Title: Central Region *E. coli* O157:H7 Outbreak, Spring 2012
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Introduction:

On Friday, March 30, 2012, the Columbia/Boone County Department of Public Health and Human Services (CBCDPHHS) received a report from a local hospital of a two-year-old female Boone County resident diagnosed with a suspected Shiga toxin-producing *Escherichia coli* (STEC) infection and hemolytic uremic syndrome (HUS). Epidemiologists at CBCDPHHS reported the case to the Missouri Department of Health and Senior Services' Bureau of Communicable Disease Control and Prevention (BCDCP), and initiated a public health investigation. The parents of the index case reported that the child regularly consumed unpasteurized cow's milk purchased from a Howard County dairyman who delivered the product directly to the family's home on a weekly basis. By Tuesday, April 3, 2012, a total of four STEC cases were identified in Central Missouri, all of whom reported consuming unpasteurized dairy products, ground beef and/or other food items purchased from the same Howard County farm (Farm A) prior to illness onset. A review of the STEC morbidity data in Central Missouri indicated the number of cases reported since the beginning of 2012 was clearly in excess of the historical five-year median for the same time period. The event was recognized as a possible outbreak and an investigation was initiated to confirm the infectious agent, determine the source of the infections, and institute control measures to prevent additional cases. The following report includes a summary of the investigation findings.

Background:

Human STEC infections, which are caused by the ingestion of *E. coli* O157:H7 or other STEC serotypes, are reportable by law to public health authorities in most states.[1] This type of bacteria secretes toxins (known as Shiga-like toxins) that inhibit protein synthesis and cause cell death in the intestinal lining and sometimes in the kidneys. Symptoms typically appear within two to ten days of ingestion of STEC bacteria, and range from mild diarrhea to heavily bloody stools (hemorrhagic colitis). Approximately 15% of pediatric cases progress to HUS (a less common occurrence in adults), half of whom require dialysis. Case fatality rates of 5% have been reported. The chief reservoir of STEC bacteria is cattle, with other ruminants and infected humans also serving as sources of exposure.

Several laboratory tests are available to diagnose STEC infections and/or characterize the organism for epidemiological purposes, including culture, polymerase chain reaction (PCR), enzyme immunoassay (EIA), pulsed field gel electrophoresis (PFGE), and Multiple –Locus-Variable number tandem repeat-Analysis (MLVA).[2,3]

Since 1982, several large STEC outbreaks associated with fecally-contaminated food products have been reported in the United States, including undercooked ground beef, alfalfa sprouts, unpasteurized apple cider, fresh spinach, and unpasteurized dairy products.[4,5] Between 1993 and 2006, STEC was the identified causative agent in nine of 73 disease outbreaks in the United States associated with unpasteurized dairy products.[6]

Central Missouri (as defined in this report) includes 24 counties, with populations ranging from approximately 15,000 to 152,000 persons. The area is predominantly rural, with the largest population centers located in Columbia (population 110,000) and Jefferson City (population 43,000). There are 17 hospitals and ten colleges and universities located in the 24-county area. In Missouri, it is legal for individuals to purchase raw milk or cream directly from a farm (or have the product delivered directly from the farm) for their own personal use.[7]

Methods:

For the purpose of this investigation, the following outbreak case definition was used to classify reports on persons with STEC infections:

- Confirmed: Any case of laboratory-confirmed *E. coli* O157:H7 with PFGE pattern MOE050/MBE006 reported in a resident of Central Missouri or person who traveled or had other type of exposure to Central Missouri during the ten days prior to onset and developed symptoms on or after March 1, 2012.
- Probable: Any clinically compatible case of *E. coli* O157:H7, reported with a symptom onset date on or after March 1, 2012 and is epidemiologically linked to a confirmed case (as described above).
- Suspect (final): Any clinically compatible case of *E. coli* O157:H7 reported with a symptom onset date on or after March 1, 2012 and is epidemiologically linked to Farm A.

The PFGE pattern MOE050/MBE006 is a common PFGE pattern in Missouri, and not all cases with this pattern reported statewide between March 1, 2012 and May 3, 2012 were outbreak-related. The PFGE results were considered in the context of available clinical and epidemiologic information to determine if the case met the outbreak case definition. Central Missouri was defined as the area including the following counties: Audrain, Boone, Callaway, Camden, Cole, Cooper, Crawford, Dent, Gasconade, Howard, Laclede, Lincoln, Maries, Miller, Moniteau, Montgomery, Morgan, Osage, Pettis, Phelps, Pulaski, Randolph, Warren and Washington. All STEC and HUS cases reported between March 1, 2012 and May 3, 2012 were evaluated to determine if they met the outbreak case definition.

Several methods were used to identify additional cases including: 1) monitoring STEC-positive laboratory reports submitted via the routine reportable disease surveillance system; 2) asking cases if they were aware of other persons with a similar illness; 3) active surveillance for additional suspect STEC cases by disseminating a DHSS Health Advisory to health care providers and, 4) sending a letter to identified customers of Farm A with a request that anyone with a diarrheal illness seek medical care and contact either the Local Public Health Agency (LPHA) or DHSS. The Farm A owner did not have a detailed customer list however, he did agree to allow DHSS staff to follow him on his delivery route to collect the names and addresses of his customers to assist with the investigation. DHSS staff followed the owner and his son as they delivered products from Farm A on four separate dates (April 9 – 11, 2012 and April 13, 2012) and collected names, street addresses and GPS coordinates of the drop-off points of the majority of their customers.

All reported or identified cases were investigated according to DHSS guidelines and interviews were conducted by the LPHA communicable disease investigators, based on the cases' respective home of residence.[8] Standard DHSS investigation forms for enteric and HUS conditions were used to collect data, as well as a supplemental questionnaire developed specifically for the outbreak (see Appendix A).

Staff from the DHSS Bureau of Environmental Health Services (BEHS) contacted the owner of Farm A on April 2, 2012, to discuss concerns regarding the reported illnesses. The owner of Farm A agreed to meet BEHS staff that day and to provide a sample of milk for laboratory analysis. Four beef and dairy product specimens originating from Farm A were collected from the homes of persons identified as cases on this date as well. The following day, April 3, 2012, four additional milk and dairy product samples were collected from customers of Farm A. These included products from at least three production dates. On April 6, 2012, BEHS Environmental Public Health Specialist Rachelle Kuster and Russell Lilly made an onsite visit to Farm A. The purpose of this visit was to gain a better understanding of the foods produced on and sold by Farm A and to assess the environmental conditions and risk factors at the farm. The BEHS staff arrived at the farm at approximately 5:30 P.M. and concluded the visit at approximately 7:00 P.M. Findings from the onsite visit detailed in this report include both observations of BEHS staff and information provided by the owner of Farm A, who was cordial and cooperative throughout the visit.

Laboratory testing of clinical specimens was conducted by: 1) private laboratories (EIA tests for Shiga toxin 1 and/or 2, or culture); 2) the Missouri State Public Health Laboratory (MSPHL), (PCR testing for the presence of Shiga toxin producing genes, culture, and PFGE); and 3) Centers for Disease Control and Prevention conducted the MLVA testing. Testing of environmental specimens was also performed by MSPHL. Laboratory testing of the food items from Farm A was conducted by the MSPHL. The test methods included PCR and culture for the presence of STEC.

Results:

A total of 38 STEC cases were reported in Central Missouri between March 1, 2012 and May 3, 2012. Nineteen (50%) of these cases met the outbreak case definition and were further classified as confirmed (10 cases), probable (6 cases) and suspect (3 cases). The isolates obtained from each of the 10 confirmed cases were identified as *E. coli* O157:H7 with the PFGE pattern MOE050/MBE006. In addition, isolates from seven of the ten confirmed cases were submitted for MLVA testing; all seven specimens had a similar MLVA pattern. Outbreak associated STEC cases were reported from five counties in Missouri (Table 1). Eleven (58%) of the cases were female, and the age of cases ranged from 1 to 47 years (median = 11 years). The reported symptom onset dates ranged from March 19, 2012 to April 17, 2012 (Figure 1). The reported symptoms included diarrhea (100%), abdominal cramps (79%), bloody diarrhea (63%), and fever (37%). Five (26%) of cases were hospitalized and one patient developed HUS; no deaths were reported (Table 2).

The supplemental questionnaire was completed on each of the 19 outbreak associated cases and the data collected are included in the final analysis (Table 3). All 19 of the cases reported consuming products from Farm A during the seven days prior to onset of symptoms. The Farm A products consumed included unpasteurized dairy (17 cases), ground beef (6 cases) and leafy greens (2 cases). Some cases consumed more than one of the products from Farm A. Of the 17 cases that reported exposure to unpasteurized dairy products from Farm A, 14 (82%) consumed whole cow's milk, 4 (24%) consumed reduced fat cow's milk and 2 (12%) consumed cream from cow's milk. Two of the 17 cases reported consuming products made from unpasteurized milk from Farm A that included cream cheese and homemade ice cream, respectively. Deliveries of dairy and non-dairy products were made on a Monday, Tuesday, Wednesday, or Friday. All 19 cases received deliveries on Monday, Tuesday or Wednesday, with no cases identified in the Friday delivery group. The reason(s) that no cases were identified in the Friday delivery group are unknown. Figure 2 depicts the total number of cases for each report week and the week of delivery of unpasteurized dairy or other products from Farm A.

The analysis of survey responses indicated 74% of cases reported shopping at a large, common, national grocery store chain. Two additional large grocery store chains were each frequented by 47% of the cases, respectively. In addition, 79% of cases reported exposure to pet animals including dogs (63%), cats (37%), and pet birds or baby poultry (26%). No single restaurant or other food establishment was reported to be commonly frequented by cases that did not live in the same household prior to onset of illness.

Food items including unpasteurized milk products (low fat milk, whole milk, and cream), beef, and leafy greens (romaine lettuce and kale) were produced at Farm A, sold, and delivered to customers homes by the owner/operators of Farm A. In addition, eggs produced on an undisclosed farm were also sold and delivered by persons associated with Farm A. A total of 244 customer households were identified though the Farm A owner by observation on his delivery route. However, the owner estimated that he may have served as many as 400 households each week in the past. The distribution of the known households by county included Boone County (42%), Howard County (30%), Randolph County (22%), and Cooper County (5%). The majority of the customers received the unpasteurized dairy products. The products were delivered to customers in a van equipped with a storage container made from Styrofoam panels, which were covered on top with blankets. When beef was delivered it was stored separated from the milk in a cooler. The leafy greens were picked in the evening and bagged, refrigerated

overnight, and then covered in heavy blankets to maintain temperature during delivery the following day. The eggs were reported to be refrigerated before delivery and the temperature maintained during delivery.

The milk was stored at Farm A in a bulk tank with an agitator that was normally left off at night. The "low fat" milk was taken from the bottom of the tank after the agitator had been turned off. The "whole milk" was obtained after the agitator had been turned on. The whole milk was the most popular product among Farm A clients. The temperature of the milk in the bulk tank at the time of the onsite visit was 32 degrees Fahrenheit. The milk was dispensed into glass jars using a homemade manifold that would fill four jars at a time. The manifold was made from PCV piping and plastic milk hose tubing. The glass jars returned from customers were washed and/or sanitized depending on appearance and smell. Jars that appeared clean were sanitized with a bleach solution. Jars that appeared soiled or had an odor were hand-washed using a chlorinated cleaning solution (in place dairy detergent), then sanitized using another type of bleach solution. The sanitized jars were capped until filling them with milk or cream for delivery. The cream was skimmed off the top of the milk in the bulk tank after the agitator was left off overnight. The Farm A owner stated that he had sold hamburger, steaks and roasts made from cattle raised on his farm to customers in the past. Approximately two head of cattle were slaughtered each month in the spring, summer and fall seasons. The cattle were then processed at an undisclosed custom exempt processing facility. Only a small quantity of beef was reported to be available at the time of the onsite visit.

At the time of the onsite visit several issues were identified in the milking parlor at Farm A. The inflations showed excessive wear with cracking. The milk pipeline leading to the bulk tank had milk residue near the back end. The owner stated he had used battery acid to clean the dirty pipeline in the past. The concrete floors of the cattle platform in the milking parlor had eroded and there was standing water in the voids. In addition, a cat was observed sitting on top of a box of unopened new glass jars used for the bottling and delivery of the milk products. Farm A uses water from a public water supply as there is no well on the farm. The owner stated the wastewater from the family home is discharged to the ground surface through a pipe as there is no onsite wastewater treatment system.

The following eight food samples from Farm A were submitted for testing at MSPHL: four raw milk samples, unprocessed; one milk sample purchased raw and then heated; one sample of whey from raw milk (processed by the consumer); one sample of cream cheese made from raw milk (processed by the consumer); and one sample of ground beef (frozen). None of the food samples from Farm A submitted to the MSPHL had positive results for *E. coli* 0157:H7 or other STEC.

Discussion:

A review of the surveillance data in the Central Region revealed the 38 STEC cases reported in 2012 was in excess of the five-year median during the two month outbreak surveillance time period (Figure 3). Laboratory testing of isolates from the reported cases consisted of six STEC serotypes, and two predominant PFGE patterns (*E. coli* O157:H7 cases only) identified. During the investigation, two predominant *E. coli* O157:H7 PFGE patters MOE050/MBE006 and MOE185/MBE006 were identified. The PFGE results were considered in the context of available clinical and epidemiologic information to determine if the case met the outbreak case definition. The PFGE pattern MOE050/MBE006 is a common PFGE pattern in Missouri, and therefore, not all cases with this pattern reported statewide between March 1st and May 3rd, 2012 were determined to be outbreak-related. The PFGE pattern MOE185/MBE006 was also not included in the final outbreak case definition. A total of six *E. coli*

O157:H7 cases with PFGE pattern MOE185/MBE006 were reported between March 1st and May 15th, 2012, three of whom were part of a household cluster. No association between the household cluster and the other three cases with this pattern was identified. Information provided by these cases or their surrogates revealed other likely risk factors for illness including exposure to cattle or cattle feces on the case's own farm, or that of a family member. In addition, five of these cases had no identified epidemiological link to cases identified as outbreak-associated. It should be noted, one of the cases reported with an isolate matching the MOE185/MBE006 was a sibling to a confirmed case of *E. coli* O157:H7, PFGE pattern MOE050/MBE006 and was classified as a probable outbreak-associated case.

The final analysis consisted of the 19 cases that met the case definition and were determined to be outbreak associated. All of the 19 outbreak associated cases were reported to have consumed products from Farm A. Eighty-nine percent of these cases reported the consumption of unpasteurized milk or cream purchased from Farm A compared to an estimated 3% of the population who report consuming unpasteurized dairy products nationally[9]. Additionally, two of the laboratory-confirmed outbreak cases resided outside of Central Missouri. One of these two "outlier" cases consumed unpasteurized milk from Farm A while visiting a friend who had some of the product delivered to her home. The second "outlier" case consumed home-made ice cream that contained unpasteurized cream from Farm A (separated from whole milk) while attending a family gathering. Unpasteurized dairy is a known high-risk food product and has previously been associated with outbreaks of STEC in the U.S.[5,6,10] .

Contact with domestic pet animals was reported by the majority of the cases, but no common exposure to the same animals was reported between case households. Grocery stores frequented by high percentages of the 19 cases were large chain operations with a national or regional presence. Monitoring of PulseNET revealed no significant increase in *E. coli* O157:H7 cases with the outbreak PFGE pattern MOE050/MBE006 (the most common pattern in the Pulse NET database) identified in other states. Significantly increased reports of MOE050/MBE006 cases in multiple states would have been expected if an adulterated nationally-or regionally-distributed product from a large grocery retailer were the source of exposure for the Missouri outbreak cases. The owner informed DHSS staff that he was going to voluntarily and permanently cease selling milk from Farm A as of Friday, April 13, 2012. DHSS staff observed him deliver photo-copies of a hand-written letter to each one of his customers on his delivery route, stating his plans to retire at the end of that week. Reports of outbreak-associated *E. coli* O157:H7 cases (and all STEC cases) decreased significantly after the cessation of milk deliveries from Farm A (see Figure 2).

The findings from the onsite visit identified several areas of concern in relation to the production of the dairy products at Farm A. The homemade manifold used to fill the bottles did not allow for disassembly for cleaning or inspection. The standing water noted in the voids of the eroded concrete floors is likely to be contaminated with fecal material, which can be introduced into the milk during the milking process. The residue observed in the milk pipeline is a concern as the pipeline is considered a direct food contact surface. Also, the reported prior use of battery acid to clean the pipeline is concerning given it is a direct food contact surface. There are commercial products available to safely clean the dairy pipelines. Many of the other findings noted pertained specifically to the overall cleanliness and maintenance of the dairy barn. The overall cleanliness of the barn did not meet standards that are expected of a Grade "A" commercial dairy. It should be noted this dairy was not under the inspection of the State Milk Board and is not a Grade "A" dairy. The milking practices at Farm A were not observed directly by public health investigators. The overall potential contribution of these specific environmental

findings to this outbreak could not be determined. In addition, the adherence to good hygienic practices during milking can reduce, but not eliminate, the risk of milk contamination. The dairy farm environment is a reservoir for illness-causing germs. No matter what precautions farmers take, and even if tests of raw milk samples for STEC or other bacterial pathogens are negative, they cannot guarantee that their milk, or the products made from their milk, is free of harmful germs.[11]

None of the eight food samples from Farm A submitted to MSPHL tested positive for *E. coli* O157:H7. However, failure to detect the pathogen in the food product does not rule out the hypothesis that products from Farm A may have caused the outbreak. Milk and ground beef from Farm A consumed by cases prior to illness onset were largely unavailable for testing, and two of the submitted dairy samples had been heated prior to testing (which could have killed any bacteria present). An analytical study (case-control or cohort) was not conducted as part of the investigation, as sufficient information about the possible source of the outbreak was gleaned by comparing descriptive data from the cases to population-based data.

Several limitations were identified during this investigation. The increase in reported STEC cases in Central Missouri included the identification of two predominant *E. coli* O157:H7 PFGE patterns MOE050/MBE006 and MOE185/MBE006. In addition, the outbreak associated *E. coli* O157:H7 PFGE pattern MOE050/MBE006 is a common pattern reported in Missouri and nationally. The increase in both patterns and the commonality of the outbreak pattern presented an initial challenge in the identification of cases associated with the outbreak. However, the similar MLVA pattern identified in all seven of the outbreak isolates supports the hypothesis that these common isolates came from the same exposure source. A limited amount of Farm A products that were consumed by cases prior to becoming ill were available for testing for the presence of *E. coli* O157:H7 due to the relatively short shelf life of unpasteurized dairy products. In addition, no environmental sampling and testing of feces from cows or from the milk production area was performed.

Prevention/Control Measures

During the course of this investigation, several prevention and control measures were implemented to prevent additional cases of STEC from occurring. The ongoing surveillance efforts of the involved local health departments and DHSS in collaboration with local medical providers identified an outbreak of *E. coli* O157:H7. The outbreak was promptly investigated by local health departments and DHSS to identify possible sources of the illnesses. Local healthcare providers were alerted of the outbreak by distribution of the DHSS Health Advisory (see Appendix). The DHSS mailed letters to the Farm A customers (identified from the milk delivery route) alerting them of the outbreak and requesting that any persons with diarrheal illness seek medical evaluation and also contact their LPHA or DHSS. The owner of Farm A agreed to voluntarily stop selling unpasteurized dairy products from his farm. The LPHAs and the DHSS will continue to rapidly investigate reported *E. coli* O157:H7 associated illness and work to promote food safety.

Conclusion

An outbreak of *E. coli* O157:H7 focused in the Central Region of Missouri was identified. The temporal and geographic clustering of these illnesses caused by an indistinguishable *E. coli* O157:H7 PFGE type is consistent with a common source of exposure. The only common risk factor identified among each of the 19 cases associated with this outbreak was the consumption of unpasteurized dairy products or ground beef originating from Farm A. Each of the cases had consumed the products within ten days prior to becoming ill, which is consistent with the known incubation period for *E. coli* O157:H7. Due to the limitations of this investigation, the source of the infections could not be confirmed. However, the findings of the investigation strongly suggest the products from Farm A were the likely source of infection for each of the cases associated with this outbreak.

It should also be noted, the consumption of unpasteurized dairy products (cows and goats) from sources other than Farm A were identified as risk factors for other non-outbreak associated STEC cases reported in Missouri during the outbreak period. Local and state public health officials should continue efforts to alert the public about the hazards of consuming unpasteurized dairy products and emphasize the lack of scientific evidence to support claims of therapeutic value (e.g., treatment for cancer, diabetes, allergies, etc.).[10] Consumers of unpasteurized dairy products should understand the risks associated with the products, especially for children and other who are at greater risk of severe disease and death associated with food-borne illnesses including those caused by STEC. Additionally, legislation designed to further restrict or regulate the sale of raw milk in Missouri should be considered.

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Table 1. Central Region *E. coli* O157:H7 Outbreak, Spring 2012 Cases Reported by County of Residence

County of Residence	Case Count (%)
Boone	8 (42%)
Cooper	6 (32%)
Howard	3 (16%)
Kansas City	1(5%)
Marion	1 (5%)
Total	19 (100%)

Table 2. Central Region *E. coli* O157:H7 Outbreak, Spring 2012: Summary of Descriptive Epidemiology Factors

Total Number of Cases	19
Confirmed	10
Probable	6
Suspect	3
Sex (% Female)	11 (58%)
Race (% white)	19 (100%)
Median Age in Years (Range)	11 (1-47 years)
Number Hospitalized (%)	5 (26%)
Number developed HUS	1 (5%)

Table 3. Central Missouri *E. coli* O157:H7 Outbreak, Spring 2012: Frequency of Exposure Type among Cases (N=19)		
Exposure Type	Frequency	Per cent of Total Cases
Animal contact (pet)	15	79%
Animal contact (cattle)	0	0%
Child care service	2	11%
Farmers market	3	16%
Health food store	9	47%
Consumed food from a restaurant	17	89%
Recreational water exposure	5	26%
Travel	7	37%
Visited/lives on a farm	4	21%
Consumed food directly from a farm	19	100%
Consumed food from Farm A	19	100%
Consumed food from Farm B	4	21%
Consumed food from Farm C	1	5%
Unpasteurized dairy	17	89%
Unpasteurized dairy from Farm A	17	89%
Ground beef from Farm A	6	32%
Leafy greens from Farm A	2	11%
Other product from Farm A	2	11%

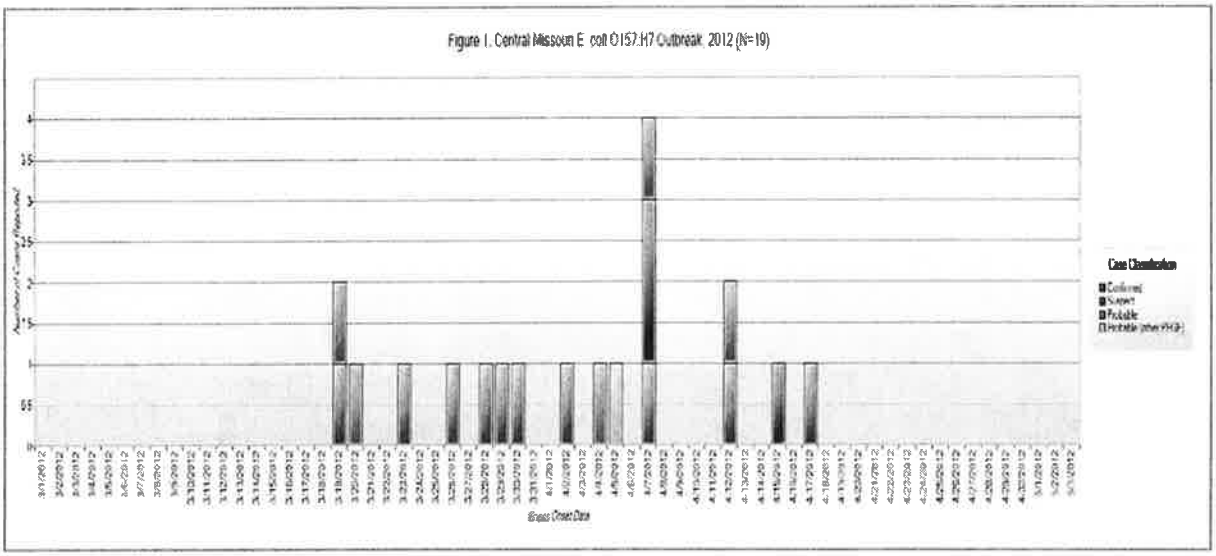
Figure 1. Central Missouri E. coli O157:H7 Outbreak, 2012 (N=19)
Case Classification
Confirmed
Suspect
Probable
Possible (after 7/1/12)

Figure 2. Central Missouri *E. coli* O157:H7 Outbreak, Spring 2012
Frequency of Cases Reported and Farm A Deliveries by Week
(confirmed, probable and suspect cases reported 3/11/12-5/3/12 , N=19)

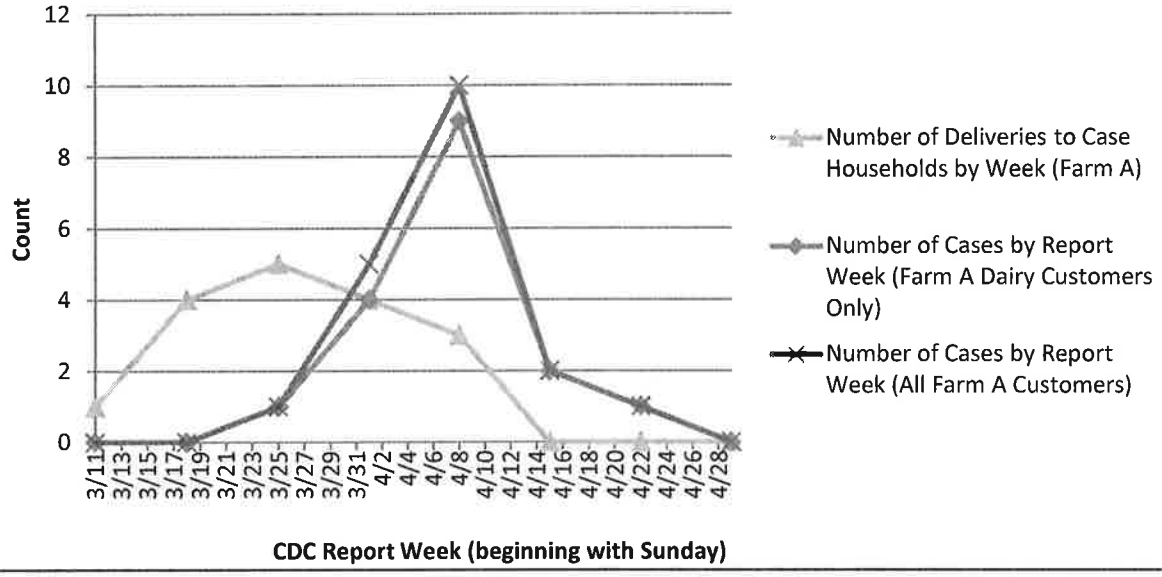

Figure 3. STEC Cases Reported in Central Missouri, 2007-2012 (Weeks 9-12)