Outbreak of *E. coli* Infections in Boulder County & other Denver-area Counties  
September-October 2008  
Report written by Nisha Alden, MPH

**Background**

On September 26, 2008, Boulder County Public Health (BCPH) received a call from the Infection Control Practitioner (ICP) at Wardenburg Student Health Center at the University of Colorado (CU) indicating that several students from one sorority were ill with symptoms of bloody diarrhea and cramping. The sorority reported that it was rush weekend on campus on September 20th-21st and at least one hundred women “rushed” through their house. Within hours of this call, another call was received from the ICP at Boulder Community Hospital indicating that there were two college-aged women presenting with bloody diarrhea and cramping and being tested for *E. coli*. Upon investigation, it was identified that these two women were also associated with the same sorority at CU.

Boulder County Public Health began an epidemiologic and environmental investigation, after the initial report on September 26th, to identify the causative agent, the source of illness, and to implement environmental controls to prevent further illness. Boulder County Public Health collaborated with the Colorado Department of Public Health & Environment (CDPHE), Tri-County Health Department (TCHD), Broomfield Health & Human Services, and the Food & Drug Administration (FDA) during the investigation. This report outlines BCPH’s investigation findings.

**Methods**

*Environmental*

Boulder County Public Health consumer protection staff conducted an onsite investigation of the sorority house’s kitchen as well as three restaurants mentioned by cases to assess food handling procedures and storage, hand washing, food temperatures, cross contamination, sanitation, and the health status of employees. Tri-County Health Department inspected an additional restaurant outside of Boulder County. Restaurants were also asked about meals catered prior to the first reported onset of illness and to provide work schedules of employees and invoices for food incoming to the restaurant.
**Epidemiologic**

As is consistent with communicable disease investigation protocol, each case of reported illness with symptoms of gastrointestinal illness, specifically bloody diarrhea and cramping, was interviewed about their illness, foods they had eaten and other exposures they may have had prior to illness onset. The following case definitions were created to classify cases:

**Probable:** A person that has diarrhea and abdominal cramping with an onset date on or after 9/15/2008 AND who is epi-linked to another case AND/OR has a history of eating Jimmy John’s food prior to onset of symptoms.

**Confirmed:** A person that has diarrhea and abdominal cramping that is culture positive for STEC (*E. coli* O157:H7) with an onset date on or after 9/15/2008 with matching pulse-field gel electrophoresis (PFGE) pattern to the outbreak pattern.

Case finding was enhanced through communication with the public and healthcare community. Health alerts were issued using the Health Alert Network (HAN) to area healthcare providers to alert them of a possible increase in *E. coli* infections. Providers were encouraged to consider *E. coli* in their differential diagnosis of *E. coli* infections. Providers were encouraged to consider any cases to BCPH. A press release was issued on October 1, 2008 to alert the public of an outbreak of *E. coli* possibly associated with CU students. Cases were also reported through the Colorado Electronic Disease Reporting System (CEDRS).

In addition, BCPH staff conducted a cohort study of the sorority women and staff at the sorority house. A foodborne illness outbreak questionnaire was developed which asked about the following: basic demographic information, a list of all known food items served at the sorority house between Saturday, September 13, 2008, and Sunday September 20, 2008, restaurants eaten at during this time, being ill or well, time of illness onset, types of symptoms, illness among housemates, and duration of illness. A list of all sorority members obtained, and members and sorority house staff were asked to complete the questionnaires.

For the purposes of analysis, in the cohort study, a case was defined as persons with bloody diarrhea and abdominal cramping who attended a rush event at the sorority house on September 20th. Frequencies of symptoms, the duration of illness, and an epidemic curve were determined. The risk of illness among sorority members was calculated for food items served at the sorority house.

Questionnaire data were downloaded into Excel and analyzed with SAS version 9.1 software. Fisher’s exact chi-square and logistic regression were used to identify potential associations and confounders; relative risks and associated 95% confidence intervals were calculated.

**Laboratory**

Rectal swab specimens were collected from suspect cases and were submitted to the CDPHE laboratory to test for *E. coli*. Specimens were collected from the kitchen staff at the sorority house. Additionally, all employees of Jimmy John’s restaurant in Boulder were asked to submit rectal swab specimens. Two negative specimens were required of each employee before working at the facility could resume. Food specimens (alfalfa sprouts, lettuce and tomatoes) were also collected for testing and submitted to the CDPHE laboratory.
Results

Environmental

Four restaurant inspections were conducted at restaurants mentioned by cases during interview. A Jimmy John’s restaurant in Adams County was inspected by TCHD (results of that inspection are available through TCHD).

It was noted that only very few cases mentioned eating food from Cheba Hut or Illegal Pete’s, whereas the majority of cases (90%) mentioned eating food from Jimmy John’s. Jimmy John’s food was also identified as the link between sorority and non-sorority cases; therefore the inspection of the Boulder Jimmy John’s is summarized below.

The Jimmy John’s restaurant in Boulder has one kitchen in the facility. An environmental inspection of the kitchen on September 29, 2008 revealed the following:

The kitchen is a small facility that prepares ready-to-eat sandwiches. Sandwiches are made to order by restaurant staff. All lunchmeat is prepared at the Jimmy John’s commissary; it is then delivered to Cisco and shipped to all Jimmy John’s facilities. The facility has no raw meats. All produce comes from Shamrock Foods, with the exception of Thursdays, on which day the produce comes from Sysco Foods. A slicer is used for both produce and meats. The slicer is taken apart, washed, rinsed and sanitized after each item.

It was noted that alfalfa sprouts and roast beef in the cold top were being held out of temperature. The inspector collected a sample of alfalfa sprouts for laboratory testing. The manager on duty did not know where a thermometer was located and was not checking food temperatures.

Employees were wearing gloves upon the inspector’s arrival, however a few employees were observed changing gloves without washing their hands. The hand sink was stocked with soap and paper towels, and warm water was appropriately available.

Management was asked to not allow anyone to work while ill and to report any staff illnesses to BCPH.

Consumer protection and disease control staff initially interviewed six food handlers from Jimmy John’s in Boulder, including two who prepared sandwiches for the catered event at the sorority house. Upon interview it was determined that two staff had vomiting and/or diarrhea while working; facility managers were informed that these employees could not work until cleared by BCPH. On October 2nd, Jimmy John’s owner informed BCPH that they were voluntarily removing alfalfa sprouts from their menu until the source of the illness was identified.

Due to the identification of ill food handlers and slow response from the facility in providing the documents requested, BCPH issued a public health order and a mandatory glove order to Jimmy John’s in Boulder on October 6, 2008. This public health order mandated that: all staff must adhere to the mandatory glove order; all staff employed prior to October 6th must submit at least two stool specimens to be cultured for E. coli; no employee with a positive stool culture shall work in any food-handling capacity until they are asymptomatic and have two consecutive
negative cultures; the facility must provide a list of catered events from September 15th to present; and, the facility must provide a complete employee work history from September 15th to present.

On October 7th, after the confirmation of a case of *E. coli* infection in a staff at the Boulder Jimmy John’s, the identification of two additional symptomatic individuals who were working while ill and the identification of at least 15 suspect cases among individuals who had eaten food from the Jimmy John’s in Boulder, BCPH determined it was necessary to issue a second public health order that mandated closure of the restaurant until: staff working at the facility are cleared by BCPH by providing two negative stool samples that have tested negative for *E. coli*; all opened and prepared food items have been discarded; all food preparation and food contact areas have been washed, rinsed and sanitized appropriately; and, BCPH consumer protection must conduct an inspection to allow the facility to reopen. A letter was also provided to employees to explain the need and process for stool testing. BCPH staff met staff at the facility (prior to cleaning and sanitizing) to interview staff and to facilitate collection of stool specimens.

On October 9th, owners of the Boulder Jimmy John’s facility determined that they had enough cleared staff to open the facility for limited service. Boulder County Public Health staff conducted an inspection that revealed that while the facility had been professionally cleaned and sanitized, a residue remained on many surfaces. Therefore, further washing, rinsing and sanitized was required prior to any food preparation. On October 10th, the facility was re-checked and determined to be ready to open. Frequent inspections continued over the next week to assure that all food workers at the facility had been cleared for work by BCPH. Daily communication was provided to the facility owners with names of cleared employees.

**Epidemiologic**

**Sorority Cohort Study**

A total of 73 out of 98 (75%) sorority members and sorority house staff completed the questionnaire, which was distributed both by email and in person. A total of 7 individuals met case definition, for an illness rate of 11%. Those who met case definition most commonly reported bloody diarrhea (100%), abdominal cramping (100%) and nausea (100%). Table 1 shows frequencies for all symptoms among sorority cases. Median duration of symptoms for sorority cases was approximately 4 days (range: 2-7 days). Sorority cases had a mean age of 19 years (range: 18-31 years); 7 (100%) were female. There was one hospitalization and no deaths associated with this group. The median time from exposure to onset of illness was 3 days (range: 0.5-5 days).

The epidemic curve for the sorority members revealed a peak onset on 10/7/2004. The earliest cases presented on 9/20/2008 (Figure 1).
Table 1. Symptoms among sorority members who met case definition (n=7)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>7</td>
<td>100.0%</td>
</tr>
<tr>
<td>Bloody Diarrhea</td>
<td>7</td>
<td>100.0%</td>
</tr>
<tr>
<td>Abdominal cramping</td>
<td>7</td>
<td>100.0%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2</td>
<td>28.6%</td>
</tr>
<tr>
<td>Fever</td>
<td>6</td>
<td>85.7%</td>
</tr>
<tr>
<td>Weight Loss</td>
<td>5</td>
<td>71.4%</td>
</tr>
<tr>
<td>Headache</td>
<td>5</td>
<td>71.4%</td>
</tr>
<tr>
<td>Aches</td>
<td>5</td>
<td>71.4%</td>
</tr>
</tbody>
</table>

Figure 1. *E. coli* Cohort Study
Illness Onset among Sorority Members, September 2008
Table 2 shows illness rates and relative risks for all questionnaire items where significance levels were less than or equal to 0.05. The sandwiches mentioned refer to sandwiches from Jimmy John’s restaurant.

The Vegetarian Sandwich and some of its ingredients were significantly associated with illness (p = 0.019). Alfalfa sprouts from any Jimmy John’s sandwich were significantly associated with illness (p=0.004).

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Illness Rate among those who ate the food (%)</th>
<th>Illness Rate among those who did not eat the food (%)</th>
<th>Relative Risk</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any food from Jimmy John’s in Boulder</td>
<td>21.2</td>
<td>0.0</td>
<td>Undefined</td>
<td>0.011</td>
</tr>
<tr>
<td>Veggie Sandwich</td>
<td>33.3</td>
<td>5.8</td>
<td>5.78</td>
<td>0.019</td>
</tr>
<tr>
<td>Lettuce</td>
<td>36.4</td>
<td>5.7</td>
<td>6.42</td>
<td>0.014</td>
</tr>
<tr>
<td>Alfalfa Sprouts</td>
<td>40.0</td>
<td>5.6</td>
<td>7.2</td>
<td>0.009</td>
</tr>
<tr>
<td>Tomato</td>
<td>37.5</td>
<td>7.1</td>
<td>5.25</td>
<td>0.036</td>
</tr>
<tr>
<td>Cucumber</td>
<td>36.4</td>
<td>5.7</td>
<td>6.42</td>
<td>0.014</td>
</tr>
<tr>
<td>Cheese</td>
<td>36.4</td>
<td>4.7</td>
<td>6.42</td>
<td>0.017</td>
</tr>
<tr>
<td>Alfalfa Sprouts (from any sandwich)</td>
<td>22.6</td>
<td>0.0</td>
<td>Undefined</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Overall Outbreak Study**

A total of 21 individuals met case definition (14 confirmed, 7 probable). Onset dates for all cases are shown in Figure 2. Those who met case definition most commonly reported diarrhea (100%), abdominal cramping (91%), and bloody diarrhea (62%). Median duration of symptoms for all cases was approximately 4 days (range: 1-7 days). Cases had a median age of 20 years (range: 18-62 years); 16 (76%) were female. There were two hospitalizations and no deaths associated with this outbreak. One case was identified in each of Arapahoe, Broomfield, Jefferson and Weld counties; the remaining 17 cases resided in Boulder County. Of the cases not in Boulder County, 2 of 4 (50%) reported eating food from Jimmy John’s restaurant—one at the sorority house and one at a Jimmy John’s restaurant located in Adams County, Colorado. All 17 cases (100%) in Boulder County reported eating food from Jimmy John’s restaurant located in Boulder.
Three food handlers were identified through the questionnaires and laboratory testing as positive for *E. coli* infection. These food workers were then thoroughly interviewed by BCPH staff. All three food handlers reported working while symptomatic. Two workers reported symptom onset on September 17th, which was the earliest onset identified during the outbreak. The third worker reported symptom onset on October 5th, the latest onset identified.

**Laboratory**

Fourteen (67%) of 21 cases had a positive culture result for *E. coli* at the CDPHE laboratory. All 14 had matching PFGE patterns. The remaining 7 were not tested. Forty-two staff members from Jimmy John’s in Boulder provide specimens for testing. Three (7%) were positive for *E. coli*; two of the three had two follow-up negative results prior to returning to work. The third individual did not provide additional specimens and did not return to work at Jimmy John’s. The remaining 39 staff had negative results.

No food specimens tested positive for the presence of *E. coli*.

**Discussion**

The symptoms, duration of illness and laboratory testing implicate *E. coli* as the contaminating agent. Transmission is most often through contaminated food, but a food handler can also contaminate food if they have the bacteria on their hands.

The two main items of concern discovered during the investigation were the alfalfa sprouts implicated in the sorority cohort study and the three ill food handlers at Jimmy John’s in Boulder who worked while ill. Of the Boulder County cases, 100% reported eating food from Jimmy John’s and alfalfa sprouts from Jimmy John’s. However, the four cases from outside
Boulder County make the picture less clear. Two of the four cases ate food from a Jimmy John’s restaurant (one at the sorority house, one at a Jimmy John’s located in Adams County). The remaining two did not have exposure to Jimmy John’s food; while one of them reported eating alfalfa sprouts during the incubation period, the other reported no known consumption of alfalfa sprouts. Overall, 95% of cases (20 of 21) reported eating alfalfa sprouts and 90% of cases (19 of 21) reported eating food from a Jimmy John’s restaurant within one incubation period of illness onset.

Due to the concern that a food item, specifically the alfalfa sprouts, might be the source of illness, BCPH consulted with CDPHE’s Consumer Protection Division and the FDA. The FDA and CDPHE coordinated a trace-back of the alfalfa sprouts. The trace-back included an inspection of the grower, Sprouts Extraordinaire in Denver, by both CDPHE and FDA. The information provided to BCPH indicated that the investigation was inconclusive. Samples of alfalfa sprouts collected at the grower were tested at the CDPHE laboratory and by the FDA; no samples had positive results for *E. coli* contamination.

This outbreak highlights the need for restaurant management to have sick policies that are strictly enforced, given that food workers continuing to work while ill likely contributed to the spread of illness. Owners of the Jimmy John’s restaurant in Boulder did not appear to fully understand the implications of the initial reports of foodborne illness associated with their restaurant. Cooperation with BCPH was somewhat slow in the beginning, and owners did not appear to have informed their staff and management of the ongoing investigation until the closure order was issued. After BCPH issued the closure order, owners, management and staff were fully cooperative with BCPH’s investigation.

This analysis indicates that while the outbreak is likely due to a common source, the exposures may have occurred at different times. The matching PFGE patterns of all the confirmed cases indicate that a common source is responsible for each of the *E. coli* infections. Despite inconclusive laboratory evidence to indicate that the alfalfa sprouts was the source of the *E. coli* illnesses, there is significant epidemiologic evidence to indicate that both alfalfa sprouts and secondary person-to-food transmission at Jimmy John’s in Boulder contributed to the spread of illness.