



Dedicated to protecting and improving the health and environment of the people of Colorado

Date: December 4, 2014

To: File, Colorado Department of Public Health and Environment (CDPHE) Communicable Disease Branch (CDB)
CDPHE Laboratory Services Division (LSD)
CDPHE Division of Environmental Health and Sustainability (DEHS)
Denver Environmental Health (DEH)
Denver Public Health (DPH)
Jefferson County Public Health (JCPH)
Tri-County Health Department (TCHD)
United States Food and Drug Administration (FDA)
Jimmy John's Gourmet Sandwich Locations:
- 2610 W Belview, Littleton, CO (Arapahoe County)
- 180 S Union, Lakewood, CO (Jefferson County)
- 1040 S Colorado Blvd, Glendale, CO (Arapahoe County)
Colo-Pac Produce, Inc., 4345 Oneida St., Denver, CO (Denver County)

From: CDPHE Communicable Disease Branch
(contact: Nicole Comstock - 303-692-2676 - Nicole.comstock@state.co.us)

Subject: Outbreak #2013-00-006 - *Escherichia coli* O157:H7 Outbreak Associated with Cucumbers Consumed at a Sandwich Restaurant Chain - Colorado, October 2013

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SUMMARY

CDPHE and several Denver metropolitan area public health departments investigated an outbreak of *Escherichia coli* O157:H7 (*E. coli* O157:H7) that occurred in October 2013. Nine cases were identified, including 1 probable case and 8 laboratory-confirmed cases with matching pulsed-field gel electrophoresis (PFGE) and multiple-locus variable number tandem repeat analysis (MLVA) patterns from *E. coli* O157:H7 isolated from stool. All 9 cases reported eating sandwiches at Denver-area Jimmy John's locations in early October 2013. The outbreak investigation consisted of case finding and interviews, 2 separate case-control

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studies, environmental investigations, produce traceback, and laboratory testing. The results of this investigation indicate that consumption of Jimmy John's sandwiches containing cucumbers imported from Mexico was the likely cause of the outbreak. To our knowledge, this is the first *E. coli* O157:H7 outbreak associated with cucumbers reported in the United States. Public health and food safety officials should be aware that cucumbers may be contaminated with *E. coli* O157:H7, which could cause sporadic *E. coli* O157:H7 infections as well as outbreaks. As of the date of this report, no other cases of *E. coli* O157:H7 with the PFGE pattern combination seen in this outbreak were reported in Colorado.

INTRODUCTION / BACKGROUND

E. coli O157:H7, a bacterium, is part of a larger group of pathogenic *E. coli* referred to as Shiga toxin-producing *E. coli* (STEC). STEC bacteria produce potent Shiga toxins that cause gastrointestinal illness. *E. coli* O157:H7 is an important cause of sporadic gastrointestinal illness as well as outbreaks in the United States. Symptoms of *E. coli* O157:H7 infection include diarrhea (often bloody) and abdominal cramping; fever and vomiting may or may not occur and some infected persons may be asymptomatic. Persons of any age can become infected with *E. coli* O157:H7 and most infected persons recover without complications; however, some persons develop serious complications such as hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP) (1, 2). HUS and TTP complications are more common in children, persons with underlying medical conditions, and the elderly, and may lead to kidney failure, nervous system damage, and death.

The natural reservoir for *E. coli* O157:H7 is in the intestines of cattle and other ruminant animals, including goats, sheep, deer, and elk; these animals shed the bacteria in their feces (2). Persons acquire *E. coli* O157:H7 infection by ingesting the bacteria in contaminated food or water, or through contact with contaminated environmental surfaces followed by hand-to-mouth behaviors. Infected persons can also spread the bacteria to others through fecal-oral transmission. The infectious dose of *E. coli* O157:H7 is very small, and the incubation period ranges from 1 to 10 days, with an average of 3 to 4 days (2). Outbreaks of *E. coli* O157:H7 have been associated with ingestion of various contaminated food products (such as beef products; produce items, especially leafy greens and sprouts; raw cookie dough; and unpasteurized and pasteurized dairy products), ingestion of contaminated water, contact with persons infected with *E. coli* O157:H7, and contact with infected animals and contaminated animal environments (3, 4).

STEC infections are a public health reportable condition in Colorado, meaning that health care providers and clinical laboratories are required to report cases to public health so investigation and follow-up can occur. STEC infections occur regularly with a peak incidence during the summer months. *E. coli* O157:H7 is the most common STEC serotype reported in Colorado. For the 5-year period from 2008 through 2012, an average of 80 laboratory confirmed cases of *E. coli* O157:H7 were reported in Colorado per year (range: 45 to 134 cases). During this time frame, an average of 14 cases of *E. coli* O157:H7 were reported in the month of October (range: 4 to 31 cases). On Friday, October 18, 2013, CDPHE CDB, CDPHE LSD, and local public health department epidemiologists noted an increase in reported STEC cases and confirmed *E. coli* O157:H7 cases in the Denver metropolitan area (5 *E. coli* O157:H7 cases reported in 1 week) and began an investigation to determine the cause of the increase.

By Monday, October 21, 2013, CDPHE LSD determined that 2 *E. coli* O157:H7 cases had matching PFGE patterns using 2 enzymes. PFGE, commonly referred to as "DNA fingerprinting", is a method of molecular subtyping that allows for discrimination between bacterial isolates. CDPHE LSD posted the PFGE patterns on PulseNet to determine if other states had recent cases with the same PFGE pattern combination. PulseNet is a national network for enteric disease surveillance established by the US Centers for Disease Control and Prevention (CDC), and allows states to compare PFGE patterns to determine if multiple states have cases that could be part of a particular outbreak. Epidemiologists at CDC noted that the PFGE pattern



combination seen in the 2 Colorado cases was commonly seen nationwide every year. In October 2013, CDC was monitoring this PFGE pattern combination as several states (AZ, MN, OR, CA, and IN) had reported recent cases. A Minnesota case with this PFGE pattern combination had leftover ground beef which tested positive for *E. coli* O157:H7; the ground beef had the same PFGE pattern combination as the patient. This ground beef was purchased from a national warehouse chain store and was determined to be the likely source of the Minnesota resident's infection; based on Minnesota's investigation findings, it was determined that ground beef was not distributed nationwide and likely not available for consumption in Colorado. Arizona's PFGE-matched cases were associated with a child care center outbreak caused by person-to-person transmission; this outbreak occurred several months before the Colorado cases. Because of the commonality of the PFGE pattern combination, MLVA testing was conducted on available *E. coli* O157:H7 isolates from states with recent cases. MLVA allows for an even finer level of comparison between isolates. On Tuesday, October 22, 2013, the MLVA testing on 4 of the Colorado cases showed that all 4 had indistinguishable MLVA patterns that had never been seen in the US to date; the MLVA pattern of the 4 Colorado cases did not match the MLVA patterns of the PFGE-matched MN and CA cases, indicating that Colorado likely had a cluster of cases not related to the PFGE-matched cases reported in other states during a similar time frame. Additional states had MLVA testing completed on *E. coli* O157:H7 cases with the matching PFGE pattern combination, none of which matched the MLVA pattern seen in the Colorado cases, further supporting that the Colorado cluster had a unique exposure.

INVESTIGATION / METHODS

The investigation was comprised of seven components:

- Case finding
- Case interviews
- Case-control study I using neighborhood controls
- Case-control study II using Jimmy John's restaurant patron controls
- Environmental investigation
- Produce traceback
- Laboratory testing

For this investigation, the following final case definitions were used:

- A **confirmed** case was defined as a Colorado resident with onset of laboratory confirmed *E. coli* O157:H7 infection with the outbreak MLVA pattern in October 2013.
- A **probable** case was defined as a Colorado resident with post-diarrheal HUS and positive *E. coli* O157 serology in October 2013.

Case Finding:

CDPHE and local public health agencies conducted routine public health surveillance in order to detect cases and determine the extent of the outbreak (i.e., monitoring cases of STEC reported by clinical laboratories and health care providers statewide into the Colorado Electronic Disease reporting system [CEDRS]). CDPHE contacted state health departments in neighboring states (Wyoming, New Mexico, and Utah) as well as the CDC to notify them of the outbreak and enhance case finding. CDPHE posted the outbreak on PulseNet to notify all states of the outbreak and to request reporting of cases that might be outbreak-associated (i.e., cases in other states with PFGE- or MLVA-matched *E. coli* O157:H7 isolates, or *E. coli* O157:H7 cases with exposure to Jimmy John's restaurants). CDPHE also provided outbreak updates on October 23, October 30, and November 26, 2013, to local public health agencies and hospital infection preventionists via the weekly CDPHE "Hot Topics in Epidemiology" email newsletter; in these updates, partners were asked to report suspected patients or cases to CDPHE for further follow-up. Case finding also occurred when public health contacted patrons of the implicated Jimmy John's locations, as described below for Case-Control Study II. In addition, this outbreak received media attention. CDPHE did not issue a press release for this outbreak as



surveillance data and the investigation findings determined that there was not ongoing transmission or risk to the public. We believe that the media was alerted to the outbreak by a Jimmy John's patron who was contacted by public health during Case-Control Study II.

Case Interviews:

Reported cases of STEC were promptly interviewed using a standard STEC questionnaire by staff at CDPHE or the local public health agency in the county where the case resided. This questionnaire collected demographic, clinical, and travel information; gathered water, animal, and food exposure data; and assessed potential transmission risks. For cases less than 18 years of age, parents or guardians were interviewed. Secondary interviews were conducted by a single CDPHE epidemiologist using the CDC hypothesis-generating questionnaire for 7 cases (cases identified later in the investigation did not need to be interviewed with this extensive questionnaire). A dynamic interviewing approach was utilized by the CDPHE epidemiologist, where cases were re-interviewed and questioned about exposures reported by other cases as those exposures were identified.

Additional public health actions were conducted based on the general and hypothesis-generating interview findings, including 2 separate case-control studies, environmental investigations, and laboratory testing. These actions were conducted to test the hypothesis that Jimmy John's was the source of the outbreak and to identify specific foods associated with illness and the outbreak.

Case-Control Study I:

Planning for the first case-control study commenced on October 23, 2013, and the study was completed by October 29, 2013. The study goal was to determine if eating at Jimmy John's was associated with illness. Cases and controls were administered a standard questionnaire that assessed possible exposures during the time frame of October 5 through October 9, including consumption of lettuce, fruit, ground beef and other foods, and eating at restaurants that at least 1 case reported eating at during case interviews, including Jimmy John's. Cases that met the confirmed case definition were eligible for this study and were interviewed by a CDPHE epidemiologist (n=8). CDPHE staff and trained public health graduate students attempted to enroll 3 age-group-, sex-, and neighborhood-matched controls for each case. The age group used in the study was 18 to 65 years of age. Potential controls were contacted using sequential digit dialing starting from the corresponding case's landline phone number. If a case did not have a landline phone number, an internet search tool (www.whitepages.com) was used to find a landline phone number associated with a residence close to where the case lived. Potential controls were contacted via telephone, with 1 attempt made to each sequential phone number. Calls were made during weekdays, evenings, and weekends. If a household contained a potential age- and sex-matched control who agreed to participate in the study, then the questionnaire was administered to that person. Controls were excluded if they had any gastrointestinal symptoms since October 7, or if they were not in Colorado for at least 1 day from October 5 through October 9. See Appendix 1 for the questionnaire used in this study. Data were analyzed using Epi Info version 7 software. Odds ratios, 95% confidence intervals, and Fisher's 2-tailed exact test p-values were calculated for each exposure.

Case-Control Study II:

Planning for the second case-control study commenced on October 23, 2013, and the study was completed by October 30, 2013. The study goal was to determine specific Jimmy John's exposures/foods that were risk factors for acquiring *E. coli* O157:H7 infection. Cases that met the confirmed case definition were eligible for this study and were interviewed by a CDPHE epidemiologist (n=8). CDPHE epidemiologists and regional epidemiologists housed at local public health agencies across the state interviewed controls. Potential controls were contacted from a list of persons who had placed phone or online orders at the same Jimmy John's location on the same date that a case ate; only records that contained phone numbers were able to be contacted as potential controls. Controls were administered a questionnaire via telephone that assessed



exposures at Jimmy John's, including sandwich(es) eaten, sandwich toppings, as well as behavioral practices. Three Jimmy John's locations where cases reported definitively eating at during their incubation period provided the list of potential controls: the Lakewood location list contained 46 records with phone numbers; the Littleton location list contained 92 records with phone numbers; and Glendale location list contained 128 records with phone numbers. Potential controls were excluded if they reported not eating at Jimmy John's, if they did not live in Colorado, or if they had any gastrointestinal symptoms since October 7, 2013. See the Appendix 2 for the questionnaire used in this study. Data were entered into a Survey Monkey data collection tool by interviewers, and data was imported into a Microsoft Access database and analyzed using SAS version 9.1 software. Summary odds ratios, 95% confidence intervals, and Fisher's 2-tailed exact test p-values were calculated for each exposure.

Environmental Investigation:

Environmental health specialists (EHS) from JCPH and TCHD conducted environmental assessments at the 3 implicated Jimmy John's locations on Wednesday, October 23, 2013 (the Lakewood location is in Jefferson County, and the Littleton and Glendale locations are in Arapahoe County). On this day, CDPHE CDB also notified Jimmy John's corporate office of our investigation. The assessments were unannounced and involved the following activities, with special focus on food items reported being consumed by multiple cases (lettuce, tomato, cucumbers, cheese, and avocado spread):

- Observing food handling and food storage activities/methods
- Collecting information about food turnover and shelf-life
- Collecting food samples common to sandwiches that the cases reported eating (lettuce, tomato, cucumber, avocado spread, and cheese) to be analyzed at CDPHE LSD
 - Samples were collected directly from the service line or from food storage areas using sterile collection equipment and were placed in sterile containers for transport to CDPHE LSD
- Collecting employee work schedules and looking for shared staff among the implicated restaurants from September 30 through October 23, 2013
- Asking about sick leave policies and illness in employees from September 30 through October 23
- Collecting information on food suppliers from September 30 through October 23
- Collecting invoices from food supply deliveries made from September 30 through October 23
- Inquiring about any foodborne illness complaints received at the restaurant from September 30 through October 23

CDPHE DEHS environmental protection specialists (EPS) conducted environmental assessments at the produce distributor that serviced all 3 Jimmy John's locations (Colo-Pac Produce, Inc.) on October 23, 24, and 25, 2013. CDPHE DEHS determined that this distributor was supplier to the Jimmy John locations by calling each of the 3 implicated Jimmy John's locations and asking where they purchased their produce. The assessments at Colo-Pac Produce, Inc. were unannounced and involved the following activities:

- Observing produce handling and produce storage activities/methods
- Collecting information about produce turnover
- Collecting produce samples
 - Samples were collected directly from food storage areas using sterile equipment and were placed in sterile containers for transport to CDPHE LSD
- Collecting environmental samples, including from delivery trucks
 - Samples were collected using sterile water moistened swabs and transported to CDPHE LSD
- Collecting employee work schedules from mid-September through October 23
- Asking about sick leave policies and illness in employees from mid-September through October 23
- Interviewing employees about illness from mid-September through October 23
- Collecting records for produce received at the facility from mid-September through October 23
- Collecting records for produce distributed by the facility from mid-September through October 23



CDPHE DEHS notified the US Food and Drug Administration (US FDA) Denver District Office on October 23, 2013, to alert them of the outbreak investigation, with additional updates provided over the course of the investigation.

Produce Traceback:

Using records collected at the Jimmy John's locations and Colo-Pac Produce, Inc., CDPHE conducted a traceback of produce items on October 28, 2013, in order to determine if a common lot of a particular produce item could have been shipped to each of the 3 implicated Jimmy John's locations. These records were also provided to FDA so they could conduct their own traceback investigation.

Laboratory Investigation:

The laboratory investigation consisted of testing clinical, food, and environmental specimens. CDPHE performed confirmatory testing on Shiga toxin positive broths sent from clinical laboratories. Shiga toxin testing was completed using a polymerase chain reaction (PCR) test. Broths were cultured, and *E. coli* isolates were serotyped using standard methods for confirmation of clinical specimens. *E. coli* O157:H7 isolates underwent PFGE testing using 2 restriction enzymes and MLVA testing to further distinguish isolates.

In addition, CDPHE LSD tested the food and environmental swab specimens obtained from Jimmy John's and Colo-Pac Produce, Inc. Food and swabs were placed in enrichment broth and plated on a selective agarose medium to identify non-lactose fermenting organisms. PCR testing for Shiga toxins 1 and 2 were also performed on these broth samples.

The CDC laboratory performed *E. coli* O157 serology on leftover blood specimens from 1 probable case.

RESULTS

Case Finding:

In total, 9 cases were determined to be part of this outbreak. Of these, 8 were confirmed cases and 1 was a probable case. The probable case ate at Jimmy John's, had a diarrheal illness, and had a stool specimen tested for Shiga toxin at a clinical laboratory, but the result was negative (however, the patient had reportedly been on antibiotic treatment for a respiratory infection when stool was collected, so that could have affected the Shiga toxin test). The patient went on to develop HUS and had multiple serum samples test IgM positive for *E. coli* O157 antigens at the CDC laboratory.

Seven (78%) of the cases were female, and the median age of cases was 23 years (range: 10 to 60 years). Eight (89%) cases reported their race and ethnicity as White/non-Hispanic, and 1 as White/Hispanic. Cases resided in the following Denver metropolitan area counties: Adams (1), Arapahoe (2), Denver (2), and Jefferson (4). All 8 confirmed cases were identified by routine disease case reporting. The probable case was identified through control calls made for Case-Control Study II (the case's dining companion was contacted by public health as part of the study and this person alerted public health to the case's illness). No cases were identified in residents of other states.

Case Interviews:

Table 1 displays the symptoms reported by the 9 cases in this outbreak. All 9 cases reported diarrhea, and 8 (89%) reported bloody diarrhea. Among 2 cases who reported fever, the maximum temperature ranged from 99 to 100 degrees F. Eight cases reported illness duration, and the median duration of illness was 6 days (range: 4 to 14 days); 1 case reported still being ill at the time of interview (the HUS case). One case developed HUS; this patient was the only hospitalized case. All cases survived.

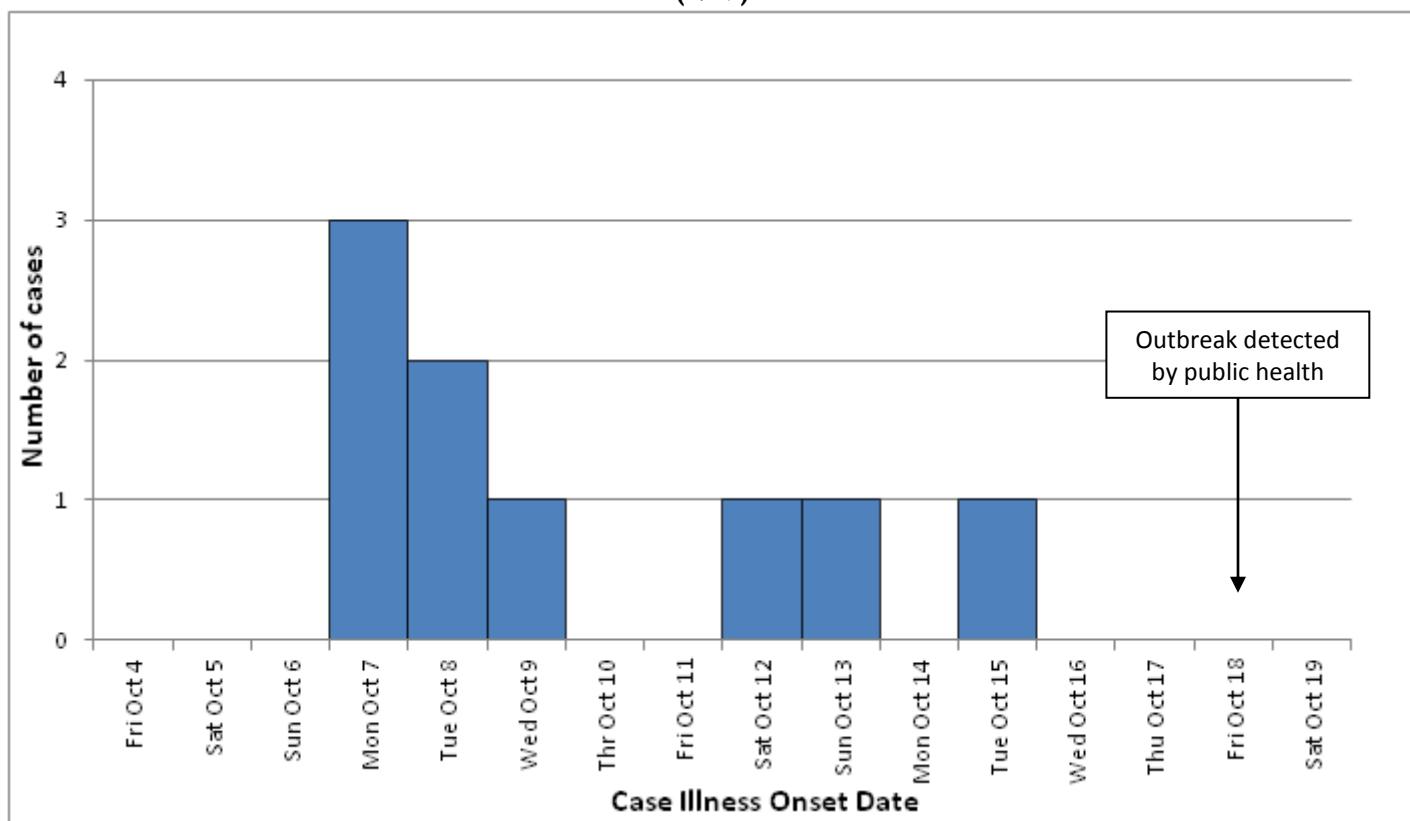


Table 1: Symptoms reported by outbreak cases (N=9)

Symptom	n (%)
Diarrhea	9 (100)
Abdominal pain/cramps	9 (100)
Bloody diarrhea	8 (89)
Vomiting	5 (56)
Body aches	4 (44)
Headache	3 (33)
Fever	2 (22)
HUS	1 (11)

Figure 1 illustrates the epidemic curve for this outbreak. Cases reported illness onset between October 7 and October 15, 2013.

Figure 1: Epidemic curve - Illness onset dates among cases, *E. coli* O157:H7 outbreak, Colorado, 2013 (N=9)



Through the dynamic interviewing process using a standard STEC questionnaire for 8 cases, a hypothesis generating questionnaire for 7 cases, and re-interviewing cases as necessary, it was determined that the common exposure among all 9 cases was consuming sandwiches from at least 3 different locations of Jimmy John's restaurants in the Denver metropolitan area. No other common exposure was detected among cases. Cases provided information about exposures commonly associated with *E. coli* O157:H7 infection, including consuming lettuce, spinach, or ground beef, and animal contact.



- Among 8 cases who provided lettuce consumption information, 5 reported consuming lettuce from sources other than Jimmy John's during their incubation period, but different sources and types of lettuce were reported among these cases, including lettuce grown in personal gardens.
- Among 8 cases who provided spinach consumption information, 4 reported consuming spinach during their incubation period and 2 cases may of have consumed spinach but could not definitively recall this exposure; there were no common sources of spinach identified among cases.
- Among 7 cases who provided ground beef consumption information, only 3 reported consuming ground beef during their incubation period; 1 case reporting being a pesco-vegetarian.
- Among 6 cases who provided animal contact information, none had contact with farm animals or a petting zoo. All 6 had contact with household dogs or dogs belonging to acquaintances; however, no common dog food or treat item was identified among the 6 cases.

Cases reported purchasing groceries at a variety of grocery stores, but no common grocery store was reported among all cases. Six cases reporting shopping for groceries at King Soopers stores in the Denver metropolitan area, so CDPHE attempted to collect shopper card numbers from these cases in order to obtain detailed shopping records. A request for shopper card data was submitted to King Soopers parent company (Kroger) for 4 of the cases; however, Kroger did not fulfill this request. CDPHE choose not to pursue this as the epidemiological data and environmental assessment data were pointing to a common source not associated with foods purchased at King Soopers.

Exact Jimmy John's location and visit date information was available from 8 cases:

- 4 ate at the Littleton location (1 on October 5, 2 on October 6, and 1 on October 7);
- 3 ate at the Lakewood location (all on October 5);
- 1 ate at the Glendale location (on October 9).

One case reported eating sandwiches prepared at several different Jimmy John's locations in the Denver area during the incubation period. This case reported that the only sandwich ever consumed was the "#12 Beach Club", regardless of the location or site eaten. Jimmy John's food items consumed by cases are listed in Table 2. Both illness onset date and Jimmy John's exposure date was known for 8 cases. The median incubation period (i.e., time period between consuming food from Jimmy John's and onset of symptoms) was 3 days (range: 2 to 8 days). All 9 cases reported consuming bread, cucumbers, and tomatoes from Jimmy John's; 8 cases reported consuming lettuce and mayonnaise from Jimmy John's.



Table 2: Jimmy John's location, date eaten, and foods consumed by cases (N=9)

CASE	Illness Onset	Jimmy John's Location	Date Eaten	Sandwich	Food Items Consumed								
					Bread	Cucumber	Tomato	Lettuce	Mayo	Turkey	Provolone	Avocado Spread	Other
A	Oct 7	Littleton	Oct 5	#6 Vegetarian	Y	Y	Y	Y	Y		Y	Y	
B	Oct 7	Lakewood	Oct 5	#12 Beach Club	Y	Y	Y	Y	Y	Y	Y	Y	
C	Oct 7	Lakewood	Oct 5	#4 Turkey Tom	Y	Y	Y	Y	Y	Y			
D	Oct 8	Lakewood	Oct 5	#12 Beach Club	Y	Y	Y	Y	Y	Y	Y	Y	
E	Oct 8	Littleton	Oct 6	#16 Club LuLu*	Y	Y	Y	Y	Y	Y			Bacon, peppers
F	Oct 9	Littleton	Oct 6	#4 Turkey Tom	Y	Y	Y	Y	Y	Y			
G†	Oct 12	Unknown	Unknown	#12 Beach Club	Y	Y	Y	Y	Y	Y	Y	Y	
H	Oct 13	Glendale	Oct 9	#12 Beach Club‡	Y	Y	Y		Y	Y	Y	Y	Dijon mustard
I	Oct 15	Littleton	Oct 7	#3 Totally Tuna	Y	Y	Y	Y					Tuna, celery, onion, sauce
					TOTALS: n	9	9	9	8	8	7	5	5
					%	100	100	100	89	89	78	56	56

* case added cucumbers and peppers to this sandwich (normally sandwich does not come with these items)

† case ate a Jimmy John's sandwich at a catered work event and during personal time and could not recall exact locations

‡ case ordered the sandwich without lettuce, which is normally included

Case-Control Study I:

Table 3 presents the results from the first case-control study. Eight cases were included in the study. Attempts were made to enroll 3 controls per case, but only 12 controls were enrolled after interviewers called 574 phone numbers; an average of 48 calls were made for each enrolled control. Of the calls made, 318 (55%) of the phone numbers were disconnected. Due to the low response rate, limited staffing resources, and epidemiological evidence that strongly supported Jimmy John's as the likely common exposure among cases, CDPHE ended the study on October 29, 2013. Several exposures assessed in the study were statistically significant: consuming food from Jimmy John's (OR undefined, $p = 0.00007$); consuming hummus (OR undefined, $p = 0.003$); consuming cucumbers (OR undefined, $p = 0.005$), and consuming tomatoes (OR undefined, $p = 0.04$). All 8 cases and only 1 control reported eating at Jimmy John's during the time frame in question. Four cases reporting consuming hummus during their incubation period, and 3 different brands were reported. All 8 cases and 4 controls reporting consuming cucumbers. All 8 cases and 6 controls reporting consuming tomatoes; the types (i.e., roma, cherry, red round, etc.) and sources of tomatoes (i.e., restaurant, grocery store, backyard garden, etc.) were different for all cases and controls.



Table 3: Results of “Case-Control Study I” (8 cases and 12 controls)
 (Note: case or control responses of “unknown” were not included in the analysis;
 statistically significant results are in bold)

Exposure	Cases Exposed n (%)	Controls Exposed n (%)	Odds Ratio	95% Confidence Interval	P-value (Fisher exact 2-sided)
Jimmy John's	8 (100)	1 (11)*	Und†	Und†	0.00007
Hummus	4 (80)	0 (0)	Und†	Und†	0.003
Cucumbers	8 (100)	4 (33)	Und†	Und†	0.005
Tomato	8 (100)	6 (50)	Und†	Und†	0.04
Green onions	3 (43)	1 (8)	8.25	0.65 - 104.20	0.12
Spinach	5 (83)	5 (42)	7.0	0.61 - 79.87	0.15
Lettuce	8 (100)	9 (75)	Und†	Und†	0.24
Sub Culture	1 (14)	0 (0)	Und†	Und†	0.37
Ground beef at restaurant	1 (13)	3 (25)	0.43	0.04 - 5.06	0.62
Bell peppers	4 (66)	6 (50)	2.0	0.26 - 15.38	0.64
Cilantro	2 (29)	4 (33)	0.80	0.10 - 6.10	1.00
Ground beef at home	2 (25)	3 (25)	1.00	0.13 - 7.89	1.00

† Und = Undefined due to 1 of the cells in the 2 x 2 table containing zero

* this control ate at the Littleton location on Oct 5 (1 case ate at this location as well) and had a ham sandwich with lettuce and cheese (no cucumbers reported)

Case-Control Study II:

Table 4 presents results from the second case-control study. Eight cases and 112 controls were included in the study. Interviewers called 175 phone numbers on October 28, 29, and 30, 2013, and enrolled 112 controls; an average of 1.6 calls were made for each enrolled control; only 2 calls were made to disconnected phone numbers. CDPHE was aware of 8 confirmed cases associated with the outbreak when this study was conducted. The ninth case (probable case) was identified through control calls made for this study (the case's dining companion and this person alerted public health to the case's illness). This study collected data on numerous food exposures at Jimmy John's, and the table shows statistically significant exposures as well as several other selected exposures. Cases were more likely than controls to have eaten sandwiches containing cucumbers (OR undefined, p = 0.0016). All of the cases and 43% of the controls reported eating sandwiches containing cucumbers. Cases were also more likely than controls to have eaten avocado spread (OR 4.76, p = 0.041); however, only 5 cases reported consuming avocado spread. Further stratification examining avocado spread exposure among persons who consumed and did not consume cucumbers was not possible since all of the cases reported eating cucumbers. Eating lettuce or tomatoes were not statistically associated with illness.



Table 4: Results of “Case-Control Study II” (8 cases and 112 controls)
 (Note: case or control responses of “unknown” were not included in the analysis;
 statistically significant results are in bold)

Exposure	Cases Exposed n (%)	Controls Exposed n (%)	Odds Ratio	95% Confidence Interval	P-value (Fisher exact 2-sided)
Cucumbers	8 (100)	46 (43)	Und*	Und*	0.002
Avocado spread	5 (63)	28 (26)	4.76	1.06 - 21.23	0.041
Tomatoes	8 (100)	89 (80)	Und*	Und*	0.349
Provolone cheese	5 (63)	46 (43)	2.17	0.49 - 9.53	0.464
Lettuce	7 (88)	102 (93)	0.55	0.06 - 5.03	0.481
Mayonnaise	7 (88)	79 (74)	2.48	0.29 - 21.07	0.678
Hot peppers	1 (13)	15 (14)	0.89	0.10 - 7.72	1.000

*Und = Undefined due to one of the cells in the 2 x 2 table containing zero

Environmental Investigation:

The 3 environmental assessments conducted by JCPH and TCHD on October 23 at the implicated Jimmy John’s restaurants had similar findings. All 3 Jimmy John’s locations received their produce items from Colo-Pac Produce, Inc. during the time frame in question; cheese, deli meat, tuna, and avocado spread were supplied by Nobel Sysco. Produce deliveries were made to the Jimmy John’s locations 2 to 3 times per week, depending on sales volume. Invoices for all deliveries were obtained. Lettuce, tomatoes, and cucumbers were all received whole and were washed, shredded or sliced, and refrigerated onsite as needed. Cucumbers were sliced and not peeled. The turnaround time for most produce items was typically around 3 days or less, but could be longer based on produce delivery schedules. No produce items were available for testing from the time period when cases ate at the restaurants. There were no shared employees among the 3 locations, and none of the locations reported ill employees during the time frame in question. Employees were not provided a free meal unless they worked more than 7 hours on a given shift; however, it was likely that employees purchased and consumed food from Jimmy John’s often. None of the locations reported receiving any complaints of illness from patrons during the prior month. Food samples were collected from all 3 locations and delivered to CDPHE LSD on the same day:

- Lakewood location - 13 samples collected: whole unwashed cucumber; sliced cucumber; unwashed head lettuce; shredded lettuce; whole unwashed Roma tomato; sliced tomato; whole onion; sliced onion; sliced peppers; avocado spread; provolone cheese; sliced turkey deli meat; and tuna mix comprised of celery, onion, mayonnaise, and soy sauce
- Littleton location - 5 samples collected: lettuce, tomato, sliced cucumber, avocado spread, and provolone cheese
- Glendale location - 10 samples collected: 2 samples of sliced cucumbers; 3 samples of shredded lettuce; 2 samples of sliced tomatoes; avocado spread; and 2 samples of sliced provolone cheese

The environmental assessments conducted by CDPHE DEHS EPS staff at Colo-Pac Produce, Inc. on October 23, 24, and 25 found no obvious sources of contamination to any of the produce items handled by this facility. Findings included:

- The facility employed 30 persons who worked in the produce distribution area and 10 office/administration staff. There were no reports of workers with gastrointestinal symptoms; several workers had been absent from work during the time period of interest but upon interview, the reasons for absence were not due to gastrointestinal illness.



- The facility delivered only to retail food establishments in Colorado and had over 200 customers (41 were various Jimmy John's locations). The facility did not deliver produce to any other restaurants that cases reported dining at during their exposure period.
- The facility consisted of 1 area for storing and sorting tomatoes, and a separate area for storing cucumbers, lettuce, and other produce items. The facility received 1-2 pallets of cucumbers per week, with 72 cases contained on each pallet. Cucumbers may be comingled from multiple suppliers, but the invoices note all lots that are received by a particular retail outlet. The general turnaround time for all produce items is 1-3 days, and refrigerated storage was available.
- There was evidence that employees had bare hand contact with some produce items. The main type of produce handled by this facility was tomatoes, as they were a major tomato supplier to a large grocery store chain in Colorado. Tomatoes were often sorted with bare hands. Cucumbers arrived in boxes and were shipped in the same box with only occasional handling occurring when checking cucumbers for product quality and repacking a box to fulfill a partial order. Lettuce was not repackaged and was handled occasionally when checking for product quality. The facility did not wash any produce items.
- The facility used 3 trucks to deliver produce to customers, including Jimmy John's; 1 was owned by the facility and was maintained, cleaned and sanitized on a regular basis by the facility; the other 2 were rented from a separate company that was responsible for all maintenance, cleaning, and sanitizing.
- From September 12-15, 2013, the facility needed to pump water that has accumulated around the buildings (this was during the unprecedented Colorado flooding event that occurred September 9-15, 2013). Water had pooled around the doors and dock area of the buildings; this water was determined to be rain water run-off rather than flood water accumulation, per the CDPHE Water Quality Control Division, so it was unlikely to be contaminated with sewage and other contaminants often found in flood waters.
- A total of 55 produce (lettuce and cucumbers) samples and environmental swabs were collected from the facility on October 23 and delivered to CDPHE LSD on the same day. On October 24, 20 environmental swabs from the delivery trucks were collected and delivered to CDPHE LSD.

Produce Traceback:

The produce traceback conducted by CDPHE on October 28, 2013, showed that all 3 implicated Jimmy John's locations received the same lot of cucumbers, and these cucumbers could have been served on the dates when cases ate at Jimmy John's. Table 5 shows the results of the traceback. The common lot of cucumbers is referred to as GR lot 19158. This lot was delivered to the Littleton location on October 4, and cases consumed cucumbers on October 5, 6, and 7. This lot was delivered to the Lakewood location on October 2, and cases consumed cucumbers on October 5. This lot was delivered to the Glendale location on October 5, and 1 case consumed cucumbers on October 9. For the Glendale location, 4 days passed between the delivery of the implicated lot of cucumbers and the date the case ate, which is outside the typical 3 day produce turn-around time reported by the restaurant. The case who ate at this location reported that the sandwich was ordered and prepared around 11:30 am (sales records obtained by public health confirm this), which is about a half hour after the restaurant opens for business. This restaurant received a new shipment of cucumbers (not from the implicated lot) on October 9, but it is unlikely that these cucumbers would have been washed and sliced in time to be served on the case's sandwich. The case likely consumed the cucumbers from the implicated lot that was delivered on October 2 or 5, or cucumbers from a non-implicated lot delivered on October 5.

There was not a common lot of tomatoes delivered to the 3 Jimmy John's locations during the time frame in question. The only lot of lettuce that was common among the 3 implicated restaurants was Church lot 19142. It is unlikely that this lettuce was available for cases to consume since it was delivered to the Littleton and Lakewood locations on October 1 (cases ate food from these restaurants on October 5, 6, and



7); with the 3 day turnaround of lettuce at the restaurants, this lettuce would have likely been used by October 4 and not available on the days the cases dined. Also, at both the Littleton and Lakewood locations, additional lots of lettuce had been received on October 4, which may indicate that the locations had exhausted their supply of the Church lot 19142 lettuce by the time the cases dined on October 5, 6, and 7. Church lot 19142 lettuce was likely available for consumption at the Glendale location on the date the case dined; however, this case stated that she specifically ordered her sandwich without lettuce.

Table 5: Results of the CDPHE Jimmy John's Produce Traceback
 (common produce lots delivered to and served at 2 or more locations are indicated by color; produce lots are shown based on the day the delivery occurred to each location)

Date	Littleton			Lakewood			Glendale		
	Lettuce	Tomatoes	Cucumbers	Lettuce	Tomatoes	Cucumbers	Lettuce	Tomatoes	Cucumbers
Tuesday 10/1/2013	CHURCH Lot 19142	PINOS Lot 19122		CHURCH Lot 19142	PINOS Lot 19122				
Wednesday 10/2/2013					PINOS Lot 19150	GR Lot 19158	CHURCH Lot 19161	PINOS Lot 19150	GR Lot 19158
Thursday 10/3/2013									
Friday 10/4/2013	CHURCH Lot 19161	PINOS Lot 19130	GR Lot 19158	CHURCH Lot 19161	PINOS Lot 19130				
Saturday 10/5/2013				DOLE Lot 19179	PINOS Lot 19150		CHURCH Lot 19142	PINOS Lot 19150	GR Lot 19158
	1 case ate			3 cases ate			DOLE Lot 19179		DISANTI Lot 19180
Sunday 10/6/2013	2 cases ate								
Monday 10/7/2013	DOLE Lot 19179	PINOS Lot 19150	DISANTI Lot 19180				DOLE Lot 19179	PINOS Lot 19150	
	1 case ate						CHURCH Lot 19142		
Tuesday 10/8/2013									
Wednesday 10/9/2013							DOLE Lot 19179	PINOS Lot 19176	GR Lot 19209
							CHURCH Lot 19211	1 case ate	



Records from Colo-Pac Produce, Inc. indicate that the implicated lot of cucumbers (GR Lot 19158) consisted of 84 cucumbers received from GR Produce, Inc. in McAllen, Texas. The cucumbers originated from a single farm in Mexico and were shipped from GR Produce, Inc. to Colo-Pac Produce, Inc. on September 26, 2013. Colo-Pac Produce, Inc. only distributed these cucumbers to Jimmy John's locations.

As of the date of this report, CDPHE had not received a final traceback report from FDA. Verbal correspondence from the FDA indicated that they agreed with our findings that cucumber was the only item available at the 3 restaurant locations that was likely served to cases. Because the implicated lot had been distributed and used by the time it was discovered by public health and FDA, no recall was issued.

Laboratory Investigation:

Confirmatory testing on 8 Shiga toxin positive broths sent from clinical laboratories confirmed that the organism was *E. coli* O157:H7 which produced both Shiga toxin 1 and Shiga toxin 2. All 8 cases had indistinguishable PFGE patterns for 2 restriction enzymes (CDPHE pattern designation 00-A for the *Xba*I enzyme, and 08-AR for the *Bln*I enzyme), and indistinguishable MLVA patterns. The *Xba*I PFGE pattern 00-A was first documented in a Colorado resident in 1999. It is a common pattern in Colorado and nationwide, so a second PFGE restriction enzyme is typically ran to help distinguish between cases. The *Bln*I PFGE pattern 08-AR is uncommon in Colorado. Prior to this outbreak, Colorado had not had any *E. coli* O157:H7 cases with this PFGE pattern combination. The MLVA pattern seen in this outbreak was unique and had not been seen in the US prior to this outbreak.

All food and environmental samples tested by CDPHE LSD were negative for Shiga toxin and *E. coli* O157:H7.

The CDC laboratory performed *E. coli* O157 serology on leftover blood specimens from 1 probable case, which were positive for *E. coli* O157 antigens, indicating likely recent infection.

DISCUSSION

We identified 9 cases in this Colorado *E. coli* O157:H7 outbreak. All 9 cases reported eating sandwiches from at least 3 Denver-area Jimmy John's locations during a 5 day period in early October 2013, which was the only common exposure discovered among cases. The results of this investigation indicate that consumption of Jimmy John's sandwiches containing cucumbers imported from Mexico was the likely cause of the outbreak. Epidemiologic and produce traceback findings support this hypothesis. While 9 identified cases may not appear to be a large outbreak, there were likely others that were ill that never sought health care, or were not tested for *E. coli* O157:H7, or were not reported to public health. It is estimated that there could be up to 26 unreported cases of *E. coli* O157:H7 for every 1 case identified by public health surveillance (8).

Jimmy John's is a nationwide franchise sandwich restaurant chain with over 40 locations in Colorado. The menu consists of sandwiches, which are typically ordered by customers by the name or number assigned to the sandwich. Patrons can order sandwiches via the internet, Smartphone app, telephone, or at the counter. Sandwiches can be delivered by the restaurant or picked up at the counter. Six of the 25 sandwich options on the menu come with cucumbers, but customers are able to add or subtract any sandwich ingredient.

Case finding efforts found that all cases resided in the Denver metropolitan area and no cases were found in other states, indicating an exposure that was likely unique to Colorado. The majority of cases (78%) were female which is often seen in produce-associated outbreaks as females tend to eat more produce than men (9). The median age of cases was 23 years indicating that younger adults were disproportionately affected. Case interviews found that all 9 cases had illness onsets within a 9 day period in October 2013, which supports a common exposure among cases. Upon extensive case interviewing, the only common exposure



discovered among cases was consuming Jimmy John's sandwiches prepared at various locations in the Denver metropolitan area within the incubation period, and the common sandwich ingredients among all cases were bread, cucumbers, and tomatoes. Eight of 9 cases reported eating lettuce and mayonnaise on Jimmy John's sandwiches, so these items were also looked into further as possible causes of the outbreak.

- Bread is not a vehicle typically associated with *E. coli* O157:H7 infections, given that it is a cooked product. In addition, all Jimmy John's menu items are served on bread, so if bread were the cause of the outbreak we would expect to see many more cases.
- The mayonnaise served at Jimmy John's is a commercially prepared product with wide distribution, so if it were the cause of the outbreak we would expect to see more cases.
- Produce items, such as cucumber, tomatoes, and lettuce have potential to be contaminated with *E. coli* O157:H7 during growing, harvest, distribution, preparation, and service. There have been many outbreaks of *E. coli* O157:H7 caused by lettuce and other leafy greens but few associated with cucumbers or tomatoes (10).

Results from Case-Control Study 1 indicate that cases were more likely than controls to have eaten at Jimmy John's in early October 2013 (OR undefined; p=0.00007). Cases were also more likely than controls to have consumed cucumbers in early October 2013 (OR undefined; p=0.005). Other statistically significant exposures in this study include consuming hummus (OR undefined; p=0.003) and tomato (OR undefined; p=0.04). Hummus consumption was only reported by 4 of 8 cases, and among those 4 cases, 3 different brands of hummus were reported, so it is unlikely that hummus caused the outbreak. The specific type and source of tomatoes that cases and controls reported consuming varied, which does not support tomatoes as the source of the outbreak.

Results from Case-Control Study 2 indicate that among Jimmy John's patrons, cases were more likely than controls to have eaten cucumbers on Jimmy John's sandwiches in early October 2013 (OR undefined; p = 0.002). Cases were also 4.8 times more likely than controls to have consumed avocado spread from Jimmy John's (p = 0.041); however, only 5 cases reported this food item and the avocado spread served at Jimmy John's is a commercially prepared product with wide distribution, so if it were the cause of the outbreak we would expect to see more cases. Consuming tomato or lettuce from Jimmy John's was not statistically associated with illness.

The fact that the cases in this outbreak reported eating food from at least 3 different Jimmy John's locations supports that it was a common contaminated food item served at all 3 locations that caused the outbreak, rather than another source such as an ill food handler or food preparation error. The environmental assessments conducted at the implicated Jimmy John's restaurants and the common produce distributor supports this, as no ill employees were discovered nor any shared employees among these entities. CDPHE considered collecting stool from all Jimmy John's and Colo-Pac Produce, Inc. to look for possible infection in food handlers, but decided not to do this given the lack of illness reported in food handlers, the amount of public health resources needed to collect and test stool from over 100 staff members from the 4 entities, and the evidence that the source of the outbreak was likely a contaminated food served at multiple Jimmy John's locations.

The produce traceback supports that a single lot of cucumbers originating from a farm in Mexico was the likely cause of the outbreak. All 9 cases consumed cucumbers on the sandwich they ate from Jimmy John's, and the implicated lot could have been available for service on the dates when the cases ate. There was not a common lot of tomatoes served at the implicated restaurants. Based on lettuce delivery records, it is unlikely that a common lot of lettuce was served to cases, and 1 case specifically ordered a sandwich without lettuce. Given that cucumbers are a perishable food item, we were unable to recover any cucumbers from the implicated lot for testing. All other food and environmental samples collected during this investigation tested negative for *E. coli* O157:H7 at CDPHE LSD.



Prior to this outbreak, there have been no documented *E. coli* O157:H7 outbreaks associated with cucumbers in the United States, per a literature search conducted by CDPHE and by searching for outbreaks on CDC's Foodborne Outbreak Online Database (<http://www.cdc.gov/foodborneoutbreaks/>), which captures foodborne outbreak reports generated by local and state health departments and the CDC from 1998 through 2012. An outbreak of *E. coli* O157:H7 associated with consumption of cucumber salad (consisting of cucumbers, hard-boiled egg, and vinaigrette dressing) affected school-age children from England who were visiting France in 2002 (5). The investigators hypothesized that the cucumbers became contaminated in the growing field. There is evidence that cucumbers can support the growth of *E. coli* O157:H7 based on a 1993 study by Abdul-Raouf *et al* (6). The researchers inoculated sliced cucumbers with *E. coli* O157:H7 and found that the bacteria load increased when storage temperatures were at 21 degrees Celcius (69.8 degrees Fahrenheit). Mukherjee *et al* conducted microbiological analyses of fresh cucumbers produced by organic and conventional farmers in Minnesota and were not able to isolate *E. coli* O157:H7 but did find that cucumbers are often contaminated with coliforms, an indicator of fecal contamination (7).

For this particular investigation, it is not clear how the cucumbers could have been contaminated. It is very unlikely that contamination occurred within the 3 implicated Jimmy John's locations, as no major food handling violations were noted during the environmental assessments and no ill food handlers were discovered. It is more likely that the implicated stores received contaminated cucumbers. Our investigation found no evidence that would support that the cucumbers became contaminated at the Denver-based produce distributor (Colo-Pac Produce, Inc.) or during shipment to the implicated Jimmy John's locations. CDPHE does not have jurisdiction to investigate outside of the state of Colorado, so we were unable to investigate if or how the cucumbers became contaminated prior to arriving in Colorado. Possible contamination points include during growing, harvest, transport, or distribution. Public health and food safety officials should be aware that cucumbers may be contaminated with *E. coli* O157:H7, and consider cucumbers as a possible vehicle in future *E. coli* O157:H7 outbreaks. More research is needed on how cucumbers can potentially become contaminated during growing, harvest, transport, or distribution.

This investigation had several limitations, lessons learned, and strengths. Regarding limitations, as with any retrospective epidemiologic study, recall bias among cases and controls might have affected the results for both case-control studies. Selection bias may have been an issue with both case-control studies. For Case-Control Study 1, controls were enrolled only if they had a land line phone number, and not all cases had a land line phone number; there could be differences among persons who have a land line phone versus only a cell phone which could have affected the results. For Case-Control Study 2, controls were enrolled if they had placed an online or phone order at an implicated Jimmy John's location and had a phone number where public health could reach them; there could be differences among Jimmy John's diners who order food in this manner versus those that order food from the counter. By not collecting stool specimens from all food handlers at the implicated Jimmy John's restaurants and Colo-Pac Produce, Inc., it is possible that we may have missed infection in food handlers; however, there was no evidence in this investigation that infected food handlers had a role in transmission, as all 9 cases exposures can be accounted for based on their history of consuming cucumbers on sandwiches prepared at Jimmy John's. Given that there were no cucumbers from the implicated lot available for testing, we were not able to definitively determine that cucumbers were the cause, and are coming to this conclusion based on the strong epidemiologic, environmental assessment, and traceback findings that converged on cucumbers being the likely source of this outbreak.

Regarding lessons learned, public health needs to continue to find innovative ways to locate and enroll controls in epidemiologic studies. As seen in Case-Control Study 1, sequential digit dialing is resource intensive, with a small yield of enrolled controls. We also had trouble obtaining shopper card records from Kroger during this outbreak. Although shopper card records were determined not to be necessary as the



investigation progressed, they are often helpful in outbreaks related to food products sold at grocery stores so it would be beneficial for us to work with Kroger so this is not an issue in the future.

Regarding strengths, we feel that this outbreak was detected and investigated quickly thanks to clinical laboratories promptly sending Shiga toxin positive stool specimens to CDPHE LSD for subtyping, and CDPHE LSD staff promptly testing those specimens and noting an increase in specimens confirming as *E. coli* O157:H7. Prompt investigation by local public health agency and CDPHE epidemiologists led to the likely source of the outbreak (consuming food from Jimmy John's) within 5 days of outbreak detection, and a likely food item (cucumbers) within 10 days of outbreak detection. The 3 Jimmy John's locations and Colo-Pac Produce, Inc. were very cooperative during this investigation and provided all of the information requested by CDPHE and local public health agencies. The collaboration and communication that occurred between different CDPHE divisions (CDB, DEHS, and LSD), local public health agencies (DEH, DPH, TCHD, JCPh), regional epidemiologists housed at local public health agencies around the state, and trained graduate student interviewers was a strength in this investigation, and everyone's efforts contributed to the success of this investigation.

As of the date of this report, no other cases of *E. coli* O157:H7 with the PFGE pattern seen in this outbreak were reported in Colorado.

ACKNOWLEDGMENTS

CDPHE would like to acknowledge and thank the numerous persons at the following agencies and organizations for their assistance in this outbreak investigation:

CDPHE Communicable Disease Branch

CDPHE Division of Environmental Health and Sustainability

CDPHE Laboratory Services Division

Denver Environmental Health

Denver Public Health

Jefferson County Public Health

Public Health Graduate Students at the Colorado School of Public Health

Regional epidemiologists based at local public health agencies across Colorado

Tri-County Health Department

United States Food and Drug Administration

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APPENDIX 1 - Questionnaire from Case-Control Study 1

A. Introduction

Hello, my name is _____ (*insert name*), and I'm calling from the state health department. We are investigating an outbreak of *E. coli* O157:H7 infections among Denver metro area residents, and we need your help. *E. coli* O157:H7 is a bacteria that causes gastrointestinal symptoms, such as diarrhea, and can cause serious illness especially in children.

To help us figure out the cause of this outbreak, we need to speak to persons who were NOT sick and compare foods that they ate to people who were sick. Do you have 5-10 minutes to answer some questions?

- NO → Thank you for your time.
(END call - record outcome on control log sheet)
 YES

B. Exclusion Criteria and Demographics

1. Do you live in Adams, Arapahoe, Denver or Jefferson county Colorado?
 NO → Thank you for your time, but we need to interview people who live in those counties.
(END call - record outcome on control log sheet)
 YES → Which county do you live in? _____
(Proceed below if from Adams, Arapahoe, Denver and Jefferson)
2. Are you between the ages of 18-65?
 YES → *(proceed below)*
 NO → Thank you for your time, but we are only obtaining information on persons between the ages of 18 and 65. *(END call - record outcome on control log sheet)*
3. Are you male or female?
If sex you are trying to reach → *(proceed below)*
If NOT sex you are trying to reach → Thank you for your time, but we are only obtaining information on [Men/Women]. *(END call - record outcome on control log sheet)*
4. Have you had any gastrointestinal symptoms, such as diarrhea, vomiting, or stomach cramps, during ____/____/____ to ____/____/____ [date range]?
 YES → Thank you for your time, but we are only obtaining information on persons who have not had gastrointestinal symptoms during that time. *(END call - record outcome on control log sheet)*
 NO → *(proceed below)*
5. Did you travel outside of the Denver metro area for more than one night during ____/____/____ to ____/____/____ [date range]?
 YES → Thank you for your time, but we are only obtaining information on persons who were in the Denver metro area during that time. *(END call - record outcome on control log sheet)*
 NO → *(proceed below)*

C. Restaurant Interview Questions



Did you eat food prepared at any of the following restaurants during ____/____/____ to ____/____/____ [date range]?

Please think about meals eaten at the restaurant, take-out meals, or delivery food from the restaurant.

Interviewers: Please try to get exact location information if available - this may assist with traceback investigations. Cross streets, cities, and neighborhoods are helpful to know.

Restaurant	Eat There?	If Yes: Date	Location	Foods & Beverages Consumed
Quizno's	Yes No Unk			
Jimmy Johns	Yes No Unk			
Sub Culture	Yes No Unk			
Wahoo's Fish Taco	Yes No Unk			

D. Food Interview Questions

1. During ____/____/____ to ____/____/____ [date range] did you eat any ground beef (hamburgers, taco meat, etc)? YES NO Unknown
If Yes, did you eat ground beef (check all that apply) At Home At a Restaurant Unknown

2. During ____/____/____ to ____/____/____ [date range] did you eat any lettuce? YES NO Unknown
If Yes, did you eat (check all that apply) Iceberg Romaine Red Leaf Green Leaf Spring Mix/Mesclun mix Other Lettuce, please specify: _____ Unknown

Did you eat the lettuce (check all that apply) At Home At a Restaurant Unknown
If at home, was the lettuce organic? YES NO Unknown
Was the lettuce purchased (check all that apply) in a head in a bunch loose from a bin
 prepackaged in a bag Prepackaged in a plastic "clam shell" container Unknown
If prepackaged, what was the brand: _____

3. During ____/____/____ to ____/____/____ [date range] did you eat any spinach? YES NO Unknown
Did you eat the spinach (check all that apply) At Home At a Restaurant Unknown
If at home, was the spinach organic? YES NO Unknown
Was it labeled as baby spinach? YES NO Unknown
Was the spinach purchase (check all that apply) in a bunch loose from a bin
 prepackaged in a bag Prepackaged in a plastic "clam shell" container Unknown
If prepackaged, what was the brand: _____

4. During ____/____/____ to ____/____/____ [date range] did you eat tomatoes? YES NO Unknown



If Yes, did you eat (check all that apply) round, red roma grape cherry tomatoes on vine
 Other tomatoes, please specify: _____ Unknown

5. During ____/____/____ to ____/____/____ [date range]did you eat green onions or scallions?
 YES NO Unknown
6. During ____/____/____ to ____/____/____ [date range]did you eat fresh cilantro, including in salsa?
 YES NO Unknown
7. During ____/____/____ to ____/____/____ [date range]did you eat cucumbers?
 YES NO Unknown
8. During ____/____/____ to ____/____/____ [date range]did you eat bell peppers?
 YES NO Unknown
9. During ____/____/____ to ____/____/____ [date range]did you eat hummus? YES NO Unknown
If yes, what was the brand: _____

E. Interview Conclusion

That is the last question. Thank you very much for your time and for answering our questions. We hope that your answers combined with the answers others provide will help us identify what happened and what can be done in the future to prevent similar outbreaks of illness.

Do you have any questions for me?

If you have questions later, please contact the Colorado Department of Public Health and Environment at 303-692-2700.

Interviewer comments:



APPENDIX 2 - Questionnaire from Case-Control Study 2

Jimmy Johns PHONE

This is [NAME] from [AGENCY].

The Colorado Department of Public Health and Environment is investigating an outbreak of E. coli O157 illnesses among people who have recently eaten at Jimmy John's and we need your help. Jimmy John's provided your phone number as someone who placed a food order on [DATE].

Jimmy John's is working closely with public health to figure out the source of illness.

In order to determine what might have made people sick and to prevent further illness, we would like to ask you some questions about what you ate from Jimmy John's and whether or not you became ill.

The survey should take less than 5 minutes to complete. All information you provide will be strictly confidential.

INTERVIEWERS: Complete one survey for EACH person who ate food from Jimmy John's.**

1. Name

First name:

2. Did you eat the food you ordered from Jimmy John's on [DATE]?

- Yes
- No - Thank you for your time and participation.
- Unsure - Thank you for your time and participation.

]} Discontinue

3. Age (in years)

For children under the age of 1, please enter 0.

4. Gender

- Female
- Male

5. County of residence:

6. Were you ill with gastrointestinal symptoms like diarrhea or vomiting in the 7 days AFTER consuming food from Jimmy John's on [DATE]?

- Yes
- No
- Unsure

→ continue to next page
3 Skip to question 21

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Jimmy Johns®;PHONE

7. If YES, would you be willing to submit a stool specimen to public health for free testing?

- Yes
 No

8. If yes, please provide a phone number where we can reach you.

9. Did you experience any of the following symptoms? Please answer Yes, No, or Unsure for each symptom.

	Yes	No	Unsure
Vomiting	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Diarrhea	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bloody diarrhea	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Abdominal cramps	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fever	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chills	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Headache	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Body aches	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fatigue	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other symptom	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

10. If "Yes" to "Other symptom", what other symptoms did you experience?

11. When did your symptoms begin? (Please be as precise as possible with date AND time)

Date:

MM DD YYYY HH MM AM/PM
Date and time / / :

12. If you are unsure of the exact onset date of your symptoms please fill in day of the week (example: "Tuesday after I ate the meal from Jimmy John's."):

13. Which symptom did you experience first?

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Jimmy Johns®PHONE

14. If you experienced diarrhea, did you have 3 or more loose stools in a 24 hour period?

- Yes
- No
- Unsure

15. If you experienced diarrhea, what is the date and time of the first episode of diarrhea?

MM DD YYYY HH MM AM/PM

Date and Time:

<input type="text"/>					
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16. If you had a fever, what was the maximum temperature (in degrees Fahrenheit)?**17. Are you still experiencing symptoms?**

- Yes
- No
- Unsure

18. How many days did your symptoms last?**19. Did you see a health care provider regarding this illness?**

- Yes
- No
- Unsure

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Jimmy Johns®PHONE

20. If you saw a health care provider for this illness, please list:

a. Name of the health care provider: _____

b. Phone number of health care provider (including area code): _____

c. Date of visit (MM/DD/YYYY): _____

d. Diagnosis given (if known): _____

e. Laboratory tests done (especially a stool sample): _____

f. Type of medication given, if any: _____

21. How did you get your food from Jimmy John's on [DATE]?

- I had my food delivered
- I picked up my food and ate it at Jimmy John's
- I picked up my food and ate it elsewhere

Other (please specify)

22. What time did you eat the food purchased at Jimmy John's?

Example: 01:30 PM

Time: HH MM AM/PM
 [] : [] []



Jimmy Johns®PHONE

23. Which sandwich(es) did you eat from Jimmy John's on [Date]?

- #1 Pepe–ham, cheese, lettuce, tomato, and mayo
- #2 Big John–roast beef, mayo, lettuce, and tomato
- #3 Totally Tuna–tuna, lettuce, tomato, cucumber
- #4 Turkey Tom–turkey, lettuce, tomato, cucumber, and mayo
- #5 Vito–salami, cheese, capicola, onion, lettuce, tomato, and Italian vinaigrette
- #6 Vegetarian–cheese, avocado spread, cucumber, lettuce, tomato, and mayo
- #7 Gourmet Smoked Ham Club–ham, cheese, lettuce, tomato, and mayo
- #8 Billy Club–roast beef, ham, cheese, mustard, lettuce, tomato, and mayo
- #9 Italian Club–salami, capicola, ham, cheese, lettuce, tomato, onion, mayo, Italian vinaigrette
- #10 Hunter's Club–roast beef, cheese, lettuce, tomato, mayo
- #11 Country Club–turkey, ham, cheese, lettuce, tomato, and mayo
- #12 Beach Club–turkey, cheese, avocado spread, cucumber, lettuce, tomato, and mayo
- #13 Gourmet Veggie Club–provolone, avocado spread, cucumber, lettuce, tomato, and mayo
- #14 Bootlegger Club–roast beef, turkey, lettuce, tomato, and mayo
- #15 Tuna Club–tuna, cheese, cucumber, lettuce, and tomato
- #16 Club Lulu–turkey, bacon, lettuce, tomato, and mayo
- #17 Ultimate Porker–ham, bacon, lettuce, tomato, and mayo
- JJ BLT–bacon, lettuce, tomato, and mayo
- JJ Gargantuan–salami, ham, capicola, roast beef, turkey, cheese, onion, lettuce tomato, mayo, and Italian viniagrette
- Slim 1–ham and cheese
- Slim 2–Roast beef
- Slim 3–Tuna
- Slim 4–Turkey
- Slim 5–Salami, capicola, cheese
- Slim 6–double cheese
- Unsure

Other (please specify)



Jimmy Johns®PHONE

24. Did you add any of these items to your order on [DATE]?

	Yes	No	Unsure
Avocado Spread	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Provolone Cheese	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dill Pickle	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hot Peppers	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Other (please specify)

25. Regardless, of what you ordered, did you eat any of the following items from Jimmy John's on [DATE]?

	Yes	No	Unsure
Lettuce	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cucumber	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tomato	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mayonnaise	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Thank you for your time in completing this survey.

Please fill out one survey for each family member or friend who ate food from Jimmy John's on [DATE].

If you have questions, please feel free to contact Rachel Jervis at the State Health Department/Communicable Disease Program: phone 303-692-2459, email Rachel.Jervis@state.co.us

