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Prevalence of *Escherichia coli* O157:H7 in White-tailed Deer from Louisiana

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ABSTRACT: *Escherichia coli* O157:H7 (EC O157) is an important zoonosis. White-tailed deer (*Odocoileus virginianus*) have been implicated in transmission of this bacterium to humans and have been suggested as reservoirs that might affect carriage in cattle populations. Our study objectives were to estimate prevalence of EC O157 in feces of hunter-harvested deer and to describe fecal shedding patterns in a captive herd sampled over 1 yr. Prevalence of EC O157 in hunter-harvested deer was 0.3% (n=338). In August 2001, EC O157 was detected in one of 55 deer (1.8%) from the captive herd. Prevalence over the 1-yr period was 0.4% (n=226). *Escherichia coli* O157:H7 was rarely isolated from hunter-harvested deer during the winter. We could not describe a seasonal shedding pattern based on one positive sample in the captive herd. These data do not support a prominent role of deer as a reservoir for EC O157 for cattle or humans.

Key words: *Escherichia coli* O157:H7, fecal shedding, foodborne disease, *Odocoileus virginianus,* prevalence, seasonal, white-tailed deer.

Escherichia coli O157:H7 (EC O157) is an important cause of human diarrheal disease. Severe manifestations include hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Griffin, 1998). An important reservoir of EC O157 is asymptomatically colonized cattle. White-tailed deer (*Odocoileus virginianus*) have been implicated in foodborne outbreaks of EC O157 and are also asymptomatically colonized (Fischer et al., 2001). Whether deer influence the epidemiology of EC O157 in cattle populations is not known.

Deer have been implicated in foodborne transmission of EC O157 in a sporadic case (Rabatsky-Ehr et al., 2002) and an outbreak (Keene et al., 1997) associated with consumption of contaminated venison. A larger outbreak involved consumption of unpasteurized apple juice, and deer were implicated as a potential source of EC O157 (Cody et al., 1999).

Escherichia coli O157:H7 occurs in cattle (Hancock et al., 1998) and has been isolated from deer and numerous other species (Chapman and Ackroyd, 1997). Cattle and deer in the same geographic area were shown to have distinct genetic subtypes of EC O157 (Fischer et al., 2001), although in one study, indistinguishable genetic subtypes were found in deer and cattle from the same farm, suggesting deer might play a role in maintaining the organism in bovine populations (Rice et al., 1995). Emerging infectious diseases have been described as zoonotic opportunists (Woolhouse, 2002) and are likely to be bidirectionally transmissible between wildlife and livestock (Bengis et al., 2002). The Center for Emerging Issues (United States Department of Agriculture) lists the significance of EC O157 in deer as "human infection" and "transmission to cattle" (USDA, 2001). Transmission of some infectious diseases between wildlife and livestock may be associated with sharing habitat, although no evidence suggests that deer play a prominent role in the epidemiology of EC O157 in cattle (Sargeant et al., 1999; Bengis et al., 2002).

White-tailed deer are colonized by EC O157 at low prevalences (Fischer et al., 2001). Rice et al. (1995) found 1.8% of deer (n=108) shed the organism. During winter surveys, prevalence of EC O157 in

Kansas was 2.4% of 212 fecal specimens collected from the ground (Sargeant et al., 1999) and 0.25% of 1,608 fecal specimens collected from hunter-harvested deer from Nebraska (Renter et al., 2001).

Experiments investigating colonization and fecal shedding of EC O157 demonstrated horizontal transmission and suggested fecal shedding in deer is transient and similar to domestic ruminant species (Fischer et al., 2001). Increased prevalence during the summer was shown in cattle (Meyer-Broseta et al., 2001) but has not been demonstrated in deer.

We conducted studies in Louisiana (USA) to estimate prevalence of EC O157 fecal shedding in hunter-harvested white-tailed deer and to describe seasonality of EC O157 fecal shedding in a herd of captive white-tailed deer. In collaboration with the Louisiana Department of Wildlife and Fisheries Deer Study Group, we collected fecal samples at hunter check stations in six wildlife management areas (WMA: 30°31′N, 91°42′W; 31°20′N, 93°48′W; 32°34′N, 93°05′W; 32°40′N, 91°30′W; 30°65′N, 92°82′W; 31°44′N, 91°63′W) during the 2001 rifle deer hunting season. Wildlife management areas were selected based on the previous year's harvest in an attempt to maximize the number of samples that we could obtain. Fecal samples (approximately 20 g) were collected per rectum with a clean glove, maintained at ambient temperature, transported to the laboratory, and placed in culture media within 24 hr. Sampling was based on convenience and hunter success.

Louisiana State University Agricultural Center (LSU AgCenter, Baton Rouge, Louisiana) maintains a herd of approximately 125 deer in high-fenced pastures which is fed a grain-based concentrate. Individual deer are identified with ear tags. Beginning in December 2000, we collected fecal samples by entering the enclosures, observing individually identified deer, and picking up freshly defecated fecal pellets from the ground (~20 g). Samples were taken every other month based on convenience to avoid handling or confining the deer.

Feces were cultured using bovine fecal culture techniques that are more sensitive (Keen and Elder, 2002) than conventional enrichment and direct plating techniques (Chapman et al., 1994; Gansheroff and O'Brien, 2000; Meyer-Broseta et al., 2001). Briefly, 10 g of fresh deer fecal pellets were placed in 90 ml of Gram-negative broth (Difco, Sparks, Maryland, USA) supplemented with cefsulodin (10 mg/l, Sigma, St. Louis, Missouri, USA), vancomycin (8 mg/ml, Sigma) and cefixime (0.05 mg/ml, Lederle, Pearl River, New York, USA) and allowed to soften for 15 min. The softened fecal pellets were then manually emulsified and incubated for 6 hr at 37 C.

Immunomagnetic separation was performed on a 1-ml aliquot using Dynabeads anti-*E. coli* O157 beads (Dynal Biotech Inc., Lake Success, New York, USA). An aliquot of 50 μl of the bead/bacteria complex was spread plated on sorbitol MacConkey agar (ctSMAC) containing cefixime (0.05 mg/l) and potassium tellurite (2.5mg/l) and incubated at 37 C for 18–24 hr. A maximum of three colonies with typical EC O157 phenotypic characteristics (1–2 mm, sorbitol-negative colonies) were selected as suspects and placed into 5 ml of MacConkey broth and 2 ml of trypticase soy broth (TSB) for 18–24 hr at 37 C.

Indirect enzyme-linked immunosorbent assay (ELISA) was performed for identification of isolate serotype (Keen and Elder, 2002). The ELISA was performed using murine monoclonal antibodies (MAb) to H7 antigen, anti-H7 MAb 2B7, and O157 antigen, anti-O157 MAb 13B3 (He et al., 1996; Westerman et al., 1997). Isolates reactive with MAb 13B3 (O157-positive ELISA) were inoculated in TSB and evaluated for motility using phase-contrast microscopy. Isolates that had the typical EC O157 phenotype on ctSMAC, fermented lactose in MacConkey broth (blue to yellow color change), positively reacted with anti-O157 MAb 13B3, and positively

reacted with anti-H7 MAb 2B7 or were nonmotile (NM) were considered to be EC O157:H7 or EC O157:NM.

Isolates were characterized by polymerase chain reaction (PCR) for *rfb*$_{O157}$ and *fliC*$_{H7}$ and the putative virulence factors, *stx*1, *stx*2, *eae*A, and *hly*A. Somatic (O157) (Paton and Paton, 1998), flagellar (H7) (Gannon et al., 1997), and virulence factor genes (Paton and Paton, 1998) were amplified using previously published primer pair sequences. Duplex reactions were run for *stx*1 and *stx*2 as well as *eae*A and *rfb*$_{O157}$. Polymerase chain reaction for *hly*A and *fliC*$_{H7}$ were run as uniplex reactions. Polymerase chain reaction cycling conditions were as previously described. Clear, well-defined bands of the correct size for *rfb*$_{O157}$ (259 base pairs [bp]), *fliC*$_{H7}$ (625 bp), *stx*1 (180 bp), *stx*2 (255 bp), *eae*A (384 bp), and *hly*A (534 bp) that were consistent with the positive control were considered positive PCR reactions.

Prevalence estimates with 95% confidence intervals (CI) were calculated as the number of positive samples divided by the total number of samples (PEPI 4.01; J. H. Abramson). Fisher's exact statistics were utilized to estimate CIs for null values.

Escherichia coli O157:H7 was isolated from one of 39 deer fecal samples (3%, CI 0.1–14.9) taken at a single WMA on 23 November 2001. Overall prevalence from all six WMAs was 0.3% (CI 0.0–1.6, n=338). The isolate had the correct phenotype but was slightly pink (partial sorbitol fermentation) on the initial ctSMAC plate. Streaking for isolation, the isolate readily fermented sorbitol. Sensititre Gram-negative (AP80) autoidentification plates (Accumed International, Westlake, Ohio, USA) confirmed that the isolate was sorbitol positive as well as beta-glucuronidase (GUD) positive, and identified it as *Escherichia coli* (100% probability). Polymerase chain reaction demonstrated clear, well-defined bands of the correct size for *rfb*$_{O157}$, *fli*C$_{H7}$, *eae*A, and *hly*A. The isolate was not characteristic of the common EC O157 clone in that it was sorbitol and GUD positive and no *stx* genes were detected.

We isolated EC O157 from one of 55 deer at the LSU AgCenter sampled in August 2001. *Escherichia coli* O157:H7 fecal shedding prevalence was 1.8% (CI 0.0–9.7) in August and 0.4% (CI 0.0–2.4, n=226) for the year. The EC O157:H7 isolate was initially confirmed by broth ELISA. However, a viable isolate was not recovered from MacConkey broth to be archived for future characterization. We performed PCR on the isolate DNA directly from the MacConkey broth and characterized the isolate as the O157:H7 serotype possessing *stx*1 and *stx*2 genes. The isolate also possessed *eae*A and *hly*A.

The prevalence of EC O157 in hunter-harvested deer is low. We sampled 338 hunter-harvested deer and found low prevalence of EC O157 during winter. The EC O157 isolates' sorbitol fermentation and positive GUD reaction were unusual and unexpected. Sorbitol-positive EC O157:NM that are also GUD positive are commonly isolated from HUS patients in Germany and have been associated with outbreaks of disease (Karch and Bielaszewska, 2001). Sorbitol-fermenting strains of EC O157:H7 are thought to be rare in the US and Canada (Strockbine et al., 1998; Bettelheim et al., 2002). Cattle, but not deer, have been reported to be a reservoir of these strains and a source of human disease in Europe (Bielaszewska et al., 2000).

Several authors in Germany have reported *stx*-deficient, sorbitol-fermenting strains of EC O157:H7/NM associated with diarrhea in humans and HUS (Schmidt et al., 1999). Furthermore, the authors hypothesize that *stx* production is not obligatory for pathogenicity in humans. The public health significance of the sorbitol-positive, stx-deficient isolate in our study is unclear.

Results of the longitudinal study conducted in the LSU AgCenter's white-tailed deer research herd did not show a seasonal trend in EC O157 fecal shedding. We ex-

pected that, if EC O157 were present and isolated in the deer herd, we would be able to describe seasonal changes in fecal shedding similar to cattle. Only one EC O157 isolate was obtained during August.

Given the sensitivity of the methods used, low prevalence demonstrated, and atypical strain isolated from the hunter-harvested deer, it appears unlikely that deer have a significant role in the epidemiology of EC O157 in cattle populations. From a public health perspective, people that consume venison that might be contaminated by EC O157 should be aware of potential health risks.

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