studies have determined the frequency of isolation of non-O157 Shiga-like toxin-producing *E. coli* from foods. In the first description of Shiga-like toxin, two strains were isolated from cheese (28). In the study in Manitoba, non-O157 Shiga-like toxin-producing *E. coli* were isolated from five (3 percent) of 165 ground beef samples (217). In the study of the processing plant in Ontario, non-O157 Shiga-like toxin-producing *E. coli* were isolated from 24 (11 percent) of 225 ground beef and nine (4 percent) of 235 ground pork samples (218). In addition, 41 percent of ground beef and 15 percent of ground pork samples had Shiga-like toxin in culture supernatants. In Thailand, non-O157 Shiga-like toxin-producing *E. coli* were isolated from eight (9 percent) of 93 beef samples, one (1 percent) of 107 chicken samples, one (1 percent) of 111 pork samples, and none of 130 vegetable samples (200).

Effect of cooking on coliform counts in beef

In one study, ground beef patties that were cooked "well done" (4 minutes per side at 149°F griddle temperature) demonstrated a reduction in the coliform count of four logs, to less than 1/gram (219). Presumably, cooking techniques that leave the center of the hamburger pink reduce the coliforms by less. Coliform counts in retail raw ground beef are variable, but may be expected to be 1,000/gram or more (219, 220). This means that high initial coliform counts or cooking that results in a rare hamburger are likely to permit the survival of coliforms after cooking.

CONCLUSION

In 1980, *E. coli* were thought to be a vanishing cause of diarrhea in developed countries. Nursery epidemics of enteropathogenic *E. coli* infections were far in the past, and disease caused by enterotoxigenic *E. coli* was diagnosed only in travelers with "turista." With the emergence and recognition of *E. coli* O157:H7, the situation has completely changed.

E. coli O157:H7 is now recognized as a common cause of bloody diarrhea in many areas; milder forms of infection, often with nonbloody diarrhea, are not uncommon. Hemolytic uremic syndrome, previously a mysterious disease, is now recognized as a complication of this infection. *E. coli* O157:H7 appears to be the major cause of the diarrheal form of hemolytic uremic syndrome in developed countries. Thrombotic thrombocytopenic purpura that has a prodrome of diarrhea or abdominal pain can also be caused by *E. coli* O157:H7. Non-O157 Shiga-like toxin-producing *E. coli* have also been detected in patients with nonbloody and bloody diarrhea and hemolytic uremic syndrome. Clinical, epidemiologic, and laboratory studies of these other Shiga-like toxin-producing *E. coli* are needed to determine which are human pathogens. Just as *E. coli* O157:H7 was an uncommon pathogen a decade ago, Shiga-like toxin-producing *E. coli* that are now relatively uncommon may be important in the future.

Many features of *E. coli* O157:H7 infections remain poorly understood. Risk factors for symptomatic infection and for progression to hemolytic uremic syndrome have been determined, but their mechanisms are incompletely explained. The effect of antimicrobial therapy—whether it is beneficial or detrimental—remains to be established. Research into the mechanism by which *E. coli* O157:H7 causes disease has demonstrated that production of attaching-effacing lesions and Shiga-like toxin are important virulence properties. It is now recognized that some other *E. coli* share similar pathogenic mechanisms, and that *E. coli* O157:H7 is the prototype of an entire class of *E. coli*. It is possible that the full pathogenic potential of these *E. coli* is not yet known. Further research using animal models is needed to determine the pathogenesis of diarrhea and hemolytic uremic syndrome, and the effect of treatment modalities. Characterization of the mechanism of adherence, determination

of the role of the toxins, and understanding of the immune response to infection may also aid in developing diagnostic and therapeutic methods.

The diagnosis of *E. coli* O157:H7 infection has been greatly aided by the use of sorbitol-MacConkey medium. Despite the availability of this simple screening technique, many laboratories, concerned about time and cost, do not even perform cultures during the peak season to determine whether this organism is an important cause of illness in their populations. Clinical laboratorians should recognize that failure to diagnose this infection may lead the physician to perform barium enema, colonoscopy, exploratory laparotomy, or hemicolectomy in an expensive and invasive search for other causes (221). In addition, failure to culture stools for *E. coli* O157:H7 may lead to delayed recognition of outbreaks and delayed institution of public health control measures.

However, culturing stool on sorbitol-MacConkey medium has limitations because *E. coli* O157:H7 is present in large numbers only in the first week of illness, and this medium is not useful in screening for other Shiga-like toxin-producing *E. coli*. Improvement and marketing of other diagnostic techniques for stool samples, such as assay for free fecal toxin and use of toxin probes and polymerase chain reaction for toxin, may help in the diagnosis of other Shiga-like toxin-producing *E. coli* infections and of hemolytic uremic syndrome, a late complication of infection. Further development of serologic tests for antibodies to O157 lipopolysaccharide and possibly to Shiga-like toxins will also aid in late diagnosis.

The increase in *E. coli* O157:H7 infections and hemolytic uremic syndrome is an important public health problem. Better surveillance for sporadic cases and outbreaks of *E. coli* O157:H7 and other Shiga-like toxin-producing *E. coli* infections is needed to better define the clinical illness, the populations at risk of infection and of severe illness, and the risks and benefits of treatment methods. Surveillance would also facilitate monitoring of the geographic spread of these organisms and changes in responsible food vehicles.

Studies of the ecology of *E. coli* O157:H7 and other Shiga-like toxin-producing *E. coli* pathogens on dairy and other farms are needed to determine risk factors for carriage of these organisms on farms and in individual animals. Studies of the mechanisms by which meat becomes contaminated with *E. coli* during slaughter and processing, and institution of methods to decrease this contamination, are critically needed. Regulations are needed to require that cooked hamburger patties and other meats be sufficiently precooked to kill pathogens. Finally, there is a need for food service personnel and consumers to be aware that all but the most well-cooked hamburger may still contain viable *E. coli* O157:H, and that consumption of insufficiently cooked ground beef can cause serious illness, especially in children and the elderly.

REFERENCES

1. Riley LW, Remis RS, Helgerson SD, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med 1983;308:681–5.
2. Levine MM, Edelman R. Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhea: epidemiology and pathogenesis. Epidemiol Rev 1984;6:31–51.
3. Gasser C, Gautier E, Steck A, et al. Hamolytisch-uramische syndrome: Bilaterale nierenrindennekrosen bei akuten erworbenen hamolytischen anamien. Schweiz med Wochenschr 1955;85:205–9.
4. Evans AS. Epidemiologic concepts. In: Evans AS, Brachman PS, eds. Bacterial infections of humans: epidemiology and control. 2nd ed. New York: Plenum Medical Book Company, 1991:3–57.
5. Riley LW. The epidemiologic, clinical, and microbiologic features of hemorrhagic colitis. Annu Rev Microbiol 1987;41:383–407.
6. Karmali MA. Infection by verocytotoxin-producing *Escherichia coli*. Clin Microbiol Rev 1989;2:15–38.
7. Griffin PM, Ostroff SM, Tauxe RV, et al. Illnesses associated with *Escherichia coli* O157:H7 infections. A broad clinical spectrum. Ann Intern Med 1988;109:705–12.
8. O'Brien AD, Holmes RK. Shiga-like toxins. Microbiol Rev 1987;51:206–20.
9. Levine MM, Xu J, Kaper JB, et al. A DNA probe to identify enterohemorrhagic *Escherichia coli* of 0157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. J Infect Dis 1987;156:175–82.
10. Tzipori S, Wachsmuth IK, Smithers J, et al. Stud-

ies in gnotobiotic piglets on non-O157:H7 *Escherichia coli* serotypes isolated from patients with hemorrhagic colitis. Gastroenterology 1988;94: 590–7.
11. Farmer JJ, Davis BR. H7 antiserum-sorbitol fermentation medium: a single tube screening medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J Clin Microbiol 1985;22:620–5.
12. Thompson JS, Hodge DS, Borczyk AA. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157. J Clin Microbiol 1990;28:2165–8.
13. Raghubeer EV, Matches JR. Temperature range for growth of *Escherichia coli* serotype O157:H7 and selected coliforms in *E. coli* medium. J Clin Microbiol 1990;28:803–5.
14. Ratnam S, March SB, Ahmed R, et al. Characterization of *Escherichia coli* serotype O157:H7. J Clin Microbiol 1988;26:2006–12.
15. Swerdlow DL, Woodruff BA, Brady RC, et al. A large waterborne outbreak of antimicrobial-resistant *E. coli* O157:H7 infections. Program and Abstracts of the Thirtieth Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, GA, 1990:239.
16. Wells JG, Davis BR, Wachsmuth IK, et al. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J Clin Microbiol 1983;18:512–20.
17. Tzipori S, Wachsmuth IK, Chapman C, et al. The pathogenesis of hemorrhagic colitis caused by *Escherichia coli* O157:H7 in gnotobiotic piglets. J Infect Dis 1986;154:712–16.
18. Toth I, Cohen ML, Rumschlag HS, et al. Influence of the 60-megadalton plasmid on adherence of *Escherichia coli* O157:H7 and genetic derivatives. Infect Immun 1990;58:1223–31.
19. Knutton S, Baldwin T, Williams PH, et al. Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. Infect Immun 1989;57:1290–8.
20. Tzipori S, Gibson R, Montanaro J. Nature and distribution of mucosal lesions associated with enteropathogenic and enterohemorrhagic *Escherichia coli* in piglets and the role of plasmid-mediated factors. Infect Immun 1989;57:1142–50.
21. Griffin PM, Olmstead LC, Petras RE. *Escherichia coli* O157:H7-associated colitis. A clinical and histological study of 11 cases. Gastroenterology 1990;99:142–9.
22. Kelly J, Oryshak A, Wenetsek M, et al. The colonic pathology of *Escherichia coli* O157:H7 infection. Am J Surg Pathol 1990;14:87–92.
23. Kelly JK, Pai CH, Jadusingh IH, et al. The histopathology of rectosigmoid biopsies from adults with bloody diarrhea due to verotoxin-producing *Escherichia coli*. Am J Clin Pathol 1987;88:78–82.
24. Hunt CM, Harvey JA, Youngs ER, et al. Clinical and pathological variability of infection by enterohaemorrhagic (Vero cytotoxin producing) *Escherichia coli*. J Clin Pathol 1989;42:847–52.
25. Richardson SE, Karmali MA, Becker LE, et al. The histopathology of the hemolytic uremic syndrome associated with verocytotoxin-producing *Escherichia coli* infections. Hum Pathol 1988;19:1102–8.
26. Morrison DM, Tyrrell DLJ, Jewell LD. Colonic biopsy in verotoxin-induced hemorrhagic colitis and thrombotic thrombocytopenic purpura (TTP). Am J Clin Pathol 1986;86:108–12.
27. Eidus LB, Guindi M, Drouin J, et al. Colitis caused by *Escherichia coli* O157:H7: a study of six cases. Can J Gastroenterol 1990;4:141–6.
28. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun 1977;18:775–9.
29. Johnson WM, Lior H, Bezanson GS. Cytotoxic *Escherichia coli* O157:H7 associated with haemorrhagic colitis in Canada. (Letter). Lancet 1983;1:76.
30. O'Brien AD, Lively TA, Chen ME, et al. *Escherichia coli* O157:H7 strains associated with haemorrhagic colitis in the United States produce a *Shigella dysenteriae* 1 (Shiga) like cytotoxin. (Letter). Lancet 1983;1:702.
31. Strockbine NA, Marques LRM, Newland JW, et al. Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. Infect Immun 1986;53:135–40.
32. Scotland SM, Smith HR, Rowe B. Two distinct toxins active on Vero cells from *Escherichia coli* O157. (Letter). Lancet 1985;2:885–6.
33. Surewicz WK, Surewicz K, Mantsch HH, et al. Interaction of *Shigella* toxin with globotriaosyl ceramide receptor-containing membranes: a fluorescence study. Biochem Biophys Res Commun 1989;160:126–32.
34. Lingwood CA, Law H, Richardson S, et al. Glycolipid binding of purified and recombinant *Escherichia coli* produced verotoxin in vitro. J Biol Chem 1987;262:8834–9.
35. Waddell T, Head S, Petric M, et al. Globotriosyl ceramide is specifically recognized by the *Escherichia coli* verocytotoxin 2. Biochem Biophys Res Commun 1988;152:674–9.
36. Jackson MP, Neill RJ, O'Brien AD, et al. Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from *Escherichia coli* 933. FEMS Microbiol Lett 1987:44:109–14.
37. Head SC, Karmali MA, Roscoe ME, et al. Serological differences between verocytotoxin 2 and Shiga-like toxin II. (Letter). Lancet 1988;2:751.
38. Schmitt CK, McKee ML, O'Brien AD. Two copies of Shiga-like toxin II-related genes common in enterohemorrhagic *Escherichia coli* strains are responsible for the antigenic heterogeneity of the O157:H- strain E32511. Infect Immun 1991;59: 1065–73.
39. Weinstein DL, Jackson MP, Samuel JE, et al. Cloning and sequencing of a Shiga-like toxin type II variant from an *Escherichia coli* strain responsible for edema disease of swine. J Bacteriol 1988;170:4223–30.
40. Endo Y, Tsurugi K, Yutsudo T, et al. Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosomes. Eur J Biochem 1988;171:45–50.
41. Hovde CJ, Calderwood SB, Mekalanos JJ, et al. Evidence that glutamic acid 167 is an active-site

residue of Shiga-like toxin I. Proc Natl Acad Sci U S A 1988;85:2568–72.
42. Boyd B, Lingwood C. Verotoxin receptor glycolipid in human renal tissue. Nephron 1989;51: 207–10.
43. Whittam TS, Wachsmuth IK, Wilson RA. Genetic evidence of clonal descent of *Escherichia coli* O157:H7 associated with hemorrhagic colitis and hemolytic uremic syndrome. J Infect Dis 1988;157:1124–33.
44. Scotland SM, Willshaw GA, Smith HR, et al. Properties of strains of *Escherichia coli* belonging to serogroup O157 with special reference to production of Vero cytotoxins VT1 and VT2. Epidemiol Infect 1987;99:613–24.
45. Ostroff SM, Neill MA, Lewis JH, et al. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J Infect Dis 1989;160:994–8.
46. Khakharia R, Duck D, Lior H. Extended phagetyping scheme for *Escherichia coli* O157:H7. Epidemiol Infect 1990;105:511–20.
47. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J Clin Microbiol 1986;23:869–72.
48. Walker CW, Upson R, Warren RE. Haemorrhagic colitis: detection of verotoxin producing *Escherichia coli* O157 in a clinical microbiology laboratory. J Clin Pathol 1988;41:80–4.
49. Chapman PA. Evaluation of commercial latex slide test for identifying *Escherichia coli* O157. J Clin Pathol 1989;42:1109–10.
50. Farmer JJ III, Griffin PM, Wells JG, et al. Diarrheal stool that yield no apparent pathogen: *Escherichia coli* O157:H7. Microbiology 1988;31:1–6. (American Society of Clinical Pathologists: Check Sample Continuing Education Program, Microbiology no. MB 88-5 (MB-176)).
51. Borczyk AA, Lior H, Thompson S. Sorbitol-negative *Escherichia coli* O157 other than H7. J Infect 1989;18:198–9.
52. Lior H, Borczyk AA. False positive identifications of *Escherichia coli* O157. (Letter). Lancet 1987; 1:333.
53. Karmali MA, Petric M, Lim C, et al. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J Infect Dis 1985;151:775–82.
54. Karmali MA. Laboratory diagnosis of verotoxin-producing *Escherichia coli* infections. Clin Microbiol Newsletter 1987;9:65–70.
55. Downes FP, Green JH, Greene K, et al. Development and evaluation of enzyme-linked immunosorbent assays for detection of Shiga-like toxin I and Shiga-like toxin II. J Clin Microbiol 1989;27:1292–7.
56. Ashkenazi S, Cleary TG. Rapid method to detect Shiga toxin and Shiga-like toxin I based on binding to globotriosyl ceramide (Gb3), their natural receptor. J Clin Microbiol 1989;27:1145–50.
57. Scotland SM, Rowe B, Smith HR, et al. Vero cytotoxin-producing strains of *Escherichia coli* from children with haemolytic uraemic syndrome and their detection by specific DNA probes. J Med Microbiol 1988;25:237–43.
58. Bettelheim KA, Brown JE, Lolekha S, et al. Serotypes of *Escherichia coli* that hybridized with DNA probes for genes encoding Shiga-like toxin I, Shiga-like toxin II, and serogroup O157 enterohemorrhagic *E. coli* fimbriae isolated from adults with diarrhea in Thailand. J Clin Microbiol 1990;28:293–5.
59. Brown JE, Echeverria P, Taylor DN, et al. Determination by DNA hybridization of Shiga-like-toxin-producing *Escherichia coli* in children with diarrhea in Thailand. J Clin Microbiol 1989; 27:291–4.
60. Pollard DR, Johnson WM, Lior H, et al. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J Clin Microbiol 1989;28:540–5.
61. Strockbine N, Popovic-Uroic T, Greene K, et al. Amplification of genes for Shiga-like toxins I and II from *Escherichia coli* in stool specimens by the polymerase chain reaction. Abstracts of the 91st General Meeting of the American Society for Microbiology, Dallas, TX, 1991:84.
62. Chart H, Scotland SM, Rowe B. Serum antibodies to *Escherichia coli* serotype O157:H7 in patients with hemolytic uremic syndrome. J Clin Microbiol 1989;27:285–90.
63. Chart H, Smith HR, Scotland SM, et al. Serological identification of *Escherichia coli* O157:H7 infection in haemolytic uraemic syndrome. Lancet 1991;337:138–40.
64. Barrett TJ, Green JH, Griffin PM, et al. Enzyme-linked immunosorbent assays for detecting antibodies to Shiga-like toxin I, Shiga-like toxin II, and *Escherichia coli* O157:H7 lipopolysaccharide in human serum. Curr Microbiol 1991;23:189–95.
65. Pai CH, Ahmed N, Lior H, et al. Epidemiology of sporadic diarrhea due to serocytotoxin-producing *Escherichia coli*: a two-year prospective study. J Infect Dis 1988;157:1054–7.
66. Ostroff SM, Kobayashi JM, Lewis JH. Infections with *Escherichia coli* O157:H7 in Washington State. The first year of statewide disease surveillance. JAMA 1989;262:355–9.
67. Spika JS, Parsons JE, Nordenberg D, et al. Hemolytic uremic syndrome and diarrhea associated with *Escherichia coli* O157:H7 in a day care center. J Pediatr 1986;109:287–91.
68. Stewart PJ, Desormeaux W, Chene J, et al. Hemorrhagic colitis in a home for the aged—Ontario. Can Dis Wkly Rep 1983;9–8:29–32.
69. Duncan L, Mai V, Carter A, et al. Outbreak of gastrointestinal disease—Ontario. Can Dis Wkly Rep 1987;13-2:5–8.
70. Belongia EA, MacDonald KL, Blaser MJ, et al. Outbreak of *Escherichia coli* O157:H7 in a day care center: patterns of transmission and control methods. Program and Abstracts of the Twenty-Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX, 1989:102.
71. Ryan CA, Tauxe RV, Hosek GW, et al. *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical, epidemiological, and pathological findings. J Infect Dis 1986;154:631–8.
72. MacDonald KL, O'Leary MJ, Cohen ML, et al. *Escherichia coli* O157:H7, an emerging gastrointestinal pathogen. Results of a one-year, prospective, population-based study. JAMA 1988;259: 3567–70.

73. Radetsky M. Laboratory evaluation of acute diarrhea. Pediatr Infect Dis J 1986;5:230–8.
74. Carter AO, Borczyk AA, Carlson JAK, et al. A severe outbreak of *Escherichia coli* O157:H7-associated hemorrhagic colitis in a nursing home. N Engl J Med 1987;317:1496–500.
75. Neill MA, Tarr PL, Clausen CR, et al. *Escherichia coli* O157:H7 as the predominant pathogen associated with the hemolytic uremic syndrome: a prospective study in the Pacific Northwest. Pediatrics 1987;80:37–40.
76. Gransden WR, Damm MAS, Anderson JD, et al. Haemorrhagic cystitis and balanitis associated with verotoxin-producing *Escherichia coli* O157:H7. (Letter). Lancet 1985;2:150.
77. Harris AA, Kaplan RL, Goodman LJ, et al. Results of a screening method used in a 12-month stool survey for *Escherichia coli* O157:H7. J Infect Dis 1985;152:775–7.
78. Krishnan C, Fitzgerald VA, Dakin SJ, et al. Laboratory investigation of outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7. J Clin Microbiol 1987;25:1043–7.
79. Bryant HE, Athar MA, Pai CH. Risk factors for *Escherichia coli* O157:H7 infection in an urban community. J Infect Dis 1989;160:858–64.
80. Karmali MA, Arbus GS, Ish-Shalom N, et al. A family outbreak of hemolytic-uremic syndrome associated with verotoxin-producing *Escherichia coli* O157:H7. Pediatr Nephrol 1988;2:409–14.
81. Remis RS, MacDonald KL, Riley LW, et al. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. Ann Intern Med 1984;101:624–6.
82. Pai CH, Gordon R, Sims HV, et al. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. Clinical, epidemiologic, and bacteriologic features. Ann Intern Med 1984;101:738–42.
83. Rowe PC, Walop W, Lior H, et al. Hemolytic anemia after childhood *Escherichia coli* O157:H7 infection: are females at increased risk? Epidemiol Infect 1991;106:523–30.
84. Rowe PC, Orrbine E, Wells GA, et al. Epidemiology of hemolytic-uremic syndrome in Canadian children from 1986 to 1988. J Pediatr 1991; 119:218–24.
85. Lior H. Incidence of hemorrhagic colitis due to *Escherichia coli* in Canada. Can Med Assoc J 1988;139:1073–4.
86. Dolislager D, Tune B. The hemolytic-uremic syndrome. Am J Dis Child 1978;132:55–8.
87. Neill MA, Tarr PI, Christie DL, et al. Clinical findings in hemorrhagic colitis with *Escherichia coli* O157:H7. (Abstract). Gastroenterology 1987; 92:1551.
88. Lopez EL, Devoto S, Woloj M, et al. Intussusception associated with *Escherichia coli* O157:H7. Pediatr Dis J 1989;8:471–3.
89. Vickers D, Morris K, Coulthard MG, et al. Anal signs in haemolytic uraemic syndrome. (Letter). Lancet 1988;1:998.
90. Neill MA, Agosti J, Rosen H. Hemorrhagic colitis with *Escherichia coli* O157:H7 preceding adult hemolytic uremic syndrome. Arch Intern Med 1985;45:2215–17.
91. Honish A. Summary of *E. coli* O157:H7 at an Edmonton extended care facility. Alberta Soc Serv Community Health 1986;10:363–8.
92. Pavia AT, Nichols CR, Green DP, et al. Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. J Pediatr 1990;116:544–51.
93. Martin DL, MacDonald KL, White KE, et al. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. N Engl J Med 1990;323:1161–7.
94. Milford DV, Taylor CM, Guttridge B, et al. Haemolytic uraemic syndromes in the British Isles 1985–8: association with verocytotoxin producing *Escherichia coli*. Part 1: Clinical and epidemiological aspects. Arch Dis Child 1990;65:716–21.
95. Cimolai N, Carter JE, Morrison BJ, et al. Risk factors for the progression of *Escherichia coli* O157:H7 enteritis to hemolytic-uremic syndrome. J Pediatr 1990;116:589–92.
96. Cimolai N, Carter JE. Gender and the progression of *Escherichia coli* O157:H7 enteritis to haemolytic uraemic syndrome. (Letter). Arch Dis Child 1991;66:171–2.
97. Rose PE, Clark AJB. Haematology of the haemolytic uraemic syndrome. Blood 1989;3:136–40.
98. Taylor CM, Milford DV, Rose PE, et al. The expression of blood group P1 in post-enteropathic haemolytic uraemic syndrome. Pediatr Nephrol 1990;4:59–61.
99. Gransden WR, Damm MAS, Anderson JD, et al. Further evidence associating hemolytic uremic syndrome with infection by verotoxin-producing *Escherichia coli* O157:H7. J Infect Dis 1986; 154:522–4.
100. Kleanthous H, Smith HR, Scotland SM, et al. Haemolytic uraemic syndromes in the British Isles, 1985–8: association with verocytotoxin producing *Escherichia coli*. Part 2: Microbiological aspects. Arch Dis Child 1990;65:722–7.
101. Ostroff SM, Kobayashi JM, Lewis JH. Epidemiology and complications of *Escherichia coli* O157:H7 infections. (Letter). JAMA 1989;262: 3408.
102. DuPont HL, Hornick RB. Adverse effect of Lomotil therapy in shigellosis. JAMA 1973;226: 1525–8.
103. Butler T, Islam MR, Azad MAK, et al. Risk factors for development of hemolytic uremic syndrome during shigellosis. Pediatrics 1987;110: 894–7.
104. Tarr PI, Neill MA, Christie DL, et al. *Escherichia coli* O157:H7 hemorrhagic colitis. (Letter). N Engl J Med 1988;318:1697.
105. Smith HR, Rowe B, Gross RJ, et al. Haemorrhagic colitis and verocytotoxin-producing *Escherichia coli* in England and Wales. Lancet 1987;1:1062–4.
106. Karch H, Strockbine NA, O'Brien AD. Growth of *Escherichia coli* in the presence of trimethoprim-sulfamethoxazole facilitates detection of Shiga-like toxin producing strains by colony blot assay. FEMS Microbiol Lett 1986;35:141–5.
107. Karch H, Goroncy-Bermes P, Opferkuch W, et al. Subinhibitory concentrations of antibiotics modulate amount of Shiga-like toxin produced by *Escherichia coli*. In: Adam D, Hahn H, Opferkuch W, eds. The influence of antibiotics on the host-parasite relationship II. Berlin, Germany:

Springer-Verlag 1985:239–45.
108. Robson WLM, Scott RB, Fick GH. Influence of antidiarrheal and antimicrobial medications on the hemorrhagic colitis associated with hemolytic-uremic syndrome. J Pediatr 1990;117:675–6.
109. Bopp CA, Greene KD, Downes FP, et al. Unusual verotoxin-producing *Escherichia coli* associated with hemorrhagic colitis. J Clin Microbiol 1987;25:1486–9.
110. Kim K-H, Suh I-S, Kim JM, et al. Etiology of childhood diarrhea in Korea. J Clin Microbiol 1989;27:1192–6.
111. Scotland SM, Day NP, Rowe B. Production of a cytotoxin affecting Vero cells by strains of *Escherichia coli* belonging to traditional enteropathogenic serogroups. FEMS Microbiol Lett 1980; 7:15–17.
112. Smith HR, Scotland SM, Chart H, et al. Vero cytotoxin production and presence of VT genes in strains of *Escherichia coli* and *Shigella*. FEMS Microbiol Lett 1987;42:173–7.
113. Gunzburg ST, Burke V, Bettelheim KA. HEp-2 cell adherence and Vero cell cytotoxin production by EPEC strains isolated from children with diarrhea in New Zealand. FEMS Microbiol Lett 1990;69:181–5.
114. Dorn CR, Scotland SM, Smith HR, et al. Properties of Vero cytotoxin-producing *Escherichia coli* of human and animal origin belonging to serotypes other than O157:H7. Epidemiol Infect 1989;103:83–95.
115. Karmali MA, Steele BT, Petric M, et al. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet 1983;1:619–20.
116. Ish-Shalom N, Arbus GS, Karmali MA, et al. Clinical and prognostic features of verotoxin (VT)-producing *E. coli* (VTEC)-associated hemolytic uremic syndrome (HUS). (Abstract). An International Symposium and Workshop on Verocytotoxin (Shiga-like toxin)-producing *Escherichia coli* (VTEC) infections, Toronto, Ontario, Canada, 1987. (Abstract no. HUS-11).
117. Pryor W, Hodson E, McIntyre P, et al. Toxigenic *Escherichia coli* in haemolytic-uraemic syndrome. Med J Aust 1990;152:221–2.
118. Stenger KO, Windler F, Karch H, et al. Hemolytic-uremic syndrome associated with an infection by verotoxin producing *Escherichia coli* O111 in a woman on oral contraceptives. Clin Nephrol 1988;29:153–8.
119. Johnson WM, Lior H. The verotoxin produced by clinical isolates of *Escherichia coli* O113 displays antigenic heterogeneity. FEMS Microbiol Lett 1987;43:15–17.
120. Robins-Browne RM. Traditional enteropathogenic *Escherichia coli* of infantile diarrhea. Rev Infect Dis 1987;9:28–53.
121. Scotland SM, Willshaw GA, Smith HR, et al. Properties of strains of *Escherichia coli* O26:H11 in relation to their enteropathogenic or enterohemorrhagic classification. J Infect Dis 1990; 162:1069–74.
122. Itoh T, Kai A, Saito K, et al. Epidemiological and laboratory investigation on an outbreak of acute enteritis associated with cytotoxin-producing *Escherichia coli* O145:H-. Ann Rep Tokyo Metropolitan Res Lab Public Health 1985;36:16–22.
123. Martin ML, Shipman LD, Wells JG, et al. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome. (Letter). Lancet 1986;2:1043.
124. McGowan KL, Wickersham E, Strockbine NA. *Escherichia coli* O157:H7 from water. (Letter). Lancet 1989;1:967–8.
125. Karmali MA, Arbus GS, Petric M, et al. Hospital-acquired *Escherichia coli* O157:H7 associated haemolytic uraemic syndrome in a nurse. (Letter). Lancet 1988;1:526.
126. Miller WT, De Poto DW, Scholl HW, et al. Evanescent colitis in the young adult: a new entity? Radiology 1971;100:71–8.
127. Clark AW, Lloyd-Mostyn RH, Sadler MR de C. "Ischaemic" colitis in young adults. BMJ 1972;4:70–2.
128. Day NP, Scotland SM, Cheasty T, et al. *Escherichia coli* O157:H7 associated with human infections in the United Kingdom. (Letter). Lancet 1983;1:825.
129. Meyers JA, Sanchez D, Elwell LP, et al. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J Bacteriol 1976;127:1529–37.
130. Pierard D, Van Etterijck R, Breynaert J, et al. Results of screening for verocytotoxin-producing *Escherichia coli* in faeces in Belgium. Eur J Clin Microbiol Infect Dis 1990;9:198–200.
131. Robaeys G, Surmont I, Lemmens P, et al. Haemorrhagic colitis and verotoxin-producing *Escherichia coli* O157 in Belgium. (Letter). Lancet 1987;1:1495–6.
132. Karch H, Wib R, Gloning H, et al. Hamolytisch-uramisches syndrom bei kleinkindern durch verotoxin-produzierende *Escherichia coli*. Dtsch Med Wochenschr 1990;115:489–95.
133. Wuthe HH. *Escherichia coli* O157:H7 bei hemorrhagischer colitis. (Letter). Dtsch Med Wochenschr 1987;112:1761–2.
134. Caprioli A, Edefonti A, Bacchini M, et al. Isolation in Italy of a verotoxin-producing strain of *Escherichia coli* O157:H7 from a child with hemolytic-uremic syndrome. Eur J Epidemiol 1990;6:102–4.
135. Sramkova L, Bielaszewska M, Janda J, et al. Vero cytotoxin-producing strains of *Escherichia coli* in children with haemolytic uraemic syndrome and diarrhoea in Czechoslovakia. Infection 1990; 18:204–9.
136. Bielaszewska M, Sramkova L, Janda J, et al. Verotoxigenic (enterohaemorrhagic) *Escherichia coli* in infants and toddlers in Czechoslovakia. Infection 1990;18:352–6.
137. Lopez EL, Diaz M, Grinstein S, et al. Hemolytic uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. J Infect Dis 1989;160:469–75.
138. Rivas ME, Voyer LE, Tous MI, et al. Detection de *Escherichia coli* O157 productor de verotoxina en pacientes con sindrome uremico hemolitico. (Letter). Medicina (B Aires) 1990;50:571.
139. Kobayashi K, Harada K. Enterohemorrhagic colitis associated with *Escherichia coli* O157:H7 in Osako prefecture. In: Saito M, Nakaya R, Matsubara Y, eds. Infectious enteritis in Japan. Tokyo, Japan: Saikon Shuppan, Showa, 1986:349–57.

140. Kain KC, Barteluk RL, Kelly MT, et al. Etiology of childhood diarrhea in Beijing, China. J Clin Microbiol 1991;29:90–5.
140a. Browning NG, Botha JR, Sacho H, et al. *Escherichia coli* 0157:H7 haemorrhagic colitis. Report of the first South African case. S Afr J Surg 1990;28:28-9.
141. Cahoon FE, Thompson JS. Frequency of *Escherichia coli* O157:H7 isolation from stool specimens. Can J Microbiol 1987;33:914–15.
142. Doyle MP, Schoeni JL. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl Environ Microbiol 1987;53:2394–6.
143. Marshall WF, McLimans CA, Van Scoy RE, et al. Results of a 6-month survey of stool cultures for *Escherichia coli* O157:H7. Mayo Clin Proc 1990;65:787–92.
144. Ratnam S, March SB. Sporadic occurrence of hemorrhagic colitis associated with *Escherichia coli* O157:H7 in Newfoundland. Can Med Assoc J 1986;134:43–6.
145. Kleanthous H, Fry NK, Smith HR, et al. The use of sorbitol-MacConkey agar in conjunction with a specific antiserum for the detection of Vero cytotoxin-producing strains of *Escherichia coli* O157. Epidemiol Infect 1988;101:327–35.
146. Chapman PA, Wright DJ, Norman P. Verotoxin-producing *Escherichia coli* infections in Sheffield: cattle as a possible source. Epidemiol Infect 1989;102:439–45.
147. Albert MJ, Bettelheim KA. Enterohaemorrhagic *E. coli* O157:H7 in central Australia. (Letter). J Diarrhoeal Dis Res 1989;7:96–7.
148. Sunthadvanich R, Chiewsilp D, Seriwatana J, et al. Nationwide surveillance program to identify diarrhea-causing *Escherichia coli* in children in Thailand. J Clin Microbiol 1990;28:469–72.
149. Giraldi R, Guth BEC, Trabulsi LR. Production of Shiga-like toxin among *Escherichia coli* strains and other bacteria isolated from diarrhea in Sao Paulo, Brazil. J Clin Microbiol 1990;28:1460–2.
150. Bhan MK, Raj P, Levine MM, et al. Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. J Infect Dis 1989;159:1061–4.
151. Seriwatana J, Brown JE, Echeverria P, et al. DNA probes to identify Shiga-like toxin I- and II-producing enteric bacterial pathogens isolated from patients with diarrhea in Thailand. J Clin Microbiol 1988;26:1614–15.
152. Ljungh A, Eriksson M, Eriksson O, et al. Shiga-like toxin production and connective tissue protein binding of *Escherichia coli* isolated from a patient with ulcerative colitis. Scand J Infect Dis 1988;20:443–6.
153. von Wulffen H, Russmann H, Karch H, et al. Verocytotoxin-producing *Escherichia coli* O2:H5 isolated from patients with ulcerative colitis. (Letter). Lancet 1989;1:1449–50.
154. Kaplan BS, Proesmans W. The hemolytic uremic syndrome of childhood and its variants. Semin Hematol 1987;24:148–60.
155. Raghupathy P, Date A, Shastry JCM, et al. Haemolytic-uraemic syndrome complicating *Shigella* dysentery in south Indian children. BMJ 1978;1:1518–21.
156. Alon U, Adler SP, Chan JCM. Hemolytic-uremic syndrome associated with *Streptococcus pneumoniae*. Am J Dis Child 1984;138:496–9.
157. Gradishar WJ, Vokes EE, Ni K, et al. Chemotherapy-related hemolytic-uremic syndrome after the treatment of head and neck cancer. A case report. Cancer 1990;66:1914–18.
158. Giroux L, Smeesters C, Corman J, et al. Hemolytic uremic syndrome in renal allografted patients treated with cyclosporin. Can J Physiol Pharmacol 1987;65:1125–31.
159. Kaplan BS. Hemolytic uremic syndrome with recurrent episodes: an important subset. Clin Nephrol 1977;8:495–8.
160. Brown CB, Clarkson AR, Robson JS, et al. Haemolytic uraemic syndrome in women taking oral contraceptives. Lancet 1973;1:1479–81.
161. Lazebnik N, Jaffa AJ, Peyeser MR. Hemolytic-uremic syndrome in pregnancy. Review of the literature and report of a case. Obstet Gynecol 1985;40:618–21.
162. Kaplan BS, Chesney RW, Drummond KN. Hemolytic uremic syndrome in families. N Engl J Med 1975;292:1090–3.
163. Hogewind BL, de la Riviere GB, van Es LA, et al. Familial occurrence of the haemolytic uraemic syndrome. Acta Med Scand 1980;207:73–7.
164. Gianantonio C, Vitacco M, Mendilaharzu F, et al. The hemolytic-uremic syndrome. J Pediatr 1964;64:478–91.
165. McLean MM, Jones CH, Sutherland DA. Haemolytic-uraemic syndrome. A report of an outbreak. Arch Dis Child 1966;41:76–81.
166. Austin TW, Ray CG. Coxsackie virus group B infections and the hemolytic-uremic syndrome. J Infect Dis 1971;127:698–701.
167. Larke RPB, Preiksaitis JK, Devine RD, et al. Haemolytic uraemic syndrome: evidence for multiple viral infections in a cluster of ten cases. J Med Virol 1983;12:51–9.
168. Chamovitz BN, Hartstein AI, Alexander SR, et al. *Campylobacter jejuni*-associated hemolytic-uremic syndrome in a mother and daughter. Pediatrics 1983;71:253–6.
169. Delans RJ, Biuso JD, Saba SR, et al. Hemolytic uremic syndrome after *Campylobacter*-induced diarrhea in an adult. Arch Intern Med 1984; 144:1074–6.
170. Tarr PI, Neill MA, Clausen CR, et al. *Escherichia coli* O157:H7 and the hemolytic uremic syndrome: importance of early cultures in establishing the etiology. J Infect Dis 1990;162:553–6.
171. Newland JW, Neill RJ. DNA probes for Shiga-like toxins I and II and for toxin-converting bacteriophages. J Clin Microbiol 1988;26:1292–7.
172. Hall SM. BPA-CDSC surveillance of hemolytic uraemic syndrome 1983–84. Commun Dis Rep Wkly Edition 1985;85/28:3–6.
173. PHLS Communicable Disease Surveillance Center. Haemolytic uraemic syndrome surveillance. British paediatric surveillance unit/CDSC surveillance scheme. Commun Dis Rep Wkly Edition 1990;90/21:1.
174. Van Wieringen PM, Monnens LAH, Schretlen EDAM. Haemolytic-uraemic syndrome. Epidemiologic and clinical study. Arch Dis Child 1974;49:432–7.
175. Kibel MA, Barnard PJ. The haemolytic-uraemic syndrome: a survey in southern Africa. S Afr Med J 1968;42:692–8.

176. Tarr PI, Hickman RO. Hemolytic uremic syndrome epidemiology: a population-based study in King County, Washington, 1971 to 1980. Pediatrics 1987;80:41–5.
177. Rogers MF, Rutherford GW, Alexander SR, et al. A population-based study of hemolytic-uremic syndrome in Oregon, 1979–1982. Am J Epidemiol 1986;123:137–42.
178. Trompeter RS, Schwartz R, Chantler C, et al. Haemolytic-uraemic syndrome: an analysis of prognostic features. Arch Dis Child 1983;58: 101–5.
179. British paediatric association-communicable disease surveillance centre surveillance of haemolytic uraemic syndrome 1983–4. BMJ 1986;292:115–17.
180. Windler F, Weh HJ, Hossfeld DK, et al. Verotoxin in thrombotic thrombocytopenic purpura. (Letter). Eur J Haematol 1988;42:103.
181. Farina C, Gavazzeni G, Caprioli A, et al. Hemolytic uremic syndrome associated with verocytotoxin-producing *Escherichia coli* infection in acquired immunodeficiency syndrome. Blood 1990; 75:2465–8.
182. Tarr PI, Neill MA, Allen J, et al. The increasing incidence of the hemolytic-uremic syndrome in King County, Washington: lack of evidence for ascertainment bias. Am J Epidemiol 1989; 129:582–6.
183. Lieberman E. Hemolytic-uremic syndrome. J Pediatr 1972;1:1–16.
184. Tune BM, Leavitt TJ, Gribble TJ. The hemolytic-uremic syndrome in California: a review of 28 nonheparinized cases with long-term follow-up. J Pediatr 1973;82:304–10.
185. Donckerwolcke RA, Kuijten RH, Tiddens HA. Haemolytic-uraemic syndrome. Paediatrician 1975;4:27–40.
186. Clarkson AR, Lawrence JR, Meadows R, et al. The haemolytic uraemic syndrome in adults. Q J Med 1969;154:227–44.
187. Gianantonio CA, Vitacco M, Mendilaharzu F, et al. The hemolytic-uremic syndrome. Nephron 1973;11:174–92.
188. Steele BT, Murphy N, Arbus GS, et al. An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple juice. J Pediatr 1982;101:963–5.
189. Chart H, Rowe B, v. d. Kar N, et al. Serological identification of *Escherichia coli* O157 as cause of haemolytic uraemic syndrome in Netherlands. (Letter). Lancet 1991;337:437.
190. Amorosi EL, Ultmann JE. Thrombotic thrombocytopenic purpura: report of 16 cases and review of the literature. Medicine 1966;45:139–59.
191. Remuzzi G, Garella S. HUS and TTP: variable expression of a single entity. Kidney Int 1987; 32:292–308.
192. Ridolfi RL, Bell WR. Thrombotic thrombocytopenic purpura: report of 25 cases and review of the literature. Medicine 1981;60:413–28.
193. Thrombotic thrombocytopenic purpura associated with *Escherichia coli* O157:H7—Washington. MMWR 1986;35:549–51.
194. Kovacs MJ, Roddy J, Gregoire S, et al. Thrombotic thrombocytopenic purpura following hemorrhagic colitis due to *Escherichia coli* O157:H7. Am J Med 1990;88:177–9.
195. Wells JG, Shipman LD, Greene KD, et al. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like toxin-producing *E. coli* from dairy cattle. J Clin Microbiol 1990;29:985–9.
196. Borczyk AA, Karmali MA, Lior H, et al. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. (Letter). Lancet 1987;1:98.
197. Clarke R, McEwen S, Harnett N, et al. The prevalence of verotoxin-producing *Escherichia coli* (VTEC) in bovines at slaughter. Abstracts of the Annual Meeting of the American Society for Microbiology, Miami Beach, FL, 1988:282.
198. Montenegro MA, Bulte M, Trumpf T, et al. Detection and characterization of fecal verotoxin-producing *Escherichia coli* from healthy cattle. J Clin Microbiol 1990;28:1417–21.
199. Mohammad A, Peiris JSM, Wijewanta EA, et al. Role of verocytotoxigenic *Escherichia coli* in cattle and buffalo calf diarrhoea. FEMS Microbiol Lett 1985;26:281–3.
200. Suthienkul O, Brown JE, Seriwatana J, et al. Shiga-like toxin-producing Escherichia coli in retail meats and cattle in Thailand. Appl Environ Microbiol 1990;56:1135–9.
201. Orskov F, Orskov I, Villar JA. Cattle as reservoir of verotoxin-producing *Escherichia coli* O157:H7. (Letter). Lancet 1987;2:276.202. Gonzalez EA, Blanco J. Serotypes and antibiotic resistance of verotoxigenic (VTEC) and necrotizing (NTEC) *Escherichia coli* strains isolated from calves with diarrhoea. FEMS Microbiol Lett 1989;60:31–6.
203. Janke BH, Francis DH, Collins JE, et al. Attaching and effacing *Escherichia coli* infection as a cause of diarrhea in young calves. J Am Vet Med Assoc 1990;196:897–901.
204. Sherwood D, Snodgrass DR, O'Brien AD. Shiga-like toxin production from *Escherichia coli* associated with calf diarrhoea. Vet Rec 1985;116:217–18.
205. Mohammad A, Peiris JSM, Wijewanta EA. Serotypes of verocytotoxigenic *Escherichia coli* isolated from cattle and buffalo calf diarrhoea. FEMS Microbiol Lett 1986;35:261–5.
206. Schoonderwoerd M, Clarke RC, van Dreumel AA, et al. Colitis in calves: natural and experimental infection with a verotoxin-producing strain of *Escherichia coli* O111:NM. Can J Vet Res 1988;52:484–7.
207. Wray C, McLaren I, Pearson GR. Occurrence of "attaching and effacing" lesions in the small intestine of calves experimentally infected with bovine isolates of verocytotoxic *E. coli*. Vet Rec 1989;125:365–8.
208. Mainil JG, Duchesnes CJ, Whipp SC, et al. Shiga-like toxin production and attaching effacing activity of *Escherichia coli* associated with calf diarrhea. Am J Vet Res 1987;48:743–8.
209. Beery JT, Doyle MP, Schoeni JL. Colonization of chicken cecae by *Escherichia coli* associated with hemorrhagic colitis. Appl Environ Microbiol 1985;49:310–15.
210. Holland RE. Some infectious causes of diarrhea in young farm animals. Clin Microbiol Rev 1990;3:345–75.
211. Salmon RL, Farrell ID, Hutchinson JGP, et al. A christening party outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7. Epidemiol Infect

1989;103:249–54.
212. Howe K, Linton AH, Osborne AD. A longitudinal study of *Escherichia coli* in cows and calves with special reference to the distribution of O-antigen types and antibiotic resistance. J Appl Bacteriol 1976;40:331–40.
213. Surveillance of *Escherichia coli* O157 isolation and confirmation, United States, 1988. MMWR 1991;40 (SS-1):1–6.
214. Stuart FA, Corbel MJ. Identification of a serological cross-reaction between *Brucella abortus* and *Escherichia coli* O157. Vet Rec 1982; 110:202–3.
215. Hockin J, Lior H, Mueller L, et al. An outbreak of *E. coli* O157:H7 diarrhea in a nursing home—Alberta. Can Dis Wkly Rep 1987:13–45:206.
216. Ostroff SM, Griffin PM, Tauxe RV, et al. A statewide outbreak of *Escherichia coli* O157:H7 infections in Washington State. Am J Epidemiol 1990;132:239–47.
217. Sekla L, Milley D, Stackiw W, et al. Verotoxin-producing *Escherichia coli* in ground beef in Manitoba. Can Med Assoc J 1990;143:519–21.
218. Read SC, Gyles CL, Clarke RC, et al. Prevalence of verocytotoxigenic *Escherichia coli* in ground beef, pork and chicken in southwestern Ontario. Epidemiol Infect 1990;105:11–20.
219. Kotula AW, Chesnut CM, Emswiler BS, et al. Destruction of bacteria in beef patties by cooking. J Anim Sci 1977;45:54–8.
220. Restaino L, Lyon RH. Efficacy of Petrifilm VRB for enumerating coliforms and *Escherichia coli* from frozen raw beef. J Food Protect 1987; 50:1017–22.
221. Tauxe RV, Griffin PM, Ostroff SM, et al. Diagnosing *Escherichia coli*. (Letter). Lab Med 1991;22:55–6.
222. Belongia EA, MacDonald KL, Parham GL, et al. An outbreak of *Escherichia coli* O157:H7 colitis associated with consumption of precooked meat patties. J Infect Dis 1991;164:338–43.
223. Foodborne outbreak caused by *Escherichia coli* O157:H7—North Dakota. MMWR 1991;40:265–7.
224. Morgan GM, Newman C, Palmer SR, et al. First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O157:H7 in the UK. Epidemiol Infect 1988;101: 83–91.
225. Tison DL. Culture confirmation of *Escherichia coli* serotype O157:H7 by direct immunofluorescence. J Clin Microbiol 1990;28:612–13.
226. Cryan B. Enterohaemorrhagic *Escherichia coli* serotype O157:H7 as an enteric pathogen in Irish children. Ir J Med Sci 1989;158:18–19.

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Pediatric Nephrology

Brief report

Pancreatic injury in the hemolytic-uremic syndrome

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Abstract. In a retrospective review of 241 cases with hemolytic-uremic syndrome, there was evidence of pancreatic involvement in 2 patients, abnormal ultrasounds in 4 patients, and pancreatic injury in 3 of 8 patients undergoing autopsy. Pancreas ultrasound examination, available in 134 cases, was very valuable in identifying those with pancreatic injury.

Key words: Hemolytic-uremic syndrome – Pancreatitis – Diabetes – Ultrasounds

Introduction

Extra-renal manifestations of hemolytic-uremic syndrome (HUS) are now well-recognized complications of the disease [1]. Since the early 1980s, pancreatic endocrine involvement has been described by several authors [2–4], often in cases with a poor or fatal outcome. We report a retrospective review of the 241 cases of HUS treated at our institution, focusing on pancreatic injury. Clinical data, as well as information from post-mortem examination performed on the 8 children who died, and acute-phase pancreas ultrasound examinations performed in 134 patients are reported.

Patients and methods

Between June 1976 and August 1996, 241 children with HUS were admitted to St. Justine's Hospital. Of these, 213 or 91% had typical childhood HUS preceded by a diarrheal syndrome. Of these 241 total patients, 136 (56%) required dialysis. The inclusion criteria for the diagnosis of HUS were the presence of microangio- pathic hemolytic anemia with evidence of fragmented red cells and schistocytes on a peripheral smear, thrombocytopenia ($<100\times10^9$/l), and acute renal failure, often associated with oligo-anuria.

All charts were obtained from the medical records department; none were excluded, as they all met the aforementioned diagnostic criteria. The study was retrospective and aimed at identifying patients who showed evidence of pancreatic involvement during the acute phase of the disease. Special attention was given to the abdominal ultrasound examination available in 134 cases, autopsies performed in all 8 patients who died, as well as the requirement for exogenous insulin. Initially, attention was also given to plasma levels of amylase and lipase, as well as blood glucose. Unfortunately, determinations were sporadic, precluding meaningful conclusions.

Results

Although hyperglycemia was not unusual during the acute phase in patients being dialyzed with hypertonic (4.25% dextrose) solutions, only 2 required insulin because of the high level of blood glucose (>20 mmol/l) and persistence of hyperglycemia (>3 consecutive days). In both these patients, insulin administration began during the 1st week after the onset of the acute illness. They were discharged from hospital with insulin. On follow-up 5 and 6 years later respectively, they still had insulin-dependent diabetes, requiring an average of 0.4 units of insulin/kg per day.

At least one abdominal ultrasound examination was available in each of the 134 cases where the pancreas was clearly visible. The examination was always performed during the acute phase of the disease, and with few exceptions during the 1st week. The main indications for performing the examination were either suspected peritonitis or severe colitis giving intense pain. Suprisingly, in all but 4 cases the pancreas size and echostructure were normal. In these 4 cases, the pancreas was substantially increased in size and the echostructure was heterogenous; 2 of these 4 patients now have insulin-dependent diabetes. A third patient with an abnormal pancreas on ultrasound examination died, and his pancreas at autopsy showed cell necrosis. The fourth patient, a survivor, has no glycoregulation problems (normal curve after oral glucose load) but has long-term sequelae of HUS, such as chronic renal

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failure and hypertension (glomerular filtration rate = 45 ml/min per 1.73 m^2).

Post-mortem examinations were performed in all 8 patients that died of HUS; 7 of these died of direct complications of their initial disease. The eighth patient died within 24 h of admission of a massive hemorrhage secondary to perforation of the aorta at the time of insertion of a rigid peritoneal catheter. The other 7 patients all had multiple complications, such as severe hemorrhagic colitis, coma, seizures, anuria, and massive bleeding. Macroscopic and microscopic examinations established significant pancreatic involvement in 3 of the 7 patients who died of direct complications of the disease. One patient had generalized necrosis involving both the exocrine and endocrine pancreas, associated with the presence of multiple arterial thrombi. In the second case arterial thrombi presented a focal distribution and were associated exclusively with the islets of Langerhans. In the third patient, generalized inflammation was seen but no necrosis was detected. All 3 patients died within 10 days of onset of the disease.

Discussion

In recent years, attention has been focused on the extra-renal manifestations of HUS, seldom described in earlier reports. Pancreatic involvement has been the subject of a number of articles, describing either the occurrence of insulin-dependent diabetes [2], pancreatic enzyme elevation during the acute phase [5], pancreatic inflammation and/or necrosis in autopsy reports [1–3, 6, 7], and even chronic exocrine insufficiency requiring enzyme replacement therapy [8].

The reported incidence of persistent hyperglycemia is quite low, with only 19 cases being reported up to 1995 [9]. In our series, the 2 cases observed of 241 patients establishes the incidence a little lower than 1%. Combining several reports from the literature an incidence of about 3% was estimated [4]. Moreover, diabetes seems to occur in very ill patients with anuria, some of whom eventually succumb to their disease [9]. Our observations in the patients who died with pancreas injury at post-mortem examination and the 2 who developed diabetes suggest that pancreas involvement may be restricted to those with the most severe forms of HUS; our results are thus in accordance with the opinions generally expressed by others. Recent information indicates that plasma cytokines may be causally involved in mediating pancreatic damage in HUS. Indeed, it was found that plasma levels of interleukin-6 (IL-6) were significantly elevated in those with severe HUS who had extra-renal manifestations, including pancreatic necrosis [10]. Levels of IL-6 were normal in all mild forms of HUS.

A fourfold elevation of plasma pancreatic enzymes, interpreted as a sign of pancreatic insult, was reported in the acute phase of HUS and in as many as 66% of patients [5]. However, the vast majority had no permanent pancreatic sequelae, suggesting that plasma pancreatic enzyme elevation is probably of little if any clinical significance in this setting. Ultrasound examination is known to be an acceptable method for examining an inflamed pancreas [11,12] and has been very accurate in precisely detecting those patients with pancreatic involvement leading to full-blown diabetes, or pancreatic necrosis in a patient who died of HUS complications. It is noteworthy that the 2 other patients who had abnormal pancreases at post-mortem examination had not had ultrasound examination. Thus, in our experience, abdominal ultrasound examination is useful in identifying at a very early stage those very-sick HUS patients who develop clinically significant pancreas injury.

In conclusion, although mild pancreas involvement in the acute phase of HUS can be frequent, severe and clinically significant pancreatic injury, such as found in patients who either die or develop diabetes, is extremely rare. Pancreas ultrasound examination seems to be an excellent tool for diagnosing this problem and alerting the physician.

References

1. Robson WLM, Leung AKC, Kaplan B (1993) Hemolytic-uremic syndrome. Curr Probl Pediatr 16: 16–33
2. Burns JC, Berman ER, Fagre J, Shikes RD, Lum GM (1992) Pancreatic islet cell necrosis: association with hemolytic-uremic syndrome. J Pediatr 100: 582–584
3. Andreoli SP, Bergstein JM (1982) Development of insulin dependent diabetes melitus during the hemolytic-uremic syndrome. J Pediatr 100: 541–545
4. Zeidan SF, Coulthard MG (1987) Transient glucose intolerance associated with the hemolytic-uremic syndrome. Pediatr Nephrol 1: 605–607
5. Grodinsky S, Telmesani A, Robson WLM, Fick G, Scott RB (1990) Gastrointestinal manifestations of hemolytic-uremic syndrome: recognition of pancreatitis. J Pediatr Gastroenterol Nutr 11: 518–524
6. Primhak RA, Taitz LS, Variend S, Webb DHK, Cser A (1984) Necrosis of the pancreas in the hemolytic uremic syndrome. J Clin Pathol 37: 655–658
7. Gallo EG, Gianantonio CA (1995) Extra-renal involvement in diarrhea associated hemolytic-uremic syndrome. Pediatr Nephrol 9: 117–119
8. Andreoli S, Bergstein J (1987) Exocrine and endocrine pancreatic insufficiency and calcinosis after hemolytic-uremic syndrome. J Pediatr 110: 816–817
9. Robson WLM, Leung AKC, Brant R, Trevenen CK, Stephvre DK (1995) Hyperglycemia in diarrhea associated hemolytic-uremic syndrome. Nephron 71: 54–58
10. Van De Kar NCAJ, Sauerwein RN, Demacker PNM, Grau GE, Can Hinsberg VNM, Monnens LAH (1995) Plasma cytokine levels in hemolytic-uremic syndrome. Nephron 71: 309–313
11. Coleman BG, Arger PH, Rosenberg HK, Mulhern CB, Ortega N, Stauffer RT (1983) Gray-scale sonographic assessment of pancreatitis in children. Radiology 146: 145–150
12. Fleischer AC, Oarker P, Kirchner SG, James AE (1983) Sonographic findings of pancreatitis in children. Radiology 146: 151–155

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THE HEMOLYTIC UREMIC SYNDROME

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Gasser and colleagues are credited with initially describing the hemolytic uremic syndrome (HUS) in 1955. Thirty years earlier, however, Moschcowitz[85] reported a female adolescent who died of multiorgan thrombotic microangiopathy and anemia. This disorder (Moschcowitz's syndrome), later known as *thrombotic thrombocytopenic purpura* (TTP), is very similar to HUS. In the years that followed, in the sentinel observations of Moschcowitz and Gasser, the great majority of childhood cases were noted to have been preceded by diarrhea that was usually bloody. More than a quarter century had passed since Gasser's original observation, however, before the association between the diarrheal prodrome and the triad of hemolytic anemia, thrombocytopenia, and acute renal failure was clarified.

Chapter two began in 1982 with the observations of Riley and coworkers[102] and Karmali and colleagues.[58] Riley and coworkers described two outbreaks of hemorrhagic colitis caused by hamburger that was contaminated with a rare *Escherichia coli* serotype known as O157:H7 the same year that Karmali and colleagues first recognized the association between cytotoxins produced by these bacteria and HUS. Since then, interest and understanding of the epidemiology, pathogenic cascade, and natural history of classic (postdiarrheal) HUS have increased exponentially. More than 300 articles from 1992 to 1995 dealt with various aspects of the syndrome.

Hemolytic uremic syndrome is now recognized as the most frequent cause of acute renal failure in infants and young children, as an important cause of stroke and chronic renal failure in the young, and as a

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substantial consumer of health care resources. Approximately 276 to 736 new cases of HUS occur yearly in the United States. An estimated $9.4 to $25.2 million are consumed each year during the acute phase of the illness[76]; each year, between 24 and 63 survivors develop advanced chronic renal failure and require chronic dialysis or transplantation at an additional cost of $7.5 to $19.1 million. Moreover, caretaker productivity losses occur subsequent to caring for a child who has received a renal transplant or is on chronic dialysis.[76]

The syndromic nature of HUS needs to be emphasized. As with any syndrome, it is merely a constellation of features. Although approximately 90% of childhood cases follow a diarrheal prodrome (classic HUS)[126] and are linked to enterohemorrhagic *E coli* (EHEC) (e.g., *E coli* O157:H7) infections, the syndrome can be secondary to a variety of drugs, malignancies, pregnancy, systemic disorders, and other glomerulopathies. In addition, there is an idiopathic variety that can be familial or sporadic and often is recurrent (Table 1).

ESTABLISHING THE DIAGNOSIS

The traditional diagnostic criteria include microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. The diagnosis usually is simple and straightforward if this triad occurs in an infant or young child following an episode of diarrhea, especially if the diarrhea is bloody. Incomplete syndromes do occur, however.[103, 105] The anemia and thrombocytopenia may be mild or absent, and the acute nephropathy may be mild to nonexistent with little or no azotemia. Incomplete

Table 1. CLASSIFICATION OF HUS IN CHILDREN AND ADOLESCENTS

Post-diarrheal (90% of All Cases)	Non-diarrheal (10% of All Cases)
Shiga-like toxin (SLT) producing bacteria (90% of SLT postdiarrheal cases in the United States are due to *E coli* O157:H7)	**Secondary** Nonenteric infections (e.g., streptococcal pneumonia) Primary glomerulopathies Drugs (e.g., cocaine, quinine, mitomycin, cyclosporine, FK-506, BCP) Pregnancy (and postpartum) Bone marrow transplant Cancer Collagen/vascular disorders AIDS Cobalamin C disease **Idiopathic** Familial* Autosomal dominant Autosomal recessive Sporadic*

*Hypocomplementemia and recurrences are frequent.

syndromes should not be diagnostically difficult if they follow a bloody diarrheal prodrome but may be a diagnostic challenge if they occur in other settings.

Hemolytic uremic syndrome can be confused with other conditions, especially disseminated intravascular coagulation (DIC) and TTP. Disseminated intravascular coagulation can usually be distinguished from HUS by the context in which it occurs (e.g., sepsis), and by the patient's prolonged prothrombin time, and partial thromboplastin time. Thrombotic thrombocytopenic purpura occurs mostly in adults, and although both can be initiated by *E coli* O157:H7 hemorrhagic colitis,[66] a diarrheal prodrome is uncommon. Moreover, no seasonal variation occurs, fever and central nervous system involvement are more frequent, renal dysfunction is less, and mortality and recurrences are greater.[103]

POSTDIARRHEAL HUS

Epidemiology

Although a variety of organisms have been implicated in the pathogenesis of postdiarrheal HUS, the association with EHEC (e.g., O157:H7) and *Shigella dysenteriae* type 1 is most compelling.

Enterohemorrhagic *E coli* are a subset of *E coli* that cause hemorrhagic colitis and produce potent cytotoxins known as *Shiga-like toxin* (SLT) or *Verotoxin.* It is called *Shiga-like toxin* because it is virtually identical to the toxin produced by *Shigella dysenteriae* type 1 (bacteria that can also cause HUS); and *Verotoxin,* because it is extremely cytotoxic to cultured Vero cells (grown from African green monkey kidneys). *E coli* O157:H7* is the prototypic EHEC and is the *E coli* serotype responsible for more than 90% of EHEC infections in the United States; dozens of other *E coli* (i.e., other EHEC) produce the same toxins, however. EHEC generally produce more than one type of SLT. Most strains produce both SLT-1 and SLT-2, and strains that produce predominately SLT-2 seem to be more virulent.[131] SLT-2 variants also exist.[95]

Abundant epidemiologic evidence links EHEC infections to classic postdiarrheal HUS. Numerous reports from North America[57, 77, 92, 111, 126] and Europe[22, 80] report a high incidence of preceding EHEC infections based on stool cultures, presence of fecal cytotoxin, or patient's antibodies against the organism's lipopolysaccharide (LPS) or toxin. Documentation of preceding EHEC infection can be achieved in 75% of cases by using a combination of stool culture, fecal cytotoxin, and antibody assays.[22, 57]

Most,[48, 63] but not all[130] studies have shown that white children are more susceptible to HUS than black children. Moreover, although the majority of studies suggest that female gender is a modest risk factor,[63, 105, 111] a recent report[25] refutes gender predisposition.

Classic (postdiarrheal) HUS occurs more frequently during warmer

*The O refers to the somatic antigen, and the H to the flagellar antigen.[42]

months[77, 105, 111, 126, 130]; the recent Pacific Northwest epidemic was a notable exception.[17] The summer disease incidence peak correlates with the higher incidence of positive EHEC fecal cultures in cattle during the warmer months.[44, 147]

Although postdiarrheal HUS occurs throughout the world, more cases have been reported from Buenos Aires than from any other region.[74] Average annual incidence figures (i.e., cases per 100,000 children) for regions in North America are 0.9 for Oregon,[105] 1.2 for King County, WA,[130] 1.4 for Utah,[126] and 1.4 for Canada.[111] The Canadian province of Alberta experiences twice the national average, however.[111] The annual incidence is highest in young children, with an age-specific incidence per 100,000 children of 3.1 for Canadian children fewer than 5 years of age,[111] and 7.1 for children in Utah, aged 1 to 2 years.[126] All regions experience highs and lows in annual incidence. The annual incidence in Utah[126] from 1971 to 1990 ranged from a low of 0.2 to a high of 3.4 cases per 100,000 children fewer than 18 years of age.

Reservoirs and Vectors of Infection

Enterohemorrhagic *E coli* asymptomatically inhabit the intestines of cattle, especially calves and heifers.[44, 145] In one study, fewer than 1% of fecal samples from randomly selected cattle tested positive for *E coli* O157:H7, and the organism was present in only 8.3% of dairy cattle herds. Within positive herds, however, 9.6% of weaned calves tested positive for the organism.[44] The Centers for Disease Control and Prevention examined 1266 fecal specimens from healthy dairy cattle in Wisconsin and Washington and found *E coli* O157:H7 in 2.8% of heifers and 0.15% of adult cows.[145] The Laboratory Center for Disease Control in Canada tested for verotoxin-producing *E coli* (VTEC) in fecal samples obtained from 336 humans, 592 calves, and 886 cows on 80 dairy farms in Ontario.[147] Thirty-six percent of cows and 57% of calves tested positive for VTEC. More surprisingly, 21 family members on 16 farms were also carriers. Carriage in both humans and cattle was transient in the great majority of cases. In a study of 896 samples of retail meat from Madison, WI, *E coli* O157:H7 was identified in 3.7% of beef, 2% of lamb, and 1.5% of pork and poultry.[29] When meat was tested for all SLT producing *E coli* (i.e., not restricted to O157:H7) in Ontario, 36.4% of beef and 10.6% of pork were contaminated.[98]

Because EHEC commonly inhabit the intestines of cattle, most outbreaks have been linked to contaminated beef products. Important to note, however, is that epidemics account for only a minority of cases; the remainder occur sporadically.[42] Hamburger is the vector that accounts for more than half of all reported epidemics.[42] Contaminated hamburger patties served in a Pacific Northwest chain of fast food restaurants left 371 children with colitis; 37 developed HUS. Fifty-one percent of those with HUS developed serious extrarenal problems, including seizures, stroke, pancreatitis, diabetes, and colonic necrosis. Three percent died.[17]

If beef contaminated during the slaughtering process is ground into hamburger, then EHEC on the surface of the meat become internalized. Thereafter, only thorough cooking kills the pathogens; few surviving bacteria are sufficient to cause disease.[42] Other cuts of beef, even if contaminated, constitute a low risk because surface contamination is controllable with conventional cooking practices.

Water or other food products contaminated by cattle feces are additional vectors of infection. Cases of EHEC colitis, HUS, or both have been linked to contaminated municipal water supplies,[129] unpasteurized milk,[94] yogurt,[83] unpasteurized apple cider made from unwashed apples,[13] potatoes,[84] and vegetables grown in a garden fertilized with cow manure.[24]

Person-to-person spread is also an important vector. Epidemics have occurred in institutions for mentally retarded persons (where the initial vector was hamburger),[93] child care centers,[8] in a small remote Canadian Eskimo community,[110] as a result of close personal contact with other individuals with diarrhea,[109] and from fecal contamination of water in a children's wading pool[18] and a small lake.[60]

An isolated report of HUS following an EHEC urinary tract infection represents a novel mode of transmission.[101] Small risk of occupational exposure also exists. A laboratory technician working with stool specimens[16] and a nurse caring for a child with postdiarrheal HUS[56] both developed colitis and HUS.

Risk Factors for Progressing From Colitis to HUS

Young age[25] and infection with strains that produce SLT-2[25, 92] seem to increase the likelihood of developing HUS. This later factor correlates with the markedly greater toxicity of SLT-2.[131] Administration of antimotility agents also may increase the risk of progression by increasing toxin contact time in the intestine, thereby facilitating absorption.[26]

Whether treating patients with EHEC diarrhea with antibiotics increases the risk of developing HUS is unknown. In an institutional epidemic, the use of trimethoprim-sulfamethoxazole seemed to increase the likelihood of developing HUS[93]; the addition of some antibiotics increases ex vivo VTEC toxin production.[144] Other clinical studies have suggested that, although prolonged use of "inappropriate" antibiotics (i.e., those shown by susceptibility testing to be ineffective) increases the risk,[21] the use of "appropriate" antibiotics might decrease risk of progression.[26] Still other investigators have concluded that the use of antibiotics has no effect on progression to HUS.[92]

Pathogenic Cascade

The pathogenic cascade (Fig. 1) that begins with the ingestion of EHEC and culminates in the thrombotic microangiopathy that characterizes HUS is becoming better understood.

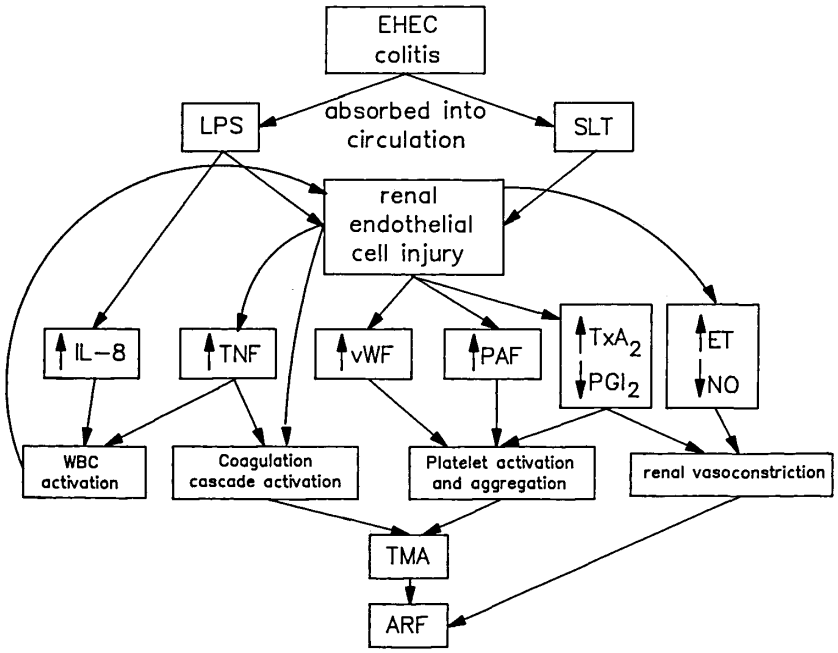

Figure 1. Proposed (abbreviated) postdiarrheal HUS pathogenic cascade. EHEC = enterohemorrhagic *E coli;* LPS = lipopolysaccharide; SLT = Shiga-like toxin; IL-8 = interleukin-8; TNF = tumor necrosis factor; vWF = von Willebrand factor; PAF = platelet activating factor; TxA_2 = thromboxane A_2; PGI_2 = prostacyclin; ET = endothelin; NO = nitric oxide; TMA = thrombotic microangiopathy; ARF = acute renal failure.

Only a small inoculum of EHEC is needed to allow enteric colonization.[42, 54] The process by which these bacteria become attached to epithelium of the distal ileum and large intestine is complex and is probably initiated by bacterial fimbrial attachment. Given EHEC's ability to elaborate potent cytotoxins, invasion is not needed for development of hemorrhagic colitis.[132]

The SLTs produced by these bacteria are proteins (molecular weight: 71 kd) comprised of one A subunit and five B subunits. The B subunits are required for binding of the holotoxin to high-affinity glycolipid (GB_3) cell surface receptors on target organs.[52] Although the toxins (i.e., SLT-1, SLT-2, and SLT-2 variants) probably are not necessary for initiation of the diarrhea,[132] they most likely cause the hemorrhagic and ulcerative intestinal lesions by damaging the microvasculature of the intestinal wall.[132] The thrombotic microangiopathy that occurs first in the gut is similar to what is later found in the kidneys.

Once the gut-blood barrier has been compromised by ischemic intestinal damage, SLTs and probably other bacterial products (e.g., LPS) presumably gain entrance to the circulation.

Lipopolysaccharide, independent of SLT, can damage endothelial cells[46] and promote thrombosis by increasing tissue factor expression, up-regulating plasminogen activator inhibitor, and down-regulating thrombomodulin expression.[107] Hemoglobin, which is abundantly present in HUS plasma, enhances the ability of LPS to induce endothelial cell tissue factor expression.[107] Moreover, LPS enhances white blood cell adhesion to endothelial cells and, in the presence of thrombin, also increases the adhesion of platelets to endothelial cells.[107] Injecting rabbits with endotoxin produces a thrombotic microangiopathy that is very similar to that seen in HUS.[12] Moreover, *endotoxemia*,[27] or antibodies to LPS,[15] have been found in patients with HUS. Shiga-like toxins absorbed into the circulation are thought to rapidly attach to GB_3 receptors in target organs. Although the major target organ is the kidney, virtually any organ can be involved.[119] Specific organ involvement is assumed to depend on the presence of GB_3 receptors; that is certainly true in the rabbit model, where the kidney is spared due to absence of renal receptors.[149] The presence and density of receptors in the various organs (e.g., brain) may vary from child to child and thus may account for the unpredictable nature of extrarenal involvement.

After the B subunits attach to the GB_3 receptors, the toxin is internalized via receptor-mediated endocytosis.[62] Once in the cytoplasm, it enzymatically inactivates ribosomes that are required for peptide elongation.[89] Thus, by inhibiting protein synthesis, the cell dies or is severely damaged.

Although most researchers agree that microvascular endothelial cell injury is pivotal for the renal thrombotic microangiopathy (TMA) that characterizes HUS, whether the renal endothelial cell is the initial target for the toxin is not yet clear. Although human endothelial cells (including glomerular endothelial cells) grown in culture possess toxin receptors,[71] studies of intact human renal tissue have been at variance with these ex vivo findings. Sections of intact human kidney show toxin binding to be predominately in the renal tubules,[71, 133] especially in those adjacent to glomeruli.[71] The mere process of culturing glomerular cells may cause them to up-regulate GB_3 receptors. In vivo, up-regulation might be caused by cytokines [e.g., tumor necrosis factor (TNF)] that are produced by perturbation of renal tubular cells[49] adjacent to glomeruli.

Inflammatory Mediators

The cytokine TNF has properties that could be important in the pathogenesis of HUS. Tumor necrosis factor acts on endothelial cells to induce procoagulant activity[14] and induces the release of von Willebrand's factor (vWF)[140] that facilitates formation of platelet thrombi. Tumor necrosis factor is elevated in the urine[59, 123] and occasionally in the serum[59, 73] of children during the acute phase of HUS. Shiga-like toxin specifically induces TNF production in the kidney but not in other tissues.[45] Moreover, TNF induces expression of GB_3 receptors on human endothelial cells.[139]

Interleukin-6 (IL-6), a cytokine produced by endothelial, mesangial, and other cells, is elevated in the serum,[59] especially in those with extrarenal involvement,[138] and in the urine[59] during the acute phase of HUS. It may be a useful marker of disease activity.

Interleukin-8, a potent activator of white blood cells, is elevated in the serum of children during the active phase of postdiarrheal HUS.[33] Moreover, leukocytosis is a consistent feature of postdiarrheal HUS, and the magnitude of the white blood cell elevation correlates with severity and outcome.[126] Evidence suggests that activated white cells participate in the pathogenesis of the disease, probably via endothelial cell damage subsequent to the release of tissue-damaging enzymes. Elastase, liberated by activated white cells, is elevated in the serum during the acute phase of HUS.[52] Also, SLT increases adhesion of white cells to endothelial cells,[150] and neutrophils from patients with HUS adhere better to endothelial cells in culture than do white cells from control patients.[36]

Activation of Platelets and the Coagulation Cascade

Platelet and coagulation cascade activation are important in the pathogenesis of HUS. Thrombocytopenia is almost always present, and the platelet release products, β-thromboglobulin, and platelet factor 4, are found in plasma.[1] Moreover, intraplatelet serotonin levels are low, and plasma levels are high.[143] In addition, platelets obtained from children with HUS are degranulated and fail to respond normally to aggregating agents, suggesting platelet "exhaustion."[35, 143] Although this may be due, in part, to the presence of a platelet-aggregating factor,[82] the presence of this factor may be infrequent.[112] vWF multimers, released from damaged endothelial cells, are often found in the plasma, and may facilitate platelet aggregation. Platelet-activating factor (PAF), a phospholipid produced by platelets and endothelial cells, is found in the urine during the acute phase of HUS[10] and may also play a role in activating platelets. Moreover, Verotoxin has also been shown to cause platelet aggregation.[106]

Although the renal microthrombi are composed largely of platelets, fibrin also is usually present. Plasma markers of coagulation activation (e.g., fibrinopeptide A) are present[81]; occasionally evidence is present of DIC.[5] The coagulation cascade is probably activated by release of tissue factor subsequent to endothelial cell damage. Moreover, fibrinolysis may be impaired owing to the presence of a plasminogen-activator inhibitor.[11] In this context, the fact that Shiga toxin decreases fibrinolysis factor expression in cultured human renal glomerular endothelial cells may be relevant.[75]

Prostaglandins

Prostacyclin (PGI_2) and thromboxane (Tx) A_2 are arachidonic acid metabolites that are of interest in HUS. Prostacyclin, produced by endothelial cells, is a vasodilator that also inhibits platelet aggregation and

adhesion. Thromboxane, released by activated platelets, on the other hand, is a vasoconstrictor substance that promotes platelet aggregation. An imbalance between these eicosanoids could cause renal vasoconstriction and promote renal microvascular thrombosis. Thromboxane biosynthesis, as a result of platelet activation, is increased during the acute phase of the syndrome.[88, 135] The role of prostacyclin in HUS is controversial. Using a variety of methods, various investigators have found either decreased or increased PGI_2 production.[118] These differences can be reconciled by recognizing that the level in a given patient at any point in time reflects the mixture of factors that both stimulate and inhibit its production.[118] One would expect endothelial perturbation to initially stimulate production as has been demonstrated following 24 hours' incubation of SLT with cultured human renal microvascular cells.[148] As the injured endothelial cells become exhausted, however, renal production of this prostanoid would likely decrease, as shown by finding reduced urinary metabolites specific for PGI_2 of renal origin in children who were several days into the acute phase of their illness.[88]

Endothelin–Nitric Oxide Axis

Some HUS patients with oliguric renal failure show little, if any, physical evidence of renal microvascular occlusion on biopsy.[70] It is therefore of interest that endothelin, a 21 amino acid peptide produced by renal endothelial, mesangial, and epithelial cells, is elevated in the urine[122] and plasma[65, 87] during the acute phase of HUS. Endothelin markedly increases renal vascular resistance and reduces glomerular filtration rate.[122] Circulating endothelin also raises blood pressure, and plasma levels have been found to be higher in hypertensive HUS patients.[87] Moreover, endothelin, by promoting increased production of PAF, facilitates white blood cell activation.[40]

Endothelin normally promotes endothelial cell production of the opposing substance nitric oxide (NO), also known as endothelium-derived relaxing factor. Nitric oxide is not only a potent vasodilator, but it also is an inhibitor of platelet adhesion and aggregation. Rats given an NO donor (nitroglycerin) are protected against LPS-induced glomerular thrombosis.[146] Free hemoglobin, which is present in HUS plasma subsequent to hemolysis, inactivates NO, however.[30] Urinary cyclic guanosine monophosphate (GMP), a marker of NO activity,[134] is decreased in the urine during the acute phase of the disease,[121] which suggests that injured renal endothelial cells are unable to produce normal amounts of NO, or that NO is inactivated by circulating free hemoglobin, or both. The resulting imbalance between endothelin and NO could partially account for the acute renal failure in HUS.

Oxidative Damage

Lipid peroxides can damage endothelial and red blood cells. Markers of lipid peroxidation are elevated in the serum or plasma of children

with HUS,[72, 127] perhaps owing to a disturbance in the metabolism of the major lipid-bound antioxidant vitamin E[127] or because of impaired antioxidant enzymatic activity.[72]

Circulating Immune Complexes and Antibodies

Immune deposits are sometimes seen in glomeruli from children with HUS.[78] Moreover, immune complexes,[90] complement-fixing antibodies that lyse endothelial cells,[68] and autoantibodies to cryptic endothelial antigens[64] all have been found in the circulation of children during the acute phase of HUS; however, whether these observations represent epiphenomena and are merely the consequence of epithelial cell injury, or whether immune complexes and antiendothelial cell antibodies play an important role in the pathogenesis of the disease is not clear.

Features of Postdiarrheal HUS

Prodrome

Although *prodromal* events are actually the initial expression of the pathogenic cascade, features that precede the diagnosis of HUS are customarily viewed as *prodromal.* Although the diarrhea is at first watery, in most cases it soon becomes bloody. Abdominal pain can be severe, and examination sometimes reveals extreme abdominal tenderness. Therefore, ulcerative colitis or acute surgical abdomen (e.g., acute appendicitis) is often the initial diagnosis. Colonoscopy reveals changes that are virtually indistinguishable from ulcerative colitis, and imaging studies (e.g., ultrasound) demonstrate bowel wall edema and ascites. On explorative laparotomy, the bowel is usually edematous and hemorrhagic, and, on rare occasion, frank bowel wall necrosis is present. The colitis usually precedes the triad of HUS by about a week, with a range of 1 to 14 days. It resolves in most cases approximately at the time the HUS appears; however, it may persist for as many as 2 months.[119] Vomiting usually accompanies the diarrhea. Lethargy and irritability are usually present; pallor and low-grade fever are common; and jaundice, petechia, or purpura occur occasionally. Oliguria, anuria, or both sometimes are recognized by the family but are difficult to appreciate in an infant with diarrhea. Anuria during the prodrome portends severe HUS.[126] In approximately 10% of cases, a seizure heralds the onset of HUS.[126]

Laboratory Studies

The great majority of *E coli* O157:H7 are unable to ferment sorbitol. This allows for easy screening; confirmation can be achieved by using comercially available agglutination tests. The yield is low, however,

unless the stool is tested within the first week of the diarrhea. Tests for free fecal SLT offer the advantage of also detecting toxins produced by non-O157:H7 EHEC and do not require the presence of viable EHEC. DNA probing for toxin genes and polymerase chain reaction techniques for toxin detection are available in research laboratories.[42]

Falling hemoglobin and hematocrit values combined with morphologic features of microangiopathic hemolysis on blood smear (i.e., fragmented red blood cells and burr cells) and high serum lactic dehydrogenase values characterize the hemolytic anemia of HUS. The anemia traditionally has been ascribed to mechanical stresses imposed on red cells as they pass through partially occluded microvessels; however, oxidative damage to red blood cells[137] and direct SLT injury[20] may contribute to the hemolysis. Serum bilirubin levels are elevated when hemolysis is brisk; reticulocytosis may be blunted by the azotemia.

Thrombocytopenia (i.e., $< 150 \times 10^9$/L), although considered to be a requisite for diagnosis of HUS, cannot be documented in approximately 5% of cases.[126] Platelet counts, on the other hand, can be very low (i.e., less than 10×10^9/L), often vacillate during the acute phase of the disease, do not reliably correlate with other features of the syndrome, and rise to normal as the anemia and renal failure resolve. The thrombocytopenia is believed to be secondary to platelet trapping in involved organs and removal of damaged platelets by the liver and spleen.[103]

Virtually all patients are azotemic and have hematuria and proteinuria. Hyperkalemia, metabolic acidosis, hyperphosphotemia, hypocalcemia, and dilutional hyponatremia are common when oliguric renal failure is present.

Liver transaminase elevation is common, serum albumin concentration is low,[115] and serum triglyceride and uric acid levels are often extremely high (i.e., > 500 mg/dL, > 20 mg/dL, respectively).[53, 55] Elevated serum amylase and lipase concentrations are observed in approximately 20% of patients, and hyperglycemia has been reported to occur in 4% to 15% of children.[119]

Clinical Features

Colitis is severe enough to cause rectal prolapse in close to 10% of cases; gangrene, requiring partial colectomy, occurs in approximately 2% of patients. Involvement of the small intestine is observed occasionally.[119]

The acute nephropathy ranges from mild, with only microscopic hematuria, modest proteinuria, and no oliguria or azotemia, to severe, with widespread cortical necrosis and irreversible anuric renal failure. The majority (60%) of patients experience oliguria that lasts, on average, approximately 1 week. Almost half are anuric for an average of 3 days[126]; however, the oligoanuria can continue for weeks. Examination of renal tissue usually reveals swollen glomerular endothelial cells that are separated from the basement membrane by fluffy fibular material. Microthrombi, composed mostly of platelets with modest amounts of fibrin,

further occlude the microvessels.[2, 70] Interlobular arteries sometimes are involved, and arterioles can be severely narrowed by concentric "onion skinning"[70]; this variant often leads to chronic renal failure and persistent severe hypertension.

Hypertension of a milder nature occurs in the majority of cases during the acute phase of the illness.[126] It is usually labile, easily controlled, and resolves before discharge from the hospital. Its pathogenesis is obscure and is not necessarily related to renin activation[97]; circulating endothelin may play a role in its cause.[87]

Although the gut and kidney are always involved in classic postdiarrheal HUS, virtually any organ can be affected.[119] The brain is most commonly involved; evidence of central nervous system (CNS) dysfunction occurs in approximately one third of cases. This includes alterations of consciousness (including complete coma) and disorders of movement, muscle tone, and posture. Seizures, usually generalized, occur commonly. In the past they were reported to occur in almost half of cases; however, now that the diagnosis of HUS is being made sooner, more attention is being paid to fluid and electrolyte balance, and dialysis is being initiated earlier, seizures occur in less than 20% of children.[119] In many cases they are metabolic in origin, although some may be due to ischemia from CNS microvascular involvement. In 3% to 5% of children, frank stroke or cerebral edema is present, and CNS involvement is the most common cause of death.[119]

Pancreatic TMA can result in pancreatitis, diabetes mellitus, or rarely, exocrine dysfunction.[119] Mild, transient hepatocellular damage occurs in approximately 40% of cases; heart, lung, muscle, skin, parotid gland, and retinal TMA occur only rarely (< 1% of cases).[119]

The 20-year population-based Utah study[126] showed that severe disease, defined as anuria lasting longer than 7 days, oliguria lasting longer than 14 days, or extrarenal structural damage (e.g., stroke), occurred in approximately 25% of cases[126] and was associated with age less than 2 years, anuria during the prodrome, and a white blood cell count greater than 20×10^9/L.

Management

Supportive Therapy

The mainstay of therapy continues to be meticulous attention to fluid and electrolyte balance; nutritional support; treatment of severe anemia; and control of hypertension, seizures, and azotemia. These management principles previously have been published in detail[117] and have not changed appreciably during recent decades; therefore, only a general overview is provided.

Once any needed repletion fluids have been given, the volume and composition of additional fluid should be limited to ongoing loss (i.e., insensible water loss plus urine and gastrointestinal loss). If the child is

both edematous and hyponatremic, no sodium should be given, and water administration is less than ongoing losses. Potassium should be withheld unless serum potassium levels reach the lower limits of normal. Vigilance must be maintained for the occurrence of hyperkalemia and, if present, treated promptly.[117]

Aggressive nutritional support is important because patients are almost always catabolic and hypoalbuminemic on admission to the hospital. If the diarrhea has resolved, enteral feeding can be tried. Poor appetite and a tendency to vomit may make nasojejunal tube feeding necessary. More often, ongoing diarrhea necessitates the use of total parenteral nutrition (TPN).

Packed red blood cell transfusions (10 mL/kg) are administered for severe anemia (i.e., hematocrit < 15%); sooner if the hematocrit is falling rapidly, or if the patient is symptomatically anemic. Platelet transfusions are restricted to those with severe bleeding or those who are about to have surgery or risky invasive vascular procedures (e.g., central-line placement). The administration of platelets may be deleterious because it provides substrate that may promote intravascular platelet aggregation and microthrombus formation.[41]

Hypertension is best treated with short-acting calcium channel blockers (e.g., nifedipine or nicardipine). The liquid contents are aspirated from a 10-mg nifedipine capsule and administered orally at 0.25 to 0.5 mg/kg/dose as needed every 2 to 6 hours. Alternatively, the powder contents of a 20-mg nicardipine capsule are suspended in 20 mL of liquid (e.g., apple juice) and administered orally at 0.5 to 1.0 mg/kg/dose as needed every 6 to 8 hours. Nicardipine is also given by constant intravenous infusion starting at a dose of 1.0 μg/kg/min.

Seizures are best treated initially with intravenous short-acting benzodiazopam medications (e.g., lorazepam), followed by intravenous phenobarbital or phenytoin. When using phenytoin, "free" levels must be monitored because azotemia decreases protein binding of the drug.

Clinicians agree that the widespread availability of dialysis largely accounts for today's lower mortality rates. There is a consensus that severe uncontrollable hyperkalemia, fluid overload with pulmonary edema, or the presence of significant uremic symptoms is an indication for starting dialysis. A wide range of opinion exists aside from these areas of agreement. Some clinicians have suggested that all patients with anuria or oliguria persisting for more than 24 hours should be started on dialysis[96] and that early peritoneal dialysis removes plasminogen activator inhibitor and thereby improves outcome.[11] Others claim that dialysis is often unnecessary, even in the context of severe azotemia.[114] A more moderate approach[117] is to initiate dialysis prophylactically for blood urea nitrogen concentrations of more than 150 mg/dL; this should be done sooner for severe hyperkalemia or fluid overload, clinical uremia (e.g., encephalopathy), or to allow initiation of full nutritional support (e.g., TPN). Whether HUS encephalopathy (e.g., seizures) is uremic in origin is often impossible to determine because it can occur indepen-

dently from severe azotemia. Even so, initiating dialysis if severe CNS dysfunction occurs is probably prudent.

Peritoneal dialysis is usually used in infants and preschool-age children unless severe colitis and abdominal tenderness are present. With the availability of small-sized hemodialysis equipment and vascular access catheters, young infants can now be hemodialyzed. Establishing vascular access sometimes is difficult and risky, however, especially if the infant is severely thrombocytopenic. Moreover, specially trained pediatric dialysis personnel must be available. High-dose intravenous furosemide therapy can be tried in an effort to decrease[9, 108] the need for dialysis, though the oliguria may be unresponsive.[9]

Specific Therapies

Several specific investigational strategies have been tried, including heparin, fibrinolytic and antiplatelet agents, prostacyclin infusions, intravenous IgG, vitamin E, and infusions of fresh frozen plasma (FFP). They have been found to be either too dangerous or noneffective.[117]

The use of plasma exchange (i.e., plasmapheresis) is an unsettled issue. It is certainly helpful in treating adults[104] with TTP. The best experience in children is being acquired in a prospective randomized multicenter study being conducted in Germany and Austria. Although their study population is not limited to classic postdiarrheal cases, plasma exchange seems to be helpful, especially in high-risk patients, in improving both the acute illness and the long-term sequelae.[86] Even so, a cautious approach is called for. Plasma exchange is expensive, requires vascular access, carries risks (especially when infusing FFP), and requires highly trained personnel.

Future Treatment Strategies

The most effective strategy would be to prevent ingestion of the organisms responsible for classic HUS. If this is not possible, effort should be directed toward interrupting the pathogenic cascade that begins with the ingestion of EHEC and culminates in damage to the kidneys and other vital organs.

One attractive approach is to inactivate the toxin before it can do harm. The Canadian Pediatric Kidney Disease Research Centre is conducting human testing of a compound (Synsorb-Pk) that contains the GB_3 receptor.[3] By administering this substance orally to children during the prodromal (diarrheal) stage of their illness, the toxins hopefully are bound to the material containing the GB_3 and thereby prevented from being absorbed into the circulation.

A related strategy would be to intravenously administer antibodies against the toxins (i.e., antitoxins) during the 5 to 7 days from the time the colitis begins until HUS develops. Antitoxin given during this window of opportunity might be able to inactivate the toxins in the circulation before they can be taken up by the GB_3 cell surface receptors.

The intravenous administration of anti-SLT-2 antibody to mice protects them from the effects of the toxin.[131] Unfortunately, commercial gamma globulin preparations usually show little neutralizing activity against SLT-2,[4] the more potent of the SLTs. Anti-SLT-1 and SLT-2 monoclonal antibodies have been developed and could be adapted for use in humans. Given the probable simultaneous participation of LPS in the pathogenic cascade, anti-LPS antibody administration might also be beneficial.

Time may still be available, even after the toxins have become attached to the cell surface receptors, to interrupt the pathogenic cascade and prevent full-blown TMA. Agents such as oxpentifylline that inhibit endotoxin-induced TNF production,[142] the ginkgolide mixture BN52063 that inhibits the effects of PAF,[43] and the recently described nonpeptide endothelin inhibitors[19] all have potential value. The new generation of platelet inhibitors (e.g., 7E3, c7E3) that are antibodies directed against the platelet surface fibrinogen receptor IIb/IIIa, and thereby block the final pathway for platelet aggregation, sound promising.[6]

Once TMA has occurred, tissue-type plasminogen activator might be helpful. Its successful use has been reported in a 4-year-old girl with HUS.[67] Because the microvascular thrombi are composed mostly of platelets (fibrin deposition is often scanty), however, the importance of facilitating fibrinolysis is uncertain.

Outcome

In the 20-year Utah population-based study,[126] 5% died, and an equal number of survivors were left with end-stage renal disease (ESRD) or chronic brain damage. Not surprisingly, severe disease during the acute phase was the best predictor of bad outcome. About one third of the 25% who experienced severe disease (i.e., prolonged oligoanuria and/or extrarenal structural damage) died or were left with severe sequelae.

Mortality in most recent series of classic postdiarrheal HUS has been approximately 5%,[17, 61, 77, 111, 126] and almost an equal number of survivors have developed ESRD.[61, 126, 141] Because renal failure can progress slowly over decades, the eventual incidence of ESRD cannot yet be determined. In a long-term (15–25 years) report from France,[37] ESRD eventually developed in four of 25 patients, 16 to 24 years after having experienced classic HUS; in two of the four, renal function had been normal at 10 years. Some preliminary data suggest that angiotensin converting enzyme inhibitors (e.g., enalapril) plus controlled intake of protein (to RDA) may help preserve renal function in those left with chronic renal damage.[28]

Recurrence of HUS in native kidneys is rare following postdiarrheal HUS and has happened in only three of 288 patients treated for postdiarrheal HUS in Utah from 1970 through 1994. One patient experienced two episodes of postdiarrheal HUS caused by *E coli* O157:H7[124] and two developed one or more nondiarrheal recurrences.[116]

Transplantation is the renal replacement modality of choice for HUS patients who develop ESRD. In a survey of six series[7, 31, 38, 47, 100, 128] that included a total of 86 transplants (range, 8–20), HUS recurred in 13% of the renal grafts (range 0%–41%); however, in three of the series (total of 41 patients) HUS did not recur.[7, 38, 100]

A substantial number of patients (30%–50%) sustain less severe chronic problems, such as hypertension, proteinuria, or azotemia.[61, 125, 126, 141] One half of the Utah survivors had chronic sequelae when evaluated one or more years (median 5.6 years) post-HUS; hypertension was present in 5.6%, approximately one third had proteinuria, and an equal number had a low glomerular filtration rate.[126] Combined proteinuria and low glomerular filtration rate was found in 12.5%, a group at high risk of eventually developing ESRD. The best predictor of chronic renal damage is the duration of anuria during the acute phase.[103, 125] In the Utah experience,[125] virtually all with anuria longer than 1 week or oliguria exceeding 2 weeks were left with chronic renal sequelae. Important to note, however, is that many who are left with chronic renal sequelae, albeit usually mild, have no history of either anuria or oliguria, and that abnormalities can sometimes appear following an interval of apparent recovery.[125]

On occasion, children may be left with chronic nonrenal related sequelae.[119] Colitis can cause bowel infarction (2%), resulting in the need for colectomy and colostomy, or stricture (3%), causing bouts of abdominal distention and diarrhea. Pancreatic involvement may result in diabetes mellitus (8%) that can be permanent; chronic exocrine dysfunction is rare (< 1%). Perhaps the most serious of the sequelae is stroke, which occurs in 3% to 5% of the HUS population.[119, 126] Sequelae resulting from the effects of TMA of the retina, heart, or lung occur very rarely (< 1%). Some preliminary evidence suggests that survivors may be at risk for chronic cognitive[91, 113] and behavioral[113] problems. These findings must be viewed cautiously because of small sample size[91, 113] and questions regarding appropriateness of the control group.[113]

NONDIARRHEAL HUS

Secondary HUS

The triad of HUS sometimes occurs secondarily to a variety of conditions shown in Table 1. The pathogenesis in most cases is obscure but is probably secondary to endothelial cell perturbation or to the direct effects on systems (e.g., PGI_2-TxA_2) that control prothrombotic-antithrombotic balance. Secondary HUS is rare in the pediatric age group. Only two cases have been reported of more than 300 HUS patients cared for in Utah during the past 25 years; both followed primary glomerulopathies.[120]

The most commonly reported examples in children follow *Streptococcus pneumoniae* infections of the lung and, rarely, the meninges.[32]

Bacterial neuroaminidase, present in the patient's plasma, exposes the cryptic Thomsen-Friedenreich antigen (T-antigen) that exists on the surfaces of red blood cells, platelets and endothelial cells. The T-antigen then reacts with anti-T antibody present in most normal sera,[79] resulting in hemolysis, thrombocytopenia, and acute nephropathy. These patients are usually found to have a positive Coombs' test during typing of blood for transfusion, and the use of blood products containing the anti-T antibody should be avoided.[32, 79]

Idiopathic HUS

Almost all nondiarrheal pediatric HUS is idiopathic, that is, unassociated with any identifiable antecedent factor or event. Some idiopathic cases are familial and can be inherited as either an autosomal dominant or recessive disorder.[51] Cases tend to be recurrent[23, 50] and are often associated with hypocomplementemia.[23, 50]

Nonfamilial (i.e., sporadic) nondiarrheal HUS probably also represents several subsets. Although many cases have been reported to follow upper respiratory infections or vomiting,[116] no firm evidence suggests a causal relationship. Moreover, nondiarrheal HUS can follow an episode of classic nondiarrheal disease.[116] Mortality rates have been higher, and ESRD more frequent, compared with nondiarrheal cases, in most,[34, 80, 99, 136] but not all,[61, 77, 116] prediarrheal series. Recurrence is frequent, however, and on average, has been reported to occur in approximately 20% of cases[34, 61, 69, 77, 99, 116, 136] (range 0%–78%). The regional variability in severity and outcome suggests that what is presently categorized as nondiarrheal HUS comprises a very heterogeneous mix of subsets.

Management

The same principles that were described for classic postdiarrheal HUS apply. It is important to remember, however, that only blood products free of T-antigen antibody should be given to children who have pneumococcal-related HUS. Because of generally poorer outcome for nondiarrheal patients, there has been enthusiasm for using therapies of unproven value. Although no randomized prospective-controlled trials have been performed, some evidence suggests that plasma exchange improves outcome but does not prevent relapse.[34] Important to remember, however, is that nondiarrheal patients do not uniformly experience bad outcome, and that one needs to know the natural history of nondiarrheal HUS in one's own region before deciding on the appropriateness of using this expensive and somewhat risky treatment modality.

SUMMARY

HUS is the most common cause of acute renal failure in infants and young children and follows a diarrheal prodrome about 90% of the time. Persuasive evidence shows that virtually all of postdiarrheal cases are caused by EHEC infections, and that the great majority of cases in the United States are caused by the EHEC serotype O157:H7.

Mortality is approximately 5%, and approximately 10% of survivors are left with severe sequelae. A much larger number (30%–50%) experience mild chronic renal damage. Public health strategies, including zero tolerance for fecal contamination in slaughter houses and additional public education on proper food handling and cooking, does much to decrease the prevalence of the syndrome.

Efforts to further dissect the postdiarrheal pathogenic cascade should continue, and an animal model needs to be developed. Only then will researchers be positioned to develop effective intervention strategies. Preventing life-threatening extrarenal complications, especially of the CNS, is a major challenge.

Idiopathic nondiarrheal HUS accounts for approximately 10% of cases and comprises a poorly understood composite of HUS subsets. Research directed toward a better understanding of these mysterious variants also is a priority for the years ahead.

References

1. Appiani AC, Edefonti A, Bettinelli A, et al: The relationship between plasma levels of the factor VIII complex and platelet release products (β-thromboglobulin and platelet factor 4) in children with the hemolytic-uremic syndrome. Clin Nephrol 17:195–199, 1982
2. Argyle JC, Hogg RJ, Pysher TJ, et al: A clinicopathological study of 24 children with hemolytic uremic syndrome. A report of the Southwest Pediatric Nephrology Study Group. Pediatr Nephrol 4:52–58, 1990
3. Armstrong GD, Rowe PC, Orrbine E, et al: Results of phase 1 study into the use of *Synsorb-PK* for preventing hemolytic-uremic syndrome (HUS). *In* Karmali MA, Goglio AG (eds): Recent Advances in Verocytotoxin-Producing Escherichia Coli Infections. Bergamo, Italy, Elsevier Science, 1994, pp 381–384
4. Ashkenazi S, Cleary TG, Lopez E, et al: Anticytotoxin-neutralizing antibodies in immune globulin preparations: Potential use in hemoytic-uremic syndrome. J Pediatr 113:1008–1014, 1988
5. Badami KG, Srivastava RN, Kumar R, et al: Disseminated intravascular coagulation in post-dysenteric haemolytic uraemic syndrome. Acta Paediatr Scand 76:919–922, 1987
6. Baker B: Agent found better than aspirin in reducing post-PTCA restenosis. Internal Medicine News & Cardiology News. November 15:2, 1992
7. Bassani CE, Ferraris J, Gianantonio CA, et al: Renal transplantation in patients with classical haemolytic-uraemic syndrome. Pediatr Nephrol 5:607–611, 1991
8. Belongia EA, Osterholm MT, Soler JT, et al: Transmission of Escherichia coli O157:H7 infection in Minnesota child day-care facilities. J Am Med Assoc 269:883–888, 1993
9. Ben-Ezer D, Veiga PA, James JA: Intravenous furosemide infusion decreases the need for dialysis in patients with hemolytic uremic syndrome (HUS). Pediatr Res 35:361A, 1994

10. Benigni A, Boccardo P, Noris M, et al: Urinary excretion of platelet-activating factor in haemolytic uraemic syndrome. Lancet 339:835–836, 1992
11. Bergstein JM, Riley M, Bang NU: Role of plasminogen-activator inhibitor type 1 in the pathogenesis and outcome of the hemolytic uremic syndrome. N Engl J Med 327:755–759, 1992
12. Bertani T, Abbate M, Zoja C, et al: Sequence of glomerular changes in experimental endotoxemia: A possible model of hemolytic uremic syndrome. Nephron 53:330–337, 1989
13. Besser RE, Lett SM, Weber JT, et al: An outbreak of diarrhea and hemolytic uremic syndrome from Escherichia coli O157:H7 in fresh-pressed apple cider. J Am Med Assoc 269:2217–2220, 1993
14. Bevilacqua MP, Pober JS, Majeau GR, et al: Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: Characterization and comparison with the actions of interleukin 1. Proc Natl Acad Sci USA 83:4533–4537, 1986
15. Bitzan M, Moeblus E, Ludwig K, et al: High incidence of serum antibodies to Escherichia coli lipopolysaccharide in children with hemolytic-uremic syndrome. J Pediatr 119:380–385, 1991
16. Booth L, Rowe B: Possible occupational acquisition of Escherichia coli O157 infection. (Letter). Lancet 342:1298–1299, 1993
17. Brandt JR, Fouser LS, Watkins SL, et al: *Escherichia coli* O157:H7-associated hemolytic-uremic syndrome after ingestion of contaminated hamburgers. J Pediatr 125:519–526, 1994
18. Brewster DH, Brown MI, Robertson D, et al: An outbreak of Escherichia coli O157 associated with a children's paddling pool. Epidemiol Infect 112:441–447, 1994
19. Brooks DP, DePalma PD, Gellai M, et al: Nonpeptide endothelin receptor antagonists. III. Effect of SB 209670 and BQ123 on acute renal failure in anesthetized dogs. JPET 271:769–775, 1994
20. Butler T, Aikawa M, Azad AK: Erythrocyte vacuolation in haemolytic anaemia during shigellosis (Letter). Lancet II(8550):111, 1987
21. Butler T, Islam MR, Azad MAK, et al: Risk factors for development of hemolytic uremic syndrome during shigellosis. J Pediatr 110:894–897, 1987
22. Caprioli A, Luzzi I, Rosmini F, et al: Hemolytic-uremic syndrome and vero cytotoxin-producing *Escherchia coli* infection in Italy. J Infect Dis 166:154–158, 1992
23. Carreras L, Romero R, Requesens C, et al: Familial hypocomplementemic hemolytic uremic syndrome with HLA-A3,B7 haplotype. J Am Med Assoc 245:602–604, 1981
24. Cieslak PR, Barrett TJ, Griffin PM, et al: Escherichia coli O157:H7 infection from a manured garden (Letter to the Editor). Lancet 342:367, 1992
25. Cimolai N, Basalyga S, Mah DG, et al: A continuing assessment of risk factors for the development of Escherichia coli O157:H7-associated hemolytic uremic syndrome. Clin Nephrol 42:85–89, 1994
26. Cimolai N, Carter JE, Morrison BJ: Risk factors for the progression of Escherichia coli O157:H7 enteritis to hemolytic-uremic syndrome. J Pediatr 116:589–592, 1990
27. Coratelli P, Buongiorno E, Passavanti G: Endotoxemia in hemolytic uremic syndrome. Nephron 50:365–367, 1988
28. Delgado N, Peñaloza J, Briones L, et al: Enalapril therapy in children with renal sequelae of hemolytic uremic syndrome (Abstract). Pediatr Nephrol 6:C165, 1992
29. Doyle MP, Schoeni JL: Isolation of Escherichia coli O157:H7 from retail fresh meats and poultry. Appl Environ Microbiol 53:2394–2396, 1987
30. Edwards DH, Griffith TM, Ryley HC, et al: Haptoglobin-haemoglobin complex in human plasma inhibits endothelium dependent relaxation: Evidence that endothelium derived relaxing factor acts as a local autocoid. Cardiovas Res 20:549–556, 1986
31. Eijgenraam FJ, Donckerwolcke RA, Monnens LAH, et al: Renal transplantation in 20 children with hemolytic-uremic syndrome. Clin Nephrol 33:87–93, 1990
32. Erickson LC, Smith WS, Biswas AK, et al: *Streptococcus pneumoniae*-induced hemolytic uremic syndrome: a case for early diagnosis. Pediatr Nephrol 8:211–213, 1994
33. Fitzpatrick MM, Shah V, Trompeter RS, et al: Interleukin-8 and polymorphoneu-

trophil leucocyte activation in hemolytic uremic syndrome of childhood. Kidney Int 42:951–956, 1992
34. Fitzpatrick MM, Walters MDS, Trompeter RS, et al: Atypical (non-diarrhea-associated) hemolytic-uremic syndrome in childhood. J Pediatr 122:532–537, 1993
35. Fong JSC, Kaplan BS: Impairment of platelet aggregation in hemolytic uremic syndrome: Evidence for platelet "exhaustion." Blood 60:564–570, 1982
36. Forsyth KD, Simpson AC, Fitzpatrick MM, et al: Neutrophil-mediated endothelial injury in haemolytic uraemic syndrome. Lancet 11:411–414, 1989
37. Gagnadoux MF, Habib R, Broyer M: Outcome of renal transplantation in 34 cases of childhood hemolytic-uremic syndrome and the role of cyclosporine. Transplant Proc 26:269–270, 1994
38. Gagnadoux MF, Habib R, Gubler MC, et al: Long-term (15–25 years) prognosis of childhood hemolytic-uremic syndrome (HUS) (Abstract). J Am Soc Nephrol 4:295, 1993
39. Gasser C, Gautier C, Steck A, et al: Hamolytisch-uramische syndrome: Bilaterale nierenindennekrosen bei akuten erworbenen hamolytischen anamien. Schweiz Med Wochenschr 85:905–909, 1955
40. Gómez-Garre D, Guerra M, González E, et al: Aggregation of human polymorphonuclear leukocytes by endothelin: Role of platelet-activating factor. Eur J Pharmacol 224:167–172, 1992
41. Gordon LI, Kwaan HC, Rossi EC: Deleterious effects of platelet transfusions and recovery thrombocytosis in patients with thrombotic microangiopathy. Seminars Hematol 24:194–201, 1987
42. Griffin PM, Tauxe RV: The epidemiology of infections caused by Escherichia coli O157:H7, other enterohemorrhage *E. coli*, and the associated hemolytic uremic syndrome. Epid Reviews 13:60–98, 1991
43. Guinot P, Summerhayes C, Berdah L, et al: Treatment of adult systemic mastocytosis with a PAF-acether antagonist BN52063 (Letter). Lancet II(8602):114, 1988
44. Hancock DD, Besser TE, Kinsel ML, et al: The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol Infect 113:199–207, 1994
45. Harel Y, Silva M, Giroir B, et al: A reporter transgene indicates renal-specific induction of tumor necrosis factor (TNF) by Shiga-like toxin: Possible involvement of TNF in hemolytic uremic syndrome. J Clin Invest 92:2110–2116, 1993
46. Harlan JM, Harker LA, Reidy MA, et al: Lipopolysaccharide-mediated bovine endothelial cell injury in vitro. Lab Invest 48:269–274, 1983
47. Hébert D, Kim EM, Sibley RK, et al: Post-transplantation outcome of patients with hemolytic-uremic syndrome: Update. Pediatr Nephrol 5:162–167, 1991
48. Jernigan SM, Waldo FB: Racial incidence of hemolytic uremic syndrome. Pediatr Nephrol 8:545–547, 1994
49. Jevnikar AM, Brennan DC, Singer GG, et al: Stimulated kidney tubular epithelial cells express membrane associated and secreted TNFα. Kidney Int 40:203–211, 1991
50. Kaplan BS: Hemolytic uremic syndrome with recurrent episodes: An important subset. Clin Nephrol 8:495–498, 1977
51. Kaplan BS: Recurrent hemolytic uremic syndrome. *In* Kaplan BS, Trompeter RS, Moake JL (eds): Hemolytic Uremic Syndrome and Thrombotic Thrombocytopenic Purpura. New York, Marcel Dekker, 1992, pp 151–162.
52. Kaplan BS, Mills M: Elevated serum elastase and alpha-1-antitrypsin levels in hemolytic uremic syndrome. Clin Nephrol 30:193–196, 1988
53. Kaplan BS, Thomson PD: Hyperuricemia in the hemolytic-uremic syndrome. Am J Dis Child 130:854–856, 1976
54. Kaplan BS, Cleary TG, Obrig TG: Recent advances in understanding the pathogenesis of the hemolytic uremic syndrome. Pediatr Nephrol 4:276–283, 1990
55. Kaplan BS, Gale D, Ipp T: Hyperlipidemia in the hemolytic-uremic syndrome. Pediatr 47:776–779, 1971
56. Karmali MA, Arbus GS, Petric M, et al: Hospital-acquired Escherichia coli O157:H7 associated haemolytic uraemic syndrome in a nurse (Letter to the Editor). Lancet 1:526, 1988
57. Karmali M, Petric M, Lim C: The association between idiopathic hemolytic uremic

syndrome and infection by verotoxin-producing *Escherichia coli.* J Infect Dis 151:775–782, 1985
58. Karmali MA, Petric M, Steele BT, et al: Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing Escherichia coli in stools. Lancet I(8325):619–620, 1983
59. Karpman D, Andreasson A, Thysell H, et al: Serum and urinary cytokines in childhood hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. *In* Karmali MA, Goglio AG (eds): Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections. Bergamo, Italy, Elsevier Science BV, 1994, pp 369–372
60. Keene WE, McAnulty JM, Hoesly FC, et al: A swimming-associated outbreak of hemorrhagic colitis caused by Escherichia coli O157:H7 and Shigella sonnei. N Engl J Med 331:579–584, 1994
61. Kelles A, Van Dyck M, Proesmans W: Childhood haemolytic uraemic syndrome: long-term outcome and prognostic features. Eur J Pediatr 153:38–42, 1994
62. Keusch GT: Receptor-mediated endocytosis of Shigella cytotoxin. *In* Middlebrook JL, Kohn LD (eds): Receptor-Mediated Binding and Internalization of Toxins and Hormones. New York, Academic Press, 1981, pp 95–112
63. Kinney JS, Gross TP, Porter CC, et al: Hemolytic-uremic syndrome: A population-based study in Washington, DC and Baltimore, Maryland. Am J Public Health 78:64–65, 1988
64. Koenig DW, Barley-Maloney L, Daniel TO: A western blot assay detects autoantibodies to cryptic endothelial antigens in thrombotic microangiopathies. J Clin Immunol 13:204–211, 1993
65. Kohan DE, Padilla EC, Siegler RL: Endothelin concentrations and endothelin stimulating activity in the plasma of children with post-diarrheal hemolytic uremic syndrome. *In* Karmali MA, Goglio AG (eds): Recent Advances in Verocytotoxin-Producing *Escherichia Coli* Infections. Bergamo, Italy, Elsevier Science, BV, 1994, pp 377–380
66. Kovacs MJ, Cameron W: Epidemiology of the hemolytic uremic syndrome (Letter). N Engl J Med 324:1065, 1991
67. Kruez W, Linde R, Becker S, et al: Successful treatment of haemolytic-uraemic syndrome with recombinant tissue-type plasminogen activator (Letter). Lancet 341:1665–1666, 1993
68. Leung DYM, Moake JL, Havens PL, et al: Lytic anti-endothelial cell antibodies in haemolytic-uraemic syndrome. Lancet II(8604):183–186, 1988
69. Levin M, Walters MDS, Barratt TM: Hemolytic uremic syndrome. Adv Pediatr Infect Dis 4:51–82, 1989
70. Levy M, Gagnadoux MF, Habib R: Pathology of hemolytic-uremic syndrome in children. *In* Remuzzi G, Mecca G, de Gaetano G (eds): Hemostatis, Prostaglandins, and Renal Disease. New York, Raven Press, 1980, pp 383–397
71. Lingwood CA: Verotoxin-binding in human renal sections. Nephron 66:21–28, 1994
72. Li Volti S, Di Giacomo C, Garozzo R, et al: Impaired antioxidant defense mechanisms in two children with hemolytic uremic syndrome. Ren Fail 15:523–528, 1993
73. López EL, Contrini MM, De Rosa S, et al: Tumor necrosis factor (TNF-α) levels in hemolytic uremic syndrome (HUS) patients and children with bloody diarrhea (BD) in Argentina. *In* Karmali MA, Goglio AG (eds): Recent Advances in Verocytotoxin-Producing *Escherichia Coli* Infections. Bergamo, Italy, Elsevier Science, BV, 1994, pp 373–376
74. López EL, Diaz M, Grinstein S, et al: Hemolytic uremic syndrome and diarrhea in Argentine children: The role of Shiga-like toxin. J Infect Dis 160:469–475, 1989
75. Louise CB, Obrig TG: Human renal microvascular endothelial cells as a potential target in the development of the hemolytic uremic syndrome as related to fibrinolysis factor expression, in vitro. Microvascular Research 47:377–387, 1994
76. Marks S, Roberts T: *E. coli* O157:H7 ranks as the fourth most costly foodborne disease. Food Review 16:51–59, 1993
77. Martin DL, MacDonald KL, White KE, et al: The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. N Engl J Med 323:1161–1167, 1990
78. McCoy RC, Abramowsky CR, Krueger R: The hemolytic uremic syndrome, with positive immunofluorescence studies. J Pediatr 85:170–174, 1974

79. McGraw ME, Lendon M, Stevens RF, et al: Haemolytic uraemic syndrome and the Thomsen Friedenreich antigen. Pediatr Nephrol 3:135–139, 1989
80. Milford DV, Taylor CM, Guttridge B, et al: Haemolytic uraemic syndromes in the British Isles 1985–8: Association with verocytotoxin producing *Escherichia coli.* Part 1: Clinical and epidemiological aspects. Arch Dis Child 65:716–721, 1990
81. Monnens L, van Aken W, de Jong M: "Active" intravascular coagulation in the epidemic form of hemolytic-uremic syndrome. Clin Nephrol 17:284–287, 1982
82. Monnens L, van de Meer W, Langenhuysen C, et al: Platelet aggregating factor in the epidemic form of hemolytic-uremic syndrome in childhood. Clin Nephrol 24:135–137, 1985
83. Morgan D, Newman CP, Hutchinson DN, et al: Verotoxin producing *Escherichia coli* O157 infections associated with the consumption of yogurt. Epidemiol Infect 111:181–187, 1993
84. Morgan GM, Newman C, Palmer SR, et al: First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O157:H7 in the UK. Epidemiol Infect 101:83–91, 1988
85. Moschcowitz E: An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries. Arch Intern Med 36:89–93, 1925
86. Müller-Wiefel DE, Tönshoff B, Bulla M, et al: The beneficial effect of therapeutic plasma exchange (TPE) on the clinical course of children with hemolytic uremic syndrome (HUS) (Abstract). Pediatr Nephrol 6:C165, 1992
87. Muramatsu Y, Oikawa T, Akashi S, et al: Plasma endothelin levels and blood pressure in the children with hemolytic uremic syndrome (Abstract). Pediatr Nephrol 7:C76, 1993
88. Noris M, Benigni A, Siegler R, et al: Renal prostacyclin biosynthesis is reduced in children with hemolytic-uremic syndrome. Am J Kidney Dis 20:144–149, 1992
89. Obrig TG, Moran TP, Brown JE: The mode of action of Shiga toxin on peptide elongation of eukaryotic protein synthesis. Biochem J 244:287–294, 1987
90. Olavarria F, Mezzano S, Ardiles L, et al: Presence of circulating immune complexes in the classic form of hemolytic uremic syndrome: A constant finding. Nephron 52:313–316, 1989
91. Orme SF, Clark E, Siegler RL: Neuropsychological sequelae of post-diarrheal hemolytic uremic syndrome encephalopathy (Abstract). Program and Abstracts of the Second International Symposium and Workshop on "Verocytotoxin (Shiga-like toxin)-producing *Escherichia coli* infections" 1994, p 81
92. Ostroff SM, Kobayashi JM, Lewis JH: Infections with *Escherichia coli* O157:H7 in Washington State: The first year of statewide disease surveillance. J Am Med Assoc 262:355–359, 1989
93. Pavia AT, Nichols C, Tauxe RV, et al: Hemolytic uremic syndrome during an outbreak of *E. coli* O157:H7 infection in institutions for the retarded: Clinical and epidemiologic. J Pediatr 116:544–551, 1990
94. Perez N, Rahman R, Zalba J, et al: Haemolytic uraemic syndrome and unpasteurized milk (Letter). Acta Paediatr 83:142, 1994
95. Pickering LK, Obrig TG, Stapleton FB: Hemolytic-uremic syndrome and enterohemorrhagic *Escherichia coli.* Pediatr Infect Dis J 13:459–476, 1994
96. Piel CF: Hemolytic uremic syndrome (Editorial). West J Med 123:486–488, 1975
97. Proesmans W, VanCauter A, Thijs L, et al: Plasma renin activity in haemolytic uraemic syndrome. Pediatr Nephrol 8:444–446, 1994
98. Read SC, Gyles CL, Clarke RC, et al: Prevalence of verocytotoxigenic *Escherichia coli* in ground beef, pork, and chicken in southwestern Ontario. Epidemiol Infect 105:11–20, 1990
99. Renaud C, Niaudet P, Gagnadoux MF, et al: Haemolytic uraemic syndrome: Prognostic factors in children over 3 years of age. Pediatr Nephrol 9:24–29, 1995
100. Repetto HA, Vazquez LA, Calvo P, et al: Renal transplantation in children with idiopathic "classic" hemolytic uremic syndrome (idioHUS) (Abstract). Pediatr Nephrol 3:C185, 1989
101. Reymond D, Burnens A, Lior H, et al: Verotoxin producing *Escherichia coli* bacteremia associated with urosepsis and hemolytic uremic syndrome (Abstract). Program and

Abstracts, Second International Symposium and Workshop on Verocytotoxin (Shiga-like toxin)-Producing *Escherichia coli* Infections, Bergamo, Italy, 1994, p 79
102. Riley LW, Remis RS, Helgerson SD, et al: Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med 308:681–685, 1983
103. Robson WLM, Leung AKC, Kaplan BS: Hemolytic-uremic syndrome. Curr Probl Pediatr 23:16–33, 1993
104. Rock GA, Shumak KH, Buskard NA, et al: Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. N Engl J Med 325:393–397, 1991
105. Rogers MF, Rutherford GW, Alexander SR, et al: A population-based study of hemolytic-uremic syndrome in Oregon, 1979–1982. Am J Epidemiol 123:137–142, 1986
106. Rose PE, Armour JA, Williams CE, et al: Verotoxin and neuraminidase induced platelet aggregating activity in plasma: Their possible role in the pathogenesis of the haemolytic uraemic syndrome. J Clin Pathol 38:438–441, 1985
107. Roth RI: Hemoglobin enhances the production of tissue factor by endothelial cells in response to bacterial endotoxin. Blood 83:2860–2865, 1994
108. Rousseau E, Blais N, O'Regan S: Decreased necessity for dialysis with loop diuretic therapy in hemolytic uremic syndrome. Clin Nephrol 34:22–25, 1990
109. Rowe PC, Orrbine E, Lior H, et al: Diarrhoea in close contacts as a risk factor for childhood haemolytic uraemic syndrome. The CPKDRC co-investigators. Epidemiol Infect 110:9–16, 1993
110. Rowe PC, Orrbine E, Ogborn M, et al: Epidemic *Escherichia coli* O157:H7 gastroenteritis and hemolytic-uremic syndrome in a Canadian Inuit community: Intestinal illness in family members as a risk factor. J Pediatr 124:21–26, 1994
111. Rowe PC, Orrbine E, Wells GA, et al: Epidemiology of hemolytic-uremic syndrome in Canadian children from 1987 to 1988. J Pediatr 119:218–224, 1991
112. Schlegel N, Moake J, Loirat C, et al: Childhood hemolytic uremic syndrome (HUS): Platelet aggregating activity (PAA) and von Willebrand factor (vWF) studies. Pediatr Nephrol 2:C118, 1988
113. Schlieper A, Rowe PC, Orrbine E, et al: Sequelae of haemolytic uraemic syndrome. Arch Dis Child 67:930–934, 1992
114. Schulman SL, Kaplan BS: Nonoliguric renal failure and significant azotemia in children with HUS: Is there a need for dialysis? (Abstract). Program and Abstracts, Second International Symposium and Workshop on "Verocytotoxin (Shiga-like toxin)-Producing *Escherichia coli* Infections," 1994, p 85
115. Serebruany VL, Christenson MJ, Pescetti J, et al: Hypoproteinemia in the hemolytic-uremic syndrome of childhood. Pediatr Nephrol 7:72–73, 1993
116. Siegler RL: Atypical hemolytic uremic syndrome: A comparison with post-diarrheal disease. J Pediatr, in press
117. Siegler RL: Management of hemolytic-uremic syndrome. J Pediatr 112:1014–1020, 1988
118. Siegler RL: Prostacyclin in the hemolytic uremic syndrome. J Nephrol 6:64–69, 1993
119. Siegler RL: Spectrum of extrarenal involvement in postdiarrheal hemolytic-uremic syndrome. J Pediatr 125:511–518, 1994
120. Siegler RL, Brewer ED, Pysher TJ: Hemolytic uremic syndrome associated with glomerular disease. Am J Kidney Dis 13:144–147, 1989
121. Siegler RL, Christofferson RD, Edwin SS, et al: Urinary cyclic GMP as a measure of endothelin derived relaxation factor (EDRF) in the hemolytic uremic syndrome (Abstract) J Am Soc Nephrol 2:274, 1991
122. Siegler RL, Edwin SS, Christofferson RD, et al: Endothelin in the urine of children with the hemolytic uremic syndrome. Pediatr 88:1063–1066, 1991
123. Siegler RL, Edwin SS, Christofferson RD, et al: Plasma and urinary cytokines in the childhood hemolytic uremic syndrome (HUS) (Abstract). JASN 2:274, 1991
124. Siegler RL, Griffin PM, Barrett TJ, et al: Recurrent hemolytic uremic syndrome secondary to *Escherichia coli* O157:H7 infection. Pediatr 91:666–668, 1993
125. Siegler RL, Milligan MK, Burningham TH, et al: Long-term outcome and prognostic indicators in the hemolytic-uremic syndrome. J Pediatr 118:195–200, 1991
126. Siegler RL, Pavia AT, Christofferson RD, et al: A 20 year population-based study of postdiarrheal hemolytic uremic syndrome in Utah. Pediatr 94:35–40, 1994

127. Situnayake RD, Crump BJ, Thurnham DI, et al: Further evidence of lipid peroxidation in post-enteropathic haemolytic-uraemic syndrome. Pediatr Nephrol 5:387–392, 1991
128. Stevenson JA, Dumke A, Glassock RJ, et al: Thrombotic microangiopathy: Recurrence following renal transplant and response to plasma infusion. Am J Nephrol 2:227–231, 1981
129. Swerdlow DL, Woodruff BA, Brady RC, et al: A waterborne outbreak in Missouri of O157:H7 associated with bloody diarrhea and death. Ann Intern Med 117:812–819, 1992
130. Tarr PI, Hickman RO: Hemolytic uremic syndrome epidemiology: A population-based study in King County, Washington, 1971 to 1980. Pediatr 80:41–45, 1987
131. Tesh VL, Burris JA, Owens JW, et al: Comparison of the relative toxicities of Shiga-like toxins type I and type II for mice. Infect Immun 61:3392–3402, 1993
132. Tesh VL, O'Brien AD: Adherence and colonization mechanisms of enteropathogenic and enterohemorrhagic *Escherichia coli*. Microbiol Pathogenesis 12:245–254, 1992
133. Tesh VL, Samuel JE, Burris JA, et al: Quantitation and localization of shiga toxin/shiga-like toxin-binding glycolipid receptors in human and baboon tissues. *In* Karmali MA, Goglio AG (eds): Recent Advances in Verocytotoxin-Producing *Escherichia Coli* Infections. Bergamo, Italy, Elsevier Science, BV, 1994, pp 189–192
134. Tolins JP, Palmer RMJ, Moncada S, et al: Role of endothelium-derived relaxing factor in regulation of renal hemodynamic responses. Am J Physiol 258:H655–H662, 1990
135. Tönshoff B, Momper R, Kühl PG, et al: Increased thromboxane biosynthesis in childhood hemolytic uremic syndrome. Kidney Int 37:1134–1141, 1990
136. Trompeter RS, Schwartz R, Chantler C, et al: Haemolytic-uraemic syndrome: An analysis of prognostic features. Arch Dis Child 58:101–105, 1983
137. Turi S, Nemeth I, Vargha I, et al: Oxidative damage of red blood cells in haemolytic uraemic syndrome. Pediatr Nephrol 8:26–29, 1994
138. van de Kar NACJ, Sauerwein R, Demacker P, et al: Plasma cytokine levels in the hemolytic uremic syndrome (Abstract). Program and Abstracts, Second International Symposium and Workshop on Verocytotoxin (Shiga-like toxin)-Producing *Escherichia coli* Infections, Bergamo, Italy, 1994, p 84
139. van de Kar NACJ, Monnens LA, Karmali MA, et al: Tumor necrosis factor and interleukin-1 induce expression of the verocytotoxin receptor globotriaosylceramide on human endothelial cells: Implications for the pathogenesis of the hemolytic uremic syndrome. Blood 80:2755–2764, 1992
140. van der Poll T, van Deventer SJ, Pasterkamp G, et al: Tumor necrosis factor induces von Willebrand factor release in healthy humans. Thromb Haemost 67:623–626, 1992
141. Van Dyck M, Proesmans W, Depraetere M: Hemolytic-uremic syndrome in childhood: Renal function ten years later. Clin Nephrol 29:109–112, 1988
142. Waage A, Sorensen M, Stordal B: Differential effect of oxpentifylline on tumour necrosis factor and interleukin-6 production (Letter). Lancet 335:543, 1990
143. Walters MDS, Levin M, Smith C, et al: Intravascular platelet activation in the hemolytic uremic syndrome. Kidney Int 33:107–115, 1988
144. Waterspiel JN, Ashkenazi S, Morrow AL, et al: Effect of subinhibitory concentrations of antibiotics on extracellular Shiga-like toxin I. Infection 20:25–29, 1992
145. Wells JG, Shipman LD, Greene KD, et al: Isolation of *Escherichia coli* O157:H7 and other shiga-like toxin producing *E. coli* from diary cattle. J Clin Micro 29:985–989, 1991
146. Westberg G, Shultz PJ, Raij L: Exogenous nitric oxide prevents endotoxin-induced glomerular thrombosis in rats. Kidney Int 46:711–716, 1994
147. Wilson JB, Clarke RC, Renwick S, et al: Verocytotoxigenic *Escherichia coli* infection on dairy farms in southern Ontario. *In* Karmali MA, Goglio AG (eds): Recent Advances in Verocytotoxin-Producing *Escherichia Coli* Infections. Bergamo, Italy, Elsevier Science BV, 1994, pp 61–64
148. Zoja C, Casiraghi F, Morigi M, et al: Shiga toxin modulates prostacyclin (PGI2) release in human renal microvascular endothelial cells (HRMEC) (Abstract). Program and Abstracts, Second International Symposium and Workshop on Verocytotoxin (Shiga-like toxin)-producing *Escherichia coli* infections, Bergamo, Italy 1994, p 90
149. Zoja C, Corna D, Farina C, et al: Verotoxin glycolipid receptors determine the

localization of microangiopathic process in rabbits given verotoxin-1. J Lab Clin Med 120:229–238, 1992

150. Zoja C, Morigi M, Foppolo M, et al: Verotoxin-1 (VT-1) increases leukocyte adhesion to endothelial cells under dynamic flow conditions. *In* Karmali MA, Goglio AG (eds): Recent Advances in Verocytotoxin-Producing *Escherichia Coli* Infections. Bergamo, Italy, Elsevier Science BV, 1994, pp 329–332

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cal activity and risk of type 2 diabetes in women: a prospective study. *JAMA.* 1999; 282:1433-1439.
9. Pate RR, Pratt M, Blair SN, et al. Physical activity and public health: a recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA.* 1995;273:402-407.
10. Lee IM, Paffenbarger RS Jr. Associations of light, moderate, and vigorous intensity physical activity with longevity: the Harvard Alumni Health Study. *Am J Epidemiol.* 2000;151:293-299.
11. Food and Nutrition Board, Institute of Medicine. *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein And Amino Acids (Macronutrients).* Washington, DC: National Academy Press; 2002.
12. Ainsworth BE, Leon AS, Richardson MT, et al. Accuracy of the College Alumnus Physical Activity Questionnaire. *J Clin Epidemiol.* 1993;46:1403-1411.
13. Wolf AM, Hunter DJ, Colditz GA, et al. Reproducibility and validity of a self-administered physical activity questionnaire. *Int J Epidemiol.* 1994;23:991-999.
14. Chasan-Taber S, Rimm EB, Stampfer MJ, et al. Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. *Epidemiology.* 1996;7:81-86.
15. Jakicic JM, Marcus BH, Gallagher KI, Napolitano M, Lang W. Effect of exercise duration and intensity on weight loss in overweight, sedentary women: a randomized trial. *JAMA.* 2003;290:1323-1330.
16. McTiernan A, Kooperberg C, White E, et al. Recreational physical activity and the risk of breast cancer in postmenopausal women: the Women's Health Initiative Cohort Study. *JAMA.* 2003;290:1331-1336.
17. Lee I-M, Oguma Y. Physical activity. In: Schottenfeld D, Fraumeni JF Jr, eds. *Cancer Epidemiology and Prevention.* 3rd ed. San Francisco, Calif: Oxford University Press. In press.
18. Thune I, Brenn T, Lund E, Gaard M. Physical activity and the risk of breast cancer. *N Engl J Med.* 1997;336:1269-1275.
19. Lee IM, Skerrett PJ. Physical activity and all-cause mortality: what is the dose-response relation? *Med Sci Sports Exerc.* 2001;33:S459-S471.

Postdiarrheal Shiga Toxin–Mediated Hemolytic Uremic Syndrome

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POSTDIARRHEAL SHIGA TOXIN–MEDIATED HEMOLYTIC uremic syndrome (HUS) is the major cause of acute renal failure in infants and young children[1] and is a substantial cause of mortality and chronic morbidity. Adolescents who develop postdiarrheal HUS fare as well as younger children[2] but adults, especially elderly individuals, experience a higher incidence of death and disability.[3]

There are no treatments of proven value, and care during the acute phase of the illness, which is merely supportive, has not changed substantially during the past 30 years. Although US mortality for infants and young children decreased markedly following the widespread availability of pediatric dialysis units and intensive care facilities, 3% to 5% of patients who develop HUS still die during the acute phase.[1] An equal number have severe brain damage or end-stage renal disease (ESRD)[1,4] and require chronic renal replacement therapy (dialysis or renal transplantation) within the first few years. A much larger proportion (30%-50%)[1,4] sustain less severe renal sequelae (proteinuria, low glomerular filtration rate [GFR], or both) but approximately 10% of this group have both overt proteinuria and low GFR[4] and are therefore at high risk[1] for experiencing a slow loss of renal function[5] and eventual ESRD due to hyperfiltration injury.[6]

Shiga toxins 1 and 2 are the most important virulence factors for development of postdiarrheal HUS and are required for disease expression. Shiga toxins produced by enterohemorrhagic *Escherichia coli* (EHEC) are very potent subunit cytotoxins that were previously referred to as Shigalike toxins because of their similarity to the prototypic Shiga toxin produced by Shigella dysenteriae type 1. Enterohemorrhagic *E coli* colonize the large intestine via a distinctive attaching and effacing lesion that provides a tight junction between the EHEC and the surface of the intestinal epithelial cells. Toxins then translocate into the circulation, probably facilitated by the influx (transmigration) of neutrophils,[7] which increases paracellular permeability.

Shiga toxin–mediated HUS is almost always preceded by colitis, which usually becomes hemorrhagic, but can occur without a diarrheal prodrome[8] or can be subsequent to a urinary tract infection caused by Shiga toxin–producing strains of *E coli*.[9] Once in the circulation, there is evidence that Shiga toxin is transported by neutrophils[10] to the kidneys, where it is transferred and bound via the toxin's B-subunits to neutral glycolipid globotriaosylceramide receptors on target cells (eg, glomerular endothelial and tubular epithelial cells). It is assumed that specific organ involvement depends on the presence of globotriaosylceramide receptors. The toxin is then internalized via receptor-mediated endocytosis and routed to the endoplasmic reticulum.[11] The single A subunit enzymatically inactivates the ribosomes via depurination of the 28S ribosomal RNA component resulting in a blockade of peptide elongation.[12] When protein synthesis is inhibited, the cell dies or is severely damaged. This leads to glomerular endothelial cell swelling and detachment from the underlying basement membrane with secondary activation of both platelets and the coagulation cascade. This sequence of events results in the classic lesions that characterize the thrombotic microangiopathy of Shiga toxin–mediated HUS. Apoptosis also may

See also pp 1337 and 1360.

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play a role in the tissue injury.[13] Although the major extraintestinal target organ is the kidney, virtually any organ (eg, brain, heart, lungs, pancreas) can be involved.[14]

Current research is slowly determining the causes of disease expression following exposure to Shiga toxin–producing EHEC. Epidemiological observations suggest that EHEC, which produces Shiga toxin 2, are more likely to lead to HUS than is infection with EHEC, which produces only Shiga toxin 1.[15] These epidemiological observations have been corroborated by cell culture,[16] mice,[17] and baboon experiments.[18] Moreover, baboon experiments suggest that disease expression depends not only on the amount and type of Shiga toxin that enters the circulation but the rapidity of absorption. An amount of toxin that causes fatal HUS if administered rapidly results in little or no illness if the same total amount of toxin is administered slowly.[19]

However, in the baboon model, the prior administration of lipopolysaccharide can prime the animal to develop severe HUS following the administration of otherwise subtoxic amounts of Shiga toxin.[20] This augmented host response is mediated by promoting the release of prothrombotic von Willebrand Factor,[21] inhibiting production of the antithrombotic prostaglandin, prostacyclin,[22] and by upregulating Shiga toxin receptor expression (R.L.S., unpublished data, 2003). There is also evidence in human postdiarrheal HUS that lipopolysaccharide enters the circulation and plays a role in the pathogenesis of the syndrome; antibodies to lipopolysaccharide can be demonstrated in up to 90% of patients with Shiga toxin–mediated HUS.[23] Lipopolysaccharide is a cell membrane product of gram-negative organisms; the lipid A portion of the molecule is responsible for its biological activity. It has a strong affinity for cell membranes and, unlike Shiga toxin that requires globotriaosylceramide, interacts with a number of eukaryotic cells, including platelets and endothelial cells.[24] There is evidence from humans[25] and baboons[20] that this priming of the host may be mediated by production of lipopolysaccharide-induced proinflammatory and procoagulant cytokines. Three such cytokines that appear to have particular relevance based on cell culture, clinical, and animal studies are tumor necrosis factor α, IL-6, and IL-8. These cytokines are produced by a number of cells including macrophages, monocytes, and renal tubular epithelial cells[26-28] in response to lipopolysaccharide, Shiga toxin, or both.

This issue of THE JOURNAL contains 2 articles that deal with postdiarrheal HUS. Trachtman and colleagues[29] report the results of a well designed and executed multicenter double-blind clinical trial designed to determine if a Shiga toxin binding agent, composed of particles of silicon linked to the globotriaosylceramide molecule, orally administered after the diagnosis of diarrhea-associated HUS had been made, could favorably affect the disease course and outcome. Their hypothesis was reasonable, namely that the intestinal absorption of Shiga toxin does not occur as a one-time event but continues even after the onset of the syndrome. Therefore, decreasing ongoing intestinal Shiga toxin absorption due to its binding with an oral agent should be beneficial.

Although the study design did not permit controlling for a number of variables between the experimental and placebo groups, the study was as well executed as reasonably possible given the number of centers involved and the many undeterminable and uncontrollable variables, especially center-to-center treatment variability. The conclusion was that the oral-binding agent was not beneficial. Trachtman et al offer a number of possible explanations for its ineffectiveness. An attractive hypothesis is that, given the nature of the tight EHEC-intestinal epithelial interface that permits absorption of Shiga toxin directly into the intestinal microvasculature, there probably is little free-floating toxin left within the intestinal lumen to bind to the agent.

The second article by Garg and colleagues[30] reports a meta-analysis of 49 published studies (3476 patients) describing long-term renal sequelae (defined as overt proteinuria, low GFR, or hypertension) in patients (aged 1-22 years) who survived an episode of postdiarrheal HUS. Their finding that death or ESRD occurs in 12% of patients with HUS is not dissimilar from outcomes reported from most single-center studies. However, their reported 25% prevalence of long-term renal sequelae is somewhat less than reported in the majority of single-center studies conducted during the past 20 years.[1]

As with all meta-analyses, this study has several limitations. For instance, the study was not limited to population-based studies and is therefore subject to referral bias. Moreover, Garg et al chose to include studies from the 1950s, a time when pediatric intensive care units and dialysis for infants were not widely available. In addition, treatment from center to center was heterogeneous and the mean time from HUS to analysis of outcome was only 4.4 years. This time interval is too short for an accurate estimation of eventual ESRD, which can evolve over decades.[5] Garg et al appropriately acknowledge that, "For all outcomes, the variability between studies was larger than would be expected by chance," and warn that the results of "mathematically pooled results should be interpreted with caution."[30]

The disappointing results of Trachtman et al[29] leave prevention as the main approach to decreasing the morbidity and mortality described by Garg et al.[30] A multifaceted approach is required that includes novel ways of decreasing the EHEC carrier rate in livestock and implementing a zero-tolerance policy for contaminated foods and beverages. This may require giving the US Department of Agriculture more resources and authority relative to the slaughtering of livestock and the processing of meat products, along with additional mandatory and verifiable safe food handling and preparation practices in restaurants (especially fast food establishments), as well as more efforts to educate the public about food safety.

Although these measures should decrease the incidence of hemorrhagic colitis and subsequent HUS, which develops in approximately 10% of those individuals with EHEC-mediated diarrhea,[31] these measures will not eliminate the

incidence completely. *Escherichia coli* that produce Shiga toxin, hemorrhagic colitis, and HUS are ubiquitous and are not limited to domestic livestock. The bacteria have been found in dozens of other animals, water supplies, and fruits and vegetables (especially those fertilized with manure). Person-to-person spread also continues to be a common mode of infection.[32] Therefore, it is imperative to continue to develop and test vaccines for both children and livestock. This will take time and there will be opposition relative to the cost-effectiveness of immunizing children and cattle against a disease that annually affects only about 1.5 children per 100000 persons younger than 18 years.[4]

Research designed to dissect and interrupt the complex pathogenic cascade that begins with ingestion of Shiga toxin-producing strains of *E coli* and culminates in HUS needs to be expanded. One novel approach in mice has been to use molecular decoys such as orally administered harmless recombinant bacteria that display a Shiga toxin receptor on its surface that in turn binds the toxin in the gut.[33] Another approach has been to subcutaneously inject mice with multivalent receptor-based inhibitors (globotriaosylceramide analogues) that bind and neutralize the biologic activity of Shiga toxin that has already gained access to the circulation.[34] Others have ameliorated disease in pigs by the intraperitoneal injection of Shiga toxin antibody as late as 4 days following the intraperitoneal injection of Shiga toxin.[35] Some investigators have focused on downstream events in the pathogenic cascade, such as blocking Shiga toxin–induced production of prothrombotic and proinflamatory factors (eg, cytokines) thought to be important in mediating disease expression. Examples have included agents that block activation of genes responsible for production of cytokines following Shiga toxin–mediated cellular perturbation (D.E. Kohan, MD, PhD, oral communication, May 7, 2003).

Although much can be learned from subprimate animal models, the use of a primate model in which disease expression and response to therapy resemble that observed in humans is essential to corroborate these intriguing subprimate observations. Without this, strategies designed to interrupt the pathogenic cascade will continue to be elusive and EHEC will remain an important cause of death and disability.

REFERENCES

1. Siegler RL. The hemolytic uremic syndrome. *Pediatr Clin North Am.* 1995;42:1505-1529.
2. Siegler R, Pavia A, Cook J. Hemolytic uremic syndrome in adolescents. *Arch Pediatr Adolesc Med.* 1997;151:165-169.
3. Melnyk AMS, Solez K, Kjellstrand CM. Adult hemolytic-uremic syndrome: a review of 37 cases. *Arch Intern Med.* 1995;155:2077-2084.
4. Siegler RL, Pavia AT, Christofferson RD, Milligan MK. A 20 year population-based study of postdiarrheal hemolytic uremic syndrome in Utah. *Pediatrics.* 1994;94:35-40.
5. Gagnadoux M-F, Habib R, Gubler MC, et al. Long-term (15-25 years) outcome of childhood hemolytic-uremic syndrome. *Clin Nephrol.* 1996;46:39-41.
6. Brenner BM, Mackenzie HS. Nephron mass as a risk factor for progression of renal disease. *Kidney Int Suppl.* 1997;63(suppl):S124-S127.
7. Hurley BP, Thorpe CM, Acheson DW. Shiga toxin translocation across intestinal epithelial cells is enhanced by neutrophil transmigration. *Infect Immun.* 2001;69:6148-6155.
8. Caprioli A, Luzzi I, Rosmini F, et al. Hemolytic-uremic syndrome and vero cytotoxin-producing *Escherichia coli* infection in Italy. *J Infect Dis.* 1992;166:154-158.
9. Starr M, Bennett-Wood V, Bigham AK, et al. Hemolytic-uremic syndrome following urinary tract infection with enterohemorrhagic *Escherichia coli*: case report and review. *Clin Infect Dis.* 1998;27:310-315.
10. Te Loo DM, van Hinsbergh VW, van den Heuvel LP, Monnens LA. Detection of verocytotoxin bound to circulating polymorphonuclear leukocytes of patients with hemolytic uremic syndrome. *J Am Soc Nephrol.* 2001;12:800-806.
11. Sandvig K, Garred O, Prydz K, et al. Retrograde transport of endocytosed Shiga toxin to the endoplasmic reticulum [letter]. *Nature.* 1992;358:510-512.
12. Obrig TG, Moran TP, Brown JE. The mode of action of Shiga toxin on peptide elongation of eukaryotic protein synthesis. *Biochem J.* 1987;244:287-294.
13. Karpman D, Hakansson A, Perez M-TR, et al. Apoptosis of renal cortical cells in the hemolytic-uremic syndrome. *Infect Immun.* 1998;66:636-644.
14. Siegler RL. Spectrum of extrarenal involvement in postdiarrheal hemolytic-uremic syndrome. *J Pediatr.* 1994;125:511-518.
15. Scotland SM, Willshaw GA, Smith HR, Rowe B. Properties of strains of *Escherichia coli* belonging to serogroup O157 with special reference to production of Vero cytotoxins VT1 and VT2. *Epidemiol Infect.* 1987;99:613-624.
16. Louise CB, Obrig TG. Specific interaction of *Escherichia coli* O157:H7-derived Shiga-like toxin II with human renal endothelial cells. *J Infect Dis.* 1995;172:1397-1401.
17. Tesh VL, Burris JA, Owens JW, et al. Comparison of the relative toxicities of Shiga-like toxins type I and type II for mice. *Infect Immun.* 1993;61:3392-3402.
18. Siegler RL, Obrig TG, Pysher TJ, et al. Response to Shiga toxin 1 and 2 in a baboon model of hemolytic uremic syndrome. *Pediatr Nephrol.* 2003;18:92-96.
19. Siegler RL, Pysher TJ, Tesh VL, Taylor FB Jr. Response to single and divided doses of Shiga toxin-1 in a primate model of hemolytic uremic syndrome. *J Am Soc Nephrol.* 2001;12:1458-1467.
20. Siegler RL, Pysher TJ, Lou R, Tesh VL, Taylor Jr FB. Response to Shiga toxin-1, with and without lipopolysaccharide, in a primate model of hemolytic uremic syndrome. *Am J Nephrol.* 2001;21:420-425.
21. Pysher TJ, Siegler RL, Tesh VL, Taylor FB Jr. von Willebrand factor expression in a Shiga toxin-mediated primate model of hemolytic uremic syndrome. *Pediatr Dev Pathol.* 2002;5:472-479.
22. Siegler RL, Pysher TJ, Tesh VL, Taylor FB. Renal prostacyclin biosynthesis in a baboon model of Shiga toxin mediated hemolytic uremic syndrome. *Nephron.* 2002;92:363-368.
23. Bitzan M, Moeblus E, Ludwig K, et al. High incidence of serum antibodies to *Escherichia coli* lipopolysaccharide in children with hemolytic-uremic syndrome. *J Pediatr.* 1991;119:380-385.
24. Morrison D, Oades Z, Di Pietro D. Endotoxin induced calcium-dependent platelet secretion: implications for generalized membrane perturbations. In: Agarwal MK, ed. *Bacterial Endotoxins and Host Response.* New York, NY: Elsevier/North-Holland Biomedical Press; 1980:311-325.
25. Karpman D, Adreasson A, Thysell H, et al. Cytokines in childhood hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. *Pediatr Nephrol.* 1995;9:694-699.
26. Pirotzky E, Delattre RM, Hellegouarch A, et al. Interleukin-6 production by tumor necrosis factor and lipopolysaccharide-stimulated rat renal cells. *Clin Immunol Immunopathol.* 1990;56:271-279.
27. Maeda K, Abello PA, Abraham MR, et al. Endotoxin induces organ-specific endothelial cell injury. *Shock.* 1995;3:46-50.
28. Hughes AK, Stricklett PK, Kohan DE. Cytotoxic effect of Shiga toxin-1 on human proximal tubule cells. *Kidney Int.* 1998;54:426-437.
29. Trachtman H, Cnaan A, Christen E, et al, for the Investigators of the HUS-SYNSORB Pk Multicenter Clinical Trial. Effect of an oral Shiga toxin–binding agent on diarrhea-associated hemolytic uremic syndrome in children: a randomized controlled trial. *JAMA.* 2003;290:1337-1344.
30. Garg AX, Suri RS, Barrowman N, et al. Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: a systematic review, meta-analysis, and meta-regression. *JAMA.* 2003;290:1360-1370.
31. Robson WL, Leung AK, Kaplan BS. Hemolytic-uremic syndrome. *Curr Probl Pediatr.* 1993;23:16-33.
32. Rowe PC, Orrbine E, Lior H, Wells GA, McLaine PN. Diarrhoea in close contacts as a risk factor for childhood haemolytic uraemic syndrome: the CPKDRC Co-investigators. *Epidemiol Infect.* 1993;110:9-16.
33. Paton A, Morona R, Paton J. A new biologic agent for treatment of Shiga toxigenic *Escherichia coli* infections and dysentery in humans. *Nat Med.* 2000;6:265-270.
34. Mulvey G, Marcato P, Kitov P, et al. Assessment in mice of the therapeutic potential of tailored, multivalent Shiga toxin carbohydrate ligands. *J Infect Dis.* 2003;187:640-649.
35. Matise I, Cornick N, Booher S, et al. Intervention with Shiga toxin (Stx) antibody after infection by Stx-producing *Eshcerichia coli*. *J Infect Dis.* 2001;183:347-350.

PREVALENCE OF NON-O157 ENTEROHAEMMORRHAGIC ESCHERICHIA COLI IN RETAIL GROUND BEEF IN THE UNITED STATES

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Purpose Enterohaemorrhagic *Escherichia coli (*EHEC) are associated with human diseases ranging from diarrhea to hemolytic-uremic syndrome (HUS). E. coli O157:H7 is the EHEC most often isolated in patients in the US causing an estimated 73,000 illnesses annually. Non-O157 EHEC also causes illness and outbreaks. More than 37,000 illnesses are attributed to non-O157 EHEC serotypes. In the US the most common non-O157 EHEC serogroups in humans are O26, O45, O10, O111, O121, and O145. The incidence of non-0157 EHEC is increasing despite limited laboratory testing. The purpose of this study was to determine the prevalence of non-O157 EHEC in retail ground beef in the US.

Materials and Methods Meat samples were selectively enriched for *E. coli* O157, EHEC, and Salmonella spp. then tested by IEH Polymerase Chain Reaction (PCR) test kits. Sample enrichments screened positive by PCR were culture confirmed by isolating the target organisms. The organisms were confirmed and samples were scored as positive.

Each sample (200g) was weighed into a sterile Whirlpak, 69oz. filter bag followed by the addition of 200-250ml IEH Enrichment Broth. The sample was macerated by hand homogenizing. An additional portion of IEH Enrichment Broth was added to bring broth volume to 500ml. The bag was mixed until uniformly homogenized. Enrichment bags were incubated at 42˚C for 24 hours. To minimize contamination, after enrichment incubation time was completed, the broth was well mixed and a 10ml aliquot was removed to sterile test tube as working stock.

Aliquots of enrichment were tested for typical gene signals using IEH-*E. coli* EHEC Test System. Presumptive positive cultures were subjected to cultural confirmation. Presumptive colonies (lactose positive, *E. coli* type colonies) were picked to a gridded Washed Sheep Blood Vancomycin-Cefsulodin-Cefixime (BVCC) plate to test for hemolysin activity. All presumptive target positive colonies were tested by PCR test kit for gene markers indicative of EHEC. Colonies testing positive for EHEC by PCR were checked for purity on MacConkey agar, confirmed as latex agglutination negative for *E. coli* O157, and confirmed by PCR for E. coli serotype specific markers. Colonies confirmed as EHEC by both PCR and negative latex agglutination were scored as positive.

Results and Discussions A total of 1216 retail ground beef samples were tested for the presence of EHEC. Twenty-three samples (1.9%) were positive for non-O157 EHEC strains. Serotypes included O26 (n=6), O103 (n=7), O113 (n=1), O121 (n=6) and O145 (n=3). All but the EHEC isolate serotype O113 were *Stx* and *eae* positive. The O113 strain was $Stx2d_{act}$ and Subtilase positive.

Prevalence of 1.9% non-9157 EHEC in the retail ground beef supply shows the need for public health agencies in the US to increase awareness regarding these pathogens. The data clearly show that clinical and public health laboratories should routinely screen human and environmental specimens for the presence non-O157 EHEC.

LEXSEE 870 F. SUPP. 143

TEXAS FOOD INDUSTRY ASSOCIATION, TEXAS INDEPENDENT MEAT PACKERS ASSOCIATION, TEXAS RETAIL ASSOCIATION FOOD COUNCIL, AMERICAN MEAT INSTITUTE, FOOD MARKETING INSTITUTE, NATIONAL AMERICAN WHOLESALE GROCERS ASSOCIATION/INTERNATIONAL FOODSERVICE DISTRIBUTORS ASSOCIATION, NATIONAL GROCERS ASSOCIATION VS. MIKE ESPY, Secretary, United States Department of Agriculture and MICHAEL TAYLOR, Administrator, Food Safety and Inspection Service of the United States Department of Agriculture

CIVIL NO. A-94-CA-748 JN

UNITED STATES DISTRICT COURT FOR THE WESTERN DISTRICT OF TEXAS, AUSTIN DIVISION

870 F. Supp. 143*; *1994 U.S. Dist. LEXIS 18038

December 13, 1994, Decided
December 13, 1994, FILED

COUNSEL: [**1] For TEXAS FOOD INDUSTRY ASSOCIATION, TEXAS INDEPENDENT MEAT PACKERS ASSOCIATION, TEXAS RETAIL ASSOCIATION FOOD COUNCIL, AMERICAN MEAT INSTITUTE, FOOD MARKETING INSTITUTE, NATIONAL-AMERICAN WHOLESALE GROCERS' ASSOCIATION/INTERNATIONAL FOODSERVICE DISTRIBUTORS ASSOCIATION, NATIONAL GROCERS ASSOCIATION, plaintiffs: David L. Orr, Johnson & Gibbs, Austin, TX. Gary Jay Kushner, Hogan & Hartson, Washington, DC. William A. Bradford, Jr., Mark D Dopp, Pierre M. Donahue, Hogan & Hartson, Washington, DC.

For MIKE SECRETARY OF THE U.S. DEPARTMENT OF AGRICULTURE, MICHAEL TAYLOR, Administrator, Food Safety and Inspection Service of the United States Department of Agriculture, defendants: Sylvia T. Kaser, Department of Justice, Washington, DC.

JUDGES: JAMES R. NOWLIN, UNITED STATES DISTRICT JUDGE

OPINION BY: JAMES R. NOWLIN

OPINION

[*144] **ORDER**

Before the Court is Plaintiffs' Motion for Preliminary Injunction, filed November 3, [*145] 1994. Defendants' filed their Opposition to Plaintiffs' Motion for Preliminary Injunction on November 16, 1994 and Plaintiffs filed their Reply Brief in Support of their Motion for Preliminary Injunction on November 23, 1994. Additionally, several trade associations have submitted an *Amici Curiae* [**2] brief in support of Plaintiffs' Motion for Preliminary Injunction and several consumer groups have submitted an *Amici Curiae* brief in opposition. After reviewing the arguments of counsel, the applicable law, and the entire record in this case, the Court is of the opinion that Plaintiffs' Motion for Preliminary Injunction should be DENIED.

I. BACKGROUND

On October 17, 1994, the United States Department of Agriculture ("USDA") announced a new *Escherichia Coli 0157:H7 ("E. Coli")* sampling program, to be conducted by the Food Safety and Inspection Service ("FSIS"). The notice announced that the FSIS would collect and test five thousand (5,000) samples of raw ground beef from federally-inspected establishments and retail stores. Any of these samples testing positive for the pathogen *E. Coli* would be treated as "adulterated" under the Federal Meat Inspection Act ("FMIA") and referred to FSIS headquarters for regulatory action. [1] Prior to this announcement, the USDA had treated patho-

gen-contaminated [2] neat as unadulterated under the FMIA.

> 1 Under the FMIA, the USDA is authorized to inspect meat and meat products to assure consumer that they are "wholesome, not *adulterated*, and properly marked, labeled, and packaged." *21 U.S.C. §§ 602-05* (emphasis added). A product is "adulterated" under the statute if "it bears or contains any poisonous or deleterious substance which may render it injurious to health; but in case the substance is not an added substance, such article shall not be considered adulterated under this clause if the quantity of such substance in or on such article does not ordinarily render it injurious to health . . . . " Id. at *§ 601(m)(1)*. When a meat food product is found to be adulterated, it shall be marked as "inspected and condemned" and "shall be destroyed for human food purposes." Id. at § 606. The statute prohibits the sale, transportation, offer for sale of transportation, or receipt for transportation of any adulterated meat food product. Id. at § 610. Violations of this restriction could result in criminal prosecution. Additionally, adulterated products are liable to be seized and condemned in a judicial proceeding. Id. at 673.

[**3]

> 2 Pathogens are food-borne bacteria such as *E. Coli* and *Salmonella.*

On November 1, 1994, several supermarket and meat-industry organizations [3] brought this action seeking to prevent the USDA from conducting its *E. Coli* sampling program. Plaintiffs argue that the USDA failed to adhere to the notice-and-comment procedure required by the Administrative Procedure Act ("APA") and move this Court for a temporary and permanent injunction. Plaintiffs also contend that the sampling program is an arbitrary and capricious exercise of agency authority and that it exceeds the USDA's statutory authority under the FMIA.

> 3 The Plaintiff's include the Texas Food industry Association, Texas Independent Meat Packers Association, Texas Retail Association Food Council, American Meat Institute, Food Marketing Institute, National-American Wholesale Grocers' Association/International Foodservice Distributors Association, and the National Grocers Association.

[**4] II. FINDINGS OF FACTS AND CONCLUSIONS OF LAW

In order to obtain a preliminary injunction, the Fifth Circuit requires the movant for a preliminary injunction to prove the following four elements:

> (1) a substantial likelihood of success on the merits; (2) a substantial threat of irreparable injury if the injunction is not issued; (3) that the threatened injury to the movant outweighs any damage the injunction might cause to the opponent; and (4) that the injunction will not disserve the public interest.

Lakedreams v. Taylor, 932 F.2d 1103, 1107 (5th Cir. 1991); see also *Enterprise International, Inc. v. Corporacion Estatal Petrolera Ecuatoriana, 762 F.2d 464, 471 (5th Cir. 1985)*. If the movant fails to sufficiently prove any of these elements, the preliminary injunction should be denied.

In evaluating the first element, whether there is a substantial likelihood of success on the merits, the nature of the Plaintiffs complaint must first be determined. Plaintiffs [*146] do not contest that the USDA has statutory authority to conduct sampling and testing; however, Plaintiffs do attack the USDA's *E. Coli* sampling program [**5] because it treats *E. Coli* as an adulterant under the FMIA. Plaintiffs claim that the USDA's decision to treat *E. Coli* as an adulterant should be enjoined because it 1) violates the APA 2) is arbitrary and capricious and 3) exceeds USDA's statutory authority. The Court will address each of these arguments in turn.

A. Violation of the APA

Under the APA, government agencies may issue rules only after the notice-and-comment procedures enumerated in the statute are completed. *5 U.S.C. § 553*. It is undisputed that the USDA's sampling program was promulgated without engaging in those procedures. However, USDA argues that notice-and-comment requirements do not apply in this case by virtue of *§ 553 (b)(3)(A)* which carves out an exception for "interpretive rules, general statements of policy, or rules of agency organization, procedure, or practice." *5 U.S.C. § 553(b)(3)(A)*. According to USDA, its *E. Coli* sampling program is nothing more than an inspection program which qualifies as a "procedural rule" under *§ 553 (b)(3)(A)*. In the alternative, USDA argues that its decision to consider *E. Coli* an adulterant [**6] under the FMIA is an interpretive rule intended to advise the meat and grocery industries of the agency's position on the law. [4]

4 In categorizing the agency's action, it is important to remember that "the court . . . must determine the category into which the rule falls. 'The label that the particular agency puts upon its given exercise of administrative power is not . . . conclusive; rather it is what the agency does in fact.'" *Brown Express, Inc. v. United States, 607 F.2d 695, 702 (5th Cir. 1979)*.

In determining whether an agency's action requires notice-and-comment, courts have recognized that there are no bright-line rules used to determine whether an agency's action fits into one of the enumerated exceptions. E.g. *Avoyelles Sportsmen's League, Inc. v. Marsh, 715 F.2d 897, 909 (5th Cir. 1983)* (stating that the "categories have 'fuzzy perimeters' and establish 'no general formula'") (citations omitted). Rather, "it has fallen to the courts to discern [**7] the line through the painstaking exercise of hopefully, sound judgment." *Community Nutrition Institute v. Young, 260 U.S. App. D.C. 294, 818 F.2d 943, 946 (D.C. Cir. 1987)*.

In the present case, the process is further complicated because the agency action in question, is actually comprised of two distinct parts. The first component is the agency's decision to test raw ground beef for the *E. Coli* pathogen and the second is its decision to treat *E. Coli* contaminated meat as "adulterated" under the FMIA. [5]

5 For ease of discussion, the Court will address each of these parts separately. Although these actions are part of the same "rule," they are theoretically distinct and could have been promulgated independently. Therefore, if each distinct part does not require notice-and-comment, there is nothing to suggest that notice-and-comment would be required merely because they were promulgated simultaneously.

As for the first part, it appears to be undisputed that USDA [**8] has the authority to test for *E. Coli* without engaging in notice-and-comment rulemaking. [6] The FMIA authorizes the USDA to conduct testing and this aspect USDA's announcement appears to be a "procedural rule" in that it merely informs the industry of the procedures it will use to conduct this testing. Furthermore, in determining whether a rule is in fact procedural, the real inquiry has been described as whether a rule will have a "substantial impact" on those regulated. *Brown Express, Inc. v. United States, 607 F.2d 695, 702 (5th Cir. 1979)*. Since the testing program is conducted and funded by FSIS, it does not compel any specific industry action. In fact, there is nothing about the agency's decision to test for *E. Coli*, by itself, that imposes any kind of burden on the industry. Therefore, USDA was not required to conduct notice-and-comment procedures before implementing its plan to test raw ground beef for *E. Coli*. See *U.S. Dept. of Labor v. Kast Metals Corp., 744 F.2d 1145, 1151-52* (5th cir. 1984) [*147] (holding that an inspection plan properly fit the "procedural rule" exception to the notice-and-comment requirement [**9] of the APA).

6 The Plaintiffs in this case do not challenge the USDA's decision to conduct sampling and testing for *E. Coli*. Indeed, the evidence indicates that such testing has been occurring for several years. Rather, Plaintiffs challenge the USDA's testing in conjunction with its treatment of *E. Coli* as an adulterant.

The more difficult question is whether the second part of USDA's announcement, its decision to treat *E. Coli* as an adulterant, violated the APA's notice-and-comment requirement. The Court agrees with the Plaintiffs that this decision may have a substantial impact on the regulated industry. Therefore, it does not qualify as a "procedural rule." Furthermore, the USDA's announcement does not appear be a "general statement of policy." See, e.g., *Brown, 607 F.2d at 701* (describing general statements of policy as "announcements to the public of the policy which the agency hopes to implement in future rulemakings or adjudications. A general statement of policy [**10] like a press release, presages an upcoming rulemaking or announces the course which the agency intends to follow in future adjudications") (citing *Pacific Gas & Electric Co. v. FPC, 164 U.S. App. D.C. 371, 506 F.2d 33, 38 (D.C. Cir. 1974)*; *Batterton v. Marshall, 208 U.S. App. D.C. 321, 648 F.2d 694, 706* (stating that an agency action was not a general policy statement because it left "no room for further exercise of administrative discretion"). Therefore, unless the Court finds that USDA's decision to consider *E. Coli* an adulterant is an "interpretive rule," the agency violated the notice-and-comment requirements of the APA.

In describing the distinction between "interpretive rules" and rules requiring notice-and-comment ("substantive rules"), courts have stated that "'substantive rules . . . are those which create law, usually implementary to an existing law; whereas interpretative rules are statements as to what the administrative officer thinks the statute or regulation means.'" *Brown, 607 F.2d at 700* (quoting *Gibson Wine Co. v. Snyder, 90 U.S. App. D.C. 135, 194 F.2d 329, 331 (D.C. Cir. 1952)*. [**11] The District of Columbia Circuit presented the best reconciliation of the case law on this issue when it stated:

> Accordingly, insofar as our cases can be reconciled at all, we think it almost exclusively on the bases of whether the purported interpretive rule has "legal effect", which in turn is best ascertained by asking (1) whether in the absence of the rule there would not be an adequate legislative basis for enforcement action or other agency action to confer benefits or ensure the performance of duties, (2) whether the agency has published the rule in the Code of Federal Regulations, (3) whether the agency has explicitly invoked its general legislative authority, or (4) whether the rule effectively amends a prior legislative rule. If the answer to any of these questions is affirmative, we have a [substantive], not an interpretive rule.

American Mining Congress v. Mine Safety & Health Administration, 302 U.S. App. D.C. 38, 995 F.2d 1106, 1112 (D.C. Cir. 1993).

In the present case, the Court concludes that the USDA's decision to consider *E. Coli* as an adulterant is an interpretive rule. The FMIA does not require the USDA to [**12] engage in substantive rulemaking as a predicate to considering a particular substance an adulterant. *Young v Community Nutrition Institute, 476 U.S. 974, 981-83, 90 L. Ed. 2d 959, 106 S. Ct. 2360 (1986).* Therefore, the "agency has the discretion to proceed through case-by-case adjudication and interpretive orders, rather than through the rulemaking process." *Marsh, 715 F.2d at 909*. Furthermore, the USDA has neither published the rule in the Code of Federal Regulations, nor invoked its general legislative authority. Finally, although the rule does constitute a change in USDA's interpretation of the FMIA, it does not effectively amend a prior legislative rule. [7]

> 7 The Plaintiffs in this action claim that the new rule implicitly amends the Safe Handling regulations recently promulgated by the USDA. However, those regulations simply require the industry to attach warning labels. Nothing in those regulations speaks to whether a pathogen contaminated product may be shipped or sold under the FMIA.

[**13] Based on the foregoing, the Court holds that the USDA did not violate the APA. Under the factors enumerated in American Mining Congress, USDA's decision to consider *E. Coli* an adulterant is an "interpretive rule." Furthermore, its decision to conduct sampling and testing constitutes a "procedural rule." Therefore, the Court concludes that, as a whole, the USDA's *E. Coli* sampling [*148] program is exempted from the notice-and-comment requirement of the APA.

B. Arbitrary and Capricious

In order to find that an agency action is arbitrary and capricious, the Court must find that there is *no rational basis* for the agency action. E.g. *American Petroleum Institute v. E.P.A., 858 F.2d 261, 264 (5th Cir. 1988)* (emphasis added). The agency's decision is entitled to a presumption of regularity and a Court is not empowered to substitute its judgment for that of the agency. *Citizens to Preserve Overton Park, Inc. v. Volpe, 401 U.S. 402, 415-16, 28 L. Ed. 2d 136, 91 S. Ct. 814 (1971).* However, the presumption "is not to shield [the agency's] action from a thorough, probing, in-depth review." [**14] Id.

Plaintiffs in this case claim that USDA's action is arbitrary and capricious because it will not achieve its intended purpose. Among other reasons, they allege that testing is prohibitively expensive and that the industry is already doing all it can to control the problem. They also argue that USDA has singled out a particular product (raw ground beef) and a particular pathogen (*E. Coli)* with no adequate explanation. They allege that other pathogens currently pose greater health risks. Finally, Plaintiffs contends that USDA's action is arbitrary and capricious because it abandons long-standing USDA policy without adequate explanation.

In response, Defendants argue that their program has already begun to achieve its intended purpose of spurring industry to use preventive measures. They also present evidence of emerging scientific and public health data which they claim justifies their focus on *E. Coli* in raw ground beef. Finally, they argue that their change in policy is due to the emerging nature of the evidence in this area.

After reviewing the evidence and arguments presented by the Parties, the Court finds that the Defendants *E. Coli* sampling program was not [**15] arbitrary and capricious. There is certainly a rational basis for the USDA to conduct some sort of testing in order to educate itself about this problem. Furthermore, the evidence indicates that the program has been at least partially successful in spurring industry to take greater preventive measures. Moreover, in light of the common cooking practices of most Americans, there is at least a rational basis for treating *E. Coli* differently than other pathogens. Finally, the Court finds that the Defendants' changing policy is a rational response to an emerging problem.

C. Statutory Authority Under the FMIA

The Plaintiffs final claim is that USDA's sampling program exceeds its statutory authority. The "'interpretation given [a] statute by the officers or agency charged with its administration' is entitled to substantial deference." *Marsh, 715 F.2d at 919* (citations omitted). "Regardless of whether the court would have arrived at the same interpretation, if the agency's interpretation is reasonable the court must respect it." Id.

Here, the Plaintiffs do not dispute the Defendants' general authority to test raw ground beef for *E. Coli*, rather [**16] Plaintiffs contest the Defendants' decision to consider *E. Coli* as an "adulterant" under the FMIA. Plaintiffs' primary argument in this regard is that *E. Coli* contaminated ground beef is not adulterated because it is only injurious to health if improperly cooked. However, after reviewing the evidence submitted by the Parties, the Court disagrees.

Under the FMIA, a product is "adulterated" if "it bears or contains any poisonous or deleterious substance which may render it injurious to health; but in case the substance is not an added substance, such article shall not be considered adulterated under this clause if the quantity of such substance in or on such article does not ordinarily render it injurious to health . . . . " *21 U.S.C. § 601(m) (1)* (emphasis added). In construing this language, Courts have held that other pathogens, such as *Salmonella*, are not adulterants. See, e.g., *American Public Health Assoc. v. Butz, 167 U.S. App. D.C. 93, 511 F.2d 331, 334 (5th Cir. 1975)*. In reaching this conclusion, the court in Butz relied upon the fact that ordinary methods of cooking and preparing [**17] food kills the *Salmonella* pathogen. See Id. (stating that "American housewives and cooks normally [*149] are not ignorant or stupid and their methods of preparing and cooking of food do not ordinarily result in salmonellosis"). However, unlike other pathogens, it is not "proper" cooking but "thorough" cooking that is necessary to protect consumers from *E. Coli.* The evidence submitted by Defendants indicates that many Americans consider ground beef to be properly cooked rare, medium rare, or medium. The evidence also indicated that *E. Coli* contaminated ground beef cooked in such a manner may cause serious physical problems, including death. Therefore, *E. Coli* is a substance that renders "injurious to health" what many Americans believe to be properly cooked ground beef. Based on this evidence, the Court finds that *E. Coli* fits the definition of an adulterant under the FMIA.

III. CONCLUSION

Pursuant to the foregoing analysis, the Court finds that Plaintiffs do not have a substantial likelihood of success on merits. Therefore, the Court is of the opinion that Plaintiffs' motion should be denied. [8]

8 Since the merits issue is determinative in this case, the Court declines to address whether Plaintiffs have satisfied the other three requirements for a preliminary injunction.

[**18] ACCORDINGLY IT IS ORDERED that Plaintiffs' Motion for Preliminary Injunction is hereby DENIED.

SIGNED AND ENTERED this 13th day of DECEMBER, 1994.

JAMES R. NOWLIN

UNITED STATES DISTRICT JUDGE