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Shiga-Toxigenic *Escherichia coli* O157:H7 Infections among Livestock Exhibitors and Visitors at a Texas County Fair

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ABSTRACT

We report an agricultural fair-associated shiga-toxigenic *Escherichia coli* O157:H7 (STEC O157) outbreak that was unusual in that it affected both livestock exhibitors and visitors. Twenty-five human cases of STEC O157 infection were detected after the Fort Bend County Fair in Rosenberg, Texas, which ran from 9/26/03 to 10/04/03. Seven cases were culture-confirmed. There were four hemolytic uremic syndrome (HUS) cases, and one thrombotic thrombocytopenic purpura (TTP) case. Cases ranged in age from 18 months to 67 years. Twenty-two (88%) cases were female. Analysis of unmatched case-control data linked STEC O157 infection with visiting fair livestock exhibit areas and with multiple fair visits. All outbreak-related isolates were of a single STEC O157 subtype. Fair Ground environmental sampling and culture for STEC O157, conducted 46 days after the end of the Fair, yielded multiple STEC O157 isolates, including the outbreak subtype. Livestock exhibitors and fair visitors should follow guidelines to reduce the risk of transmission of STEC O157 at agricultural fairs.

INTRODUCTION

State and county fairs attract >125 million visitors annually in the United States (LeJeune and Davis 2004) and represent the only contact that much of the general public has with livestock. Fairs afford an opportunity for people to have close direct contact with livestock, including contact with saliva and fecal-contaminated hides.

STEC O157 causes human disease ranging from mild diarrhea to hemorrhagic colitis, HUS, TTP, and death. At least seven U.S. fair-associated STEC O157 outbreaks since 1998 have resulted in >1200 human illnesses and >300 culture-confirmed infections, including 34 cases of HUS-associated renal failure and two deaths (LeJeune and Davis 2004; Bender and Shulman 2004). Cattle are an STEC O157 reservoir. Previous fair outbreaks have been linked to direct contact with ruminants such as cattle, sheep, or goats (Centers for Disease Control and Prevention 2001; Chapman et al. 2000; Crump et al. 2002) or exposure to contaminated water supplies (Bopp et al. 2003; Centers for Disease Control and Prevention 1999) and contaminated buildings (Varma et al. 2003). Fair attendees are also at increased risk of acquiring STEC O157 (Crump et al. 2003).

Between September 26 and October 4, 2003, 170,307 persons visited the Fort Bend County (FBC) Fair in Rosenberg, Texas, including

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19,500 persons who attended any of five rodeos during the nine-day fair. Furthermore, 385 exhibitors in the livestock barns showed >700 4-H and/or FFA animal projects. On October 8, FBC public health officials were notified of three local persons hospitalized with hemorrhagic diarrhea and initiated an outbreak investigation that rapidly implicated visiting the FBC Fair as a common exposure. On October 10, three more persons, all children who had attended the FBC Fair, were hospitalized with bloody diarrhea. County health officials expanded the investigation to evaluate ongoing risks, implement control measures, determine the scope of the outbreak, and investigate infection risk factors. Suspected fair outbreak causes included contaminated food or water, livestock contact, and sewage contamination from a backup in the restrooms that flooded part of the fairgrounds on opening day.

MATERIALS AND METHODS

Case definition and case finding and outbreak investigation

A case was defined as a person who attended the FBC Fair and had onset of bloody diarrhea and abdominal cramping, HUS, or TTP within 2 weeks of the start of the Fair. The FBC Fair Association, local hospitals, school nurses, patient word-of-mouth, and the local media facilitated case finding. The case-control study enrolled two unmatched controls per case. Controls were excluded if they did not attend the Fair or if they experienced any diarrheal symptoms in the 2 weeks after the start of the Fair. Controls were recruited by word-of-mouth and included family members and well companions of cases.

Potential cases were interviewed via a questionnaire which asked about food and animal fair exposures, including whether the person had attended the fair as an exhibitor or a visitor, the number of days of fair attendance, fair areas visited, and possible animal exposures. Food histories and demographic information were also collected. Controls were administered the same questionnaire as cases. Stool samples for STEC O157 culture were collected from ill persons by physicians and hospitals. Isolates were sent to the Houston Department of Health and Human Services Bureau of Laboratory Services (HDHHS) for laboratory confirmation. Pulsed field gel electrophoresis (PFGE) of STEC O157 isolates was performed by the HDHHS using standardized procedures (Swaminathan et al. 2001).

Post-fair environmental sampling and microbiology

Fairground environmental samples ($n = 62$) were collected on November 20, 2003 (46 days after the end of the FBC Fair) from the sewage back-up area, show arena, livestock pens, holding areas and wash racks, drainage ditches, the Ag-tivity and petting zoo areas and the rodeo arena (Fig. 1). Samples were composed of soil, dried livestock feces, livestock bedding, standing water, and surface swabs taken at ground level, above ground on railings and from building beams. Samples were aliquotted (10 g) into sterile Whirl-Pak bags to which 90 ml of 1.5× (60 g/L) brilliant green bile broth (BGB) was added for enrichment. Environmental swabs (two – 3 × 3-in. gauze pads per sample) were enriched in 20 ml of 1.5× BGB. A duplicate sample set was prepared as described above, but with an antibiotic cocktail of vancomycin (8 mg/L), cefixime (0.05 mg/L), and cefsulidan (10 mg/L) added to the BGB enrichment broth. All enrichment samples were statically incubated for 6 h at 37°C, followed by immunomagnetic separation using 1 ml of enrichment broth and 20 μl of Dynal anti-O157 paramagnetic beads (Dynal Biotech, Brown Deer, WI). Washed beads were spread-plated onto ChromAgar O157 plates (CHROMagar, Paris, France) containing 18 μl per liter of 3.5% potassium tellurite solution (TCA) and incubated for 24 h at 37°C. Suspect mauve-pink isolates were serologically confirmed as STEC O157 by enzyme immunoassay using anti-*E. coli* O157 and anti-*E. coli* H7 monoclonal antibodies (Elder et al. 2000) and by PCR detection of *stx*1, *stx*2 (shiga-toxin), *eae* (intimin), *hly* (hemolysin), rfb_{O157} (O157 O-antigen) and $fliC_{H7}$ (H7 flagellum) genes (Paton and Paton 1998). Up to three confirmed STEC O157 isolates were selected from each positive sample and PFGE subtyped using *XbaI* restriction enzyme.

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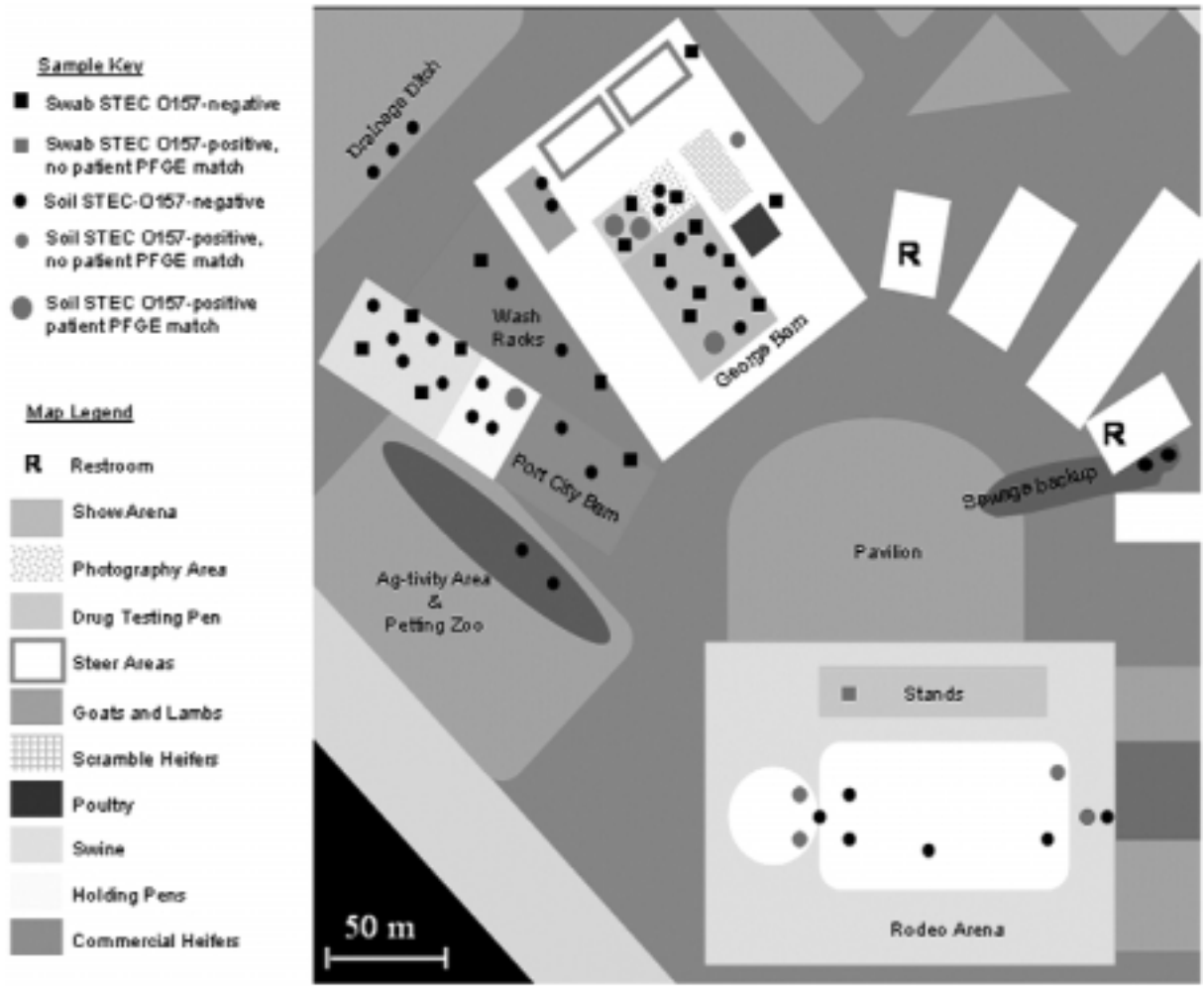

FIG. 1. Map of 2003 Fort Bend County Fairgrounds showing 61 soil and swab sampling locations and STEC O157 culture results.

Data analysis

The outbreak data were analyzed as an unmatched case-control study by exact logistic regression using the LOGISTIC procedure of SAS 9.1 (SAS Institute, Inc., Cary, NC). The binary response variable (outcome) of interest was the probability of being a human STEC O157 case vs. control. Fair exposure and demographic data (gender, age, and race) were converted into categorical or continuous variables. The association of these potential explanatory or confounding variables with the likelihood of being a case was examined by generating univariate exact odds ratios (OR) with exact 95% OR confidence intervals (CI) and corresponding p values. Exact logistic regression was used because the outbreak data set was small, with sparse data or with complete data separation (i.e., variables which perfectly predicted the outcome response) so that asymptotic logistic regression estimation was not possible. The subset of fair exposure or demographic variables associated with the outcome at $p < = 0.20$ in the univariate analysis were used as candidate explanatory variables to develop a multivariable exact logistic regression model.

RESULTS

Descriptive epidemiology

Sixty-eight persons were identified with illness onset within the case finding enrollment period. Of these, 58 (85%) were interviewed

and 25 (37%) met the case definition for presumed STEC O157 infection. Of these 25 cases, 24 (96%) experienced bloody diarrhea. In addition, 21 (84%) cases experienced abdominal cramping, 12 (48%) were treated with antibiotics, and 19 (76%) required hospitalization. Seven cases (28%) were laboratory confirmed by STEC O157 isolation from the stool (Fig. 2). All patient STEC O157 isolates had indistinguishable PFGE patterns. The patient STEC O157 PFGE subtype was *stx*2-positive and *stx*1-negative. Median case age was 17 years (range 18 months–67 years). The four HUS cases were 1.5, 6, 8, and 14 years old, respectively, and the TTP case was 59 years old. Twenty-two (88%) of 25 cases were female (Fig. 3), including all HUS and TTP cases. Three cases were fair livestock exhibitors, and the remaining 22 cases were visitors. On average, cases spent 3.0 (range 1–8) days at the fair, compared to 1.9 days (range 1–5) for controls. Based on total fair attendance of 170,307, the crude attack rate was 0.015%.

Case-control study

Table 1 shows the categorical risk factor distribution and univariate unmatched case-control analysis results. Food and beverage consumption at the fair were not risk factors for STEC O157 infection. Food histories showed that some cases (8/25) ate at the fair; most only had a soda or bottled water (OR 0.49; 95% CI 0.11–1.86). Only five cases ate hamburgers (OR 0.26; 95% CI 0.06–0.85). All cases had some contact with the fair livestock exhibits and 12/25 cases had contact with swine areas (OR 2.59; 95% CI 0.85–8.07). Two cases (8%) showed a pig while ten cases (40%) accompanied or visited a person showing a pig.

Two continuous variables were examined as risk factors for STEC O157 infection: age (in years) and number of days of fair attendance. Age was not associated with STEC O157 infection (OR 0.996, 95% CI 0.97–1.03, $p = 0.836$) while number of days of fair attendance was significantly associated with being an STEC O157 case (OR 1.42, 95% CI 1.07–1.97; $p = 0.0155$). In the multivariable logistic regression model developed from univariate risk factor screening, only the categorical variable "visiting livestock areas of the fair" (OR 28.71; 95% CI 4.53–infinity, $p < 0.0001$) and the continuous variable "number of days of fair attendance" (OR 1.51; 95% CI 1.06–2.36, $p = 0.0185$) were associated with fair STEC O157 infection. Forced inclusion of the potential demographic confounders "age" (in years), "gender" (male or female), and "race" (white or non-white) in this multivariable model had little effect on the magnitude of the exact ORs for "visiting livestock areas of the fair" (exact OR 25.73; 95% CI 4.00–∞) and in "number of days of fair attendance" (exact OR 1.57; 95% CI 1.09–2.55).

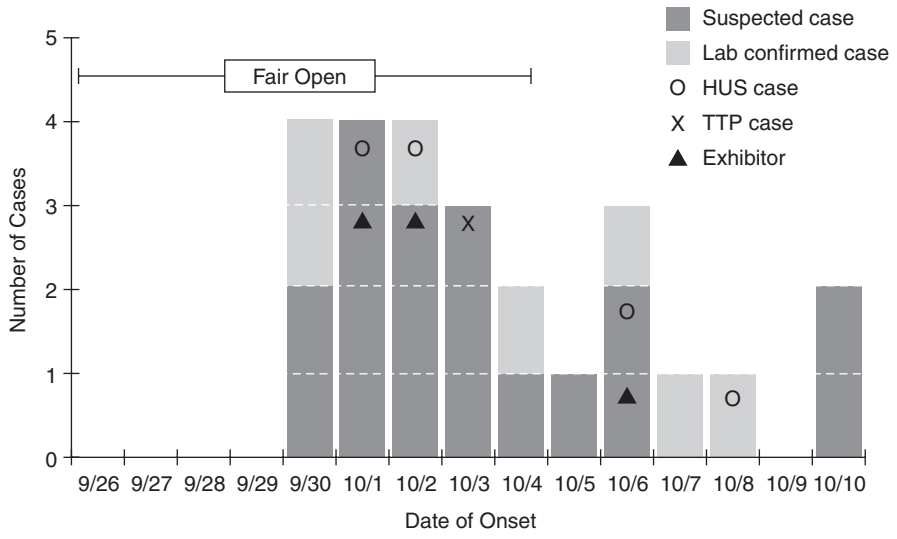

FIG. 2. Date of symptom onset for 25 cases of STEC O157 infection enrolled in case-control study, Fort Bend County Fair, 2003.

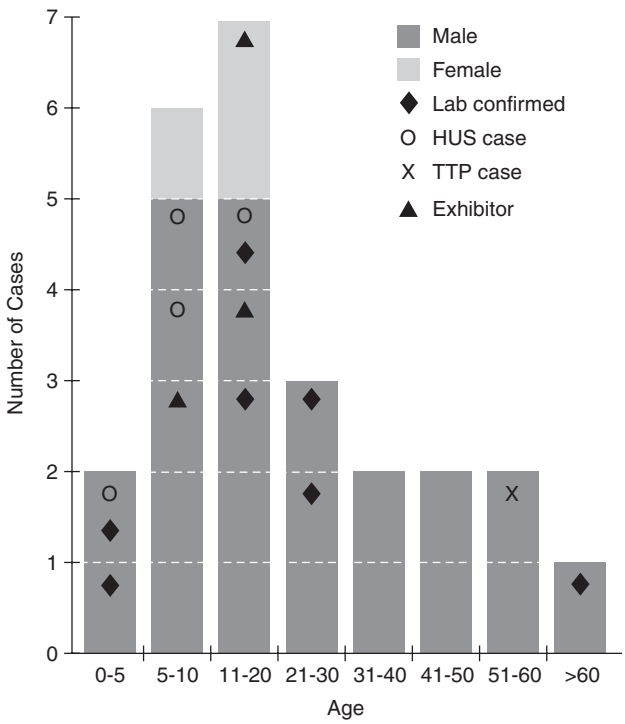

FIG. 3. Age and gender profiles for 25 cases of STEC O157 infection enrolled in case-control study, Fort Bend County Fair, 2003.

Environmental and microbiological investigation

STEC O157 was isolated from 10 of 62 environmental sites sampled 46 days after the Fair's end (Fig. 1). Five of 46 livestock exhibit sites were culture-positive as were 5 of 11 rodeo arena sites. STEC O157 was not isolated from the sewage overflow area. One positive site was a swab of the hand railings in the rodeo arena seating section. We characterized 28 STEC O157 isolates derived from the 10 positive environmental sites by serologic and molecular methods (Table 2). All isolates were serologically *E. coli* O157:H7 and PCR-positive for *stx*2, *eae* and *hly*. Five of 28 isolates were also *stx*1-positive. Seven different STEC O157 PFGE subtypes were identified. STEC O157 isolates derived from a given positive sample were indistinguishable except for one rodeo arena pooled dirt sample which produced two distinct PFGE subtypes. The STEC O157 isolate PFGE pattern from four of the five livestock exhibit sites matched the human outbreak pattern (Fig. 1). The rodeo arena STEC O157 isolates had five unique PFGE subtypes, none of which matched the human isolate PGFE subtype. T2

DISCUSSION

The STEC O157 outbreak at the FBC Fair in September–October 2003 affected 25 persons, including three livestock exhibitors. This is the

TABLE 1. CATEGORICAL RISK FACTOR DISTRIBUTION AND UNIVARIATE ANALYSIS OF UNMATCHED CASE-CONTROL STUDY OF STEC O157 INFECTION AT FORT BEND COUNTY FAIR, 2003

Risk factor	*Cases* (n = 25)	*Controls* (n = 50)	*Odds ratio*	*95% CI*	p *value*
Food exposures					
Hot dogs	0 (0%)	2 (4%)	0.82	0.00–10.71	0.883
Hamburger	5 (20%)	25 (50%)	0.26	0.06–0.85	0.022
Soda or bottled water	4 (16%)	14 (28%)	0.49	0.11–1.86	0.393
Funnel cake	2 (8%)	6 (12%)	0.64	0.06–3.97	0.925
Turkey leg	2 (8%)	4 (8%)	1.00	0.09–7.58	1.000
Animal exposures					
Contact with livestock or pet	25 (100%)	22 (44%)	42.25	6.79–∞[b]	<0.0001
Showing animal	3 (12%)	11 (22%)	0.49	0.08–2.13	0.471
With someone showing animal	11 (44%)	14 (28%)	2.00	0.65–6.15	0.261
Visited livestock area of fair	25 (100%)	27 (54%)	28.50	4.57–∞	<0.0001
Pig[a]	12 (48%)	13 (26%)	2.59	0.85–8.07	0.102
Demographic and other factors					
Travel	2 (8%)	2 (4%)	2.07	0.14–30.16	0.815
Water-recreational exposure	0 (0%)	1 (2%)	2.00	0.00–78.00	1.000
Attended large gatherings	3 (12%)	7 (14%)	0.84	0.13–4.14	1.000
Race white (vs. non-white)	23 (92%)	45 (90%)	1.27	0.19–1.43	1.000
Gender female (vs. male)	22 (88%)	40 (80%)	1.82	0.41–11.36	0.604

CI, confidence interval; ∞, positive infinity.
[a]Includes showing a pig, being a family member of someone showing a pig, or visiting someone who was showing a pig.

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TABLE 2. STEC O157 FAIRGROUND ISOLATION LOCATIONS AND PROPERTIES FOR ENVIRONMENTAL SAMPLES COLLECTED NOVEMBER 20, 2003 FROM THE FORT BEND COUNTY FAIRGROUNDS

			ELISA serotyping		*PCR confirmation*						
			α-O157	*α-H7*							*PFGE*
Type of sample	*Location*	*Isolate ID*	*MAb*[a]	*MAb*[b]	stx*1*	stx2	hly*A*	Rfb_{O157}	eae*A*	$fliC_{H7}$	*subtype*
Droppings	In scramble heifer area	FB4-1	+	+	Neg	+	+	+	+	+	B
	George Barn	FB4-2	+	+	Neg	+	+	+	+	+	B
		FB4-3	+	+	Neg	+	+	+	+	+	B
Pooled ground	Drug testing pen	FB9-1	+	+	Neg	+	+	+	+	+	A
cover—sawdust	George Barn	FB9-2	+	+	Neg	+	+	+	+	+	A
		FB9-3	+	+	Neg	+	+	+	+	+	A
Pooled ground	Drug testing pen	FB10-1	+	+	Neg	+	+	+	+	+	A
cover—sawdust	George Barn	FB10-2	+	+	Neg	+	+	+	+	+	A
Pooled dirt	Overflow pen area-	FB35-1	+	+	Neg	+	+	+	+	+	A
	goats/lambs/pigs	FB35-2	+	+	Neg	+	+	+	+	+	A
		FB35-3	+	+	+	+	+	+	+	+	A
Pooled dirt	Show arena	FB41-1	+	+	Neg	+	+	+	+	+	A
	George Barn	FB41-2	+	+	Neg	+	+	+	+	+	A
Pooled dirt	Rodeo arena, east end,	FB52-1	+	+	+	+	+	+	+	+	D
	bull and horse area,	FB52-2	+	+	+	+	+	+	+	+	D
	bull chute	FB52-3	+	+	+	+	+	+	+	+	D
Pooled dirt	Rodeo arena, northeast	FB53-1	+	+	Neg	+	+	+	+	+	E
		FB53-2	+	+	+	+	+	+	+	+	D
		FB53-3	+	+	Neg	+	+	+	+	+	E
Pooled dirt	Rodeo arena, west end	FB59-1	+	+	Neg	+	+	+	+	+	C
	holding area, calves,	FB59-2	+	+	Neg	+	+	+	+	+	C
	steers	FB59-3	+	+	Neg	+	+	+	+	+	C
Pooled dirt	Rodeo arena, west end	FB60-1	+	+	Neg	+	+	+	+	+	F
	holding area, calves	FB60-2	+	+	Neg	+/−[c]	+	+	+	+	F
	and steers	FB60-3	+	+	Neg	+	+	+	+	+	F
Pooled swab	Rodeo arena railings	FB61-1	+	+	Neg	+	+	+	+	+	G
	and seating area	FB61-2	+	+	Neg	+	+	+	+	+	G
		FB61-3	+	+	Neg	+	+	+	+	+	G

[a]α-O157 MAb 13b3.
[b]α-H7 MAb 2B7.
[c]+/− = weak positive reaction.

first report, to our knowledge, of an STEC O157 fair outbreak in which both animal exhibitors and visitors became ill. Additionally, the STEC O157 case age and gender profiles were atypical compared to previous fair-associated STEC O157 outbreaks in that this outbreak had a predominance of female cases and a broad age spectrum.

Two ill exhibitors showed pigs, and the third showed a lamb. STEC O157 was not isolated from the swine area environment. However, the adjacent holding pens did yield an environmental STEC O157 matching the outbreak strain (Fig. 1). These holding pens were a common use area for many fair livestock, and traffic patterns were such that swine exhibitors frequently walked through the holding pen area. The goat and lamb area environmental samples were negative. However, the drug testing pen and show arena both yielded STEC O157 isolates that matched the patient PFGE subtypes. Although STEC O157 has been cultured from pigs on farms, at fairs, and in feedlots (Callaway et al. 2004; Feder et al. 2003) and from lambs in pastures and at slaughter facilities (Kudva et al. 1996; McCluskey et al. 1999), pig and lamb exposures are not commonly associated with human STEC O157 infections. While both livestock exhibitors and visitors were infected by STEC O157 in this outbreak, it is typical that only visitors are sickened at agricultural fair STEC O157 outbreaks for reasons that are not yet understood. Farm family studies suggest that subclinical STEC O157 infection following frequent exposure may be protective (Rahn et al. 1998; Reymond et al. 1996; Wilson

et al. 1996). Since a large proportion of U.S. fair visitors are urban and suburban residents, immunologic naivety to zoonotic enteric bacteria such as STEC O157 may account for higher fair visitor (vs. livestock exhibitor) susceptibility to these infections upon exposure.

Cattle were implicated as the likely source of human STEC O157 infections in several recent fair outbreaks (LeJeune and Davis 2004; Bender and Shulman 2004). However, we uncovered no specific evidence supporting or absolving cattle as the STEC O157 source in the FBC Fair outbreak. Historically, hamburger consumption is a consistent risk factor for human STEC O157 infection (Kassenborg et al. 2004), yet our univariate case-control analysis found eating hamburger to be mildly protective (OR = 0.26). One possible explanation for this is that some fair visitors only attended the carnival section, which was immediately adjacent to the food vendors. Only 8/25 cases reported eating food or drinking water obtained at the fair.

All STEC O157 cases in this outbreak visited the Fair livestock areas or exhibited livestock, and all reported livestock contact. STEC O157 is potentially transmittable from animals-to-humans via direct animal contact or by indirect contact with animal-contaminated environments (Varma et al. 2003). Fair environments allow intimate livestock–people contact and permit multiple opportunities for exposure to microbe-contaminated dust and dirt. The FBC Fair included a rodeo, an "Ag-tivity" area, a petting zoo and a variety of 4-H, Future Farmers of America, and community livestock competitions. STEC O157-positive environmental samples were collected from the rodeo and livestock areas but not from the petting zoo. All four STEC O157-positive environmental samples with PFGE subtypes matching human isolates came from livestock barns. Cases may have been STEC O157-exposed by touching fecal material directly on animals or from indirect contact with the livestock environment. For example, one case was observed doing handstands in the livestock areas of the fairgrounds. In addition, STEC O157 is readily isolated from the hide and mouth of cattle (Keen and Elder 2002) so human STEC O157 infection may have been acquired by either petting livestock or by allowing animals to lick people's hands.

Both univariable and multivariable case-control analyses identified livestock exhibit exposure as a significant STEC O157 infection risk factor (exact OR = 28.5 and 28.7, respectively). Furthermore, the estimated 51% increased risk of being a case per additional day visiting the fair is biologically consistent with elevated infection likelihood with greater exposure to the STEC O157-contaminated fair environment, perhaps in a dose–response manner.

One swab of the rodeo arena observation stands was culture-positive. Direct contamination of the stands with livestock feces was unlikely. This positive swab may have resulted from fecal-contaminated dust which settled on or was tracked into the stands. Interestingly, none of five STEC O157-positive rodeo arena samples yielded isolates with PFGE patterns that matched the human isolates. The rodeo was well-attended (19,500 visitors over five rodeos), and many people were potentially indirectly exposed to these STEC O157 strains, yet did not become ill enough to meet the case definition. Many persons also attended the fair livestock exhibitions, but did not become ill.

Interestingly, 22/25 cases, including all HUS and TTP cases, were female. All adult cases (older than 20 years) were female. Gender-specific fair attendance data was unavailable, but if we assume that males and females attended the fair in equal numbers, then the exact probability of 22 of 25 cases being female is 6.8×10^{-5}. More women than men may have attended the fair in their role as caregivers or women might have been were more likely than men to become contaminated through contact with children's shoes or clothes. While no attempt was made to match on gender in the case-control study, the controls also contained many more females than males, prohibiting a gender-based analysis. Controls were recruited mainly by word-of-mouth, and perhaps females were more likely than males to volunteer to talk to public health officials.

All STEC O157-positive environmental samples came from roof-covered areas protected from both direct sunlight and rainfall. It is unclear how natural exposure to sunlight and rainfall affect STEC O157 survival or their iso-

lation efficiency. The fairgrounds received heavy rainfall the week before sample collection, and ambient temperatures exceeded 40°C. Under these conditions, environmental STEC O157 might have been killed by the sunlight or heat, washed away by rain or diluted to below the detection limit of the culture methods employed. Since the area where sewage backup occurred was of particular public concern, samples were collected close to the restroom building where the overflow originated that had some protection from sun and rain, but these were also STEC O157 culture-negative. We found no epidemiologic or microbiologic evidence to suggest that sewage overflow played any role in this outbreak.

The sampling scheme and laboratory methods that we employed may explain why STEC O157 was isolated from the fair environment 46 days after the fair's end. In STEC O157-infected people, the pathogen is excreted in relatively high numbers in the stool (March and Ratnam 1986) so that direct plating of fecal material is suitable for detection. In contrast, infected livestock STEC O157 is commonly a minor component of the animal's fecal flora (Pearce et al. 2004), and it can be easily outgrown by competing flora during bacterial culture unless both positive- and negative-selective enrichment and plating are used. Agricultural soil samples also contain high numbers of competing microorganisms, requiring use of selective culture conditions to permit STEC O157 detection. In our experience, methods optimized for STEC O157 recovery from livestock feces differ from, and are inappropriate for, STEC O157 recovery from soil, water, and other ambient environmental samples. We find that TCA plates are preferable to the widely used sorbitol MacConkey agar (SMAC) for livestock feces and agricultural environmental samples. STEC O157 colonies on TCA are usually bright pink, while most completing microflora are white, blue or grey, making it easier to spot an isolated STEC O157 colony on a crowded TCA plate compared to a crowded SMAC plate. Furthermore, the pink TCA STEC O157 colony phenotype is stable over time (unlike the sorbitol reaction on SMAC plates) so that TCA plates can be accurately read days or weeks post-plating.

CONCLUSIONS

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Seven different PFGE subtypes were found at the fair and rodeo grounds, yet only a single PFGE subtype was found in culture-confirmed human cases. All environmental STEC O157 isolates were *stx*2-positive, indicating that they had human pathogenic potential. Our findings suggest that while STEC O157 may be common in the fair environment, perhaps only a subset of STEC O157 subtypes are transmissible to or virulent for humans (LeJeune et al. 2004).

In conclusion, our investigation implicated livestock contact at the FBC Fair as an important STEC O157 infection risk factor. Fair exhibitors and visitors should follow precautions and available guidelines (Eidson et al. 2005) to prevent or minimize the risk of acquiring zoonotic STEC O157 infections.

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DURSO

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