

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. 09-03

SUPPLEMENTAL STATEMENT
OF ADDITIONAL GROUNDS

CITIZEN PETITION

Submitted by:

Marler Clark LLP, PS

Outbreak, Inc.

The Family of June Dunning

Megan Richards

Shiloh Johnson

May 7, 2010

Philip Derfler, Assistant Administrator
Office of Policy and Program Development
USDA-Food Safety and Inspection Service
1400 Independence Avenue, SW
Room 350-E Jamie Whitten Building
Washington, DC 20250

I. INTRODUCTION

A petition was filed on October 5, 2009 that requested 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). What follows is a statement of additional grounds in support of the petition. As stated in our letter to FSIS dated February 12, 2010, our intention was to submit supplemental information as it became available or was identified so that you would have as much information as possible in acting on the petition. Please let us know if you need any further clarification or guidance regarding the following materials.

II. STATEMENT OF ADDITIONAL GROUNDS

As noted in the petition, numerous studies have been published in recent years that detail the dangers of non-O157:H7 EHEC. It has come to our attention, however, that research documenting the dangers of non-O157:H7 EHEC is not a new development. Two studies listing these dangers were authored nearly a decade and a half ago by former Assistant Commissioner for Food Protection for the U.S. Food & Drug Administration, David Acheson. A third study was published by the Centers for Disease Control and Prevention in 2007.

A. Study Recognizing non-O157:H7 STEC as a Looming Danger¹

In 1996, David Acheson and Gerald Keusch published a paper entitled “Which Shiga Toxin-Producing Types of *E. coli* Are Important?” The paper focused on the dangers of Shiga Toxin-Producing *E. coli* as a whole, recognizing that *E. coli* O157:H7 is not the only potentially deadly strain of *E. coli*. Addressing the limited focus of other studies, Dr. Acheson and Dr. Keusch stated that “we cannot let ourselves be complacent in thinking that *E. coli* O157:H7 is the only Shiga toxin-producing microorganism that can cause problems.” They went on to state that “while the emphasis in the United States has been on serotype O157:H7, approximately 100 other *E. coli* serotypes not only produce Shiga toxins but have been isolated from patients with [hemorrhagic colitis] or [hemolytic uremic syndrome].”

The paper then details European studies that, even in the early 1990s, showed that non-O157:H7 STEC infections were occurring at alarmingly high rates. According to the paper:

In [a] Belgian study group, the overall rate for STEC [was] about 1% among the 10,241 stool specimens that were analyzed in this study. In that positive subset, 38% were O157:H7 but 62% were non-O157 serotypes (comprising 22 different O:H types).

...

Elsewhere in Europe, several non-O157 serotypes including O1:NM, O5:NM, O18:H?, O26:H11, and O111:NM, have been isolated from patients with HUS in the Czech Republic. Another serotype, O103:H2 was isolated from patients following an outbreak of HUS in France. Meanwhile, in Italy, O111:H– and O26 were isolated from similar patients.

...

[T]he variety of non-O157:H7 strains responsible for North American outbreaks that investigators are isolating could well rival that seen throughout Europe, South America, and elsewhere.²

¹ Acheson, D. W. K., Keusch, G. 1996. Which Shiga Toxin-Producing Types of *E. coli* Are Important?. ASM News. 62:6:302-06.

² *Id.* at 303.

Even in 1996, the source of STEC was no mystery. As stated in the paper, “Cattle are considered to be the major reservoir of EHEC, including O157:H7 and other serotypes.” The paper also recognized the need for expanded testing programs. As stated by Dr. Acheson and Dr. Keusch, “reliance on a test which detects only a single serotype does not seem wise.”

The paper concludes with an ominous recognition of the pathogenicity of various *E. coli* serotypes. It states:

Studies from around the world support the notion that many of the non-O157:H7 serotypes lead to HUS and death. For example, the O111 serotypes seem to be as virulent as O157:H7...[I]t seems prudent to assume that any patient infected with Shiga toxin-producing infections is at risk for developing HUS or TTP.

B. STEC Detection Study³

Shortly following his paper detailing the importance of non-O157 STEC, Dr. Acheson published a paper entitled “Detection of Shiga-Like Toxin-Producing *Escherichia coli* in Ground Beef and Milk by Commercial Enzyme Immunoassay.” The paper detailed the feasibility of testing for STEC in ground beef and milk, stating that “it is possible to detect low levels (approximately 1 SLTEC⁴ per g of ground beef) in both small-scale (2 g of beef per 5 ml) and standard large-scale (25 g of beef per 225 ml) food microbial cultures.”

In this paper—published 14 years ago—Dr. Acheson voiced concern regarding the danger of non-O157 STEC. He stated:

In view of the large number of non-O157 SLTEC serotypes associated with both sporadic and outbreak disease in humans, and because the expression of SLTs is the one common factor among all SLTEC, it is logical to identify a way to detect the toxins in a sensitive, specific, and practical manner.⁵

³ Acheson, D. W. K., Breucker, S., Keusch, G., Lincicome, L. 1996. Detection of Shiga-Like Toxin-Producing *Escherichia coli* in Ground Beef and Milk by Commercial Enzyme Immunoassay. *J Food Protect.* 59:4:344-49.

⁴ In this paper, “SLTEC” refers to Shiga-like toxin-producing *Escherichia coli*.

⁵ *Id.* at 344.

The methods detailed in the paper described just such a means of detection, concluding by stating:

In the present study we have demonstrated that it is possible to detect low levels of multiple SLTEC serotypes in either ground beef or milk by undertaking overnight cultures in an appropriate broth and testing the resultant cultures in a simple EIA.⁶ While it is clear that proper food handling and cooking remain the most reliable ways of preventing infection with SLTEC from food sources, the EIA described in the present study offers a reliable method of detecting low levels of Shiga-like toxin in food samples produced not only by O157:H7 serotypes but by non-O157:H7 SLTEC as well, which must be considered potentially just as dangerous for humans as the O157:H7 serotype.

C. Non-O157:H7 STEC Study in Idaho⁷

In 2007, members of the Idaho Bureau of Laboratories published a study detailing the prevalence of non-O157:H7 STEC infections in regional medical center patients. As stated in the study, data indicated that “more than half of Idaho Shiga toxin-producing *Escherichia coli* (STEC) illnesses are caused by non-O157:H7 serotypes.”

The study concluded with an indication of the need for increased testing for non-O157:H7 STEC:

Our findings suggest that perceptions of low non-O157 STEC incidence in Idaho are probably artificial and due to overemphasis on culture methods for O157 STEC.

III. NEW DEVELOPMENTS RELATED TO NON-O157 EHEC

Recently, the national spotlight has focused on non-O157:H7 EHEC due to an outbreak stemming from tainted romaine lettuce. In late April 2010, the Centers for Disease Control and Prevention (CDC) began investigating reports of illnesses in Michigan, New York, and Ohio that

⁶ Enzyme immunoassay.

⁷ Ball, C.L., Hudson, R. F., Lockary, V. M. 2007. Shiga Toxin-producing *Escherichia coli*, Idaho. 13 Emerging Infect Dis. (No. 8) (Aug. 2007).

were caused by *E. coli* O145.⁸ As of May 5, 2010, the CDC counted a total of 19 confirmed and 10 probable cases related to the outbreak. Among the 29 reported cases, 12 (41%) were hospitalized. Additionally, three patients developed HUS. Fortunately, there have not yet been any reports of deaths linked to this outbreak.

The CDC's own investigation report noted the dangers associated with the lack of testing for non-O157 STEC. According to the report:

Currently, there are limited public health surveillance data on the occurrence of non-O157 STECs, including *E. coli* O145, therefore *E. coli* O145 may go unreported.⁹

Currently, the CDC believes that one processing facility was the source for the entire national outbreak.

IV. CONCLUSION

Although many persons in the food safety community are currently fixated on the dangers of non-O157:H7 EHEC due to the nation-wide romaine lettuce outbreak, the risks associated with Shiga Toxin-producing *E. coli* have long been known. These materials are just a few of the literally hundreds of documents and studies that indicate the dire need for EHEC detection beyond the O157:H7 serotype. These bacteria present a dire and immediate danger to the safety of this nation's food supply. It is for this reason that we again urge you to quickly act on the requests listed in petition number 09-03, following the expedited review procedures, so that we can be one step closer to avoiding the unfortunate illnesses that so many persons have endured due to non-O157:H7 EHEC.

⁸ CDC. May 6, 2010. Investigation Announcement: Multistate Outbreak of Human *E. coli* O145 Infections Linked to Shredded Romaine Lettuce from a Single Processing Facility. *available at* http://www.cdc.gov/ecoli/2010/ecoli_o145/index.html.

⁹ *Id.*

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. Acheson, D. W. K., Breucker, S., Keusch, G., Lincicome, L. 1996. Detection of Shiga-Like Toxin-Producing *Escherichia coli* in Ground Beef and Milk by Commercial Enzyme Immunoassay. *J Food Protect.* 59:4:344-49.
- Attachment No. 2. Acheson, D. W. K., Keusch, G. 1996. Which Shiga Toxin-Producing Types of *E. coli* Are Important?. *ASM News.* 62:6:302-06.
- Attachment No. 3. Ball, C.L., Hudson, R. F., Lockary, V. M. 2007. Shiga Toxin-producing *Escherichia coli*, Idaho. *Emerging Infect Dis.* (No. 8) (Aug. 2007).
- Attachment No. 4. CDC. May 6, 2010. Investigation Announcement: Multistate Outbreak of Human *E. coli* O145 Infections Linked to Shredded Romaine Lettuce from a Single Processing Facility. *available at* http://www.cdc.gov/ecoli/2010/ecoli_o145/index.html.

Detection of Shiga-Like Toxin-Producing *Escherichia coli* in Ground Beef and Milk by Commercial Enzyme Immunoassay

DAVID W. K. ACHESON,* LISA L. LINCICOME, SABINE DE BREUCKER, and GERALD T. KEUSCH

Tupper Research Institute, Division of Geographic Medicine and Infectious Diseases, New England Medical Center, 750 Washington Street, Boston, Massachusetts 02111, USA

(MS# 95-180: Received 21 July 1995/Accepted 2 October 1995)

ABSTRACT

Shiga-like toxin (SLT)-producing *Escherichia coli* (SLTEC) is the leading cause of acute renal failure among children. SLTEC are most commonly ingested from contaminated food, and because cattle are a major reservoir, ground beef and milk have been a significant source of contamination associated with multiperson outbreaks. While serotype O157:H7 has been principally identified in the United States there are many other SLTEC serotypes associated with human disease. We have therefore examined the utility of an enzyme immunoassay (EIA) for Shiga-like toxins as a means of detecting the presence of low levels of multiple SLTEC serotypes in ground beef and milk. In the present study we demonstrated that it is possible to detect low levels (approximately 1 SLTEC per g of ground beef) in both small-scale (2 g of beef per 5 ml) and standard large-scale (25 g of beef per 225 ml) food microbial cultures. The EIA was also capable of allowing detection of SLTEC in nonspiked retail ground beef samples: we were able to recover SLTEC isolates (O113:H_u; O22:H₋; O82:H₈) from 3 of 12 ground beef samples. The EIA detected SLTs produced in spiked milk samples when as few as 1 SLTEC per ml was added. Overall the EIA proved to be a highly sensitive way to detect the presence of SLTEC in either ground beef or milk samples after overnight enrichment culturing in an appropriate broth and should provide a rapid and convenient method for the detection of multiple pathogenic SLTEC serotypes.

Key words: Shiga-like toxins, Shiga-like toxin-producing *E. coli*, enzyme immunoassay, milk, ground beef

Shiga-like toxin (SLT)-producing *E. coli* (SLTEC), also known as enterohemorrhagic *Escherichia coli* (EHEC) has been associated with a variety of diseases in humans, including bloody and nonbloody diarrhea, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (19). SLTEC is now the major cause of acute renal failure in children in the United States and is generally among the top three bacterial causes of diarrhea. *E. coli* O157:H7 was the first serotype associated with HC and HUS in the United States; it was

identified following two outbreaks in 1982 (28). Over the following decade additional outbreaks due to *E. coli* O157:H7 were reported (15), the largest of which occurred in 1993 on the West Coast of the United States with over 700 individuals becoming sick, including 56 cases of HUS and 4 deaths (9). While O157:H7 has been the predominant serotype identified in the United States, with 60 documented outbreaks (13), this predominance is probably due in part to the fact that it is the only serotype which is routinely looked for. Currently over 50 serotypes of SLT-producing *E. coli* have been associated with human disease in North America (19) and many non-O157:H7 serotypes have been associated with foodborne outbreaks in different parts of the world, including O111 in Australia, Japan, Italy, and Germany (7, 8, 17, 23), O103 in France (24), and O104 in the United States (4). There are also multiple sporadic cases associated with non-O157:H7 strains in many parts of the world (1, 20, 32).

Cattle are the major reservoir of SLTEC (5, 25); however, these bacteria have also been isolated from pork, poultry, and lamb (12), and a variety of domestic animals (3). While ground beef and milk have been the major vehicles of transmission of SLTEC (11, 12, 15), there have been several recent reports of transmission in acidic foods such as dry fermented sausage (10), apple cider (2), and mayonnaise (31). Transmission via water (21, 30) and by both person-to-person and cattle-to-person routes have also been reported (27, 29). Despite the different vehicles and modes of spread many of the outbreaks have been associated with contamination of food with bovine fecal bacteria. Studies from Ontario have shown that ground beef and pork from meat-processing plants were heavily contaminated with SLTEC, with the prevalence of SLTEC being 36.4% and 10.6% in beef and pork respectively (26). A study from London showed 17% contamination of raw beef products with SLTEC (33).

Many of the assays for the detection of SLTEC have focused on the specific detection of serotype O157. In view of the large number of non-O157 SLTEC serotypes associated with both sporadic and outbreak disease in humans, and because the expression of SLTs is the one common factor among all SLTEC, it is logical to identify a way to detect the toxins in a sensitive, specific, and practical manner. The use

* Author for correspondence. Tel: 617-636-7001; Fax: 617-636-5292; Email: DACHESON@OPAL.TUFTS.EDU.

of Vero cell cytotoxicity assays and genetic methods including DNA probes and polymerase chain reaction (PCR) fulfill the first two criteria but are time consuming and expensive. We have previously developed an enzyme immunoassay (EIA) designed to detect SLTs, and have shown that this assay is able to detect free SLTs in human fecal samples and SLTEC from overnight broth cultures of fecal samples (1). The purpose of the present study was to examine the feasibility of using the same EIA for the detection of SLTEC in ground beef and milk.

MATERIALS AND METHODS

Detection of Shiga-like toxin-producing E. coli in ground beef

Small-scale spiking experiments. Two-gram aliquots of ground beef obtained from retail stores were spiked with various numbers of SLT-producing *E. coli* (SLTEC) of various serotypes (see Table 1) from 5-ml overnight cultures grown at 37°C on a culture wheel in Luria broth (10 g of Bacto-tryptone, 5 g of Bacto yeast extract, and 10 g of NaCl, all per liter). All SLTEC were human isolates from patients with either hemorrhagic colitis or hemolytic uremic syndrome. The precise number of colony-forming units added in these and later experiments were determined by plating aliquots of the overnight cultures onto Luria broth agar plates and counting colonies following overnight culture. The small-scale spiking experiments were set up by adding 5 ml of MacConkey broth (Difco) to 2-g meat samples and then adding the various SLTEC. Following culture (16 h at 37°C with agitation), aliquots of the broth were tested for the presence of SLTs using a commercially available EIA, Premier EHEC (Meridian Diagnostics Inc., Cincinnati, OH). The EIA is designed to detect both SLT-I and II (including SLT-IIc but not SLT-IIe) in the same well and does not differentiate between the two toxins. The assay was performed by adding 100 µl of the meat culture directly to the wells in the EIA. Following a 1-h incubation at room temperature, the plates were washed. The bound toxin was detected with rabbit polyclonal antibodies to SLT-I and II followed by anti-rabbit horseradish peroxidase conjugate and substrate provided with the kit. The plates were read at 450 nm (Bio-tech. Instruments, Model EL 308) after the addition of substrate. Apart from the fact that sample diluent was not used in these assays, the remainder of the assay procedure was as described in the protocol supplied with the EIA kit.

Large-scale spiking experiments. Twenty-five-gram aliquots of ground beef were spiked with various numbers of SLT-producing *E. coli* of different serotypes (see Table 2) from an overnight Luria broth culture. The precise number of bacteria added was determined as in the small-scale experiments. The meat (either spiked or nonspiked) was then added to 225 ml of either MacConkey broth or modified EC broth containing novobiocin (Sigma Chemical Co., St. Louis, MO) (20 µg/ml) (mEC). The constituents of the mEC were as follows: 20 g of tryptone, 5 g of lactose, 4 g of K₂HPO₄, 1.5 g of KH₂PO₄, and 5 g of NaCl, made up to 1 liter with distilled water). Following overnight culture with shaking at 37°C, aliquots of the broth were tested for the presence of SLTs by EIA as above.

Testing of retail ground beef samples for the presence of SLT-producing E. coli. Twelve different samples of ground beef of approximately 1 lb (ca. 0.45 kg) each were obtained from a variety of retail food markets. Fifteen subsamples (2 g each) were removed from each sample and placed separately in 5 ml of broth. Three different broths (MacConkey, mEC, and brain-heart infusion (BHI)) were used (5 separate subsamples in each of the three broths for

each meat sample). Following overnight culture, aliquots of the culture were removed and tested for the presence of SLTs by EIA as described above.

Cultures which were considered to be positive in the EIA (A_{450} greater than 0.15) were processed for colony isolation. If multiple subsample cultures from the same meat sample were EIA positive no more than three were processed for colony isolation, which was undertaken using a colony blot immunoassay developed in our laboratory (16). Briefly, aliquots of the overnight cultures were plated on a double layer of nitrocellulose filters on Luria plates containing mitomycin C (100 ng/ml) at a density to give individual colonies following overnight culture. After overnight culture the lower filter was processed in the immunoassay with monoclonal antibodies directed toward SLT-I and II. Any positive signals on the lower filter were then matched up with colonies on the upper filter. All positive colonies were further analyzed by growing the isolates overnight and assessing the resultant culture for the production of cytotoxins in a tissue-culture assay (22) and for the presence of SLT-I and II genes by PCR using the primers and protocol described by Brian et al. (6). All positive isolates were confirmed as *E. coli* using the Rapid E Enterobacteriaceae ID kit (BioMérieux, Vitek Inc., Hazelwood, MO) and then serotyped.

Up to 5 EIA-positive samples and up to 5 EIA-negative samples in the case of a batch being totally EIA negative had DNA extracted from them for PCR. DNA was extracted from various overnight meat cultures from each batch of meat by adding 250 µl of culture to 1 ml of 50 mM Tris (pH 8.0). The procedure described for fecal DNA extraction (14) was then followed. Extracted DNA was analyzed for the presence of SLT genes by PCR as above.

Detection of Shiga-like toxin-producing E. coli in milk

Modified Trypticase soy broth was used in all milk experiments and was prepared by adding the following: 30 g of TSB, 1.5 g of bile salts #3, 6 g of Na₂HPO₄, 1.35 g of KH₂PO₄, 10 g of Casamino Acids, and water to 1 liter as described by the manufacturers for the EHEC-TEK system (Organon Teknika, Durham, NC). Following autoclaving, acriflavine (Sigma) was added to a final concentration of 10 µg/ml.

In order to determine the appropriate proportion of milk to use, initial experiments were undertaken in which milk (2% milkfat) was added in various proportions (from 5% to 90%) to the medium and the mixture then spiked with low numbers (11 bacteria) of *E. coli* O157:H7. After overnight culture the mixture was then assayed in the EIA. In subsequent experiments we spiked different strains of SLTEC at very low levels (in triplicate) into a 10% milk solution (4.5 ml of broth, 0.5 ml of 2% milk). The precise numbers of bacteria added to each sample were determined by plating similar aliquots of the spiking mixture (in duplicate) onto Luria plates and culturing overnight. The level of spiking was deliberately kept at a low level and we anticipated that some of the samples tested would not be inoculated with SLTEC.

RESULTS

Detection of Shiga-like toxin-producing E. coli in ground beef

In the initial experiments, we examined 25 different Shiga-like toxin-producing *E. coli* isolates with various SLT phenotypes from human cases of either hemorrhagic colitis or hemolytic uremic syndrome. Various numbers of bacteria were added to 2-g meat samples and then grown overnight in MacConkey broth. The same meat was used for all small-scale spiking experiments and gave EIA A_{450} readings of

TABLE 1. Detection of Shiga-like toxins from overnight cultures of 2-g ground beef samples spiked with different numbers of SLT-producing *E. coli*: data from a representative experiment

Strain of <i>E. coli</i> ^a	SLT genotype	Bacteria added/g beef	A ₄₅₀	Bacteria added/g beef	A ₄₅₀	Bacteria added/g beef	A ₄₅₀	Level of detection (SLTEC/g)
O26:H11	I	200	>3	26	>3	2.5	>3	<2.5
O6:H31	I + II	123	>3	12	>3	1	>3	<1
Ou:Hu	II	119	>3	15	>3	<1	>3	<1
Ou:H16	II	82	>3	10	>3	1	>3	<1
O26:H11	I	180	>3	24	>3	2.5	>3	<2.5
Ou:H16	II	77	>3	6	>3	1.5	>3	<1
OX3:H21	II	76	2.9	9	>3	<1	0.55	<1
O4:NM	I + II	47	0.13	4	0.13	<1	0.14	ND ^b
O165:H25	II	78	>3	10	>3	<1	0.17	<1
O45:H2	I	150	>3	19	2.67	<1	0.84	<1
O128:H27	I	63	>3	5	2.83	<1	0.37	<1
O121:H19	I + II	119	>3	12	0.16	2.5	0.17	<10
O91:H21	II	74	>3	9	>3	3	2.16	<3
O146:H21	I	165	>3	17	>3	2.5	2.99	<2
O137:H41	I + II	160	>3	14	>3	3.5	>3	<3
O50:H7	I	74	2.99	8	0.99	1	2.02	<1
O145:NM	II	111	2.98	8	0.37	1	0.15	<2
O103:H2	I	145	>3	18	>3	2	>3	<2
O165:NM	I + II	106	2.88	9	>3	<1	0.32	<1
O125:NM	I	54	>3	3	0.21	<1	0.15	<1
O111:NM	I + II	100	2.89	7	2.94	<1	1.88	<1
O157:H7	II	38	>3	4	>3	<1	0.10	<4
O157:H7	I + II	70	>3	9	>3	1	0.68	<1
O157:H7	II	62	>3	6	>3	1	0.28	<1
O157:H7	II	87	>3	9	0.61	<1	0.15	<1
Negative control provided with EIA kit (A ₄₅₀)					0.089			
Positive control provided with EIA kit (A ₄₅₀)					1.175			
Meat without added bacteria (A ₄₅₀)					0.130 & 0.127			

^a The first six strains were clinical isolates from Meridian Diagnostic, Inc.; the remainder were from the Centers for Disease Control.

^b ND, no toxin detected.

0.13 and 0.127 in duplicate experiments when grown without added SLTEC. We therefore used an EIA A₄₅₀ reading of 0.15 as our lower limit of positivity for the remainder of the small-scale experiments. There was only one sample in which we were unable to demonstrate any SLT by the EIA even with the highest bacterial inoculation. Subsequent analysis of this isolate in separate cultures revealed it to have lost the capacity to produce SLTs. Of those samples in which we were able to detect SLTs, all were detectable at <10 bacteria per g of meat, 92% were detectable at <4 bacteria per g of meat, and 60% were detectable at <1 bacteria per g of meat (Table 1).

Following the small-scale spiking experiments using MacConkey broth we examined the ability of the EIA to detect SLTs in a larger-scale culture grown either in MacConkey broth or mEC. We spiked 25-g aliquots of ground beef with various numbers of bacteria of seven different SLTEC serotypes and grew them overnight in 225 ml of each of the two broths. Different meat samples were used during these large-scale experiments; therefore a nonspiked control flask was run with each spiking experiment. Again we were able to detect low numbers of the different serotypes as in the small-scale cultures. We also found that the sensitivity of the assay, in terms of the number of bacteria needed to detect toxin in the EIA, varied between the different serotypes

(Table 2). These experiments revealed that low levels of bacteria were more readily detected when MacConkey broth was used rather than mEC broth (Table 2). All bacteria were detected at some level in MacConkey broth, whereas growing the spiked meat in mEC resulted in failure to detect toxin production by four of the seven serotypes tested, even at the highest spiking levels used. We also noted positive EIA readings (taking an A₄₅₀ of 0.15 as a cutoff) in 2 of the nonspiked meat samples grown in MacConkey broth.

Having demonstrated that it was possible to detect low levels of various serotypes of SLTEC when they were spiked into ground beef, we then systematically examined 12 different ground beef samples obtained from various retail food markets for the presence of SLTEC. We compared three different media (MacConkey, mEC, and BHI) in which a 2-g subsample of meat taken directly from the packet was added to five separate tubes containing 5 ml of broth. The EIA results from these experiments are shown in Table 3 and demonstrate that there was a high prevalence of positive assays amongst the various samples. The EIA readings from any single ground beef batch were variable, even in the same medium, suggesting that SLT-producing bacteria were not evenly distributed within these samples of ground beef. There were no clear differences between the three types of media in terms of sensitivity and SLT was detected in all

TABLE 2. Detection of SLTs following overnight culture of spiked 25-g meat samples grown in either MacConkey (Mac) or modified EC (mEC) broth: data from a representative experiment

Strain of <i>E. coli</i>	SLT genotype	Bacteria added/g beef	MAC EIA (A ₄₅₀) ^a	mEC EIA (A ₄₅₀)
O157:H7	I + II	1.6	1.50	0.54
		0.1	0.12	0.13
		0	0.09	0.09
O91:H21	II	2.0	0.41	0.24
		0.2	0.13	0.90
		0	0.15	0.07
O111:NM	I + II	22.3	2.38	0.09
		2.6	0.88	0.13
		0	0.11	0.10
O26:H11	I	16.8	1.09	0.10
		2.0	0.49	0.09
		0	0.33	0.09
O45:H2	I	12.5	1.31	0.10
		1.6	1.41	0.10
		0	0.11	0.10
O6:H31	I + II	15.7	2.602	0.11
		1.7	2.36	0.10
		0	0.19	0.11
O103:H2	I	13.2	1.67	1.46
		1.8	0.53	0.12
		0	0.13	0.09

^a Any EIA A₄₅₀ value above 0.150 is considered to be positive.

three. Overall 9 (75%) of the ground beef samples were positive in the EIA (using an A₄₅₀ of 0.15 as the cutoff). By using colony immunoblot techniques we were able to isolate SLTEC from 3 of the EIA-positive samples. All 12 ground beef samples were also analyzed by PCR for the presence of SLT-I and/or II genes. Eight (66.6%) of the 12 were PCR positive for SLT-I and/or II, including the 3 in which we obtained SLTEC isolates (Table 3).

Detection of Shiga-like toxin-producing *E. coli* in milk

Initially tubes containing media with different proportions of 2% milk were spiked with the same number of *E. coli* O157:H7 (11 bacteria). After overnight culture the level of SLTs was determined by EIA. The results from this experiment are shown in Table 4 and demonstrate that the bacteria produced detectable SLTs at all concentrations of milk, even in 100% milk. Solutions of 10% milk not spiked with SLTEC gave a low background EIA A₄₅₀ reading of 0.085. On the basis of this experiment we decided to conduct the remaining spiking experiments using 10% milk in medium. When 0.3 to 2.3 bacteria from seven different strains of SLTEC were added to broth containing 10% milk, all seven strains produced high levels of detectable SLTs. We deliberately spiked at a very low level of bacteria with the intention of placing only one bacterium in each tube. This inevitably resulted in some tubes not being spiked. For this reason each experiment was run in triplicate. Within each set of three, either one, two, or all three replicates were positive for SLTs as determined by the EIA. As can be seen from the data in Table 5 there is a clear division between the tubes in which bacteria were added, grew, and produced toxin, and

bacteria. When larger numbers of bacteria were added to milk in medium we consistently found them to produce toxin and to be positive in the EIA (data not shown).

DISCUSSION

Shiga-like toxin-producing *E. coli* are now a significant cause of morbidity and mortality in the United States. While it is clear that the Shiga-like toxins themselves are epidemiologically associated with the development of disease, it is not known exactly how the toxins cause hemorrhagic colitis

TABLE 3. Detection of Shiga-like toxins following culture of retail ground beef in three different broths

Meat sample	SLT (PCR)	Broth ^a	EIA A ₄₅₀ of subsample:					Positives
			1	2	3	4	5	
1	I + II	Mac	0.091	0.134	0.134	0.093	0.124	0
		mEC	2.615 ^b	0.082	0.068	0.075	0.083	1
		BHI	0.094	0.076	0.106	0.085	0.089	0
2	I + II	Mac	0.266	0.154	0.165	0.261	0.544 ^c	5
		mEC	0.200	0.204	0.578	0.382	0.361	5
		BHI	0.309	0.350	0.455	0.218	0.354	5
3	II	Mac	0.135	0.182	0.171	0.084	0.090	2
		mEC	0.081	0.086	0.093	0.86	0.079	1
		BHI	0.078	0.074	0.078	0.081	0.105	0
4	Neg	Mac	0.102	0.087	0.109	0.113	0.156	1
		mEC	0.094	0.089	0.091	0.083	0.092	0
		BHI	0.116	0.092	0.138	0.137	0.090	0
5	Neg	Mac	0.096	0.092	0.109	0.085	0.091	0
		mEC	0.093	0.091	0.107	0.093	0.086	0
		BHI	0.085	0.082	0.083	0.082	0.102	0
6	Neg	Mac	0.245	0.205	0.234	0.263	0.270	5
		mEC	0.205	0.193	0.201	0.196	0.142	4
		BHI	0.150	0.161	0.162	0.175	0.146	3
7	II	Mac	0.157	1.634 ^d	0.199	0.174	0.222	5
		mEC	0.087	0.082	0.077	0.088	0.079	0
		BHI	0.080	0.091	0.071	0.066	0.071	0
8	Neg	Mac	0.087	0.083	0.076	0.092	0.085	0
		mEC	0.086	0.077	0.082	0.071	0.078	0
		BHI	0.083	0.084	0.081	0.089	0.085	0
9	II	Mac	0.089	0.085	0.080	0.077	0.080	0
		mEC	0.085	0.089	0.078	0.095	0.871 ^e	1
		BHI	0.081	0.081	0.094	0.081	0.079	0
10	II	Mac	0.074	0.075	0.067	0.068	0.069	0
		mEC	0.081	0.127	0.135	0.112	0.082	0
		BHI	0.067	0.071	0.285	0.076	0.074	1
11	I + II	Mac	0.063	0.063	0.063	0.069	0.066	0
		mEC	0.065	0.063	0.062	0.06	0.061	0
		BHI	0.060	0.061	0.059	0.062	0.065	0
12	I + II	Mac	0.065	0.078	0.066	0.060	0.064	0
		mEC	0.064	0.066	0.064	0.350 ^f	0.061	1
		BHI	0.157 ^f	0.700 ^f	0.410	1.011	0.067	4

^a Mac, MacConkey broth; mEC, modified EC broth with novobiocin (20 µg/ml); BHI, brain heart infusion broth.

^b Five isolates of O113:Hu producing SLT-II.

^c Five isolates of O22:H producing SLT-I and II.

^d SLT-II positive by PCR: no isolates obtained.

^e SLT-II positive by PCR: no isolates obtained.

^f Five isolates of O82:H8 producing SLT-I and II.

TABLE 4. Production of Shiga-like toxins from *E. coli* O157:H7 in media containing different proportions of milk

% milk in medium	Culture dilution ^a	A ₄₅₀ in EIA
5	100	2.307
10	100	2.308
20	100	2.236
40	100	2.048
50	100	1.966
60	100	1.752
70	100	1.553
80	10	2.384
90	10	2.222
100	10	0.763

^a The overnight enrichment cultures were diluted in the diluent supplied with the EIA kit to either 1:100 or 1:10 for testing in the EIA.

and hemolytic uremic syndrome. Many different serotypes of SLTEC have been associated with human disease; however, detection methods designed to identify only a single serotype, such as O157:H7, will fail to detect many human SLTEC pathogens. The purpose of this study therefore was to examine the feasibility of using a commercially available toxin-based enzyme immunoassay for the detection of any serotype of SLTEC in ground beef and milk samples.

Our initial small-scale experiments showed that it was possible to spike ground beef with different SLTEC serotypes at levels of less than 1 bacterium per g of meat and still detect the presence of SLTs following overnight culture, irrespective of whether SLT-I or II was produced. Only one strain (O4:NM) was negative after overnight culture. This may have been due to loss of the SLT-encoding bacteriophage in this strain (18). When we scaled up to 25-g ground beef samples in 225 ml of broth we saw a similar pattern and demonstrated the ability to detect SLTEC at low spiking levels, in the range of 1 bacterium added per g of ground beef for all the serotypes grown in MacConkey broth. In contrast, when we used modified EC broth the sensitivity of the assay dropped off dramatically and we failed to detect SLT production in 4 (57%) of the sets of cultures. Thus there

TABLE 5. Detection of various Shiga-like toxin-producing *E. coli* in modified TSB broth with 10% milk by EIA

Strain of <i>E. coli</i>	Number of bacteria added ^a	A ₄₅₀ of sample:		
		1	2	3
O6:H31	1.2	0.063	2.431	2.272
O103:H2	0.7	0.088	2.863	0.177
O26:H11	2.3	0.074	2.678	0.067
O157:H7	0.3	2.737	0.072	0.071
O91:H21	1.5	2.612	2.700	2.759
O111:NM	0.5	2.866	2.818	2.829
O45:H2	0.67	2.746	0.065	0.066

^a The number of bacteria added was determined by plating 600 μ l of the bacterial inoculation solution. Only 100 μ l was added to the broth containing milk.

was a clear advantage of using MacConkey broth over modified EC broth for the production of SLTs in this system.

We next examined the utility of this assay system for the detection of SLTEC in retail ground beef samples. Several observations can be made regarding the data obtained from the ground beef samples. First, there appeared to be variability in the distribution of SLTEC within a contaminated meat sample, as evidenced by variation in the level of detectable SLTs in the samples following overnight culture. For example, in meat sample no. 1 only one positive subsample was found, with an EIA A₄₅₀ reading of 2.615, and from which we isolated an SLTEC (O113:Hu); as compared with meat sample no. 2 in which all 15 subsamples were positive at various levels (Table 3). Second, there were no major differences in the ability of the various broths to detect SLTEC, although the heterogeneity within the meat subsamples for SLTEC does not provide a thorough test of the different broths, unlike the experiments with the large-scale cultures shown in Table 2. Third, we isolated SLTEC that were confirmed to be *E. coli* capable of producing Shiga-like toxin as shown by both EIA and cytotoxicity (data not shown) from 3 of 12 (25%) purchased ground beef samples. None of the isolates were strains we had been working with in the spiking experiments, so it is very unlikely that they were laboratory contaminants, and all of the SLTEC serotypes isolated have been associated with disease in humans. The EIA was positive in a further 5 ground beef samples, and although we were unable to isolate SLTEC from these samples, we found 3 of the 5 to be positive for SLT genes by PCR. This suggests that up to 58% of the ground beef samples may have contained live SLTEC.

The enzyme immunoassay was also able to detect all SLTEC serotypes spiked into milk samples in these studies after overnight culture. We deliberately used very low SLTEC inocula in the milk experiments (less than 1 bacterium per ml of milk) and so anticipated that some of the milk in spiking experiments would be SLT negative, because no SLTEC would have actually been added. Based on these data, we predict that as few as one SLTEC in a milk sample, irrespective of serotype, will grow in an overnight culture to produce a readily detectable level of Shiga-like toxin. None of the nonspiked milk samples tested were positive in the EIA, and we found no difference in SLT production in skimmed milk, 2% milk, or whole milk (data not shown).

Others have shown that SLTEC of multiple serotypes other than O157:H7 are present in meat samples (7, 26, 33); however, the previous assays have either been dependent on cumbersome and expensive genetic methods such as DNA probes or PCR, or required time-consuming tissue-culture assays. In the present study we have demonstrated that it is possible to detect low levels of multiple SLTEC serotypes in either ground beef or milk by undertaking overnight cultures in an appropriate broth and testing the resultant cultures in a simple EIA. While it is clear that proper food handling and cooking remain the most reliable ways of preventing infection with SLTEC from food sources, the EIA described in the present study offers a reliable method of detecting low levels of Shiga-like toxin in food samples produced not only by O157:H7 serotypes but by non-O157:H7 SLTEC as well,

which must be considered potentially just as dangerous for humans as the O157:H7 serotype.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI-16242 from the NIAID, P30 DK-34928 for the Center for Gastroenterology Research on Absorptive and Secretory Processes from the NIDDK, National Institutes of Health, Bethesda, MD; and a Health Sciences for the Tropics Grant from The Rockefeller Foundation; by the Charles H. Hood Foundation; and by Meridian Diagnostics Inc.

REFERENCES

- Acheson, D. W. K., S. DeBreucker, A. Donohue-Rolfe, K. Kozak, A. Yi, and G. T. Keusch. 1994. Development of a clinically useful diagnostic enzyme immunoassay for EHEC infection, p. 109-112. *In* M. A. Karmali and A. G. Goglio (ed.), Recent advances in verocytotoxin producing *Escherichia coli* infections. Elsevier, Amsterdam.
- Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barrett, J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA* 59:2526-2530.
- Beutin, L., D. Geire, H. Steinruck, 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.
- Bibb, W. F., C. Whitman, J. Green, N. Banatvala, D. Swerdlow, P. M. Griffin, and D. Abbott. 1995. Application of an ELISA for antibodies to *Escherichia coli* O104 lipopolysaccharide for identification of cases during an outbreak, abstr. P-12, p. 384. *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1995. American Society for Microbiology, Washington, D.C.
- Borczyk, A. A., M. A. Karmali, H. Lior, and M. C. Duncan. 1987. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. *Lancet* i:98.
- Brian, M. J., M. Frosolono, B. E. Murray, A. Miranda, E. L. Lopez, H. F. Gomez, and T. G. Cleary. 1992. Polymerase chain reaction for diagnosis of EHEC infection and HUS. *J. Clin. Microbiol.* 30:1801-1806.
- Cameron, S., C. Walker, M. Beers, N. Rose, E. Aneer. 1995. Enterohaemorrhagic *Escherichia coli* outbreak in south Australia associated with the consumption of mettwurst. *Communicable Dis. Intelligence* 19:70-71.
- Caprioli, A., I. Luzzi, F. Rosmini, C. Resti, A. Edefonti, P. Francesco, 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.
- Centers for Disease Control and Prevention. 1993. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers—western United States, 1992-1993. *Morbidity Mortal. Weekly Rep.* 42:258-263.
- Centers for Disease Control and Prevention. 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami—Washington and California, 1994. *Morbidity Mortal. Weekly Rep.* 44:157-160.
- Centers for Disease Control. 1995. *Escherichia coli* O157:H7 outbreak at a summer camp—Virginia, 1994. *Morbidity Mortal. Weekly Rep.* 44:419-421.
- 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.
- Feng, P. 1995. *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. *Emerg. Infect. Dis.* 1:47-52.
- Frankel, G., L. Riley, J. A. Giron, J. Valmassoi, A. Friedmann, N. Strockbine, S. Falkow, and G. K. Schoolnik. 1990. Detection of *Shigella* in feces using DNA amplification. *J. Infect. Dis.* 161:1252-1256.
- 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.
- Hull, A. E., D. W. K. Acheson, P. Echeverria, A. Donohue-Rolfe, and G. T. Keusch. 1993. Mitomycin immunoblot colony assay for detection of Shiga-like toxin-producing *Escherichia coli* in fecal samples: comparison with DNA probes. *J. Clin. Microbiol.* 31:1167-1172.
- Karch, H. Verocytotoxin-producing *Escherichia coli* infection in Europe: diagnostic and public health perspectives, p. 13-16. *In* M. A. Karmali and A. G. Goglio (ed.), Recent advances in Verocytotoxin producing *Escherichia coli* infections. Elsevier, Amsterdam.
- Karch, H., T. Meyer, H. Russmann, and J. Heeseman. 1992. Frequent loss of Shiga-like toxin genes in clinical isolates of *E. coli* upon subcultivation. *Infect. Immun.* 60:3464-3467.
- Karmali, M. A. 1989. Infection by Verocytotoxin-producing *Escherichia coli*. *Clin. Microbiol. Rev.* 2:15-38.
- 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., 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.
- Keusch, G. T., Donohue-Rolfe, A., Jacewicz, M., and A. V. Kane. 1988. Shiga toxin: production and purification. *Meth. Enzymol.* 165:152-162.
- Kudoh, Y., A. Kai, H. Obata, J. Kusunoki, C. Monma, M. Shingaki, Y. Yanagawa, S. Yamada, S. Matsushita, T. Itoh, and K. Ohta. 1994. Epidemiological surveys on Verocytotoxin-producing *Escherichia coli* infections in Japan, p. 53-56. *In* M. A. Karmali and A. G. Goglio (ed.), Recent advances in verocytotoxin producing *Escherichia coli* infections. Elsevier, Amsterdam.
- Mariani-Kurkdjian, P., E. Denamur, A. Milon, B. Picard, H. Cave, N. Lambert-Zechovsky, C. Lohat, P. Goullet, P. J. 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. Arnold, and J. Tilleli. 1986. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome. *Lancet* ii:1043.
- 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.
- Renwick, S. A., J. B. Wilson, R. C. Clarke, H. Lior, A. A. Borczyk, J. Spika, K. Rahn, K. McFadden, A. Brouwer, A. Copps, N. G. Anderson, D. Alves, and M. A. Karmali. 1993. Evidence of direct transmission of *Escherichia coli* O157:H7 infection between calves and a human. *J. Infect. Dis.* 168:792-793.
- Riley, L. W., R. S. Remis, S. D. Helgeson, H. B. McGee, J. G. Wells, 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. *New Engl. J. Med.* 308:681-685.
- Spika, J. S., J. E. Parsons, D. Nordenberg, J. G. Wells, R. A. Gunn, and P. A. Blake. 1986. Hemolytic uremic syndrome and diarrhea associated with *Escherichia coli* O157:H7 in a day care center. *J. Pediatr.* 109:287-291.
- Swerdlow, D. L., B. A. Woodruff, and R. C. Brady. 1992. A water borne outbreak in Missouri of *Escherichia coli* O157:H7 associated with blood diarrhea and death. *Ann. Intern. Med.* 117:812-819.
- Weagant, S. D., J. L. Bryant, and D. H. Bark. 1994. Survival of *Escherichia coli* in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. *J. Food. Prot.* 57:629-631.
- Willshaw, G. A., S. M. Scotland, H. R. Smith, and B. Rowe. 1992. Properties of Vero cytotoxin-producing *Escherichia coli* of human origin of O serogroups other than O157. *J. Infect. Dis.* 166:797-802.
- Willshaw, G. A., H. R. Smith, D. Roberts, J. Thirlwell, T. Cheasty, and B. Rowe. 1993. Examination of raw beef products for the presence of Vero cytotoxin producing *Escherichia coli*, particularly those of serogroup O157. *J. Appl. Bacteriol.* 75:420-426.

Which Shiga Toxin-Producing Types of *E. coli* Are Important?

Although diagnostic tests are geared to E. coli O157:H7, other strains also cause serious infections and deserve attention

DAVID W. K. ACHESON and GERALD T. KEUSCH

Escherichia coli pathogens that produce Shiga toxins first gained broad notoriety in the United States in 1982 following two outbreaks of bloody diarrhea involving patrons of a fast food restaurant chain. Subsequently, investigators learned that the culprit pathogen is *E. coli* O157:H7, a serotype not previously associated with disease in humans. *E. coli* O157:H7 is now blamed for several additional outbreaks of bloody diarrhea as well as serious complications, including hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Acknowledging the impact this microorganism has on its host, investigators often refer to this and similar pathogenic strains as enterohemorrhagic *E. coli* (EHEC).

In the decade since 1982, some 30 outbreaks of *E. coli* O157:H7 were recorded in the United States. In 1993, for instance, a very large outbreak affecting 732 people occurred along the west coast of the United States: 195 were hospitalized; 55 developed HUS, which is a serious disorder involving kidney function; and 4 died.

Since the 1993 outbreak, at least another 30 such outbreaks have been identified, with the dramatic increase due in part to increased awareness of this pathogen. However, some of this increase can be attributed to the changing behavior of this bacterium and to a growing understanding that the pattern of this disease is far more complex than is generally realized.

Importantly, many different serotypes of *E. coli* besides O157:H7—and a few non-*E. coli* species of bacteria—produce Shiga toxins and cause serious human disease. Multiple serotypes exist and have caused serious disease and death in many parts of the world.

David W. K. Acheson is an assistant professor and Gerald T. Keusch is a professor in the Division of Geographic Medicine and Infectious Diseases, New England Medical Center, Boston, Mass.

We cannot let ourselves be complacent in thinking that *E. coli* O157:H7 is the only Shiga toxin-producing microorganism that can cause problems.

Enterohemorrhagic Strains Produce Key Toxins

E. coli O157:H7 and other EHEC strains can produce bacteriophage-encoded cytotoxins that closely resemble toxins produced by highly potent pathogens, the *Shigella dysenteriae* type 1 strains. These *E. coli* strains produce at least two immunologically distinct Shiga toxins, designated Shiga toxin 1 and Shiga toxin 2. With the importance of these toxins in mind, investigators often use the term STEC in referring to Shiga toxin-producing *E. coli*.

This recently developed terminology is used interchangeably—and often confusingly—with older terms developed to describe research results from the late 1970s. In those studies of two decades ago, several isolates of *E. coli* produced toxins that killed Vero cells in tissue culture. Confusion arises because the more recently described Shiga toxins proved to be the same as those verotoxins. To overcome this confusion, we and other microbiologists recently proposed a way of renaming the toxins (*ASM News*, March 1996, p. 118).

Regardless of these nomenclature problems, the role of these toxins is central to explaining the spectrum of clinical disease associated with STEC. Thus, infections with such *E. coli* strains may lead to mild or severe gastrointestinal symptoms, including bloody or nonbloody diarrhea and HC, as well as life-threatening complications such as HUS and thrombotic thrombocytopenic purpura (TTP).

Shiga Toxins May Be the Major Virulence Factor

Although many questions remain, the Shiga toxins themselves appear to be responsible for causing many pathological effects associated with STEC infections.

Specific endothelial cell beds in the gut, kidney, and brain are the main targets of these Shiga toxins. Thrombotic microangiopathy (TMA), the formation of tiny clots and other damage in capillary beds within the kidney, is the pathological hallmark of Shiga toxin-related disease. How the toxins move from the intestinal tract lumen to these sites where they damage the kidney is another unanswered question.

How important specific serotypes may be is not fully understood. However, for some reason, serotypes O157:H7 and O111:NM have caused major outbreaks more often and led to higher rates of systemic disease than have any of the other serotypes. Factors such as a lower infectious dose with these serotypes, higher production of toxin, greater gastrointestinal colonization, or enhanced delivery of toxins to endothelial cell targets may account for some of these differences.

Whatever the serotype, if a bacterium is making Shiga toxins in the gastrointestinal tract of an infected individual, that person is at risk for developing HC and HUS. Although the overall risk for many STEC serotypes is unknown, the risk is in the 5 to 10% range for individuals infected with *E. coli* O157:H7 strains, based on estimates from incidence data.

Several instances of non-*E. coli* Shiga toxin-producing infections are now documented. One outbreak in Germany involved a strain of *Citrobacter freundii* that can express Shiga toxin 2. Some patients infected with this organism developed HUS. In another case reported in 1995, a patient in Australia with severe diarrhea that led to HUS was infected with a strain of Shiga toxin 2-producing *Enterobacter cloacae*.

Geographic Patterns of STEC

While the emphasis in the United States has been on serotype O157:H7, approximately 100 other *E. coli* serotypes not only produce Shiga toxins but have been isolated from patients with HC or HUS. Among these strains, several are considered responsible for significant outbreaks in various parts of the world. However, developing an overall picture remains an elusive undertaking.

A demographic study conducted in Belgium sheds some light on the patterns of STEC infection and other diarrhea-inducing bacterial diseases in a European-based population. Researchers report that STEC was the third most common enteric pathogen isolated from these patients, coming after *Campylobacter* and *Salmonella* strains. Approximately half of those in each group had uncomplicated diarrhea.

In this Belgian study group, the overall rate for STEC is about 1% among the 10,241 stool specimens that were analyzed in this study. In that positive subset, 38% were O157:H7 but 62% were non-O157 serotypes (comprising 22 different O:H types). Only two patients infected with O157 developed HUS, whereas none infected with the non-O157 strains developed HUS. However, one patient from each of these two groups presented with HC, and one of the patients in the non-O157 group developed TTP.

Elsewhere in Europe, several non-O157 serotypes, including O1:NM, O5:NM, O18:H?, O26:H11, and O111:NM, have been isolated from patients with HUS in the Czech Republic. Another serotype, O103:H2 was isolated from patients following an outbreak of HUS in France. Meanwhile, in Italy, O111:H- and O26 were isolated from similar patients.

STEC outbreaks are being reported outside Europe. For instance, an outbreak due to an O111:NM strain recently led to 23 cases of HUS and one death in Australia. Among the STEC strains characterized so far in Australia, O111:H-, O6:H31, O98:H-, and O48:H21 are the predominant serotypes. STEC strains are also found in certain South American countries, especially Argentina, where a large variety of STEC serotypes, including O157:H7, cause serious disease. Moreover, in Japan O111:H- and O145:H- strains have caused outbreaks.

Implications of Global STEC Patterns for North America

With so many non-O157:H7 serotypes causing disease outbreaks around the world, what is the status of non-O157:H7 strains in North America in general and the United States in particular? Although the picture is in flux, the variety of non-O157:H7 strains responsible for North American outbreaks that investigators are isolating could well rival that seen throughout Europe, South America, and elsewhere.

Over 100 different STEC serotypes have been isolated in Canada from patients with either HC or HUS. For example, one 2-year prospective study was conducted a decade ago in Alberta. Analysis of stool specimens from 5,415 patients indicated that 130 patients were infected with O157:H7 strains, whereas 29 patients were infected with a broad range of non-O157:H7 strains, including O26:H11, O103:H2, O91:H-, O145:H-, O111:H-, O38:H21, O6:H-, O5:H-, and O?:H21. Severity of disease varied from one strain to another, with O157:H7 consistently the most virulent. Thus, only 42% of those individuals infected with the non-O157:H7 strains had bloody diarrhea, compared with 98% of those infected with O157:H7.

In a similar survey involving 9,449 individuals in British Columbia, 67 stool specimens contained STEC, including seven non-O157:H7 strains divided among O26, O128, and O103 serotypes. Two patients in this group had bloody diarrhea, and both of them were infected with *E. coli* O26.

Surveys of U.S. population groups also indicate that many non-O157 strains are showing up in patients with severe diarrhea. For instance, according to a prospective study conducted in Seattle, Wash., 13 of 445 patients with diarrhea had O157:H7 *E. coli* infections, whereas 5 were infected with various non-O157 strains, including O26:H-, O153:H2, O68:H-, and O-:H11. Similar to results from other studies, the non-O157 strains were associated with bloody diarrhea less frequently than were the O157:H7 strains.

In another U.S. report, an analysis of 95 fecal

samples obtained from state health laboratories in Minnesota from patients with severe diarrhea was conducted. Of those samples, 14 proved to be STEC positive. Eight of the 14 contained O157:H7 strains, and the remainder contained various non-O157 serotypes, including O26:H11, O6:H31, and O?:H16.

At least two other recent reports indicate that non-O157:H7 strains have been causing severe cases of diarrhea and other complications in the United States. The first involved a single family in which infection with an O111:NM strain caused diarrhea and led to one case of HUS. The second report describes a small outbreak in Montana involving *E. coli* O104:H21. The outbreak involved 11 confirmed and 7 suspected cases, most of which included bloody diarrhea.

It is difficult to imagine that large outbreaks of disease due to non-O157 serotypes are occurring but not being reported. However, small outbreaks similar to the one in Montana could easily go undiagnosed and unrecognized. Moreover, the number of sporadic cases of disease caused by non-O157:H7 STEC in the United States is unknown.

Infectious Bacteria in Various Foods Trace to Cattle

E. coli O157:H7 has a low infectious dose, on the order of a few hundred organisms, which contributes to the spread of the infection in outbreak situations.

Cattle are considered to be the major reservoir of EHEC, including the O157:H7 and other serotypes. Although EHEC does not generally cause illness among cattle, the microorganisms are associated with fecal matter. Release of those contaminants during slaughter is considered the primary route by which EHEC enters the food supply. Safeguards are particularly ill suited for dealing with ground beef products because they are prepared from meat obtained from many animals. Thus, even if contamination occurs from only one animal, grinding usually disperses the bacteria throughout the lot.

EHEC has also been transmitted in dry fermented sausage, milk, apple cider, mayonnaise, and various salads, as well as in water and by direct person-to-person and cattle-to-person contacts. In most cases, however, the ultimate source of the infection has been traced to cattle.

If one considers the number of documented cases of non-O157:H7 disease in Canada, combined with the observation that cattle in the United States carry many STEC serotypes and the fact that residents of the United States consume beef products from Australia and Argentina, where non-O157:H7 serotypes cause significant disease, it is hard to believe that non-O157 serotypes are not causing more cases of disease in the United States than are being documented. Although the necessary methodology for detecting such cases is available, current diagnostic and screening efforts focus principally on O157.

Current Main Diagnostic Test for STEC Has Shortcomings

Unique biochemical characteristics of *E. coli* O157:H7—it ferments sorbitol very slowly and usually does not make β -glucuronidase—make it relatively easy for microbiologists to differentiate this strain from other enteric *E. coli* strains. The standard procedure involves growth of suspect organisms on differential media in the clinical diagnostic laboratory.

The biochemical characteristics provide a basis for a number of diagnostic tests. For instance, when the sugar lactose in MacConkey plates is replaced with sorbitol (SMAC plates), growth of O157:H7 leads to white colonies, whereas other strains of *E. coli* usually produce pink colonies. To confirm suspicions based on appearance, white colonies usually are tested with specific antisera.

However, this scheme is by no means foolproof. For instance, sorbitol-fermenting *E. coli* O157:H7 isolates are found in Europe, β -glucuronidase-producing strains are found in the United States, and non-sorbitol-fermenting STEC serotypes other than O157:H7, such as O26, O55, and O111, are also sometimes found. In its favor, this plate test is relatively inexpensive and easy to perform. Although it is limited to O157:H7, this serotype arguably is predominant in the United States and currently seems to account for the highest frequency of, and possibly also the severest, disease outbreaks. However, reliance on a test which detects only a single serotype does not seem wise.

To address this shortcoming, a number of alternative methods based on the detection of the Shiga toxins themselves using an enzyme immunoassay format have been developed. At least one of these has been approved by the U.S. Food and Drug Administration for testing stool samples directly for the presence of Shiga toxins, as well as for testing individual colonies or broth cultures of stool. These toxin-based tests are totally independent of the serotype of the bacteria producing the toxins and so offer significant advantages over SMAC plates in being able to detect any Shiga toxin-producing strain which may cause HC or HUS.

Approach to Diagnostic Testing Needs Improvements

Currently in the United States approximately half of the clinical microbiology laboratories are screening either all stools or all bloody stools for *E. coli* O157:H7 using the SMAC-based test. Undoubtedly screening bloody stool samples for O157:H7 is better than doing nothing. Should we be doing more? One framework for addressing this question involves considering the consequences of missing a case involving an infection with a Shiga toxin-producing bacterium.

Because these toxins can cause gastrointestinal disease and also lead to systemic complications such as HUS and TTP, delayed diagnosis may enable a bad situation to move unhindered to serious, perhaps life-threatening complications. Of course, many patients

who harbor O157:H7 may develop only mild cases of diarrhea and no overt systemic complications. The long-term effects of having mild infections involving exposure to Shiga toxins are unknown.

STEC infections can induce partial or incomplete HUS. Moreover, some patients apparently recover from HUS but later develop renal disease. These observations raise the intriguing possibility that even mild cases involving infections with Shiga toxin-producing microorganisms are able to trigger long-term pathological changes that lead to serious renal disease.

Studies from around the world support the notion that many of the non-O157:H7 serotypes lead to HUS and death. For example, the O111 serotypes seem to be as virulent as O157:H7, whereas several other serotypes may be more likely to be associated with non-bloody diarrhea and a lower incidence of complications.

Until the bacterial factors other than the Shiga toxins themselves or the host factors which are likely to lead to systemic complications can be identified, however, it seems prudent to assume that any patient infected with Shiga toxin-producing infections is at risk for developing HUS or TTP.

Several Reasons for Detecting, Monitoring Shiga Toxins

For patients, much of the value from diagnostic procedures depends on whether or what kind of therapy is available. In the case of infections with STEC, little besides symptomatic or supportive treatment is currently available. Whether antibiotic treatment does any good is still a matter of considerable disagreement.

Various antibiotics at subinhibitory concentrations, especially trimethoprim sulfamethoxazole, can increase Shiga toxin expression *in vitro*. This observation, combined with the concern that STEC which are lysing in the gastrointestinal tract lumen may actually release more toxin than do intact bacterial cells, has raised doubts about the value of antibiotic therapy in such infections. However, this issue has not yet been evaluated by randomized controlled clinical studies. An important specific question to address clinically is whether administration of antibiotics early during an infection would benefit patients.

Other approaches to therapy are being considered, including oral administration of a Shiga toxin receptor analog to absorb free toxin in the intestinal tract before it can be absorbed. This approach is currently being evaluated in Canada.

Even without specific treatment strategies, however, knowing that a patient with diarrhea is infected with a Shiga toxin-producing organism can be helpful in a clinical setting. Importantly, such information may save a patient from unnecessary surgery or inap-

propriate medical treatment. STEC infections can mimic appendicitis, diverticulitis, ischemic colitis, intussusception, and inflammatory bowel disease. In some STEC cases, patients have had unnecessary appendectomy or laparotomy procedures.

There is another important reason for having a clear diagnosis of whether Shiga toxins are present. With such information, physicians will know to monitor patients closely for signs of incipient TMA syndromes such as thrombocytopenia, rising blood urea nitrogen, or anemia. Awareness of such developments will facilitate earlier intervention with supportive therapy if required.

Knowing that a patient is carrying STEC is also epidemiologically important. However, deciding which samples to test, from which patients, and for what type of diarrhea frequently is driven by economics rather than by public health concerns. With assays available for testing stool samples for STEC of multiple serotypes or for free Shiga toxin, it is time to begin prospective studies to determine the incidence of such infections from all serotypes. Only by undertaking such studies can we determine the true level of both O157:H7 and non-O157:H7 disease in the United States. □

Suggested Reading

- Armstrong, G. D., P. C. Rowe, P. Goodyear, E. Orrbine, T. P. Klassen, G. Wells, A. MacKenzie, A. Lior, C. Blanchard, F. Auclair, B. Thompson, D. J. Rafter, and P. N. McLaine. 1995. 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.* 171:1042-1045.
- Bokete, T. N., C. M. O'Callahan, C. R. Clausen, N. M. Tang, N. Tran, S. L. Moseley, T. R. Fritsche, and P. I. Tarr. 1993. Shiga-like toxin-producing *Escherichia coli* in Seattle children: a prospective study. *Gastroenterology* 105:1724-1731.
- Boyce, T. G., A. G. Pemberton, J. G. Wells, and P. M. Griffin. 1995. Screening for *Escherichia coli* O157:H7—a nationwide survey of clinical laboratories. *J. Clin. Microbiol.* 33:3275-3277.
- Boyce, T. G., D. L. Swerdlow, and P. M. Griffin. 1995. *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. *N. Engl. J. Med.* 333:364-368.
- Brotman, M., R. A. Giannella, P. F. Alm, H. Bauman, A. R. Bennett, R. E. Black, C. M. Bruhn, M. B. Cohen, S. L. Gorbach, J. B. Kaper, M. R. Roberts, J. L. Stanek, S. Taylor, and H. F. Trout. 1995. Consensus conference statement: *Escherichia coli* O157:H7 infections—an emerging national health crisis, July 11-13, 1994. *Gastroenterology* 108:1923-1934.
- Feng, P. 1995. *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. *Emerg. Infect. Dis.* 1:47-52.
- Griffin, P. M. 1995. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*, p. 739-761. In M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant (ed.), *Infections of the gastrointestinal tract*. Raven Press, New York.
- Lopez, E. L., M. M. Contrini, S. Devoto, M. F. De Rosa, M. G. Grana, L. Aversa, H. F. Gomez, M. H. Genero, and T. G. Cleary. 1995. Incomplete hemolytic-uremic syndrome in Argentinean children with bloody diarrhea. *J. Pediatr.* 127:364-367.

Lukas Fenner,*¹
Véronique Roux,*
Pascal Ananian,†
and Didier Raoult**†

*Hôpital de la Timone, Marseille, France;
and †Hôpital de la Conception, Marseille,
France

References

1. Rautio M, Lonroth M, Saxen H, Nikku R, Väisänen ML, Finegold SM, et al. Characteristics of an unusual anaerobic pigmented gram-negative rod isolated from normal and inflamed appendices. *Clin Infect Dis*. 1997;25(Suppl 2):S107–10.
2. Rautio M, Saxen H, Siitonen A, Nikku R, Jousimies-Somer H. Bacteriology of histopathologically defined appendicitis in children. *Pediatr Infect Dis J*. 2000;19:1078–83.
3. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*. 1980;16:111–20.
4. Rautio M, Eerola E, Väisänen-Tunkelrott ML, Molitoris D, Lawson P, Collins MD, et al. Reclassification of *Bacteroides putredinis* (Weinberg et al., 1937) in a new genus *Alistipes* gen. nov., as *Alistipes putredinis* comb.nov., and description of *Alistipes finegoldii* sp. nov., from human sources. *Syst Appl Microbiol*. 2003;26:182–8.
5. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*. 1991;173:697–703.
6. Fenner L, Roux V, Mallet MN, Raoult D. *Bacteroides massiliensis* sp. nov., isolated from blood culture of a newborn. *Int J Syst Evol Microbiol*. 2005;55:1335–7.
7. Rigottier-Gois L, Rochet V, Garrec N, Suau A, Doré J. Enumeration of *Bacteroides* species in human faeces by fluorescent in situ hybridisation combined with flow cytometry using 16S rRNA probes. *Syst Appl Microbiol*. 2003;26:110–8.
8. Brook I. Clinical review: bacteremia caused by anaerobic bacteria in children. *Crit Care*. 2002;6:205–11.
9. Wareham DW, Wilks M, Ahmed D, Brazier JS, Millar M. Anaerobic sepsis due to multidrug-resistant *Bacteroides fragilis*: microbiological cure and clinical response with linezolid therapy. *Clin Infect Dis*. 2005;40:67–8.

¹Current affiliation: University Hospital Basel, Basel, Switzerland

10. Feuillet L, Carvajal J, Sudre I, Pelletier J, Thomassin JM, Drancourt M, et al. First isolation of *Bacteroides thetaiotaomicron* from a patient with a cholesteatoma and experiencing meningitis. *J Clin Microbiol*. 2005;43:1467–9.

Address for correspondence: Didier Raoult, Hôpital de la Timone, 264 rue Saint-Pierre, 13385 Marseille, France; email: didier.raoult@medecine.univ-mrs.fr

Shiga Toxin-producing *Escherichia coli*, Idaho

To the Editor: Data collected from expanded surveillance study suggest that more than half of Idaho Shiga toxin-producing *Escherichia coli* (STEC) illnesses are caused by non-O157 serotypes. Using data from a regional medical center whose stool culture protocol included Shiga toxin testing, we predicted Idaho's STEC incidence to be significantly higher if non-O157 STEC *E. coli* were routinely detected by immunoassay. Recent findings suggest that the prediction was accurate in an expanded surveillance area.

Several studies have shown an increased incidence of non-O157 STEC infections in the United States. For example, a community hospital in Virginia detected non-O157 serotypes in 31% of patients with STEC from 1995–2002 (1). A 1998 Nebraska study that analyzed 30,000 diarrheal stool samples found that non-O157 and O157:H7 STEC were equally prevalent (2). Additionally, findings from a Connecticut study of laboratory-confirmed cases (3), STEC surveillance results from Montana (4), and a recent study from Michigan (5) indicate that non-O157 serotypes

comprise a substantial percentage of STEC cases.

In other countries, nonculture-based methods are routinely used for STEC detection (6). However, *E. coli* O157:H7 culture methods remain the focus in the United Kingdom, Canada, and the United States (6). Reliance on culture methods can result in misleading interpretations of STEC prevalence. For example, 93% of STEC infections in Canada are reported to be *E. coli* O157:H7, yet a Manitoba 1992 study showed that when toxin assays were used, 35% of the recovered STEC isolates were non-O157 serotypes (6).

Analysis of reported non-O157 STEC cases in Idaho showed a similar trend. From 2002–2004, 66% of Idaho's non-O157 cases originated in Health District 7, where >70% of stool cultures are screened by enzyme immunoassay (EIA) for Shiga toxin (Premier EHEC, Meridian Bioscience, Cincinnati, OH, USA). This rate was disproportionately higher than that of the remaining 6 health districts, which primarily use culture methods to screen for *E. coli* O157:H7. We hypothesized that this disproportion was due to differences in stool culture protocol. To test this premise, we conducted enhanced surveillance for 16 months in a “low” STEC incidence area, Health District 5. A total of 2,065 stools submitted for culture were screened for Shiga toxin by EIA. With this approach, reported non-O157 STEC incidence rose from <1 case/year/100,000 population to 11 cases/year/100,000 population. Additionally, 56% of recovered STEC isolates were non-O157 serotypes, mirroring the proportion of non-O157 detected in District 7. Notably, this appears to be the endemic rate for District 5 because no non-O157 STEC outbreaks or matching pulsed-field gel electrophoresis patterns were detected during the surveillance period. Although our study captured only a portion of stool cultures in Idaho, our findings

demonstrated increased prevalence of non-O157 STEC in the region when nonculture methods were used.

Two barriers cited for not routinely screening diarrheal stools for Shiga toxin are cost and perception of low non-O157 STEC incidence. While toxin testing is more expensive than culture testing, the potential effects of misdiagnosis may outweigh cost concerns. A study estimating the financial repercussions of *E. coli* O157 infections in the United States suggested that annual cost associated with this pathogen is \$405 million, with the cost per case varying from \$26 for those who do not seek medical care to \$6.2 million for a patient with fatal hemolytic uremic syndrome (HUS) (7). Non-O157 STEC infections have been an important cause of HUS in many countries. For example, a 3-year prospective study in Germany and Austria reported that non-O157 serotypes comprised 90 (43%) of 207 STEC isolates from stools of 394 pediatric patients with HUS (8). Further, a 6-year Danish study of 343 registered STEC patients found that 76% of STEC and 48% of HUS cases were attributable to non-O157 serotypes (9). In the United States, continued reliance on O157 STEC culturing hinders our ability to determine the financial effects and the

proportion of HUS cases attributable to non-O157 STEC.

Some evidence suggests that the testing focus may be changing in the United States. We used US Census Bureau population statistics to translate reported O157:H7 and non-O157 STEC cases for each state into incidence data. Despite widespread variation in STEC testing and incidence among states, there has been a significant statistical decline in the proportion of *E. coli* O157:H7 among total STEC cases every year since 2001 (Figure; $p < 0.001$) (10). Consistent with this trend, the incidence of non-O157 STEC in the United States has increased (10). This may indicate that more laboratories are adopting Shiga toxin testing protocols, as we are advocating in Idaho. Our findings suggest that perceptions of low non-O157 STEC incidence in Idaho are probably artifactual and due to overemphasis on culture methods for O157 STEC. Our ongoing EIA-based surveillance highlights the need for continued investigation of the epidemiology of non-O157 STEC disease. We conclude that O157 STEC culturing has limited usefulness in areas like the Idaho health districts investigated, where non-O157 serotypes accounted for 55% of STEC illnesses. The true involvement of non-

O157 in STEC disease will remain obscured as long as screening methods focus on traditional culture methods.

Acknowledgments

We thank Richard Gelok and staff at Eastern Idaho Regional Medical Center in Idaho Falls and Janie Palmer and staff at St. Luke's Magic Valley Regional Medical Center in Twin Falls for their participation.

Partial support came from the Centers for Disease Control and Prevention, Epidemiology and Laboratory Capacity grant PA-01022.

**Vivian Marie Lockary,*
Richard Frederick Hudson,*
and Christopher Lawrence Ball***

*Idaho Bureau of Laboratories, Boise, Idaho, USA

References

1. Park CH, Kim HJ, Hixon DL. Importance of testing stool specimens for Shiga toxins [letter]. *J Clin Microbiol.* 2002;40:3542-3.
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. 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.
4. Jelacic JK, Damrow T, Chen GS, Jelacic S, Bielaszewska M, Ciol M, et al. Shiga toxin-producing *Escherichia coli* in Montana: bacterial genotypes and clinical profiles. *J Infect Dis.* 2003;188:719-29.
5. Manning SD, Madera RT, Schneider W, Dietrich SE, Khalife W, Brown W, et al. Surveillance for Shiga toxin-producing *Escherichia coli*, Michigan, 2001-2005. *Emerg Infect Dis.* 2007;13:318-21.
6. Kaper JB, O'Brien AD, eds. *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* Strains. Washington: ASM Press; 1998. p. 26, 55.
7. Frenzen PD, Drake A, Angulo FJ; Emerging Infections Program FoodNet Working Group. Economic cost of illness due to *Escherichia coli* O157 infections in the United States. *J Food Prot.* 2005;68:2623-30.

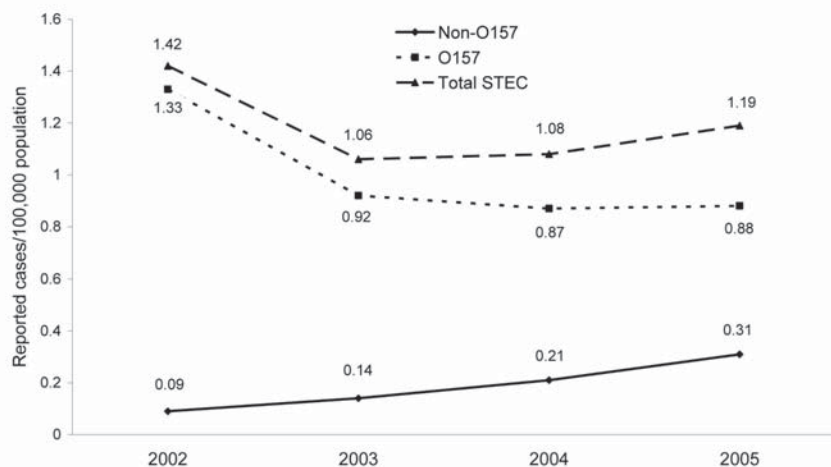


Figure. Shiga toxin-producing *Escherichia coli* (STEC) incidence trends, United States, 2002-2005.

8. 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.
9. Ethelberg S, Olsen KE, Scheutz F, Jensen C, Schiellerup P, Engberg J, et al. Virulence factors for hemolytic uremic syndrome, Denmark. *Emerg Infect Dis*. 2004;5:842–7.
10. Centers for Disease Control and Prevention. Summary of notifiable diseases, United States. [cited 2007 Jan 11]. Available from <http://www.cdc.gov/mmwr/summary.html>

Address for correspondence: Vivian Marie Lockary, Idaho Bureau of Laboratories, 2220 Old Penitentiary Rd, Boise, ID 83712, USA; email: lockaryv@dhw.idaho.gov

Imported Chikungunya Infection, Italy

To the Editor: Chikungunya virus (CHIKV) infection is a self-limiting illness characterized by fever, headache, weakness, rash, and arthralgia. Some patients have prolonged weakness or arthralgia lasting several months. In 2006, several Indian Ocean states and India had an outbreak of CHIKV infection (1,2). During the epidemic's peak, some European and American travelers returning from these areas were infected (3–6).

Because the foci of *Aedes albopictus*, 1 of the 2 main vectors of CHIKV, are now in Italy and many travelers visit CHIKV-epidemic areas, surveillance for imported cases is mandatory in Italy (7). From July to September 2006, a total of 17 confirmed cases of CHIKV infection were observed in travelers at 5 Gruppo di Interesse e Studio delle Patologie di

Importazione (GISPI) centers (Italian network of Institutes of Infectious and Tropical Diseases). Serologic diagnosis was performed with a hemagglutination-inhibition test and confirmed by a plaque-reduction neutralization test (8). Demographic and epidemiologic characteristics of these patients are reported in the Table.

Cases were distributed throughout the year with a peak from March to May 2006 (n = 10). Nine patients (53%) were men. Median age was 43 years (range 31–66 years). Several reasons for travel were reported: tourism (64.6%), visits to relatives or friends (11.8%), business (11.8%), and missionary work (5.9%). One patient was a resident in the disease-epidemic area. The median exposure time in the CHIKV-endemic area for the 15 travelers was 15 days (range 9–93 days) (missionary and resident patients were excluded). The median delay before being seen at a clinic after return was 2 days (range 0–73 days). Only 7 patients (41.2%) were hospitalized. The remainder were outpatients.

All patients had fever; arthralgia (88.2%, n = 15), weakness (70.6%, n = 12), headache (11.8%, n = 2), diarrhea (11.8%, n = 2), and gum bleeding and epistaxis (5.9%, n = 1) were other reported symptoms. The median duration of fever was 5 days (range 2–12 days). Only 7 of 16 patients (43.8%) were still febrile when first seen. Physical examination showed diffuse macular erythematous rash in 13 patients (76.5%), a similar rate to that reported among French travelers (4). Hepatomegaly was found in 2 patients (11.8%), splenomegaly in 2 (11.8%), and peripheral lymphadenopathy in 2 (11.8%).

Twelve acute-phase patients were admitted to the hospital for blood testing within 3 days of the initial examination. In contrast with results of other studies, leukopenia and thrombocytopenia were uncommon in our study. Leukopenia (leukocyte count $\leq 4,000/\mu\text{L}$) was present in 4 patients

(33.3%) and thrombocytopenia (platelet count $\leq 150,000/\mu\text{L}$) in 1 patient (8.3%). This finding may help distinguish CHIKV infection from dengue fever (4). Anemia (hemoglobin level ≤ 12 g/dL) was found in only 1 patient (8.3%). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) determination were available for 12 patients. ALT and AST levels were elevated (>40 IU/L) in 5 (41.7%) and 2 (16.7%) patients, respectively. Seven (46.7%) of 15 patients fully recovered within 1 month; 8 patients (53.3%) reported persistent arthralgia.

Because the GISPI network provides regional coverage only, the number of imported CHIKV cases in all of Italy in 2006 was likely higher. Moreover, most patients probably did not seek medical care, and when they did, physicians may have failed to recognize the disease because of lack of familiarity with it or limited diagnostic facilities. Differential diagnosis with other arthropodborne viruses of the *Alphavirus* genus (Ross River, Barmah Forest, o'nyong nyong, Sindbis, and Mayaro viruses) is difficult, but these are comparatively rare. In contrast, dengue and CHIKV epidemics may overlap, and potential patients should be screened for both.

The potential risk for introduction and establishment of CHIKV reservoirs in areas with mosquito vectors was discussed in March 2006 by a multidisciplinary European expert panel (9). In Italy, *A. albopictus* was first recorded in 1990; it has since quickly spread across the country. Scattered foci are now reported in almost all regions, mainly along the coastal plains, from the sea to the inlands, up to an altitude of ≈ 500 –600 m (7).

The ability of *A. albopictus* to colonize new areas and its adaptability to the mild Italian climate allow vector populations to be active throughout the year (10). The patient is thought to be viremic for only 6–7 days (shortly before and during the febrile period) (6). We were unable to directly assess



[E. coli \(www.cdc.gov/ecoli/\)](http://www.cdc.gov/ecoli/) > [E. coli Outbreak Investigations \(www.cdc.gov/ecoli/outbreaks.html\)](http://www.cdc.gov/ecoli/outbreaks.html) > Multistate Outbreak of Human *E. coli* O145 Infections Linked to Shredded Romaine Lettuce from a Single Processing Facility

Contact CDC

800-CDC-INFO
888-232-6348 (TTY)
cdcinfo@cdc.gov
(<mailto:cdcinfo@cdc.gov>)

Report a Foodborne Illness

(<http://www.cdc.gov/ncidod/dbmd/reportfi.htm>)

Investigation Announcement: Multistate Outbreak of Human *E. coli* O145 Infections Linked to Shredded Romaine Lettuce from a Single Processing Facility

Updated May 6, 2010

Local and state public health officials in Michigan, New York, and Ohio are investigating human illnesses caused by *E. coli* O145. CDC is supporting these investigations and facilitating regular communication and information sharing between the states and with the U.S. Food and Drug Administration (FDA).

As of **May 5, 2010**, a total of 19 confirmed and 10 probable cases related to this outbreak have been reported from 3 states since March 1, 2010. The number of ill persons identified in each state with this strain is : MI (10 confirmed and 3 probable), NY (2 confirmed and 5 probable), and OH (7 confirmed and 2 probable).

Among the confirmed and probable cases with reported dates available, illnesses began between April 10, 2010 and April 26, 2010. Infected individuals range in age from 13 years old to 29 years old and the median age is 19 years. Sixty-nine percent of patients are male. Among the 29 patients with available information, 12 (41%) were hospitalized. Three patients have developed a type of kidney failure known as hemolytic-uremic syndrome, or HUS. No deaths have been reported.

The outbreak can be visually described with a chart showing the number of persons who became ill each day. This chart is called an **epidemic curve or epi curve** (www.cdc.gov/ecoli/2010/ecoli_O145/0506_chart.html). Of note, it takes an average of 2 to 3 weeks from the time a person becomes ill to the time when the illness is confirmed by laboratory testing and reported. Please see the [E. coli Outbreak Investigations: Timeline for Reporting Cases \(www.cdc.gov/ecoli/reportingtimeline.htm\)](http://www.cdc.gov/ecoli/reportingtimeline.htm) for more details.

The bacteria responsible for this outbreak are referred to as Shiga toxin-producing *E. coli*, or STEC. STECs have been associated with human illness, including bloody diarrhea and a potentially fatal kidney condition called hemolytic-uremic syndrome (HUS). STEC bacteria are grouped by serotypes (e.g., O157 or O145). The STEC serotype found most commonly in U.S. patients is *E. coli* O157. Other *E. coli* serotypes in the STEC group, including O145, are sometimes called "non-O157 STECs." Currently, there are limited public health surveillance data on the occurrence of non-O157 STECs, including *E. coli* O145, therefore *E. coli* O145 may go unreported. Because it is more difficult to identify than *E. coli* O157, many clinical laboratories do not test for non-O157 STEC infection.

Investigators are using pulsed-field gel electrophoresis (PFGE), a type of DNA fingerprint analysis of *E. coli* bacteria obtained through diagnostic testing to identify cases of illness that might be part of this outbreak. This testing is done in public health laboratories as part of the [PulseNet \(http://wwwdev.cdc.gov/pulsenet/\)](http://wwwdev.cdc.gov/pulsenet/) network. Investigators have established a common definition of confirmed and probable cases related to this outbreak.

Confirmed cases are persons with:

- (1) *E. coli* O145 infection, or *E. coli* infection with O Group pending, AND

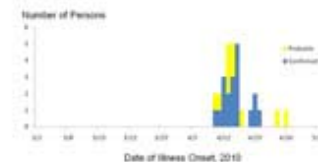
Confirmed cases of *E. coli* O145 Infection, United States, by state



(www.cdc.gov/ecoli/2010/ecoli_O145/0506_map.html)

[Click to view a larger image. \(www.cdc.gov/ecoli/2010/ecoli_O145/0506_map.html\)](http://www.cdc.gov/ecoli/2010/ecoli_O145/0506_map.html)

Infections with the outbreak strain of *E. coli* O145, by date of illness onset (n=19 for whom information was reported as of May 5, 2010)



(www.cdc.gov/ecoli/2010/ecoli_O145/0506_chart.html)

[Click to view a larger image. \(www.cdc.gov/ecoli/2010/ecoli_O145/0506_chart.html\)](http://www.cdc.gov/ecoli/2010/ecoli_O145/0506_chart.html)

- (2) an illness onset on or after March 1, 2010, AND
- (3) a DNA fingerprint matching the outbreak strain; AND
- (4) an epidemiologic link to the outbreak.

Probable cases are persons with an epidemiologic link to the outbreak and

- (1) *E. coli* O145 infection with an illness onset on or after March 1, 2010 regardless of DNA fingerprint pattern, AND/OR
- (2) hemolytic-uremic syndrome; AND/OR
- (3) a laboratory isolate positive for Shiga toxin 2 [*stx2*] or isolate positive for Shiga toxin, but toxin type is unknown or pending.

Current Status of the Investigation

Multiple lines of evidence have implicated shredded romaine lettuce from one processing facility as a source of infection in this outbreak. This evidence includes the identification of *E. coli* O145 from an unopened package of shredded romaine lettuce obtained from a facility associated with the outbreak. DNA testing to confirm the link to ill persons is pending at this time. The lettuce processing company has issued a [recall](http://www.freshwayfoods.com/about/press/20100506.php) (<http://www.freshwayfoods.com/about/press/20100506.php>) of lettuce produced at their facility as a result of the evidence obtained to date.

This investigation is ongoing. At this time, local, state, and federal health officials are involved in many different types of investigative activities including:

- Conducting surveillance for additional illnesses that could be related to the outbreak.
- Conducting epidemiologic studies that includes gathering detailed information from persons who were ill persons (cases) and from healthy persons (controls) about foods recently eaten and other exposures.
- Gathering and testing food products that are suspected as potential sources of infection to see if they are contaminated with bacteria.
- Following any epidemiologic leads gathered from interviews with patients, food purchase information, or from patterns of processing, production and/or distribution of suspected products.
- FDA is working closely with its state partners in the investigations at the food processor and at the farm level to determine where in the distribution chain the point of contamination likely occurred.

Public health and agriculture officials in Michigan, New York, and Ohio, along with CDC and FDA, are actively engaged in this investigation. Updates on the progress of this investigation will be shared as information becomes available.

Clinical Features/Signs and Symptoms

Most people infected with *E. coli* develop diarrhea (often bloody) and abdominal cramps 2-8 days (average of 3-4 days) after swallowing the organism, but some illnesses last longer and can be more severe. Infection is usually diagnosed by culture of a stool sample. Many clinical laboratories do not test for non-O157 STEC, such as *E. coli* O145, because identifying it is more difficult than for *E. coli* O157. Most people recover within a week, but some develop a severe infection. A type of kidney failure called hemolytic uremic syndrome (HUS) can begin as the diarrhea is improving; HUS can occur in people of any age but is most common in children under 5 years old and the elderly.

General Information

- [General Information: *E. coli* \(STEC\) \(\[www.cdc.gov/nczved/divisions/dfbmd/diseases/ecoli_o157h7/index.html#what_shiga\]\(http://www.cdc.gov/nczved/divisions/dfbmd/diseases/ecoli_o157h7/index.html#what_shiga\)\)](http://www.cdc.gov/nczved/divisions/dfbmd/diseases/ecoli_o157h7/index.html#what_shiga)
- [Description of the Steps In a Foodborne Outbreak Investigation \(<http://www.cdc.gov/outbreaknet/investigations/investigating.html>\)](http://www.cdc.gov/outbreaknet/investigations/investigating.html)
- [CDC's Role During a Multistate Foodborne Outbreak Investigation \(\[http://www.cdc.gov/salmonella/typhimurium/cdc_role_outbreak.html\]\(http://www.cdc.gov/salmonella/typhimurium/cdc_role_outbreak.html\)\)](http://www.cdc.gov/salmonella/typhimurium/cdc_role_outbreak.html)

Page last modified: May 7, 2010

Content source: [National Center for Zoonotic, Vector-Borne, and Enteric Diseases \(ZVED\) \(\[www.cdc.gov/nczved/\]\(http://www.cdc.gov/nczved/\)\)](http://www.cdc.gov/nczved/)