

**Outbreak of Shiga toxin producing *E. coli* (STEC)
infections associated with a petting zoo at the
North Carolina State Fair – Raleigh, North Carolina,
November 2004**

Final Report

by

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Final Report: Outbreak of Shiga toxin producing *E. coli* (STEC) infections associated with a petting zoo at the North Carolina State Fair – Raleigh, North Carolina, November 2004.

BACKGROUND

Escherichia (E.) coli O157:H7 and other enterohemorrhagic *E. coli* (EHEC) infections cause hemorrhagic colitis and hemolytic uremic syndrome (HUS). An estimated 73,000 EHEC related infections and 61 deaths occur annually in the United States. Contaminated foods, beverages, water and livestock contact have caused outbreaks.

In the past 5 years, two foodborne outbreaks of *E. coli* O157:H7 occurred in North Carolina as well as sporadic and more isolated cases. In 2003, there were 34 cases of Shiga Toxin-producing *E. coli* (STEC) infection reported in North Carolina, a rate of 0.4 cases per 100,000. Excluding the foodborne outbreaks, an annual mean of 55.8 cases were reported between 1994 and 2003. 20 cumulative cases of HUS or TTP were reported in North Carolina from 1998 through 2003 (Figure 1).

On November 1st, 2004, the North Carolina Department of Health and Human Services (NCDHHS), Division of Public Health reported to the Foodborne and Diarrheal Diseases Branch, the Centers for Disease Control and Prevention (CDC), 15 cases of culture-confirmed *E. coli* O157:H7 infections among residents of North Carolina, including four patients diagnosed with hemolytic uremic syndrome (HUS). Many of those reporting illness had a history of attending the North Carolina State Fair, which took place in Raleigh, North Carolina from October 15th to October 24th, 2004. The North Carolina State Fair is an annual event run by the North Carolina Department of Agriculture and Consumer Services (NCDACS). Over 800,000 visitors attended the state fair between October 15th and October 24th, 2004, at the state fairgrounds in Raleigh.

On November 2nd, 2004, the North Carolina Department of Health and Human Services' Epidemiology Section requested further assistance with the investigation of this outbreak because of growing numbers of HUS cases, and because of the large number of visitors to the State Fair. On November 3rd, 2004, John Dunn, DVM, PhD, and Ciara O'Reilly, PhD, EIS Officers at the Foodborne and Diarrheal Diseases Branch, Katie Fullerton, MPH, Surveillance Epidemiologist at the Foodborne and Diarrheal Diseases Branch and Stacey Smith, epidemiology elective medical student joined Brant Goode to assist with the investigation of the outbreak. Kira Christian, MPH, epidemiology elective veterinary student, and Lisa Morgan, MPH, CDC Emerging Leaders Fellow joined the Epi-team on November 16th, 2004. Investigation objectives included working collaboratively with NCDHHS to determine the magnitude of the outbreak, identifying risk factors for *E. coli* O157:H7 infection, and preventing additional cases.

MATERIALS AND METHODS

Case finding and hypothesis generation

On October 30th, 2004, the North Carolina Division of Public Health (DPH) initiated active surveillance for diarrheal illness after an alert using North Carolina's Health Alert Network or HAN was received from Wake County's Community Health Director. The HAN alert described three cases of hemolytic uremic syndrome (HUS) clustered in time and location, all with histories of attending a petting zoo at the North Carolina State Fair. This first of three HAN alerts was sent to all North Carolina county health department directors, other state health department staff and managers, and a variety of designated contacts in the state's largest medical systems. It informed recipients of the outbreak soon after it was recognized, and asked recipients to alert their local networks of clinical providers to increase suspicion for STEC illnesses in their differential diagnoses of diarrheal illnesses. The initial Epi-X notification about this outbreak was posted November 1st.

On November 3rd, 2004, you, as State Epidemiologist, activated the North Carolina Public Health Command Center (NCPHCC) to organize outbreak investigation and control. As standard operating procedure, the NCPHCC held daily conference calls with county health department partners. During these calls, Command Center and Epi-team staff briefed state and local officials on the outbreak status and control measures, including requests to continue case-finding and expedite reporting using fax capabilities. Additional channels utilized to alert and inform medical providers about the outbreak, STEC infections and clinical care included DPH website postings and mailings to licensed family practice and pediatric physicians in the state. Child-care related risks and control measures were addressed with local health departments.

Initially, all patients reported with diarrheal illness were interviewed using CDC's *Standard Foodborne Disease Outbreak Case Questionnaire* available online at http://www.cdc.gov/foodborneoutbreaks/standard_questionnaire.htm. Information from this questionnaire was entered into a line list. As the case count grew, administering and entering data from this 16-page questionnaire became impractical. Investigators began using a 2-page *E. Coli , Shiga Toxin-Producing Infection* report form available online through the state public health website, <http://www.epi.state.nc.us/epi/gcdc/manual/forms/Ecoli4039.pdf>. An Epi-Info questionnaire matching this form was rapidly developed to manage and analyze data. Revisions of the two-page report form reflected descriptive analysis of early case reports which noted the relevance of fair and petting zoo attendance. Staff entered information from case report forms into Epi-Info record files, then appended these files into a Microsoft Access database.

Initial surveillance case definitions for suspect, probable and confirmed cases were broad and did not include attendance at the fair:

Suspect: Illness in a person who was in North Carolina from 10/8/2004 or later with onset of diarrhea (3 or more loose stools in a 24 hour period) since 10/15/2004 that lasted 2 days or longer without known cause, e.g. lab + for Salmonella.

Probable: Suspect case with epidemiological link to a confirmed case.

Confirmed: Suspect plus one of the following:

- 1) Lab confirmed Enterohemorrhagic *E. coli*, or
- 2) HUS or Thrombotic Thrombocytopenic Purpura (TTP) after 10/15/2003, even if culture negative.

Preliminary interviews suggested that illness was associated with attending the North Carolina State Fair and thus surveillance case-patient definitions were refined as follows:

A **suspect case** was defined as illness in a person who attended the North Carolina State Fair between 10/15/2004 and 10/24/2004 with onset of diarrhea (three or more loose stools in a 24 hour period) on or after 10/15/2004 through 11/09/2004 for a duration of 2 days or longer and without another known cause, e.g. laboratory confirmed *Salmonella* infection.

A **probable case** was defined as a suspect case with an epidemiological link to a confirmed case.

A **confirmed case** was defined as a suspect case with laboratory confirmed shiga toxin producing *E. coli* (STEC), or HUS or TTP clinically diagnosed after 10/15/2003 (even if culture negative).

A **secondary case** was defined as diarrhea (three or more loose stools in a 24 hour period) for 2 or more days in a person in contact (e.g. household member, caregiver, daycare classmates) with a suspect, probable, or confirmed case with either of the following criteria (a) onset 3 or more days after the primary case's onset but within two weeks of the primary case's onset, or (b) onset on or after 11/2/2004 (to exclude secondary cases with fair exposure).

To develop hypotheses about possible sources of infection at the state fair the Epi-team reviewed case-patients' reports and conducted face-to-face hypothesis generation interviews with parents of three lab confirmed *E. coli* O157:H7 HUS patients who became ill after attending the state fair.

Clinical Laboratory Investigation

Clinical laboratories in North Carolina routinely send *E. coli* O157 clinical isolates to the North Carolina State Laboratory for Public Health (SLPH) for confirmation, toxin assay, and pulsed-field gel electrophoresis (PFGE) molecular sub-typing. *E. coli* O157:H7 isolates from case-patients were submitted for further characterization. PFGE enzyme patterns were uploaded to PulseNet, the National Molecular Subtyping Surveillance System for foodborne diseases.

Early in the investigation, five clinical isolates were sent from the SLPH to CDC's Outbreak Response and Surveillance Laboratory for further characterization. These included four *E. coli* O157:H7 isolates, including three fair-associated isolates with indistinguishable PFGE patterns, and one fair-associated isolate with a unique PFGE pattern. The fifth was a fair-associated *E. coli* O45 isolate from a patient diagnosed with HUS.

Ten case-patient serum samples were also sent to CDC for O antigen serology, including specimens from HUS cases with serum available. These included *E. coli* O157 culture negative and positive HUS patients, and an *E. coli* O45 positive HUS patient.

Environmental Investigation

To further assist with hypotheses generation, the team visited the state fairgrounds on several occasions and consulted with NCDACS fair officials regarding specific activities that might support an outbreak. We obtained maps of the area and lists of activities which took place each day. We obtained information about all animals exhibited at the fair, the number of each species at each of the areas where persons could have direct contact with animals, health certificates, dates of rotation of animals in and out of specific exhibits, and the layout of animals and pens in each of the animal areas.

Because of concerns about a potential food- or water-borne outbreak, we asked the fairgrounds manager to provide information about all foods and beverages served at the fair. The fairgrounds manager reported that over 200 food and beverage vendors served items. We received a list of all registered food and beverage vendors and generated a coded list of foods and beverages. We interviewed the Wake County sanitarian supervisor who oversaw inspections and investigations of the permitted food and beverage vendors at the fair. We requested copies of inspections and complaints about food or beverage vendors from Wake County Health Department, and findings from their investigations. To address the water-borne outbreak potential, we requested coliform and residual chlorine testing records for the municipal water supply that served the fairgrounds.

Information on cleaning methods and the composition of the cleaning agents used for cleaning facilities and hand sanitizer used in dispensers during the fair were also compiled and examined.

Investigators collected composite ground samples and surface swab samples from structures from ten separate areas where animals were present and two other areas of interest (Figure 2). After the fair had ended, shavings and manure remained in six of eight animal areas where ruminant farm animals had been present during the fair. Between eight and 12 composite samples were taken from each of these sites. Materials sampled from animal areas included bedding, shavings and manure. Swab samples were taken inside permanent structures. NCDACS fairgrounds staff cleaned the interiors of the Kelley and Graham buildings which prior to sampling. Swab samples were taken from floors of these structures, and dust was sampled from elevated surfaces in these buildings including exhaust fans in the Graham Building. In addition to composite ground samples and surface swabs, investigators captured flies in nets at sites where they were present. Flies were transferred alive to specimen cups for shipment. Samples collected from non-animal areas of interest included swabs from a cider press used during the fair, and a fountain in front of a large arena. Investigators systematically re-sampled the Crossroads Farm Petting Zoo area where 10 of 11 initial ground composite samples grew *E. coli* O157:H7.

Fair visitors and cases offered other items to aid the investigation. These included a pair of shoes worn by a 2 year-old child who visited the Crossroads Farm Petting Zoo. This child's mother said her child's shoes became contaminated with manure in this petting zoo. Other samples taken included bedding and shavings collected from a case-patient's stroller seat, and

a soiled lint remover strip used to clean the stroller. Case-patients offered digital photographs and short movie clips of fair activities during case-patient interviews. These images were also collected to help illustrate human-animal interactions at the fair.

Environmental Laboratory Investigation

Environmental samples from fairground sites and facilities were cultured using sensitive methods that included selective broth enrichment, immuno-magnetic separation, and plating on selective media at a United States Department of Agriculture (USDA) laboratory. Isolates from samples positive for STEC were sent to CDC for PFGE analysis.

Case-Control Study

Our review of case finding data supported the hypothesis that the outbreak occurred among persons who attended the State Fair. Based on information gathered during hypothesis generation and results from preliminary environmental sampling, we suspected a petting zoo as the primary exposure site for this outbreak. Between November 14-21, 2004, we conducted a case-control study of fair attendees to test the hypothesis that human to animal interaction or human exposure in the animal areas at the state fair was the source of illness, and to identify the specific risk factors for STEC infection. To reduce the risk of including patients with a secondary infection or other diarrheal illness, we constructed a narrow case definition.

For the case-control study we defined a case as follows:

*A **case** was defined as laboratory-confirmed STEC infection, HUS/TTP clinically diagnosed after 10/15/2003, or bloody diarrhea in a person who attended the North Carolina State Fair between 10/15/2004 and 10/24/2004, who developed diarrhea (three or more loose stools in a 24 hour period) on or after 10/15/2004 through 11/07/2004, without a known cause other than STEC infection and determined not to have acquired infection through secondary transmission.*

For the case-control study we defined controls as follows:

*A **control** was defined as a person who attended the North Carolina State Fair between 10/15/2004 and 10/24/2004 and did not develop diarrhea (three or more loose stools in a 24 hour period) after attending the state fair through 11/07/2004.*

We developed a questionnaire focusing on 10 areas where human contact with animals could occur, hand-washing practices at the fairgrounds, foods and beverages consumed, and other activities at the fair. We also included items about knowledge, attitudes and beliefs about contact with animals, and hand-hygiene at the fairgrounds. A section on health status included questions about medical conditions that reduce resistance to infections, *E. coli* O157:H7 case-patient signs and symptoms, and their medical care.

We recruited controls from a list provided by fair officials of 23,972 persons who purchased tickets to the fair online, at kiosks or in malls. The list included names, addresses and phone

numbers of persons who made advance ticket purchases. Duplicates were removed from the list and only residents of North Carolina and persons for whom telephone numbers were available were included in the study. We generated a randomized list of 14,974 names and phone numbers to use for enrolling controls. Only one person per household was used as a control. Controls were frequency matched by age group (1-5 years, 6-17 years and 18 years and above). The youngest member of each household who met the control definition was enrolled as the control from that household. When enrolling controls less than 18 years of age, we asked to interview the parent, guardian, or other adult who had attended the fair with the child and was most familiar with the child's activities at the fair. For children aged 6-17, with the parent or guardians consent, we asked both the adult and the child to participate in the interview. We sought three controls per case.

The epi-team received key assistance with interviews from the CDC's Epidemiology Program Office, epidemiology elective students, staff from the Foodborne and Diarrheal Diseases Branch, CDC; faculty, staff and students from Team Epi-Aid at the University of North Carolina at Chapel Hill's Center for Public Health Preparedness, epidemiologists in North Carolina's hospital-based Public Health Epidemiology program, leaders and Public Health Preparedness and Response Teams from North Carolina's Office of Public Health Preparedness and Response, and the Occupational and Environmental Epidemiology Branch, Epidemiology Section, North Carolina Division of Public Health.

Statistical Analysis

Investigators entered data collected in case-control study questionnaires into a Microsoft Access database. Preliminary statistical analysis was performed using SAS version 9.1. Analysts computed odds ratios (OR), with 95% confidence intervals (CI) and p-values. A nested analysis of one of two petting zoos was also performed. Mr. Goode conducted further analysis using Stata software (version 8.2) after validating preliminary findings.

RESULTS

Case finding and Hypothesis Generation

Initial case finding using the most sensitive case definition resulted in 187 diarrheal illness case reports, including 15 with HUS. Cases were reported from 23 counties, with most originating from Wake, Durham and Orange Counties (Table 1). No fatalities were reported. After revised case definitions with criteria for State Fair attendance and illness onset after 10/15/2004 were applied, 108 cases were classified as outbreak-related. These included 43 confirmed, 6 probable and 59 suspect cases. Distribution of case-patients' illness onset dates was consistent with typical *E. coli* O157:H7 incubations after presumed exposures during the fair (see Figure 3). We further classified cases as primary or secondary cases. Eighteen

(9.6%) case-patients were classified as secondary cases. Age distribution of outbreak-related cases was skewed: nearly 80% were less than 18 years-old, and 50.5% were under 6 years of age (Figure 4). 93% of cases reported attending a fair, and 89% reported petting zoo contact.

Laboratory Results: Clinical specimens

By November 23rd, 2004, 42 outbreak-related patients with laboratory confirmed STEC infection or clinically diagnosed HUS had been reported to the North Carolina Division of Public Health. Forty-one of the patients had laboratory confirmed *E. coli* O157:H7 infection. One had clinically diagnosed HUS and laboratory confirmed *E. coli* O45 infection. 38 of the 41 *E. coli* O157:H7 isolates shared an indistinguishable PFGE XbaI and BlnI patterns. This pattern was designated Pattern A. The three remaining *E. coli* O157:H7 isolates demonstrated unique PFGE patterns. These isolates were designated Patterns G, H and J. The *E. coli* O45 isolate also had a unique pattern, designated pattern C. Table 2 presents PFGE XbaI and BlnI designations for these patterns.

Complete serotyping at the CDC laboratory determined the O45 isolate to be *E. coli* O45:H19. This *E. coli* O45:H19 isolate was negative for shiga toxin (stx), *eaeA* and *Ehly* virulence genes. An isolate negative for all three virulence genes is unlikely to cause disease, although serial culture may have diminished capacity to produce stx. A serum sample from the HUS patient with confirmed *E. coli* O45:H19 infection had elevated O157 IgM antibodies, suggesting co-infection with O157. One other serum sample from an O157 culture negative HUS patient examined at CDC was also found to have O157 IgM titer of 1:180. Table 3 presents characterization of the five clinical isolates from the outbreak sent to CDC for analysis. The four O157:H7 isolates characterized were positive for virulence genes stx 2, *eae* and *Ehly* by Polymerase Chain Reaction (PCR).

Environmental Investigation

Over 5,400 fair exhibits included animals. While exhibit types varied extensively, livestock exhibits presented the greatest opportunities for people to have contact with the most animals. The most common animals exhibited were goats, sheep, pigs and cattle. Persons could see large animals in the Kelley and Graham Buildings, milk a cow in the State Fair Ark exhibit, and touch and see other livestock in Children's Barnyard and other venues. Animals were exhibited in a total of 10 sites, including two commercial petting zoos, Commerford and Sons Petting Zoo, and Crossroads Farm Petting Zoo.

Commerford and Sons Petting Zoo housed animals in pens. Visitors could reach over or through railings to touch the animals in this exhibit, i.e. they could touch animals and pen structures. Another petting zoo, Crossroads Farm Petting Zoo, allowed persons to walk among and have extensive direct contact with sheep and goats, and touch various exotic species held in pens in the rear of the exhibit. Both petting zoos allowed feeding. Hand-

sanitizer was available at both petting zoos. Handwashing facilities were not located next to either petting zoo, but persons could find soap and running water handwashing facilities elsewhere at the fairgrounds.

Fairgrounds' managers reported that exhibitors or fairgrounds contract staff removed contaminated bedding from various areas which held animals. Fairgrounds staff closed the Graham Building twice during the fair to remove contaminated bedding or manure, and to apply chemical disinfection. Accumulated debris from animal exhibits was collected and placed in refuse containers away from public access.

Fair policy stated that all animals exhibited must have valid Certificates of Veterinary Inspection issued by an USDA accredited veterinarian within 60 days before exhibition. The fair did not require actual health records. If questions or concerns arose, a regulatory veterinarian determined whether an animal could stay at the fair. We obtained copies of inspection certificates provided to NCDACS Animal Health Program staff who inspected the certificates and the animals before exhibits commenced. No deficiencies were noted.

The Wake County Health Department Sanitarian supervisor who oversaw food and beverage vendors' inspections and temporary food service permits described sporadic deficiencies which were corrected before permitting and operation began, i.e. before foods or beverages were served at the fair. Wake County Health Department sanitarians reported receiving only one complaint about food served at the fair. Sanitarians investigated the complaint that smoked turkey legs had pink meat and found that the product did have pink meat but was not undercooked.

The laboratory supervisor from the City of Raleigh E.M. Johnson Water Treatment Plant, which serves the municipal area including the fairgrounds, provided heterotrophic plate count (HPC), residual chlorine level, and Colilert® test records for water specimens tested during the NC State Fair. Staff microbiologists from the water plant noted that *E. coli* O157:H7 would be detected as total coliform by Colilert® methodology, but not as *E. coli*. If *E. coli* O157:H7 bacteria were present in water, they would likely travel with other *E. coli* bacteria that would be detected by the method. Colilert® test results from 210 compliance distribution samples obtained during October, 2004, showed absence of total coliforms in all but three sites. None of these three total coliform positive sites were located in the fairgrounds vicinity. Residual Chlorine levels for samples obtained in the fairgrounds vicinity were within recommended ranges. One water sample obtained October 19th, 2004, from a site near the fairgrounds had an elevated HPC of 80 colonies per ml—within the acceptable range of less than 500 colonies per ml.

Laboratory Results: Environmental samples

Eighteen of 96 environmental samples collected at the fairgrounds during initial testing November 3rd yielded cultures positive for *E. coli* O157:H7. One of the ten fly pools was positive. Results are summarized in Table 4. Ten of 15 samples obtained from the Crossroads

Farm Petting Zoo site yielded positive cultures. Investigators systematically re-sampled this site on November 9th to further elucidate these findings. Nineteen of 30 samples from the second sampling of the Crossroads Farm Petting Zoo site yielded *E. coli* O157:H7; these samples were obtained from locations in an area within the Crossroads Farm Petting Zoo site where people could apparently have direct contact with sheep and goats (Figure 5). All 19 positive cultures had PFGE patterns indistinguishable from Pattern A. Figure 6 shows a pulsed-field gel electrophoretogram from the PulseNet national database aligning clinical and environmental PFGE Pattern A results.

Eight of 74 samples obtained during initial sampling from sites other than Crossroads Farm Petting Zoo yielded *E. coli* O157:H7 isolates. These eight samples were obtained from 3 sites: the Ark, Sheep and Goat tent, and from a rail used in the Graham Building. Five isolates from these cultures were sent to CDC for PFGE. These 5 isolates demonstrated PFGE patterns undistinguishable from each other, but different from Pattern A (Table 4). This pattern was indistinguishable from the PFGE pattern from a confirmed *E. coli* O157:H7 case-patient who denied visiting Crossroads Farm Petting Zoo, but did report contact with animals in the Ark and Graham Building.

E. coli O157:H7 was also isolated from a pair of shoes worn by a child who had visited Crossroads Farm Petting Zoo, from debris collected in a stroller seat used at the fair, and the lint remover strip used to clean this stroller. These *E. coli* O157:H7 isolates had PFGE patterns indistinguishable from Pattern A.

Case-Control Study

At the time of the study, 46 patients met the case-control study case definition. We excluded one patient after interviewing them and reviewing clinical and laboratory data showed that they did not meet either the clinical or laboratory case definition. We enrolled 45 cases and 188 controls at the end of the case control study, an overall control-to-case ratio of greater than four to one.

Females comprised 57.8% of case-patients and 58.1% of controls. Case-patients' ages ranged from one year-old to 61 years (median 3.0 years); controls' ages ranged from 11 months-old to 60 years (median 4.8 years). Mean case and control ages were 8.8 and 12.1 years respectively. Distributions of case-patients' and controls' ages overall were similarly skewed ((Figure 7). Overall age distributions were not similar (rank-sum $p=0.02$), despite appropriate age-group frequency matching. In cases and controls younger than 6 years of age, median case age was 2.2 years and median control age was 3.4 years. Among the 100 visitors to Crossroads Farm Petting Zoo, case and control age distributions were similarly skewed and were statistically similar (median: 2.8 and 3.7 years respectively, rank-sum $p=0.35$). Age distribution was similar as well among the 76 visitors to Crossroads Farm Petting Zoo who were younger than 6 years old (rank-sum $p=0.11$).

Table 5 presents features of the 45 case-patients' illnesses. Reported signs and symptoms included diarrhea (100%), bloody diarrhea (87%), abdominal cramps (80%), fatigue (82%)

and fever (62%). Median duration of illness reported in the case control study was 10 days (range 1-28 days). Median duration of diarrhea was 7 days; median length of bloody diarrhea was 3 days. Fifteen (33%) patients developed HUS. No patients died. 44% of patients were hospitalized for their illness. Median hospitalization reported was 8 days. The median number of health care visits among all cases was 3 visits. 56% of patients received intravenous fluids. 20% of patients took antibiotics in the seven days after illness onset, and 31% took anti-diarrheal medication.

Illness onset dates of the patients in the study ranged from October 18th to November 4th, 2004 (Figure 8), with dispersion of onset dates similar to onset dates reported by all case-patients during case finding (N=108). The incubation period was calculated using the date of the case-patient's first visit to the fair. Median incubation period was 5 days.

Investigators examined consumption of specific foods and beverages at the fair, including hamburgers, hotdogs, ice cream, cotton candy, fresh pressed apple cider, and fresh lemonade. Analysis showed no association between illness and any foods or beverages consumed at the fair (Table 6). We also examined exposures to recreational water at the fair, including lake water, two recreational exhibits with water, and water and spray from the fountain at the fairgrounds for association with illness. These water exposures were not significantly associated with illness.

When comparing all cases and controls, analysis of the distinct animal areas showed significant associations between visits in 4 animal exhibit areas (Crossroads Farm Petting Zoo, the Children's Barnyard, the Graham Building, and the Poultry Tent) and illness (Table 7).

Many exposures in Crossroads Farm Petting Zoo were statistically associated with illness (Table 8). These include falling down or sitting on the ground (OR = 7.6; CI 3.2-18.1; p = 0.001), petting or touching the sheep and goats (OR = 7.8; CI 3.6-16.8; p = 0.02), feeding the sheep and goats (OR = 3.5; CI 1.7-6.9; p = 0.001), the sheep or goats rearing up and putting their front feet on a person, and touching or stepping in manure (OR = 9.2; CI 1.4-21.6; p = 0.001). Feeding and touching the exotic animals in the back of the tent was also associated with becoming ill (OR = 4.8; CI 2.3-10). Case-patients sucked their thumb, a pacifier or drank from a sippy cup significantly more than controls (OR = 26, CI 5.2-129.2, p<0.001).

In the Children's Barnyard which was located next to Crossroads Farm Petting Zoo, no exposure variables were positively associated with illness among cases and controls who reported visiting this exhibit. However, controls reported hand-hygiene practice upon exiting this exhibit more often than case-patients (OR = 0.2; CI 0.1-0.8; p = 0.03). In the Graham Building, a large animal exhibit hall, no exposure variables were positively associated with illness among cases and controls who reported visiting this exhibit, although thumb or pacifier-sucking, or use of a sippy cup and hand-hygiene practice upon exiting this exhibit were marginally significant. In the Poultry Tent, case-patients sucked their thumb, a pacifier or drank from a sippy cup significantly more than controls in this exhibit (OR = 6.3, CI 1.3-31.1, p=0.04).

Because many significant findings were associated with age, we examined the youngest age-group, cases and controls younger than 6 years of age. Tables 9 and 10 present significant associations for visits to various exhibits, and significant exposures within those exhibits. Visits to Crossroads Farm Petting Zoo, the Children's Barnyard, and the Graham Building were again significantly associated with illness. Visits to Crossroads Farm Petting Zoo had an odds ratio of 9 for this age group (95% CI 3.0-27.3), while visits to the Children's Barnyard and the Graham Building had odds ratios of 3.3 (CI 1.4-7.6) and 2.8 (CI 1.2-6.9) respectively. No specific exposures within the Children's Barnyard were significantly associated with illness for this age group. Cases spent significantly more time in Crossroads Farm Petting Zoo and the Graham Building than controls (Table 9). Further, cases reported significantly more thumb-sucking, pacifier use or drinking from a sippy cup in the Graham Building and Crossroads Farm Petting Zoo than controls (OR=3.2 and 10.6, respectively; CIs: 1.0-10.3; 2.0-55.0). Cases who visited Crossroads Farm Petting Zoo also reported significantly more time spent in the exhibit (20 minutes vs 15, $p=0.02$), exposure to manure (OR 6.9; CI 2.2-21.9), and falling or sitting on the ground compared to controls (OR 3.2; CI 1.1-9.1).

Because of concerns that age modified the effect of exposures, we further stratified age-groups to assess for effect modification and confounding. Results of these analyses are presented in tables 11 and 12. Lower odds ratios and narrower confidence intervals support finding age as an effect modifier—not unexpected given the age distribution of cases, but did not support evidence of confounding. Tables 13 and 14 present findings for cases and controls in the two older age groups, 6-17, and 18 years and older.

Because of the predominance of significant associations between illness and exposures at Crossroads Farm Petting Zoo as well as the young age of most cases, we present findings for cases and controls aged < 3 years who reported visiting this location (Table 15). The significant associations among visitors less than 3 years of age included: falling down or sitting on the ground (OR = 5.43; CI 1.4-21.6; $p = 0.02$), and touching or stepping in manure (OR = 7.5; CI 1.9-30.1; $p = 0.005$). Marginal associations were noted for having sheep or goats rear up and put their front feet on a person (OR = 4.7; CI 1.1-21.0; $p = 0.08$), and sucking one's thumb, a pacifier or drinking from a sippy cup (OR = 5.7; CI 1.0-31.5; $p = 0.05$).

Hand hygiene is recommended for persons who visit petting zoos or who have contact with animals in other settings. In Crossroads Farm Petting Zoo, signs recommending hand hygiene were present, and most cases and controls who visited this exhibit reported use of hand hygiene on exiting—32 of 36 cases (89%), and 52 of 64 controls (81%). Most visitors who practiced hand-hygiene used alcohol-based hand-sanitizing gel: 29 of 32 cases (91%) and 43 of 52 controls (83%). However, reporting use of hand sanitizing gel after visiting this petting zoo was not significantly protective when compared to not practicing hand-hygiene overall or for age-groups (see Table 16) all ages: OR=1.9, 95%CI=0.5-6.4; 0-5 y/o: OR=1.9, 95% CI=0.5-10.2; 6 y/o and older: OR=2.2, 95%CI=0.3-15).

Table 17 presents findings of a protective effect from reporting awareness of potential disease transmission from farm animals to humans. Reported awareness was associated

with a protective effect on the odds of becoming ill among all cases and controls (OR = 0.14; 95% CI 0.1-0.3; $p < 0.001$), as well as among the cohort of 100 visitors to Crossroads Farm Petting Zoo (OR=0.2, 95%CI=0.08-0.6), including the youngest Crossroads Farm Petting Zoo visitors (those under age 6) (OR=0.1, 95% CI= 0.03-0.5).

CONCLUSIONS

Findings from the case-control study, laboratory investigation and environmental sampling consistently associate most outbreak illnesses with exposures in Crossroads Farm Petting Zoo. These exposures probably resulted from a confluence of 1) animals shedding *E. coli* O157:H7, 2) intensive and extensive contact with animals, and 3) behaviors associated with very young ages. The age distribution of cases in this outbreak supports this conclusion given the level of contact possible and encouraged between young children and animals in this exhibit. Characteristics of young children, e.g. their propensity to insert non-food items into their mouths, their higher surface area-to-body mass ratio, and their reduced resistance to infections relative to older children and adults offer additional support for this contention.

High numbers of animals in an enclosed area likely concentrated *E. coli* O157:H7 within Crossroads Farm Petting Zoo. The zoo owner reported that as many as 100 individual sheep or goats were present in the exhibit which measured 40 by 60 feet. The owner reported limiting the number of persons admitted to the exhibit due to overcrowding during especially busy fair days. Animals were stressed at times during the fair, and were noted by the owner to have had loose stools.

Hand-hygiene, regardless of its well-known public health benefits, was either ineffective or inadequately timed to prevent infection in this outbreak. While hand-sanitizers were available and were used at Crossroads Farm Petting Zoo, exposure sufficient for infection may have occurred prior to use, or contamination of young children's skin areas other than their hands, e.g. their faces may have led to infection. Other findings that may explain the lack of protection from hand-hygiene include cases' reports of spending more time in this petting zoo along with more contact with manure or the ground.

Respondents' awareness of zoonotic disease risk was a notable protective factor. Persons with such knowledge may have voluntarily taken measures to reduce such risks by limiting time and extent of contact with animals in the petting zoo.

Fomite transmission of *E. coli* O157:H7 from contaminated bedding may also have been important in this outbreak given that *E. coli* O157:H7 was isolated from a pair of shoes worn by a child at the fair, from material obtained from a stroller used at the fair, and from the lint roller strip used to clean the stroller. Should similar outbreaks occur, an attempt should be made to take environmental samples from items which may act as fomites.

Limitations

Selection bias due to recruitment of controls from a list of persons who purchased tickets online may have distorted findings if this group was different from cases. Misclassification bias may also have distorted findings due to including probable cases in the definition of cases in the case control study.

Although interviewers were instructed to prompt control respondents to try to recall with precision, recall bias may have differentially affected case-control study respondent's ability to provide specific answers to items. While age groups were appropriately matched, actual age distribution of controls and cases varied, particularly within the youngest age-group (0-5 y/o).

Cases may have valued animal contact more than controls, and this may have led them to practice more risk behaviors than controls. This was not fully explored in the case-control study. Both cases and controls reported that direct contact with animals was important to them. However, given their illnesses and because we did not ask how respondents felt about such contact specifically during their fair visit, case respondents may have underreported the importance of such contact given their illness experience. Cases may have sought contact more than controls, as evidenced by the finding noted above that cases spent more time in Crossroads Farm Petting Zoo.

Finally, further analysis may offer new or clarify existing findings.

RECOMMENDATIONS

Many case-patients in this outbreak reportedly used hand-hygiene gels and nonetheless became ill. This suggests that fair managers and petting zoo owners should consider additional prevention and control measures at petting zoos to reduce risks of zoonotic disease transmission beyond providing hand-sanitizers. The following measures are provided to inform policy and practice for petting zoo exhibits. Evidence to support the recommendations are based on findings from this investigation as well as from the *Compendium of Measures to Prevent Disease and Injury Associated with Animals in Public Settings, 2004* by the National Association of State Public Health Veterinarians (NASPHV), available at http://www.avma.org/pubhlth/comp_animals_public_settings.asp. Findings have been reviewed and incorporated into the latest *Compendium* document, available at <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5404a1.htm> or <http://www.cdc.gov/mmwr/PDF/rr/rr5404.pdf>.

Fair managers and petting zoo operators should prohibit or discourage direct or extensive interaction between farm animals and very young children or persons known to have reduced resistance to infection. Engineered controls such as physical barriers between animals and people can reduce such direct contact, and perhaps altogether eliminate contact with contaminated animal bedding and manure. Direct supervision by persons willing and able to control interactions associated with illness is recommended. For example, petting zoo staff should be trained to prohibit contact between infants and livestock, to promptly remove

contaminated bedding, and to assure proper maintenance and service of hand-washing facilities and their use by petting zoo visitors to decontaminate hands.

Persons attending petting zoos should receive easily understandable information about zoonotic disease risks prior to animal contact. Specific messages about zoonotic transmission risks, particularly to very young children or others with lower resistance to infections, should be provided to all potential visitors. Such messages should be communicated effectively with adequate signage as well as through other media channels, and with consideration of literacy levels and appropriate language.

To reduce potential exposures, fair managers and petting zoo operators should work together to reduce large crowds of people and dense numbers of animals in petting zoos at any one time. Providing more space and limiting the length of interactions between people and animals might reduce risks by lowering the environmental microbial burden and opportunity for human exposure.

Fair managers and petting zoo operators should monitor for and assure removal of any animals ill with diarrhea from public access. Contaminated materials should be handled as described in the *Compendium of Measures to Prevent Disease and Injury Associated with Animals in Public Settings, 2005* by the National Association of State Public Health Veterinarians (NASPHV).

However, STEC is also shed by asymptomatic animals; all contaminated materials should be handled similarly, regardless of animal health status. Petting zoo operators should assure frequent and sufficient removal of contaminated bedding. Such removal should reduce contamination burden and risk of cross-contamination. Contaminated materials should be removed per guidelines noted above. Fair management or other DACS staff may need to periodically inspect petting zoo premises in a similar fashion as sanitarians inspect food service establishments, with a similar goal of healthful public interaction.

In this outbreak, hand-hygiene opportunities relied on hand sanitizers near the petting zoo. Sanitizers have limited efficacy in the face of gross contamination with organic material, may have become contaminated themselves, and can provide a sense of security not based on evidence. If gross contamination is likely, hand-washing stations with running water and soap should be available to more effectively reduce such contamination. Devices designed to reduce cross-contamination between soiled hands and fixtures are preferred. Hand-washing facilities should be located close to animal areas to encourage hygiene measures immediately after leaving the petting zoo. Stations should be cleaned routinely to reduce cross-contamination, and maintained to assure continued service. State Fair management should separate animal areas from areas where foods and beverages are prepared, served or consumed. Design of exhibits should provide areas with hand hygiene stations as people move from animal areas to non-animal areas.

Extensive environmental sampling aided this investigation and helped refine the outbreak hypothesis. Future suspected petting zoo or zoonotic outbreaks should include rapid

environmental testing for potentially implicated pathogens. Animal testing should also be considered, as careful sampling may determine which animals were infected and their potential roles in transmission. For example, detecting pathogens on animal coats or in their saliva may suggest other routes of transmission besides well-recognized fecal-oral risks. While it is clear that many animals carry STEC, the course of infection or carriage is notably variable. Further description of such variability may help identify species- or situation-specific prevention or control measures.

*This Epi-Aid Report summarizes the field investigation.
Additional analysis might present results, interpretation, and recommendations
that differ from those contained in this document.*

Appendix: Figures and Tables

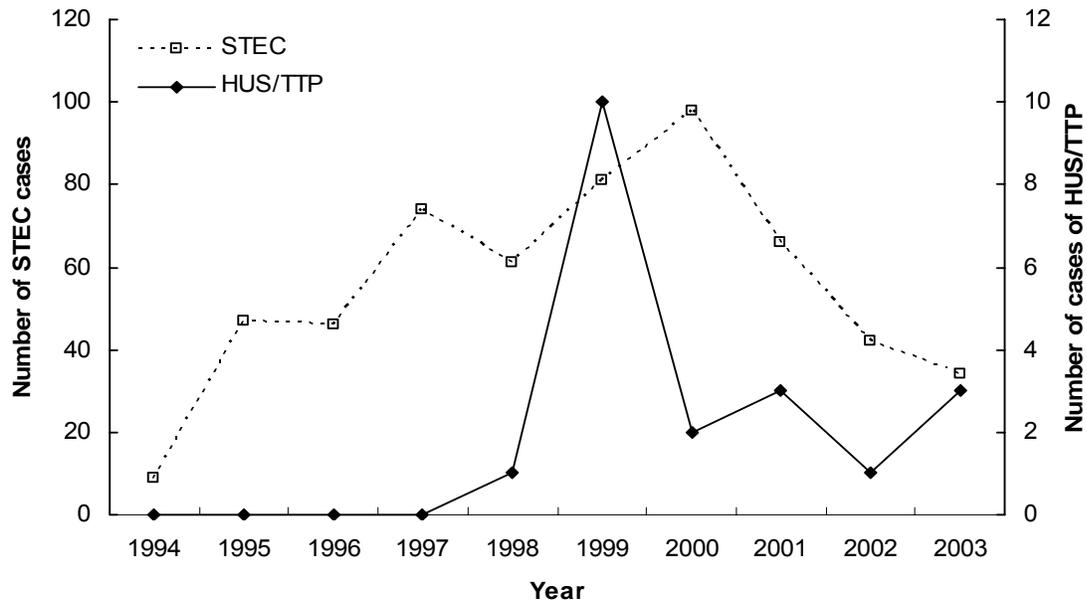


Figure 1. *E. coli* O157, STEC and HUS/TTP reports, North Carolina 1994-2003 (excluding two large outbreaks in 1998 and 2001).



Figure 2. Environmental sampling locations, North Carolina State Fairgrounds.

<u>Key</u>	
<u>Location</u>	<u>Type of Exhibit</u>
A. Commerford and Sons Petting Zoo	Commercial petting zoo
B. Crossroads Farm Petting Zoo	Commercial petting zoo
C. Children's Barnyard	Livestock exhibit
D. State Fair Ark	Livestock exhibit
E. Sheep and Goat tent	Livestock exhibit
F. Kelley Buidling	Livestock exhibit
G. Graham Building	Livestock exhibit
H. Apple Cider making	Fresh juice exhibit
I. Livestock tie up	Animal tie up area—non-exhibit
J. Rabbit Barn	Small animal livestock exhibit
K. Fountain	Water feature
L. Horse Arena	Horse arena
M. Poultry tent	Poultry exhibit

Table 1. Case-patients' county of residence.

County	Confirmed	Probable	Suspect	Total Cases
Alamance	1	0	4	5
Buncombe	1	0	0	1
Caswell	0	0	4	4
Chatham	1	0	3	4
Cumberland	0	0	1	1
Durham	5	0	3	8
Forsyth	1	0	0	1
Granville	0	0	1	1
Guilford	0	0	2	2
Harnett	1	0	0	1
Johnston	1	0	5	6
Lee	1	0	0	1
Mecklenburg	1	0	0	1
Moore	1	0	0	1
Orange	4	1	7	12
Pasquotank	0	0	1	1
Person	2	2	3	7
Randolph	0	0	2	2
Robeson	0	0	2	2
Rockingham	1	1	1	3
Scotland	0	0	5	5
Wake	19	2	14	35
Wilson	3	0	1	4
Total	43	6	59	108

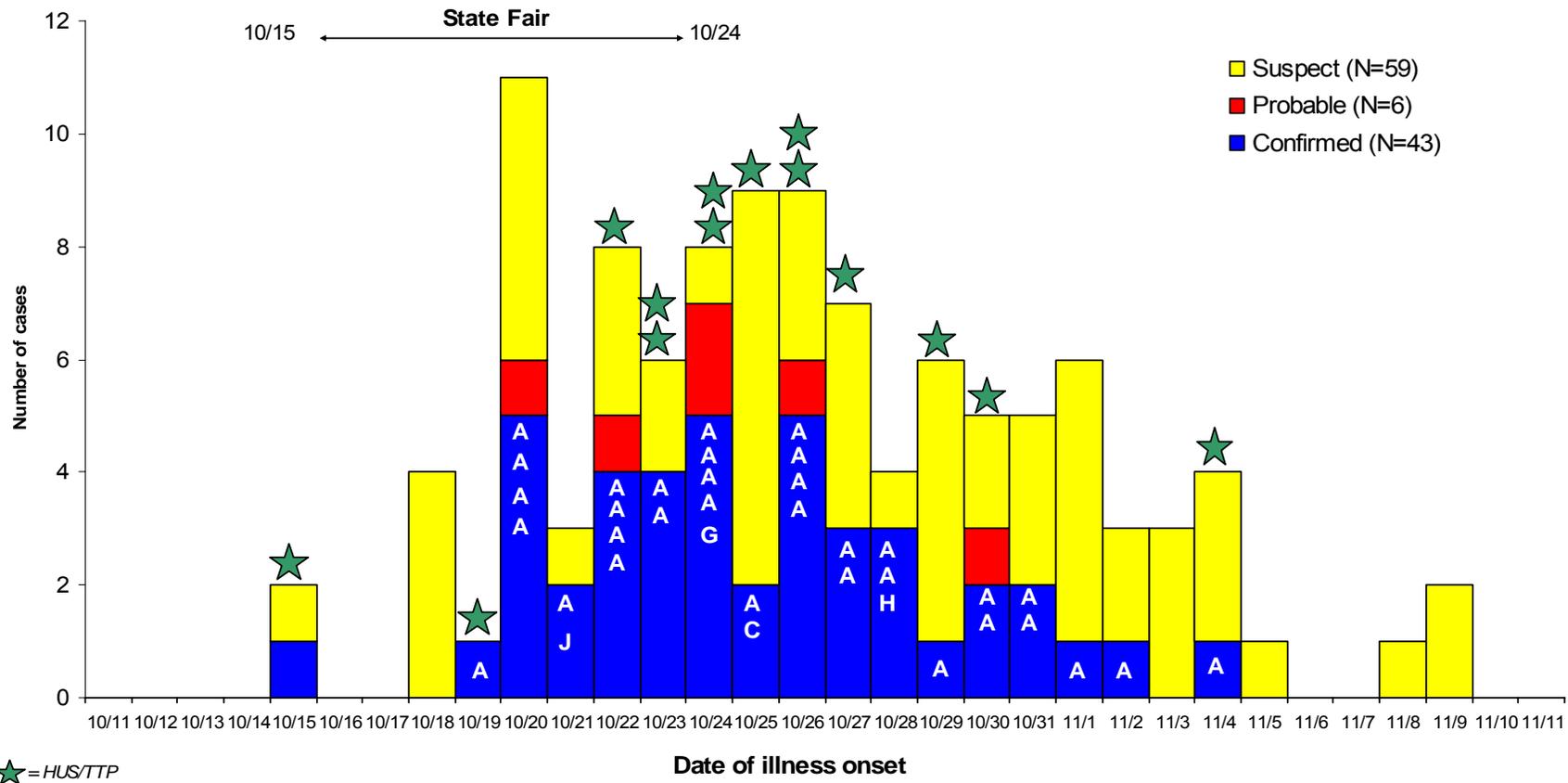


Figure 3. Illness onset dates for persons reporting diarrhea to North Carolina State Health Department after attending the North Carolina State Fair – Raleigh, North Carolina, November 2004. (Note: A, C, G and H on the chart denote different PFGE patterns.)

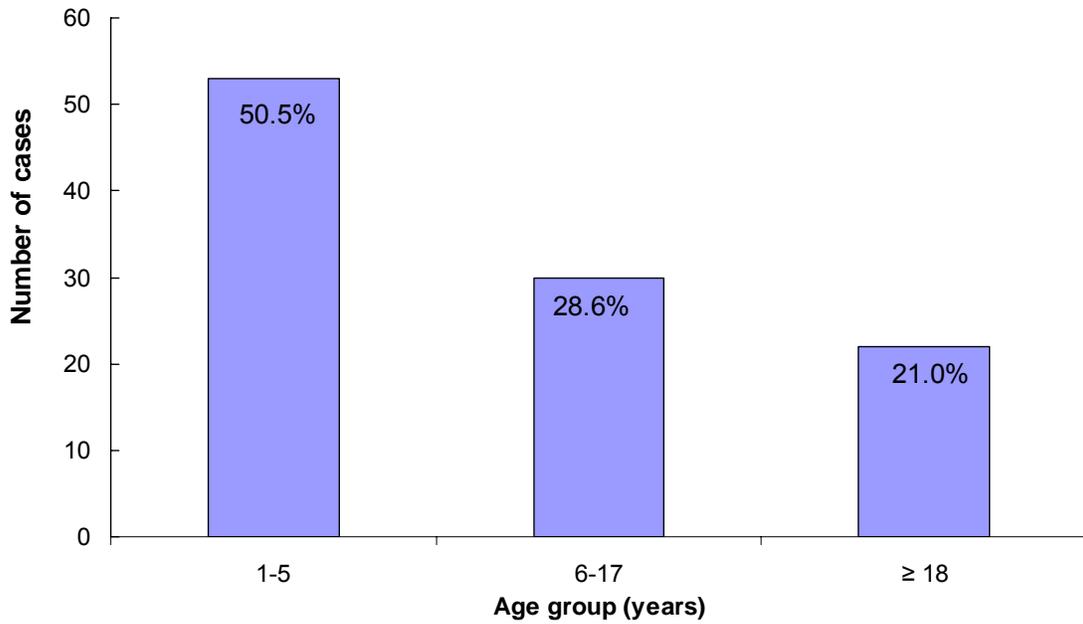


Figure 4. Case-patient's age group frequencies.

Table 2. Clinical isolates, North Carolina State Fair outbreak, 2004.

PFGE Pattern designation	Number of cases	Serotype	PFGE-XbaI-pattern	PFGE-BlnI-pattern	Epidemiological Information
Pattern A	35	<i>E. coli</i> O157:H7	EXHX01.0224	EXHA26.0536	All cases attended the NC State Fair
Pattern A	3	<i>E. coli</i> O157:H7	EXHX01.0224	EXHA26.0536	Secondary cases (primary case attended the NC State Fair)
Pattern C	1	<i>E. coli</i> O45:H19	EH2X01.0002	EH2A26.0001	Attended the NC State Fair
Pattern G	1	<i>E. coli</i> O157:H7	EXHX01.0802	EXHA26.0717	Attended the NC State Fair
Pattern H	1	<i>E. coli</i> O157:H7	EXHX01.2224	EXHA26.0718	Attended the NC State Fair
Pattern J	1	<i>E. coli</i> O157:H7	EXHX01.0129	pending	Attended the NC State Fair

Table 3. Characterization of clinical isolates at the Centers for Disease Control Outbreak Response and Surveillance Laboratory.

Isolate	Serotype	NC PFGE Pattern	NC Shiga Toxin/Premier EHEC	CDC Shiga Toxin/Premier EHEC	PCR (stx1, stx2, eae, Ehly)
Isolate 1	O157:H7	A	+	Not Tested	Stx 2, eae, Ehly
Isolate 2	O157:H7	A	+	Not Tested	Stx 2, eae, Ehly
Isolate 3	O157:H7	A	-	+	Stx 2, eae, Ehly
Isolate 4	O45:H19	C	-	-	-
Isolate 5	O157:H7	G	-	+	Stx 2, eae Ehly

Table 4. Results of environmental testing from the North Carolina State Fairgrounds, November 3rd and 9th, 2004.

Source Location	Type ¹	# samples Nov. 3 rd , 2004	Positive for <i>E. coli</i> O157:H7	# samples Nov. 9 th , 2004	Positive for <i>E. coli</i> O157:H7	PFGE pattern ²
Commerford and Sons Petting Zoo	C	12	0	4	0	
	F	4	0			
Crossroads Farm Petting Zoo	C	11	10	30	19	Nov. 3 rd , 2004: 8 Pattern A, 1 with 1 Pattern A enzyme. One unique pattern. Nov. 9 th , 2004: All 19 were Pattern A
	W	1	0			
	F	3	0			
Children's Barnyard	C	8	0	2 (fan)	0	
	S	7	0			
State Fair Ark	C	5	2			2 nd Pattern ²
	S	3	1			
	F	2	0			
Sheep and Goat tent	C	8	3			2 nd Pattern
	S	3	0			
	F	1	1			
Kelley Building	S	9	0	6	0	
Graham Building	S	12	1	4	0	2 nd Pattern
Apple Cider Exhibit	S	2	0			
Livestock Tie-up	C	5	0			
Rabbit Barn	C			2	0	
	S			4	0	
Fountain near arena	S			2	0	
Horse Arena	S			2	0	
Total		96	18	56	19	

¹Sample type: C= composite ground sample (feces, shavings, soil), S=surface swab, F= fly pool, W= water.

²2nd PFGE Pattern enzyme designation: Xbal EXHX01.0272, BlnI EXHA26.0684.

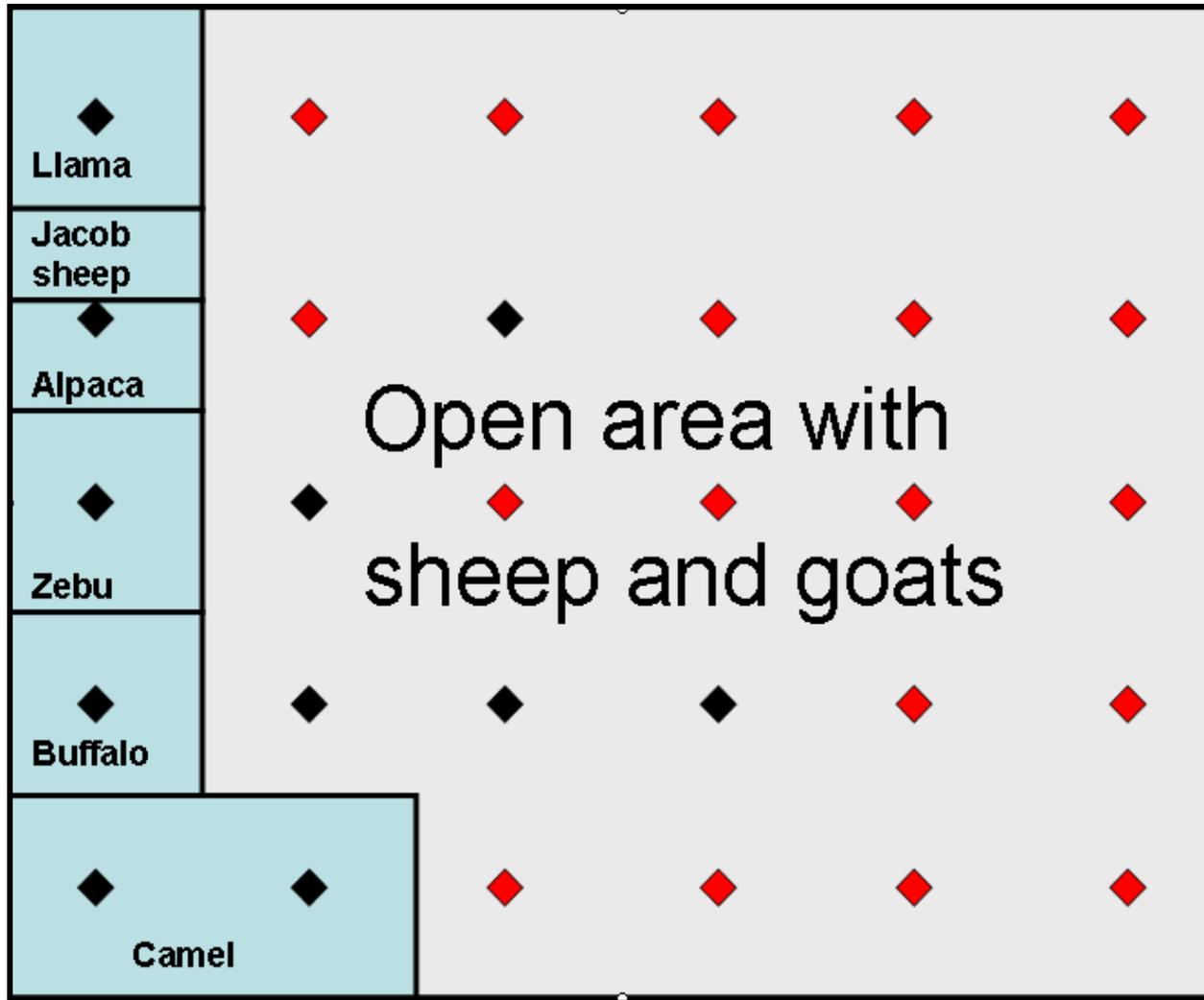


Figure 5. Sampling schematic from Crossroads Farm Petting Zoo locations, November 9, 2004. Fence locations are approximate. Red diamonds indicate locations that yielded *E. coli* O157:H7.

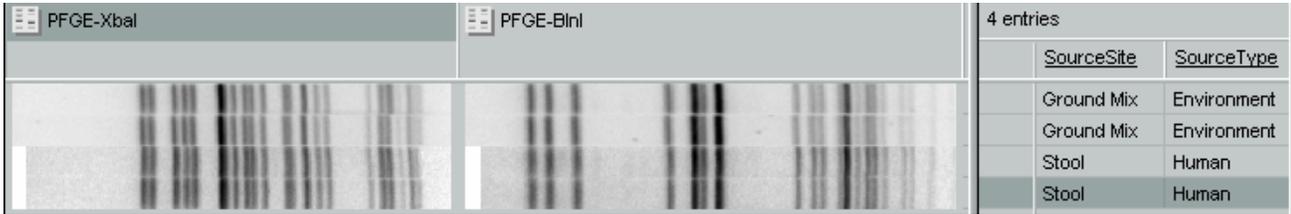


Figure 6. Pulsed-field gel electrophoretogram of *E. coli* O157:H7 outbreak Pattern A from human and environmental isolates.

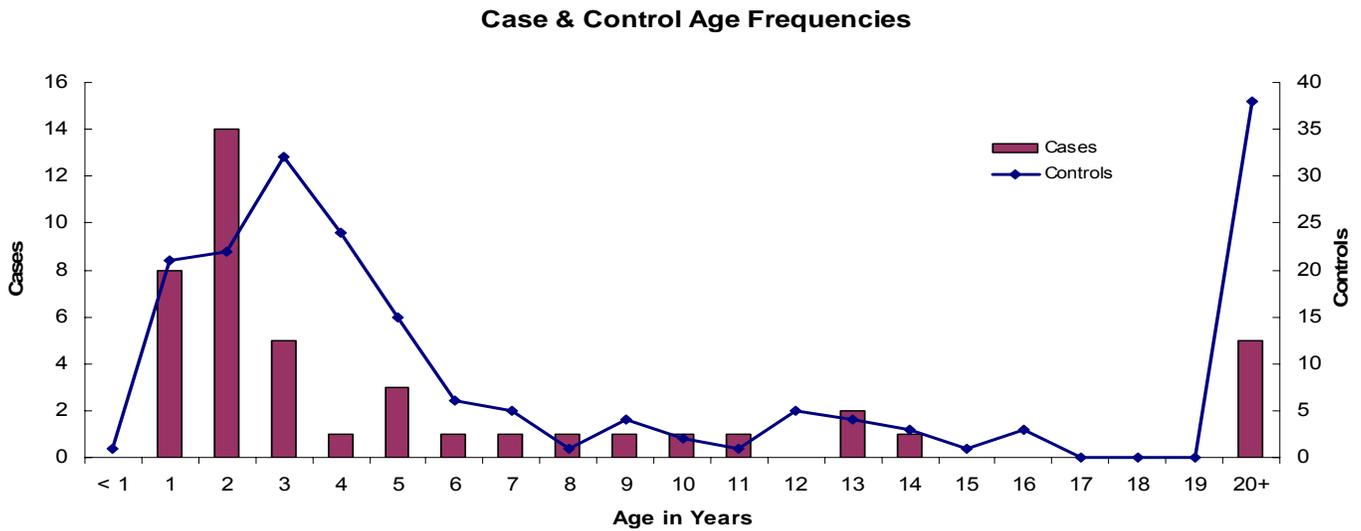


Figure 7. Case-control study participant age frequencies.

Table 5. Clinical information among case-control study cases (N=45)

Characteristic	Cases n/N (%)
Female	26/45 (57.8)
Median age in years (range)	2.9 (1-61)
≤ 5 years old	31/45 (68.9)
Median illness onset dates (range)	10/26 (10/18-11/4)
Median duration of illness in days (range)	10 (1-28)
Median days incubation from 1 st visit to fair	5
<i>Symptom</i>	
Diarrhea	45/45 (100)
Medium number of stools in a 24-hour period (range)	22 (2-72)
Median days duration of diarrhea (range)	7 (1-22)
Bloody diarrhea	39/45 (86.7)
Median days duration of bloody diarrhea (range)	3 (1-17)
Nausea	21/45 (46.7)
Vomiting	22/45 (48.9)
Abdominal cramps	36/45 (80.0)
Fever	28/45 (62.2)
Maximum temperature (°F)	104
Chills	10/45 (22.2)
Headache	8/45 (17.8)
Body aches	13/45 (28.9)
Fatigue	37/45 (82.2)
Constipation	6/45 (13.3)
Other symptoms	14/45 (32.6)
HUS	15/45 (33.3)
TTP	1/45 (2.2)
<i>Health care providers</i>	
Median number of health care visits (range)	3 (0-12)
Hospitalization	20/45 (44.4)
Median days duration of hospitalization (range)	8 (2-21)
Treated with intravenous fluid	25/45 (55.6)
<i>Medication taken in the seven days after illness began</i>	
Antibiotics	9/45 (20.0)
Anti-diarrhea medication	14/45 (31.1)

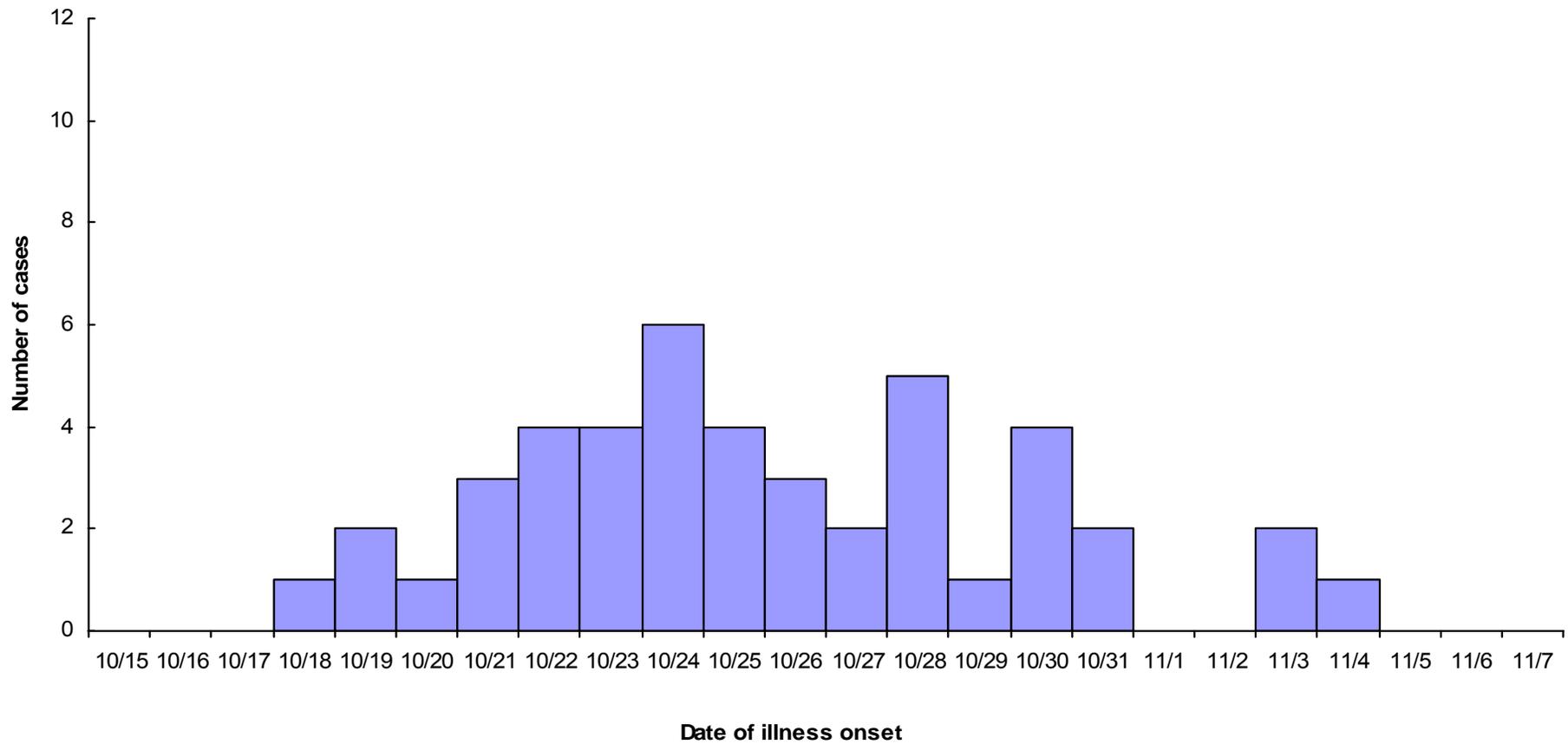


Figure 8. Dates of illness onset for all cases (N=45) enrolled in case-control study, November 2004.

Table 6. proportion of all cases and controls who visited and were exposed to various foods and beverages.

Exposure	Cases (%) n=45	Controls (%) n=188	Odds Ratio (95%CI)	p-value
<i>Food and Drinks</i>				
Eat or drink at the fair	44/45 (98%)	183/187 (98%)	0.96 (0.1-8.82)	1.000
Eat any hamburgers or cheeseburgers	2/45 (4%)	22/183 (12%)	0.34 (0.08-1.5)	0.179
Eat any pink or undercooked hamburger	1/2 (50%)	2/21 (10%)	9.5 (0.41-217.61)	0.249
Eat any hotdogs	20/45 (44%)	58/183 (32%)	1.72 (0.89-3.35)	0.117
Eat any other rare or undercooked meat	0/45 (0%)	5/180 (3%)	-	0.586
Eat any raw vegetables	1/45 (2%)	4/184 (2%)	1.02 (0.11-9.38)	1.000
Eat any salad	1/45 (2%)	1/184 (1%)	4.16 (0.26-67.8)	0.355
Visit the cider press	2/45 (4%)	24/188 (13%)	0.32 (0.07-1.4)	0.184
Drink fresh pressed apple cider	1/45 (2%)	13/188 (7%)	0.31 (0.04-2.4)	0.316
Eat any cotton candy	18/45 (40%)	80/184 (44%)	0.87 (0.45-1.68)	0.738
Eat any ice cream	13/45 (29%)	61/183 (33%)	0.81 (0.4-1.66)	0.600
Drink fresh squeezed lemonade	11/41 (27%)	43/183 (24%)	1.19 (0.55-2.58)	0.688
Drink any other drink purchased at the fair	22/43 (51%)	129/184 (70%)	0.45 (0.23-0.88)	0.021
Eat any other food purchased at the fair	30/43 (70%)	150/184 (82%)	0.52 (0.25-1.11)	0.097

Table 7. Associations with illness by visits to various animal exhibits, North Carolina State Fair, 2004.

Exhibit visited	Cases (%) n=45	Controls (%) n=188	OR	CI	p
Commerford and Sons Petting Zoo	5/44 (11%)	23/188 (12%)	0.9	0.3 - 2.6	1
Crossroads Farm Petting Zoo	36/45 (80%)	64/187 (34%)	7.7	3.5 - 17.0	<0.001
Children's Barnyard	21/44 (48%)	50/186 (27%)	2.5	1.3 - 4.9	0.01
State Fair Ark	12/45 (27%)	65/188 (35%)	0.7	0.3 - 1.4	0.4
Sheep and Goat tent	8/42 (19%)	32/186 (17%)	1.1	0.5 - 2.7	0.8
Kelley Building	8/44 (18%)	52/186 (28%)	0.6	0.2 - 1.3	0.3
Graham Building	31/44 (70%)	94/187 (50%)	2.3	1.1 - 4.8	0.02
Rabbit Barn	11/44 (25%)	49/187 (26%)	0.9	0.4 - 2.0	1
Poultry tent	19/44 (43%)	49/187 (26%)	2.1	1.1 - 4.2	0.04
Pony Ride	9/45 (20%)	27/187 (14%)	1.5	0.6 - 3.4	0.4

Table 8. Proportion of all cases and controls who visited various animal areas with significant associations for such visits, other exposures. Bold text denotes odds ratios that do not include 1.0 and p values < 0.05. (All variables examined for Crossroads Farm Petting Zoo presented; only significant findings and hygiene variables from Children's Barnyard, Graham Building and Poultry tent presented.)

Exposure	Cases (%) n=45	Controls (%) n=188	Odds Ratio (95%CI)	p-value
<i>Crossroads Farm Petting Zoo</i>				
Visit Crossroads Farm Petting Zoo	36/45 (80%)	64/187 (34%)	7.69 (3.49-16.95)	<0.001
Drink beverage while in petting zoo	0/44 (0%)	1/186 (1%)	-	1.00
Eat foods while in petting zoo	0/45 (0%)	1/186 (1%)	-	1.00
Fall down or sit on the ground	14/41 (34%)	12/187 (6%)	7.56 (3.16-18.07)	<0.001
Pet or touch the sheep or goats	35/45 (78%)	58/187 (31%)	7.78 (3.61-16.78)	<0.001
Feed the sheep and goats	21/44 (48%)	39/187 (21%)	3.46 (1.74-6.9)	<0.001
Pet/touch animals in the pens at the back of the tent	19/43 (44%)	26/184 (14%)	4.81 (2.32-9.99)	<0.001
Feed animals in pens at the back of the tent	9/42 (21%)	15/184 (8%)	3.07 (1.24-7.61)	0.022
Pick up or hold any sheep or goats	6/45 (13%)	10/187 (5%)	2.72 (0.93-7.94)	0.093
Kiss any animals	4/42 (10%)	6/187 (3%)	3.18 (0.85-11.8)	0.089
Sheep or goats nuzzle, nibble or lick	30/43 (70%)	48/184 (26%)	6.54 (3.15-13.56)	<0.001
Pick up any object from the ground	1/44 (2%)	3/187 (2%)	1.43 (0.14-14.05)	0.573
Touch or step in manure	21/38 (55%)	22/186 (12%)	9.21 (4.23-20.07)	<0.001
Sheep or goats rearing up	16/43 (37%)	17/187 (9%)	5.93 (2.68-13.11)	<0.001
Pick up any shavings/bedding from the ground	6/40 (15%)	17/187 (9%)	1.76 (0.65-4.8)	0.256
Chew gum, eat candy, or use a toothpick	0/45 (0%)	1/187 (1%)	-	1.000
Suck thumb, pacifier or drink from sippy cup	8/34 (24%)	2/171 (1%)	26 (5.23-129.24)	<0.001
Carry a toy or blanket	0/35 (0%)	1/171 (1%)	-	1.000
Wash hands upon exiting petting zoo	32/45 (71%)	52/186 (28%)	6.34 (3.09-13.03)	<0.001
Walk through petting zoo	34/44 (77%)	47/174 (27%)	9.19 (4.21-20.05)	<0.001
<i>Children's Barnyard</i>				
Visit Children's Barnyard	21/44 (48%)	50/186 (27%)	2.48 (1.27-4.88)	0.011
Wash hands upon exiting	4/17 (24%)	28/50 (56%)	0.2 (0.1-0.8)	0.03
<i>Graham Building</i>				
Visit Graham Building	31/44 (71%)	94/187 (50%)	2.36 (1.16-4.79)	0.018
Suck thumb, pacifier or drink from sippy cup	8/17 (47%)	13/56 (23%)	2.9 (0.9-9.2)	0.07
Wash hands upon exiting	3/29 (10%)	26/91 (29%)	0.2 (0.1-1.0)	0.05
<i>Poultry Tent</i>				
Visit Poultry Tent	19/44 (43%)	49/187 (26%)	2.14 (1.08-4.22)	0.042
Suck thumb, pacifier or drink from sippy cup	6/10 (60%)	5/26 (19%)	6.3 (1.3-31.1)	0.04

Table 9. Proportions of cases and controls younger than 6 who visited various animal areas. Bold text denotes Odds Ratios that do not include 1.0 and p values < 0.05

Exhibit visited	Cases (%) n=31	Controls (%) n=115	OR	95 % CI	p
Commerford & Sons Petting Zoo	0/31	11/115 (10%)	-		0.12
Crossroads Farm Petting Zoo	27/31 (87%)	49/114 (43%)	9	3.0 - 27.3	< 0.001
Children's Barnyard	19/30 (63%)	39/113 (35%)	3.3	1.4 - 7.6	0.006
State Fair Ark	7/31 (23%)	44/115 (38%)	0.5	0.2 - 1.2	0.14
Sheep and Goat tent	5/30 (17%)	19/113 (17%)	1	0.3 - 2.9	1
Kelley Building	6/30 (20%)	33/113 (29%)	0.6	0.2 - 1.6	0.37
Graham Building	22/30 (73%)	56/114 (49%)	2.8	1.2 - 6.9	0.02
Rabbit Barn	8/30 (27%)	33/114 (29%)	0.9	0.3 - 2.2	1
Poultry Tent	11/30 (37%)	28/114 (25%)	1.8	0.75 - 4.2	0.25
Pony Ride	8/31 (26%)	24/114 (21%)	1.3	0.5 - 3.3	0.63

Table 10. Significant associations for cases and controls younger than 6 who visited the Graham Building and Crossroads Farm Petting Zoo.

Exposure	Cases (%)	Controls (%)	OR	CI	p
<u>Graham Building</u>					
Median time in Graham Building (minutes)	20	14			0.01
Suck thumb, pacifier, or drink from a sippy cup	8/16 (50%)	13/55 (24%)	3.2	1.0 - 10.3	0.06
<u>Crossroads Farm Petting Zoo</u>					
Median time in petting zoo (minutes)	20	15			0.02
Touch or step in manure	19/24 (79%)	17/48 (35%)	6.9	2.2 - 21.9	0.001
Sit or fall on ground	11/23 (48%)	11/49 (22%)	3.2	1.1 - 9.1	0.05
Suck thumb, pacifier or drink from sippy cup	8/25 (32%)	2/47 (4%)	10.6	2.0 - 55.0	0.002

Table 11. Significant associations for cases and controls under the age of 3 who visited various animal areas.

Exposure	Cases (%) n=23	Controls (%) n=45	Odds Ratio (95%CI)	p-value
Visit Crossroads Farm Petting Zoo	21/23 (91%)	24/45 (53%)	9.19 (1.92-43.9)	0.002
Fall down or sit on the ground in Crossroads Farm Petting Zoo	10/19 (53%)	5/45 (11%)	8.89 (2.44-32.43)	<0.001
Sheep or goats nuzzle, nibble or lick in Crossroads Farm Petting Zoo	18/21 (86%)	19/45 (42%)	8.21 (2.11-31.92)	0.001
Sheep or goats rearing up in Crossroads Farm Petting Zoo	8/22 (36%)	3/45 (7%)	8 (1.86-34.39)	0.004
Suck thumb, pacifier or drink from sippy cup in Crossroads Farm Petting Zoo	7/22 (32%)	2/44 (5%)	9.8 (1.83-52.5)	<0.005
Pet or touch the sheep or goats in Crossroads Farm Petting Zoo	20/23 (87%)	21/45 (47%)	7.62 (1.98-29.31)	0.002
Wash hands upon exiting Crossroads Farm Petting Zoo	19/23 (83%)	18/45 (40%)	7.13 (2.08-24.43)	<0.001
Walk through Crossroads Farm Petting Zoo	19/22 (86%)	19/43 (44%)	8 (2.06-31.12)	0.001
Touch or Step in Manure in Crossroads Farm Petting Zoo	15/21 (71%)	8/45 (18%)	11.56 (3.42-39.04)	<0.001

Table 12. Significant and marginally significant associations for cases and controls 3 to 5 years who visited various animal areas.

Exposure	Cases (%) n=8	Controls (%) n=70	Odds Ratio (95%CI)	p-value
Visit Children's Barnyard	6/8 (75%)	19/68 (28%)	7.74 (1.43-41.75)	0.013
Touch or lean on the cages in Children's Barnyard	5/8 (63%)	14/68 (21%)	6.43 (1.37-30.21)	0.020
Touch or step in manure in Crossroads Farm Petting Zoo	4/7 (57%)	9/68 (13%)	8.74 (1.67-45.65)	0.015
Pet or touch the sheep or goats in Crossroads Farm Petting Zoo	6/8 (75%)	23/69 (33%)	6 (1.12-32.09)	0.047
Walk through Crossroads Farm Petting Zoo	6/8 (75%)	22/66 (33%)	6 (1.12-32.2)	0.047
Wash hands or using hand sanitizer after exiting Crossroads Farm Petting Zoo	6/8 (75%)	24/68 (35%)	5.05 (1.03-29.39)	0.052

Table 13. Significant associations for cases and controls 6 to 17 years of age who visited various animal areas.

Exposure	Cases (%) n=9	Controls (%) n=35	Odds Ratio (95%CI)	p-value
Touch or step in manure in Commerford and Sons Petting Zoo	4/9 (44%)	4/35 (11%)	6.2 (1.16-33.17)	0.042
Visit Crossroads Farm Petting Zoo	6/9 (67%)	7/35 (20%)	8 (1.59-40.2)	0.012
Sheep or goats nuzzle, nibble or lick in Crossroads Farm Petting Zoo	5/9 (56%)	6/34 (18%)	5.83 (1.2-28.41)	0.034
Sheep or goats rearing up in Crossroads Farm Petting Zoo	4/9 (44%)	2/35 (6%)	13.2 (1.9-91.91)	0.011
Pet/touch the animals in the pens at the back of Crossroads Farm Petting Zoo	6/9 (67%)	4/35 (11%)	15.5 (2.74-87.74)	0.002
Pet or touch the sheep or goats in Crossroads Farm Petting Zoo	6/9 (67%)	7/35 (20%)	8 (1.59-40.2)	0.012
Walk through Crossroads Farm Petting Zoo	6/9 (67%)	5/34 (15%)	11.6 (2.16-62.22)	0.004
Visit the Poultry Tent	5/9 (56%)	5/35 (14%)	7.5 (1.48-37.91)	0.018
Feed, pet or touch any poultry in the Poultry Tent	5/9 (56%)	3/35 (9%)	13.33 (2.27-78.2)	<0.005

Table 14. Significant associations for cases and controls over age 18 who visited various animal areas.

Exposure	Cases (%) n=5	Controls (%) n=38	Odds Ratio (95%CI)	p-value
Feed the sheep and goats in Crossroads Farm Petting Zoo	2/5 (40%)	1/38 (3%)	24.67 (1.7-357.36)	0.032
Sheep or goats rearing up in Crossroads Farm Petting Zoo	2/5 (40%)	1/38 (3%)	24.67 (1.7-357.36)	0.032
Pet/touch the animals in the pens at the back of Crossroads Farm Petting Zoo	2/5 (40%)	2/38 (5%)	12 (1.22-118.1)	0.060
Wash hands upon exiting Crossroads Farm Petting Zoo	3/5 (60%)	4/38 (11%)	12.75 (1.61-100.7)	0.024
Walk through Crossroads Farm Petting Zoo	3/5 (60%)	1/31 (3%)	45 (3.09-654.9)	0.005

Table 15. Comparison of cases and controls under the age of 3 who visited Crossroads Farm Petting Zoo. Bold text denotes OR > 1.0 with 95% CI that does not include 1.0.

Exposure	Cases (%) n=21	Controls (%) n=24	Odds Ratio (95%CI)	p-value
Fall down or sit on the ground	10/17 (59%)	5/24 (21%)	5.43 (1.37-21.57)	0.021
Feed the sheep and goats	10/20 (50%)	13/24 (54%)	0.85 (0.26-2.78)	1
Feed the animals in pens at the back of the tent	4/18 (22%)	3/23 (13%)	1.9 (0.37-9.87)	0.679
Pick up or hold any sheep or goats	5/21 (24%)	5/24 (21%)	1.19 (0.29-4.85)	1
Kiss any animals	2/18 (11%)	4/24 (17%)	0.63 (0.1-3.86)	0.685
Sheep or goats nuzzle, nibble or lick	18/19 (95%)	19/24 (79%)	4.74 (0.5-44.57)	0.205
Pick up any object from the ground	1/20 (5%)	0/24 (0%)		0.455
Touch or step in manure	15/19 (79%)	8/24 (33%)	7.5 (1.86-30.16)	0.005
Sheep or goats rearing up	8/20 (40%)	3/24 (13%)	4.67 (1.04-21.01)	0.078*
Pick up any shavings/bedding	5/16 (31%)	5/24 (21%)	1.73 (0.41-7.33)	0.482
Suck thumb, pacifier or drink from sippy cup	7/20 (35%)	2/23 (9%)	5.65 (1.02-31.48)	0.059*
Pet/touch the animals in the pens at the back	8/19 (42%)	10/23 (44%)	0.95 (0.28-3.23)	1
Pet or touch the sheep or goats	20/21 (95%)	21/24 (88%)	2.86 (0.27-29.8)	0.611
Wash hands upon exiting	19/21 (91%)	18/24 (75%)	3.17 (0.56-17.78)	0.252
Walk through	19/20 (95%)	19/22 (86%)	3 (0.29-31.48)	0.608

*95% CI does not include 1.0, p > 0.05.

Table 16. Comparison of Hand Hygiene Practices.

	Cases (%)	Controls (5)	Odds Ratio (95%CI)	p-value
Hand-hygiene vs no hand-hygiene				
0-5 y/o	25/27 (93%)	42/49 (86%)	0.9 (0.5-1.6)	0.7
6 and older	7/9 (78%)	10/15 (67%)	1.8 (0.3-11.7)	0.6
Total	32/36 (89%)	52/64 (81%)	0.9 (0.5-1.5)	0.7
Hand-Gel vs no hand-hygiene				
0-5 y/o	22/24	35/41	1.9 (0.3-10.2)	0.5
6 and older	7/9	8/13	2.2 (0.3-15)	0.4
Total	29/33 (88%)	43/54 (80%)	1.9 (0.5-6.4)	0.3

Table 17. Awareness of disease transmission risk from contact with livestock.

Awareness of disease transmission risk	Cases (%) n=45	Controls (%) n=188	OR	95% CI	p
All cases and controls	29/44 (66%)	175/188 (93%)	0.1	0.06-3.3	<0.001
Cases and controls who visited Crossroads Farm Petting Zoo	23/36 (64%)	57/64 (89%)	0.2	0.08-0.6	0.004
Cases and Controls <6 years old who visited Crossroads Farm Petting Zoo	17/27 (63%)	46/49 (94%)	0.1	0.03-0.5	0.001