APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2004, p. 7578–7580 Vol. 70, No. 12
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.12.7578–7580.2004

Association of *Escherichia coli* O157:H7 with Houseflies on a Cattle Farm†

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Received 14 May 2004/Accepted 10 August 2004

The ecology of *Escherichia coli* O157:H7 is not well understood. The aims of this study were to determine the prevalence of and characterize *E. coli* O157:H7 associated with houseflies (HF). *Musca domestica* L. HF (n = 3,440) were collected from two sites on a cattle farm over a 4-month period and processed individually for *E. coli* O157:H7 isolation and quantification. The prevalence of *E. coli* O157:H7 was 2.9 and 1.4% in HF collected from feed bunks and a cattle feed storage shed, respectively. *E. coli* O157:H7 counts ranged from 3.0×10^1 to 1.5×10^5 CFU among the positive HF. PCR analysis of the *E. coli* O157:H7 isolates revealed that 90.4, 99.2, 99.2, and 100% of them (n = 125) possessed the *stx1*, *stx2*, *eaeA*, and *fliC* genes, respectively. Large populations of HF on cattle farms may play a role in the dissemination of *E. coli* O157:H7 among animals and to the surrounding environment.

Enterohemorrhagic *Escherichia coli* O157:H7 is a well-known causative agent of hemorrhagic colitis and hemolytic uremic syndrome in humans. Outbreaks of the food-borne illness caused by *E. coli* O157:H7 have been reported throughout the northern hemisphere, most frequently in the United States, Canada, Japan, and the United Kingdom. In the United States alone, *E. coli* O157:H7 causes more than 73,000 cases of human infection every year (11). *E. coli* O157:H7 strains commonly carry verotoxins (encoded by the *stx1* and *stx2* genes) and factors for the attachment to the host mucosa, including intimin (encoded by the *eaeA* gene) (11). The low infectious dose and high virulence of *E. coli* O157:H7 make infections severe and life-threatening, particularly for young children, the elderly, and those with weakened immune systems (11). The main reservoir for *E. coli* O157:H7 is the intestinal tracts of healthy cattle. Individual cattle are transiently colonized and shed *E. coli* O157:H7 in their feces (1). The sources of *E. coli* O157:H7, which colonizes cattle, are not well understood, and little is known about the ecology of *E. coli* O157:H7 in the environment (1). Additionally, the high variability in the prevalence of *E. coli* O157:H7 among cattle suggests the possibility of a reservoir of *E. coli* O157:H7 external to cattle. However, other than the detection of *E. coli* O157:H7 in nonbovine animals, including sheep, horses, dogs, and wild birds (1), the ecology of this pathogen has not been extensively studied.

One of the potential modes of dissemination of this pathogen in the environment is by insects that are associated with animal feces and manure, primarily houseflies (HF; *Musca domestica* L.). HF larvae develop in animal feces, including cattle manure. Consequently, HF commonly build up very large populations on cattle farms and in other animal facilities. Previously, a laboratory-based study demonstrated that *E. coli* O157:H7 ingested by HF remained viable in the fly excreta and that the HF were able to carry and disseminate *E. coli* for several days (9). In Japan, HF were implicated in the transmission of *E. coli* O157:H7 from reservoir animals to other animals and humans (10).

The objectives of this study were (i) to assess the temporal prevalence and concentration of *E. coli* O157:H7 associated with HF collected on a beef cattle farm, (ii) to characterize *E. coli* O157:H7 isolates by screening for the virulence factors genes and antibiotic resistance, and (iii) to determine the concentration of fecal coliforms carried by HF in the cattle farm environment as an indicator of fecal contamination.

HF were collected by sweep net from two sites (feed bunks and a storage shed containing steam-flaked corn) on a cattle feedlot in northeastern Kansas four times per week from mid-June until the end of October 2003. From each collection, 50 HF were randomly selected and processed for *E. coli* O157 isolation and quantification. Individual HF (n = 3,440) were homogenized in 1 ml of phosphate-buffered saline (pH 7.2; ICN Biomedicals) and serially diluted. Dilutions were plated by a direct drop plate technique onto sorbitol MacConkey agar (Becton Dickinson) with cefixime (25 μg/liter) and tellurite (1.25 mg/liter). Plates were incubated overnight at 37°C, and sorbitol-negative colonies with a morphology characteristic of *E. coli* O157 were tested for the O157 antigen by the latex agglutination assay (Oxoid Limited, Basingstoke, England). Colonies positive for the O157 serogroup were counted, and up to three positive colonies per sample were subcultured on Trypticase soy agar (Becton Dickinson) and identified by the API Rapid 20E test (Biomerieux Vitek). Isolated *E. coli* O157 strains were tested for the virulence genes (*stx1*, *stx2*, and *eaeA*) and the flagellar H7 gene (*fliC*) by PCR as described previously (4, 6). The sensitivity of *E. coli* O157:H7 isolates (n = 108) to 10 antibiotics (ampicillin, apramycin, ceftiofur, chlortetracycline, enrofloxacin, gentamicin, neomycin, oxytetracycline, spectinomycin, and trimethoprim-sulfamethoxazole) was measured by determination of the MIC in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (12) with a diagnostic system (TREK Diagnostic Systems, Cleveland, Ohio) with the Sensititre AutoInoculator,

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† Contribution no. 05-21-J from the Kansas Agricultural Experiment Station.

TABLE 1. Prevalence and concentration of *E. coli* O157:H7 associated with HF on a beef cattle farm

Mo (2003)	Feed bunk		Corn storage	
	No. positive/ no. tested (%)	*E. coli* O157:H7 count range (CFU/fly)	No. positive/ no. tested (%)	*E. coli* O157:H7 count range (CFU/fly)
June	3/65 (4.6)	8.0×10^1–3.0×10^2	2/75 (2.7)	3.0×10^2–1.2×10^4
July	32/525 (6.1)	3.0×10^1–9.0×10^3	9/375 (2.4)	3.0×10^1–1.5×10^5
August	9/425 (2.1)	3.0×10^1–4.8×10^4	3/375 (0.8)	3.0×10^1–1.5×10^2
September	4/450 (0.9)	3.0×10^1–1.2×10^3	4/450 (0.9)	3.0×10^1–1.2×10^2
October	4/350 (1.1)	3.0×10^1–1.2×10^2	5/350 (1.4)	3.0×10^1–3.0×10^2
Total	52/1,815 (2.9)	3.0×10^1–4.8×10^4	23/1,625 (1.4)	3.0×10^1–1.5×10^5

sensitivity antibiotic plates, and the SensiTouch plate reader. Fecal coliform counts in randomly selected individual fly homogenates ($n = 350$) were determined by a drop plate technique with mFC (membrane fecal coliform) agar (Oxoid Limited) incubated at 44.5°C for 18 to 24 h. Fecal coliform colonies were counted from dilutions and calculated per fly.

The prevalence of *E. coli* O157:H7 associated with HF was 2.9 and 1.4% in feed bunks and a steam-flaked corn storage shed, respectively, with peaks in June and July (Table 1). *E. coli* O157:H7-positive HF were detected every week throughout the screening period. *E. coli* O157:H7 counts ranged from 3.0×10^1 to 1.5×10^5 CFU per fly (Table 1). The high concentration of *E. coli* O157:H7 associated with individual HF is an important factor in potential transmission. Cattle manure and other components of the cattle environment usually do not contain a high number of *E. coli* O157:H7 bacteria (10^5 CFU) per gram of sample (5, 13). Interestingly, about 30% of the positive HF ($n = 125$) were collected from a storage shed containing flaked corn. This indicates that HF carry *E. coli* O157:H7 in different parts of a farm and potentially contaminate various substrates on which they feed or rest. Johnson et al. (8) outlined various habitats of *E. coli* O157:H7 in the farm environment, including manure heaps, ponds, dams and wells, barns, calf hutches, straw and other beddings, feed and feed troughs, water, soil, and farm equipment. They also noted that, once present in the environment, this pathogen can be transferred to other sites by rainwater, wind, and spreading of manure, as well as by animals and humans. Unfortunately, most studies report only qualitative data on *E. coli* O157:H7. Our results indicate that HF can be an important vector for *E. coli* O157:H7 in the cattle farm environment.

PCR analyses of *E. coli* O157:H7 isolates from HF ($n = 125$) revealed that 90.4, 99.2, 99.2, and 100% of the latex agglutination-positive isolates (O157) possessed the *stx1*, *stx2*, *eaeA*, and *fliC* genes, respectively (Table 2). Most of the isolates (90.4%) possessed both toxin genes and the intimin gene, indicating that these strains are highly virulent. Resistance of *E. coli* O157:H7 to ampicillin (4.6%), ceftiofur (10.2%), chlortetracycline (97.2%), gentamicin (17.6%), neomycin (2.7%), oxytetracycline (100%), spectinomycin (12%), and trimethoprim-sulfamethoxazole (17.6%) was detected. No resistance to apramycin or enrofloxacin was detected. Twenty-six percent of the isolates showed multidrug (three or more antibiotics) resistance.

Little is known about the association of *E. coli* O157:H7 with insects, including HF. Previously, *E. coli* O157:H7 was reported from HF in two studies from Japan (7, 10). Although both studies implicated HF in the transmission of this pathogen, the conclusions reached were based on a very limited data set. Sasaki et al. (14) addressed the role of regurgitation and excretion by HF in the dissemination of *E. coli* O157:H7 and suggested that these processes enhance the potential transmission of the pathogen. In addition, Kobayashi et al. (9) reported that HF may not be simple mechanical vectors of *E. coli* O157:H7 and that this pathogen likely multiplies in the gastrointestinal tract of HF. The high numbers of *E. coli* O157:H7 bacteria detected in our HF samples strongly support this hypothesis.

Fecal coliforms were detected in 334 (95.4%) out of 350 HF screened. Counts of fecal coliforms ranged from 3.0×10^1 to 3×10^6 CFU/fly with a mean count of 2.1×10^5 CFU/fly and a median count of 2.4×10^4 CFU/fly (Table 2). The large number of fecal coliforms in HF indicates a potential to harbor other pathogens.

The very large populations, suitable habitats (feces, manure), unrestricted movement, and mode of feeding (regurgitation) of HF represent a likely mechanism for the spread of *E. coli* O157:H7 on cattle farms and into the surrounding environment. The infectious dose of *E. coli* O157:H7 is very low,

TABLE 2. Characteristics of *E. coli* O157:H7 isolates and the number of fecal coliforms associated with HF collected from feed bunks and a corn storage shed

Site	No. of isolates positive for virulence genes detected in *E. coli* O157:H7 isolates/no. tested (%)				Fecal coliform count per fly ($n = 334$)		
	stx1	*stx2*	*eaeA*	*fliC*	Mean	Median	Range
Feed bunks	84/95 (88.4)	94/95 (98.9)	94/95 (98.9)	95/95 (100)	2.4×10^5	3.6×10^4	3.0×10^1–3×10^6
Corn storage	29/30 (96.7)	30/30 (100)	30/30 (100)	30/30 (100)	1.8×10^5	1.6×10^4	3.0×10^1–3×10^6
Total	113/125 (90.4)	124/125 (99.2)	124/125 (99.2)	125/125 (100)	2.1×10^5	2.4×10^4	3.0×10^1–3×10^6

probably fewer than 100 cells (17). Humans can become infected through the consumption of contaminated food, particularly inadequately cooked ground beef (often in the form of beef burgers) and milk (nonpasteurized or contaminated postpasteurization). Other sources of infection include contaminated nonpasteurized apple cider, water (drinking and swimming), vegetables, mayonnaise, lamb, venison, deer jerky, cured salami, and direct contact (animal to person or person to person) (2, 15, 16). Because of the attraction of HF to places where food is prepared, stored, and consumed, these insects present a potential route for the contamination of food and drinks. The dispersal range of HF is usually 0.5 to 2 miles, although distances as great as 10 to 20 miles have been reported (3). Human infections associated with *E. coli* O157:H7 are more common during summer months, with the majority of cases occurring from May to September. This correlates with an increased shedding of *E. coli* O157:H7 by cattle, as well as with very large populations of HF on cattle farms and in other animal operations.

In summary, our study demonstrated that HF carry virulent *E. coli* O157:H7 in the farm environment primarily during the summer and may play an important role in the ecology and transmission of this pathogen among individual cattle and potentially to the surrounding farm and urban environment. Information on the association of *E. coli* O157:H7 with HF will assist in developing more comprehensive and quantitative risk assessments, as well as formulating *E. coli* O157:H7 intervention strategies that should include an effective HF management program.

We thank Jan Metlevski, Jon-Joseph Armstrong, and Jennifer Hurley for collecting HF and S. Kambhampati for reviewing the manuscript. This study was funded in part by USDA grant 34359-13008.

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