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ESCHERICHIA COLI O157:H7 IN FREE-RANGING DEER IN NEBRASKA

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ABSTRACT: In order to determine the prevalence and distribution of the human pathogen, *Escherichia coli* O157:H7, in free-ranging deer, hunters were asked to collect and submit fecal samples from deer harvested during a regular firearm season (14–22 November 1998). Prior to the season, 47% of the hunters with permits in the southeastern Nebraska (USA) study area indicated a willingness to participate in the study. Approximately 25% of successful hunters in the area submitted deer fecal samples. *Escherichia coli* O157:H7 was cultured from four (0.25%) of 1,608 total samples submitted. All of the fecal samples that were properly identified (1,426) and all that were positive for *E. coli* O157:H7 were from white-tailed deer (*Odocoileus virginianus*). We were unable to detect a statistically significant geographic distribution pattern of *E. coli* O157:H7. The presence of *E. coli* O157:H7 in the feces of free-ranging deer has implications not only for hunters, consumers of venison, and others in contact with deer or deer feces, but also for the development of strategies aimed at reducing and/or controlling this pathogen in water sources and domestic livestock.

Key words: *Escherichia coli* O157:H7, hunter sample submission, *Odocoileus virginianus*, response rate, survey, zoonosis.

INTRODUCTION

Escherichia coli O157:H7 and other enterohemorrhagic *E. coli* have emerged as important zoonoses leading to major public health concerns in many areas of the world (Coia, 1998). These bacteria can cause disease in humans ranging from bloody diarrhea to hemolytic uremic syndrome and can even result in death (Griffin and Tauxe, 1991). Although the exact source of infection often is undetermined, fecal contamination of water and food is an important source of *E. coli* O157:H7 in outbreaks and sporadic infections (Armstrong et al., 1996). Cattle feces are the most widely studied source of enterohemorrhagic *E. coli*, but other species also are known to shed these bacteria in their feces (Hancock et al., 1998). Most emerging infectious diseases in people initially result from exposure to zoonotic pathogens; wildlife may play a key role in emergence by providing a "zoonotic pool" of the infectious agents (Daszak et al., 2000). *Escherichia coli* O157:H7 has been detected in the feces of white-tailed deer (*Odocoileus virginianus*), but the extent of direct or indirect zoonotic risk of this source of *E. coli* O157:H7 has yet to be determined (Sargeant et al., 1999). In 1995, an outbreak of *E. coli* O157:H7 infections in people was traced to jerky made from deer meat (Keene et al., 1997). Deer feces also were identified as a possible source of contamination in an outbreak of *E. coli* O157:H7 from unpasteurized commercial apple juice (Cody et al., 1999). Wild deer may be involved in the natural ecology of *E. coli* O157:H7 on rangelands. Both cattle and deer occasionally shed *E. coli* O157:H7 in their feces, and cattle and deer feces from the same pasture can have identical genetic strains of this pathogen (Rice et al., 1995). A previous study of free-ranging deer has estimated the fecal prevalence of *E. coli* O157:H7 (2.4%) to be similar to the prevalence in water and cattle fecal samples from the same region (Sargeant et al., 1999).

The presence of *E. coli* O157:H7 in the feces of deer has implications not only for the zoonotic risk to hunters, people consuming venison, and others in contact with deer and deer feces, but also in the development of programs for controlling this pathogen at the farm level. For *E. coli* O157:H7 reduction and/or control programs, the focus should be on the ecosystem rather than on individual farm operations, and programs designed for pathogen control must account for fecal shedding by wildlife (Sargeant et al., 1999). The National Animal Health Monitoring System's (NAHMS) Beef '97 survey found that nearly 70% of cow-calf ranchers reported seeing deer at least four times per month within a 1-mile radius of the cattle, and 85% of cattle ranchers reported seeing deer near the cattle at least once a month (NAHMS, 1998). Because the potential exists for interaction between cattle and free-ranging deer in pasture situations and cattle may not be the only source of *E. coli* O157:H7, interspecies transmission may occur (Rice et al., 1995). Currently not enough is known about the ecology of *E. coli* O157:H7 in rangeland situations to identify critical control points for farm-level programs or to quantify the direct or indirect risk associated with deer shedding these organisms in their feces.

The objective of this observational study was to establish the prevalence and distribution of *E. coli* O157:H7 in the feces of free-ranging white-tailed deer in a large agricultural area. As a result of the sample collection methods, we also were able to assess the degree of deer hunter participation for an investigation into a zoonotic pathogen that has been the subject of extensive coverage by national and local media.

MATERIALS AND METHODS

During the regular firearm season of 1998 (November 14–22), hunters collected fecal samples from deer harvested in the Blue and Wahoo Management Units of southeastern Nebraska, USA. Nebraska deer hunters are required by the Nebraska Game and Parks Commission (NGPC, Lincoln, Nebraska) to register all harvested deer at one of the "check stations" in/near the area. The approximately 25,600 km^2 study area (centered at 40°39′N, 96°39′W) included 28 local check stations and all or part of 25 counties (Fig. 1). A mailing list from the NGPC was used to contact hunters issued a permit in the study area for the regular firearm season. Approximately 12 wks before the start of the season, each hunter was mailed a letter explaining the study, a fact sheet explaining *E. coli* O157:H7, and a postage-paid response card. Information on the project also was distributed in the area via local extension agents, newspapers, and radio stations. Nebraskaland Magazine, a publication of the NGPC, included a brief synopsis of the study in its October 1998 issue. Hunters who had not responded to the initial mailing in 3 wks were sent a second mailing. As a response facilitator, hunters submitting samples were eligible for a drawing of gift certificates from a national hunting outfitter.

FIGURE 1. *Escherichia coli* O157:H7 investigation in free-ranging deer in Nebraska. The hatched area in southeastern Nebraska denotes the area from which hunters submitted fecal samples from harvested deer.

Hunters who agreed to participate in the study were sent a collection packet approximately 2 wks before the start of the season. These packets contained a letter thanking them for participating and providing step-by-step instructions for sample collection. After field dressing the deer, the hunters were asked to use a pair of disposable nonlatex gloves, locate the rectum of the animal, collect fecal pellets directly from the rectum by using a plastic spoon, place the feces in a plastic bag, and seal the bag. All of the necessary supplies were included in the packet. The hunters submitted the deer fecal samples as their deer were registered at a check station. The sample collection bags were labeled with the unique regis-

tration number that is assigned routinely to each deer. The fecal samples were stored on ice immediately after submission and during transport to the bacteriological laboratory of the Food Animal Health & Management Center (Kansas State University, Manhattan, Kansas, USA). Samples were transported to the lab no more than 4 days after sample submission.

We used microbiological culture to identify the presence of *E. coli* O157:H7. A 10-g sample of feces was homogenized in a stomacher (Stomacher 400, Seward Inc., London, UK) for 30 secs with 90 ml of trypticase soy broth supplemented with 400 μg/ml novobiocin, 0.1 μg/ml cefexime, 10 μg/ml cefsulodin, and 8 μg/ml vancomycin (mTSB). An identical (9:1) ratio of broth to feces was used for samples of less than 10 g. The broth then was incubated at 37C for 12 hr. After incubation, 1 ml of the broth was removed and added to 20 μg anti-O157 coated magnetic beads (Dynabeads® Anti-*E. coli* O157, Dynal Inc., New Hyde Park, New York, USA). The bead/broth mixture was mixed at room temperature for 30 min, after which it was placed in a magnetic block for 5 min. The broth then was removed and the beads were washed in 1 ml phosphate buffered saline (PBS, pH 7.5) and then vortexed. The beads were separated again on the magnetic block and washed two more times with PBS. The final resuspension was in 100 μl PBS. The bead suspension was added to 9 ml of mTSB and incubated for an additional 18 hr at 37C. After incubation, a swab sample was removed from the broth, plated on sorbitol MacConkey agar supplemented with 5 μg/ml potassium tellurite and 0.1 μg/ml cefixime (CT-SMAC), and incubated overnight. After incubation, all non-sorbitol-fermenting (grey/white) colonies were plated on both blood agar and Levine Eosin Methylene blue agar and incubated overnight at 37C. Individual isolates were tested for the presence of the O157 and the H7 antigens using latex agglutination following the manufacturer's instructions (Rim *E. coli* O157:H7; Remel, Lenexa, Kansas, USA). Isolates were confirmed biochemically as *E. coli* with an API 20E biochemical test strip (bioMerieux Vitek Inc., Hazelwood, Missouri, USA).

The prevalence of *E. coli* O157:H7 was determined and 95% confidence intervals (CI) were calculated (Snedecor and Cochran, 1989). We also calculated the proportion of positive samples for each check station. An exact test for the homogeneity of proportions was used to determine if the proportion *E. coli* O157:H7 positive samples submitted to each check station differed (Agresti, 1996). With the check station point as a proxy variable for the deer location, Moran's I coefficient (spatial autocorrelation test for point locations) was used to test the null hypothesis of random spatial distribution of the proportion of positive samples (Moran, 1950).

RESULTS

The mailing list of permit holders in the study area for the 1998 regular firearm season included 9,996 hunters. Over half of the hunters returned response cards (5,024, 50.3%) and, with nearly all of these indicating a willingness to participate, the total positive response rate was 47.3% (4,731/9,996). We received 3,055 of the positive response cards from the first mailing (64.6%), and the remaining 1,676 from the second mailing (35.4%). There were 4,669 collection kits successfully delivered to hunters. Hunters submitted 1,608 deer fecal samples from the study area. Therefore, 34.4% (1,608/4,669) of hunters sent kits submitted a sample. The NGPC issued approximately 4,000 permits for the Wahoo Unit and 6,000 for the Blue Unit, and the overall hunter success rates were 65% and 63%, respectively (K. Menzel, pers. comm.). Therefore, approximately 25% of the estimated 6,400 successful hunters submitted samples (1,608/6,400). If the 4,669 hunters who were sent kits had a similar success rate as those in the rest of the study area, then an estimated 3,000 of these hunters were successful. Therefore, the submission rate for successful hunters who were sent kits was approximately 54% (1,608/3,000). Hunters did occasionally indicate that they attempted to collect a sample, but no fecal matter was available in the animal.

E. coli O157:H7 was cultured from four (0.25%) of 1608 samples (95% CI: <0.01–0.49%). We were unable to determine the source of origin (check station or permit number) for 172 samples due to improper labeling. Two of the check stations on the periphery of the collection area had no sample submissions during the study period. Therefore, 1,436 samples were identified to 26 check stations. Of the four samples positive for *E. coli* O157:H7, two

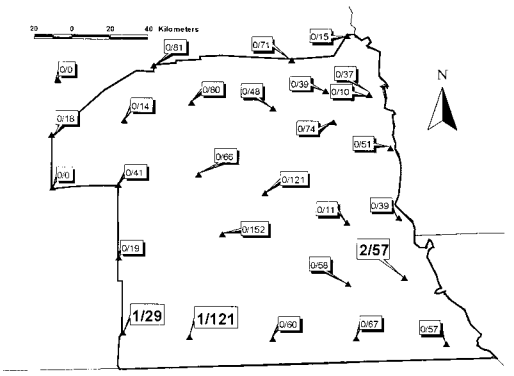

FIGURE 2. Distribution of samples of free-ranging deer feces examined for *Escherichia coli* O157:H7 in southeastern Nebraska. Legends include check station location (▲) and the corresponding number of positive samples over the total number of samples submitted. The heavy line denotes the boundaries of the study area.

were submitted at one check station (2 of 57 submitted; 3.51% positive) and the others at two different stations (1/121, 0.83%; 1/29, 3.45%) (Fig. 2). All of the samples that could be identified (1,436) including those positive for *E. coli* O157:H7 were from white-tailed deer. Few mule deer (*Odocoileus hemionus*) were harvested in the Wahoo and Blue Units, 7 and 13 respectively.

The exact test for detecting differences in proportion positive between check stations was not significant ($P = 0.166$). The Moran's I coefficient (0.10) for testing the null hypothesis of random spatial distribution of check station proportions also was not significant ($P = 0.42$).

DISCUSSION

The 54% estimated submission rate for successful hunters sent sampling kits was influenced by the fact that these hunters already had indicated a willingness to cooperate in the study. Nearly half of the hunters in the area indicated a willingness to participate, and an estimated 25% of the successful hunters cooperated in sample collection. Hunter responses to previous big game research in Nebraska have ranged from 49.6% to 66.8% for mail surveys, 19% to 41% for submission of feathers from harvested birds, and 6.1% to 30.7% for submission of incisors from harvested deer at check stations, with the highest cooperation generally occurring in the first year of a program (K. Menzel, pers. comm.).

We feel the 1,608 fecal samples collected from harvested deer allow us to provide a good estimate of the prevalence of *E. coli* O157:H7 in the study area. Although harvested deer may not be completely representative of the deer population, it is unlikely that the fecal flora of deer harvested by hunters willing to collect a sample would differ from that of other harvested deer in the area. Although we cannot be assured that the deer were free from disease, there is no evidence that *E. coli* O157:H7 causes disease in deer (Sargeant et al., 1999) or in other ruminant species (Whipp et al., 1994). The 0.25% prevalence estimate (95% CI: <0.01–0.49%) of *E. coli* O157:H7 reported here was low, but it is comparable to the 2.36% (95% CI: 0.32–4.40) previously estimated for deer (Sargeant et al., 1999) and to estimates for cattle under similar pasture conditions (Hancock et al., 1994). By collecting fecal samples from harvested animals, repeated sampling of positive (or negative) animals could not have occurred in this investigation; an admitted weakness of the previous study (Sargeant et al., 1999). In addition, the results presented here are from a much larger number of samples (1,608) than the previous study (212), which should result in a more precise estimate of the prevalence.

In cattle feces, the shedding of *E. coli* O157:H7 appears to be transient; therefore, estimates at a single point in time may underestimate prevalence (Sargeant et al., 2000). If fecal shedding is also transient in deer, a cross-sectional survey study such as the one reported here may not accurately assess the extent of *E. coli* O157:H7 in deer. Longitudinal monitoring of deer populations for *E. coli* O157:H7 by sampling individuals, simultaneously monitoring cattle populations, water, and other

possible *E. coli* O157:H7 sources in the area, and comparing the genetic strains of positive isolates from all sources could provide greater insight into the prevalence and natural ecology of the organism.

Although all four positive samples were from the southern part of the study area, we found no significant differences in the proportion of positive samples submitted at each check station, and the geographic distribution of these proportions was not statistically different from random (i.e., not significantly clustered). The lack of statistical significance may be real or may be due to the low power to detect a difference because of the small number of positive samples. Detection of statistically meaningful trends or geographic clustering in studies of the distribution of *E. coli* O157:H7 may be limited by the low prevalence of the organism.

The voluntary submission of samples at check stations could have resulted in a delay in sample processing. Some hunters may not have registered their animals immediately after harvest. Therefore, hunters could have harvested their deer and collected the fecal samples early in the season, but not submitted them until later in the season (up to 9 days). Survival of *E. coli* O157:H7 in deer fecal pellets has not been determined. However, Kudva et al. (1998) showed that *E. coli* O157:H7 can survive in ovine fecal pellets for months under environmental conditions.

Even if the presence of *E. coli* O157:H7 in the feces of free-ranging deer is infrequent, any water or food sources contaminated by deer feces should be considered potentially infectious. Hunters, those consuming venison, or otherwise in contact with deer or deer feces should be made aware of the potential risk and educated as to safe handling and proper food preparation. In addition, strategies aimed at controlling this organism in water sources and domestic livestock should account for fecal shedding by deer. Therefore, farm level programs should be targeted towards ecological systems rather than individual operations. The complex interactions between wildlife, food-producing livestock species, and humans are critical issues in ensuring biosecurity and food safety (Gillespie, 2000). The emergence of infectious diseases of wildlife and domesticated species can be driven by the "spill over" and "spill back" phenomena by which agents are transmitted between domestic and wild animal populations (Daszak et al., 2000). Therefore, efforts to control, decrease, and/or eradicate emerging zoonotic and foodborne disease organisms such as *E. coli* O157:H7 should focus on the ecology of the organism and recognize the complex relationships between wildlife, domestic animal, and human populations.

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