

Experimental and Field Studies of *Escherichia coli* O157:H7 in White-Tailed Deer

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Studies were conducted to evaluate fecal shedding of *Escherichia coli* O157:H7 in a small group of inoculated deer, determine the prevalence of the bacterium in free-ranging white-tailed deer, and elucidate relationships between *E. coli* O157:H7 in wild deer and domestic cattle at the same site. Six young, white-tailed deer were orally administered 10⁸ CFU of *E. coli* O157:H7. Inoculated deer were shedding *E. coli* O157:H7 by 1 day postinoculation (DPI) and continued to shed decreasing numbers of the bacteria throughout the 26-day trial. Horizontal transmission to an uninoculated deer was demonstrated. Although *E. coli* O157:H7 bacteria were recovered from the gastrointestinal tracts of deer necropsied from 4 to 26 DPI, attaching and effacing lesions were not apparent in any deer. Results are similar to those of inoculation studies in calves and sheep. In field studies, *E. coli* O157 was not detected in 310 fresh deer fecal samples collected from the ground. It was detected in feces, but not in meat, from 3 of 469 free-ranging deer in 1997. In 1998, *E. coli* O157 was not detected in 140 deer at the single positive site found in 1997; however, it was recovered from 13 of 305 dairy and beef cattle at the same location. Isolates of *E. coli* O157:H7 from deer and cattle at this site differed with respect to pulsed-field gel electrophoresis patterns and genes encoding Shiga toxins. The low overall prevalence of *E. coli* O157:H7 and the identification of only one site with positive deer suggest that wild deer are not a major reservoir of *E. coli* O157:H7 in the southeastern United States. However, there may be individual locations where deer sporadically harbor the bacterium, and venison should be handled with the same precautions recommended for beef, pork, and poultry.

Cattle are considered a major reservoir of *Escherichia coli* O157:H7 because consumption of undercooked meat products from cattle has been associated with many human infections and because the bacterium has been detected in bovine feces throughout the United States, Canada, and Europe (7). However, the possible role of wild deer in the epidemiology of *E. coli* O157:H7 began to receive attention as early as 1988 when a human infection was associated with undercooked venison from which *E. coli* O157:H7 was isolated (N. A. Strockbine, personal communication). In 1995, *E. coli* O157:H7 was isolated from persons in Oregon who had consumed venison jerky. Pulsed-field gel electrophoresis (PFGE) patterns of genomic DNA of isolates from the patients, jerky, and the source deer carcass were identical. *E. coli* O157:H7 was cultured from deer feces collected from the ground in the Oregon area where hunters had taken the source deer; however, PFGE patterns differed from those of the outbreak isolates (12).

Surveys of deer for *E. coli* O157:H7 have been largely limited to samples of feces collected from the ground. *E. coli* O157:H7 was found in deer feces collected in cattle pastures in Texas (17) and Kansas (19). Deer were investigated as a pos-

sible source of *E. coli* O157:H7 in unpasteurized apple juice associated with a large outbreak in 1996 in the western United States and Canada (3), and the bacterium was cultured from deer feces collected from the ground near an orchard that was one source of apples for the juice. However, the deer fecal isolate differed genetically from outbreak isolates and produced a different Shiga toxin (J. Farrar, personal communication). *E. coli* O157:H7 was not isolated from an unspecified small number of individual deer sampled during a study of Wisconsin cattle (20), nor from 100 deer in 1992 in the southeastern United States (W. R. Davidson, V. F. Nettles, and N. A. Strockbine, unpublished data).

Inoculation studies have been used to characterize *E. coli* O157:H7 infections of calves, steers, and adult cattle; however, no information is available regarding *E. coli* O157:H7 in white-tailed deer, the most common wild ruminant in the United States. Neonatal calves inoculated with 10¹⁰ *E. coli* O157:H7 bacteria developed diarrhea as well as attaching and effacing enteric lesions (5). However, diarrhea or other signs of disease have not been observed in most calves inoculated after 1 week of age (2, 18, 21), and inoculated adult cattle have rarely exhibited clinical signs (4, 11). Fecal shedding of *E. coli* O157:H7, although variable, was generally of greater magnitude and longer duration in calves than in adults, and shedding persisted in some individual animals for several months (2, 4, 11, 21). *E. coli* O157:H7 bacteria were recovered in the greatest num-

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bers from the large intestine, although pathological lesions were not apparent in steers and adult cattle (4), and from the forestomachs and colon of weaned calves in which lesions also were absent (2).

The objectives of our laboratory studies were to evaluate the clinical response, fecal shedding, sites of bacterial localization, and associated lesions in a small group of young white-tailed deer inoculated with *E. coli* O157:H7. The protocols of a previous bovine trial (2) were followed to facilitate comparison of deer inoculations with calf inoculations. In the field study, we cultured feces of free-ranging white-tailed deer in the southeastern United States, primarily in Georgia. Deer fecal samples were collected from the ground during summer, which, in some reports, is the season of highest prevalence of fecal shedding of *E. coli* O157:H7 by domestic cattle (8, 9) and sheep (13). Samples also were collected directly from deer killed by hunters during the fall hunting season, which is the time of greatest human exposure to wild deer. Additionally, we cultured uncooked venison from deer with *E. coli* O157:H7 in their feces. During the second year of the study, wild deer and domestic cattle at the single positive site found in the first year were cultured for *E. coli* O157:H7, and genomic subtypes of deer and cattle isolates were compared.

[Portions of this work were presented at the Third International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections (J. R. Fischer, M. P. Doyle, T. Zhao, C. A. Brown, and C. T. Sewell, abstr. V233/II, 1997).]

MATERIALS AND METHODS

Experimental design. Nine 3-month-old white-tailed deer were hand raised on milk replacer prior to their arrival at the University of Georgia. They subsequently were weaned and were fed a commercial pelleted deer ration (Checkers; Purina Mills, St. Louis, Mo.) and hay ad libitum during the 9-day acclimation period and throughout the study. Two deer each were housed in separate bio-safety level 2 concrete rooms with floor drains to facilitate daily cleaning with water. The animals remained clinically normal during the acclimation period. All deer were screened twice and found negative by culture for fecal *E. coli* O157:H7 during the acclimation period. Blood samples were collected the day after arrival, and a sandwich enzyme-linked immunosorbent assay (ELISA) was performed on serum from each deer for antibodies against *E. coli* O157:H7. On day 10 following arrival, six deer were orally administered 10^8 CFU of nalidixic acid-resistant *E. coli* O157:H7. Two deer received a similar inoculum of nalidixic acid-resistant, nontoxigenic *E. coli*, and one received no inoculum. To assess contact transmission, the uninoculated deer was cohoused with an inoculated deer shedding *E. coli* O157:H7 from 12 to 26 days postinoculation (DPI). All deer were examined daily for signs of infection including diarrhea, lethargy, and anorexia. Fecal samples were collected per rectum from all deer at 1- to 3-day intervals throughout the 4-week trial. Blood samples were collected for serologic testing at 7-day intervals following the day of inoculation and at the time of euthanasia.

Deer were necropsied at intervals during the trial. Of deer inoculated with *E. coli* O157:H7, two were necropsied at 4 DPI to determine whether attaching and effacing lesions were present, one was necropsied at 11 DPI, two were necropsied at 25 DPI, and one was necropsied at 26 DPI along with the uninoculated contact transmission animal 14 days postcontact (DPC). One deer inoculated with nontoxigenic *E. coli* was necropsied at 7 DPI, and the other was necropsied at 26 DPI.

Bacterial inoculum. Deer received 10^8 CFU of a five-strain mixture of enterohemorrhagic *E. coli* O157:H7 consisting of one venison isolate (E0139), three cattle isolates (E009, E0018, and E0122), and one human isolate (932). Inoculum preparation was similar to that of a previous study (2). Briefly, individual *E. coli* O157:H7 strains and the nontoxigenic strain were selected for nalidixic acid resistance. Nontoxigenic strains did not produce Shiga toxins and were negative by fluorescent antibody tests for pilus antigens F41 and K99. Each strain of nalidixic acid-resistant *E. coli* was grown individually in tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.) containing nalidixic acid (50 µg/ml), sedi-

mented by centrifugation, and washed three times as described elsewhere (2). Phosphate-buffered saline (PBS) was added to sedimented bacteria in an amount needed to obtain an optical density of 0.5 at 630 nm, representing approximately 10^8 CFU/ml, and 0.8 ml of each of the five strains of *E. coli* O157:H7 was mixed in 16 ml of sterilized skim milk immediately prior to deer inoculation via individual nursing bottles. The concentration of each strain and the final *E. coli* O157:H7 concentration in the skim milk were confirmed by standard dilution plating on tryptic soy agar and MacConkey sorbitol agar (Difco) containing 50 µg of nalidixic acid per ml (MSA-NA) in duplicate.

Necropsy of deer. Animals were euthanized with intravenous sodium pentobarbital, and a complete necropsy was performed. Two deer that died spontaneously were necropsied shortly after death. At necropsy of all deer, the gastrointestinal tract (GI tract) was clamped at the esophagus and rectum and removed in toto. Five-centimeter lengths of the duodenum, midjejunum, ileum, cecum, spiral colon, and descending colon were double clamped with plastic bands to allow enumeration of *E. coli* O157:H7 bacteria in the tissues and contents of each section without cross-contamination. Sections and contents of rumen, reticulum, omasum, and abomasum also were collected for culture and enumeration of *E. coli* O157:H7 bacteria. Samples of all GI tissues as well as visceral organs and lymph nodes were placed in 10% buffered formalin for histologic examination.

Histopathology and immunohistochemistry. Formalin-fixed tissues were embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin, and examined by light microscopy. Paraffin-embedded, formalin-fixed sections of GI tract tissues from which large populations ($>3 \log_{10}$ CFU/g) of *E. coli* O157:H7 were cultured were selected and treated in an automated immunohistostaining machine to identify *E. coli* O157:H7. A positive control slide consisted of an enteric section from a neonatal calf with experimental *E. coli* O157:H7 infection (5), and a negative control consisted of an intestinal section from an uninoculated deer. Tissues were deparaffinized in xylene for 15 min, rehydrated through graded alcohols, rinsed in diluted citrate buffer (Antigen Retrieval Citra 10× concentrate; Biogenex Laboratories, San Ramon, Calif.), and then steamed in citrate buffer in a covered steam chamber for 20 min. Sections then were placed into an automated immunostaining machine, rinsed in citrate buffer, covered with blocking antibody (rabbit serum; Vector Laboratories, Burlingame, Calif.) for 5 min, rinsed in buffer, and then covered with *E. coli* O157:H7-specific goat antiserum (0.1 µg/ml; Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.) and incubated for 30 min. Sections were rinsed in buffer and covered with rabbit biotinylated antibody (Supersensitive link; Biogenex Laboratories) for 25 min. Sections were rinsed in buffer and covered with 3% hydrogen peroxide for a total of 7.5 min, rinsed, and then covered with avidin-biotin complex (Elite ABC kit; Vector Laboratories) according to the manufacturer's instructions. After rinsing, sections were treated with 3,3'-diaminobenzidine (DAB peroxidase substrate; Sigma Chemical Co., St. Louis, Mo.) per the manufacturer's instructions for a total of 12 min, rinsed, and stained with Gills 2 hematoxylin (Surgipath Medical Industries, Richmond, Ill.) for 3 min. Sections were rinsed, dehydrated, cleared, mounted (Flotex; Lerner Laboratories, Pittsburgh, Pa.), and examined by light microscopy.

Serologic testing of experimental deer. ELISA was performed in 96-well polystyrene enzyme immunoassay-radioimmunoassay plates (GIBCO, Grand Island, N.Y.). Each well was coated with 10^6 formaldehyde-killed *E. coli* O157:H7 whole cells in 100 µl of 50 mM carbonate buffer, pH 9.6, by incubation at 25°C overnight with agitation (150 rpm). The plate was washed four times with 50 mM Tris, pH 7.4, containing 150 mM NaCl (Tris-buffered saline [TBS]), and remaining binding sites were blocked with milk diluent-blocking solution (Kirkegaard and Perry Laboratories, Inc.) for 1 h at 37°C. The blocking agent was removed, and 100 µl of double-diluted deer serum per well was added and incubated at 37°C for 1 h. Serum from a fetal deer was used as a negative control. The wells were washed four times with TBS containing 0.05% Tween 20 (TBS-T), and 100 µl of goat anti-deer immunoglobulin M and G conjugated to alkaline phosphatase (diluted 1:800 in TBS-T; Kirkegaard and Perry Laboratories, Inc.) per well was added and incubated at 37°C for 1 h. Wells were washed four times with TBS-T, and 100 µl of *p*-nitrophenylphosphate in 1 M 2-amino-2-methyl-1-propanol (Sigma), pH 9.9 (1 mg/ml), was added to each well. The plate was incubated at 37°C for 1 h, and the optical density at 405 nm of each well was determined with an EL 312e microplate reader (Bio-Tek Instrument, Winooski, Vt.). An optical density reading of 0.1 above the negative control was considered positive for that dilution.

Collection of samples from deer and cattle in the field. In 1997, approximately 60 fresh deer fecal samples were collected from the ground in July-August at each of five public wildlife areas and directly from hunter-killed deer in October to December at the same five areas plus an additional site (sites GA1 to GA6). The wildlife areas were widely scattered throughout Georgia. Fecal samples also

were collected directly from 98 deer from April to December 1997 in Arkansas, Georgia, Mississippi, South Carolina, Tennessee, and West Virginia. Age and sex were recorded for all individually sampled deer. Frozen meat samples were obtained from deer that had *E. coli* O157:H7 in feces.

In November and December of 1998, fecal samples were collected per rectum from hunter-killed deer at site GA4, where *E. coli* O157:H7 was detected in deer in 1997. Site GA4 consisted of an approximately 20,000-acre public wildlife area adjacent to property on which a dairy herd and a beef herd were present. From November 1998 to February 1999, fecal samples were obtained per rectum from cattle belonging to the two herds at site GA4.

Isolation of *E. coli* O157:H7 from feces and tissue samples. Techniques used to enumerate *E. coli* O157:H7 bacteria in deer feces and GI tissues were similar to those previously described (2). Briefly, 10 g of feces was placed in 15 ml of Cary-Blair medium (Remel Co., Lenexa, Kans.), held at 5°C, and transported to the laboratory within 72 h. One gram of feces was serially diluted to 10^{-5} CFU/g, and each dilution was plated in duplicate on MSA-NA and incubated. GI tissue samples were weighed and washed gently in sterilized PBS to remove the contents of the tubular organs. The rinsed tissue samples were resuspended in PBS and homogenized. Tissue sample suspensions and content samples were plated separately in quadruplicate on MSA-NA plates. When the direct plating technique was negative, selective enrichment was employed (2).

For samples collected from wild deer and domestic cattle, isolation techniques were similar to those used in previous surveys of domestic cattle (22). Briefly, fecal samples were placed in Cary-Blair medium as described above and then plated and incubated on sorbitol-MacConkey agar plates (Unipath, Oxoid Division, Ogdensburg, N.Y.) containing 0.1% 4-methylumbelliferyl- β -D-glucuronide (Sigma Chemical Co.). For enrichment, samples were placed in TSB containing novobiocin and incubated with agitation. Following incubation, a sandwich ELISA using a specific monoclonal antibody against *E. coli* O157:H7 was performed on each enriched sample as described elsewhere (22). For immunomagnetic selective isolation, 1 ml of enrichment medium from ELISA-positive samples was incubated with 20 μ l of immunomagnetic beads coated with antibody to *E. coli* O157 (anti-*E. coli* O157 Dynabeads; Dynal, Oslo, Norway) and processed according to the manufacturer's instructions. Sixteen samples of frozen ground venison, cubed steak, roast, and hindquarter from three fecal culture-positive deer were assayed for *E. coli* O157:H7. For each sample, 25 g of thawed meat was placed in 225 ml of enrichment broth and processed as described above for wild deer and cattle fecal samples.

Characterization of *E. coli* O157:H7 isolates from laboratory and field studies. Isolates were identified as *E. coli* O157 as described elsewhere (22). Latex agglutination assay-positive colonies were further characterized by an H7 antiserum motility inhibition assay and by biochemical tests with the API 20E miniaturized diagnostic test (Analytab Products, Division of Sherwood Medical, Plainview, N.Y.). For determination of Shiga toxin production by the 16 cattle and deer *E. coli* O157 isolates obtained in field studies, isolates were tested by PCR using oligonucleotide primers for Stx1a (GAAGAGTCCGTGGATTAC G), Stx1b (AGCGATGCAGCTATTAATAA), Stx2a (TTAACACACCCAC GGAGT), and Stx2b (GCTCTGGATGCATCTGGT). The primers were based on published sequences for the *stx1* and *stx2* genes (16). The sizes of the amplified products were 130 bp for Stx1 primers and 346 bp for the Stx2 primers.

Genetic fingerprints of eight *E. coli* O157:H7 isolates obtained at necropsy on day 26 from the GI tracts of the two deer in the contact transmission trial were determined, as were fingerprints of the 3 deer isolates and 13 cattle isolates of *E. coli* O157 obtained in field studies. Genomic subtyping of *E. coli* O157 isolates was done by PFGE of *Xba*I restriction endonuclease digests of genomic DNA as previously described (15). PFGE patterns of the isolates from the experimental animals were compared with those of the inoculum strains, and patterns of the field isolates from wild deer were compared with those of cattle.

RESULTS

Serologic and clinical response of experimental deer. Antibodies against *E. coli* O157:H7 were not detectable by ELISA in deer prior to the trial, at weekly intervals following inoculation, or at the time of euthanasia. Deer did not exhibit anorexia or lethargy following inoculation with enterohemorrhagic or nontoxigenic *E. coli*. Two deer that received *E. coli* O157:H7 developed mild nonhemorrhagic diarrhea. One deer (deer 9) had mild diarrhea from 1 to 14 DPI and subsequently had soft, unformed feces through 25 DPI. The other deer (deer 13) had mild diarrhea at 3 and 10 DPI and died spontaneously

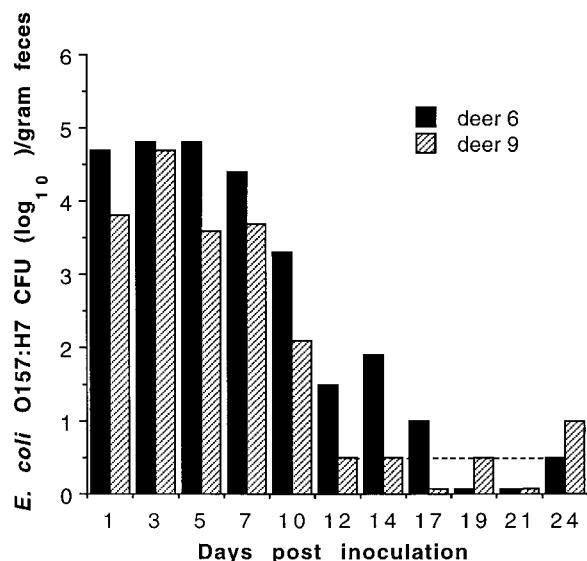


FIG. 1. Recovery of *E. coli* O157:H7 from feces of two inoculated deer. Deer were inoculated with 10^8 CFU of *E. coli* O157:H7 on day 0. Bars ending at the dashed line represent fecal samples positive only by enrichment culture. Bars ending beneath the dashed line represent samples that were negative for *E. coli* O157:H7.

of a noninfectious digestive problem (pyloric stenosis) at 11 DPI. Additionally, one deer (deer 10) that received nontoxigenic *E. coli* died spontaneously of a similar noninfectious digestive problem at 7 DPI. The spontaneous deaths of these animals underscore the husbandry difficulties associated with this species. The other six deer were clinically normal during the trial.

Patterns of fecal shedding of *E. coli* O157:H7 by experimental deer. Inoculated deer were shedding 3.5 to 5.1 (average, 4.3) \log_{10} CFU of *E. coli* O157:H7/g of feces by 1 DPI. Fecal shedding of *E. coli* O157:H7 by deer decreased substantially during the first 10 DPI but was consistently or intermittently detectable in three of three deer for the remainder of the 26-day study (Fig. 1 and 2). Two cohoused deer (deer 1 and 2) were euthanized and necropsied at 4 DPI after shedding 3.4 to 4.8 \log_{10} CFU of *E. coli* O157:H7/g of feces at 1 and 3 DPI. Two cohoused deer (deer 9 and 6) shed decreasing numbers of *E. coli* O157:H7 bacteria until 12 and 17 DPI, respectively, and intermittent shedding of low numbers of *E. coli* O157:H7 bacteria (<10 CFU/g) was detectable only by enrichment culture through 25 DPI (Fig. 1).

In the contact transmission trial, shedding by the two inoculated deer decreased during the first 10 DPI (Fig. 2). One deer (deer 13) died spontaneously of unrelated causes at 11 DPI. The remaining deer was shedding 1.9 \log_{10} CFU/g of feces at 12 DPI when an uninoculated deer was introduced to the room for the remainder of the trial. By 2 DPC, the uninoculated deer was shedding 2.1 \log_{10} CFU/g of feces, and it continued to shed from 1.6 to 3.2 \log_{10} CFU/g through 14 DPC. After introduction of the uninoculated deer, fecal shedding of *E. coli* O157:H7 by the inoculated animal increased (from 1.9 to 3.9 \log_{10} CFU/g), and it remained detectable by standard plating throughout the trial (Fig. 2).

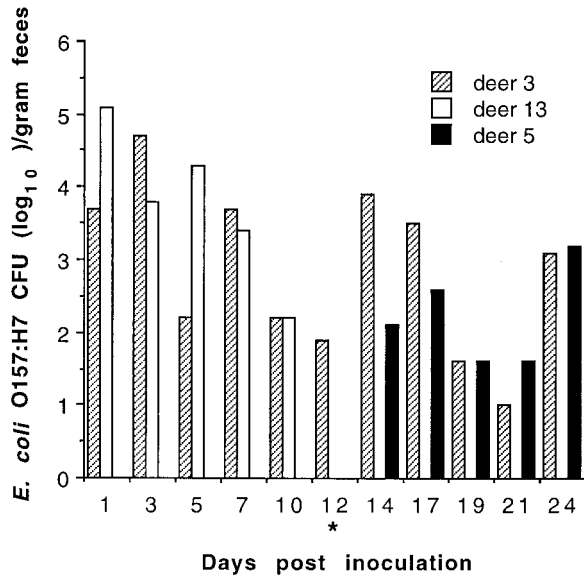


FIG. 2. Recovery of *E. coli* O157:H7 from feces of deer in horizontal transmission trial. Deer 3 and 13 were inoculated with 10⁸ CFU of *E. coli* O157:H7 on day 0. Deer 13 was removed on day 11, and deer 5 (uninoculated contact animal) was cohoused with deer 3 from day 12 (asterisk) through day 26 of the study.

Recovery of *E. coli* O157:H7 from experimental deer at necropsy. Inoculated deer were necropsied 4, 11, 25, and 26 DPI, and the uninoculated contact deer was necropsied 14 DPC. *E. coli* O157:H7 bacteria were enumerated in contents and washed tissues at 10 sites of the GI tract of all deer (Table 1). Moderate or high (1 to 3 or >3 log₁₀ CFU/g, respectively) numbers of enterohemorrhagic or nontoxigenic *E. coli* were recovered from the contents and tissues of the forestomachs and the intestines of deer necropsied 4 to 11 DPI. Low populations (<10 CFU/g) of *E. coli* O157:H7 were detected using enrichment culture in the contents but not in the mucosa of the large intestines of two deer (deer 6 and 9) necropsied at 25 DPI (Table 1). Moderate to high populations of *E. coli* O157:H7 were found in contents of all sections of the GI tract except in the abomasal contents of the inoculated deer in the contact transmission trial at 26 DPI and in all sections except the abomasum, duodenum (detectable only using enrichment

culture), and spiral colon of the uninoculated deer (Table 1). A consistent finding for all deer was smaller populations of *E. coli* O157:H7 bacteria in washed tissue than in the contents of the corresponding GI tract site (data not shown). This difference ranged from 0.1 to 2.9 log₁₀ CFU/g.

Gross, histological, and immunohistological findings for experimental deer. Two deer that died spontaneously had distended forestomachs and abomasums, and there was moderate pyloric hypertrophy and stenosis with a paucity of digesta distal to the pylorus. The deer inoculated with *E. coli* O157:H7 that died at 11 DPI also had mild multifocal intracorneal microabscessation of the reticulum mucosa. Additional gross or microscopic lesions, including attaching and effacing lesions suggestive of enterohemorrhagic *E. coli* infection, were not apparent in these animals. The gross and microscopic findings for these animals are consistent with pyloric stenosis that most likely was caused by dietary changes in the animals as they were weaned. Gross lesions were not apparent in deer that were euthanized and necropsied per protocol. Adherent bacteria were not observed on mucosal surfaces of hematoxylin-and-eosin- or immunohistochemically stained sections of the GI tract. Attaching and effacing lesions were not observed in any deer.

Characterization of *E. coli* O157:H7 isolates from experimental deer. PFGE patterns of *Xba*I endonuclease digests of genomic DNA from eight *E. coli* O157:H7 isolates obtained at necropsy on day 26 from two deer of the contact transmission trial were indistinguishable. This pattern matched the fingerprint of the venison strain (E0139) of the inoculum.

Prevalence and characteristics of *E. coli* O157:H7 in free-ranging deer and cattle. *E. coli* O157:H7 was isolated from 3 of 919 deer fecal samples. The bacterium was detected in feces of 3 of 609 (0.5%) individually sampled deer, but it was not found in 310 fresh fecal samples collected from the ground. *E. coli* O157:H7 was isolated from feces of 3 of 469 individually sampled deer in 1997 and in 0 of 140 deer at GA4 in 1998. The three positive animals (2.5- and 3.5-year-old males and a 0.5-year-old female) were among 77 hunter-killed deer sampled during three consecutive days in November 1997 at site GA4. Isolates were obtained only after enrichment indicating less than 5 CFU/g of feces. All three *E. coli* O157:H7 isolates were positive for *stx*1 and *stx*2 genes. *E. coli* O157:H7 was not isolated from 16 venison samples from the three positive deer.

E. coli O157 was isolated from feces of 13 of 305 (4.3%)

TABLE 1. *E. coli* O157:H7 recovery from GI contents of experimental deer at necropsy

Sample site	No. of <i>E. coli</i> O157:H7 bacteria (log ₁₀ CFU)/g at:				
	4 DPI (deer no. 1/no. 2)	11 DPI (deer no. 13)	14 DPC (deer no. 5)	25 DPI (deer no. 6/no. 9)	26 DPI (deer no. 3)
Rumen	2.8/4.6	3.3	3	0/0	3.6
Reticulum	2.5/4.3	2.3	2.7	0/0	3.4
Omasum	2.0/4.3	2.6	2.6	0/0	3.2
Abomasum	1.8/2.9	0	0	0/0	0
Duodenum	1.9/E+ ^a	E+	E+	0/0	1.6
Jejunum	4.5/6.5	E+	2.3	0/0	3.5
Ileum	4.6/6.6	4.4	3.7	0/0	1.3
Cecum	4.5/7.5	3.6	3.5	E+/0	3.5
Spiral colon	4.6/7.6	3.4	0	E+/0	3.5
Descending colon	4.6/6.4	3.7	2.3	0/E+	3.4

^a E+, enrichment culture positive.

TABLE 2. Characteristics of enterohemorrhagic *E. coli* O157 isolates obtained from deer and cattle at the same site in Georgia

Source	Serotype	PFGE pattern ^a	Shiga toxin gene ^b	
			<i>stx1</i>	<i>stx2</i>
Deer 594	O157:H7	1	+	+
Deer 569	O157:H7	1	+	+
Deer 602	O157:H7	2	+	+
Cattle 186	O157:NM	3	+	+
Cattle 486	O157:NM	4	+	-
Cattle 128	O157:H7	5	-	+
Cattle 249	O157:H7	5	-	+
Cattle 302	O157:H7	5	-	+
Cattle 472	O157:H7	5	-	+
Cattle 538	O157:H7	5	-	+
Cattle 541	O157:H7	5	-	+
Cattle 547	O157:H7	5	-	+
Cattle 57	O157:H7	6	-	+
Cattle 117	O157:H7	6	-	+
Cattle 197	O157:H7	6	-	+
Cattle 317	O157:H7	6	-	+

^a PFGE pattern of genomic DNA following endonuclease digestion with *Xba*I.

^b Presence of gene sequences of *Stx1* or *Stx2*.

cattle in 1998-1999 at site GA4. The isolates were obtained from seven dairy and five beef cattle in November 1998 and from one beef animal in February 1999. Eleven isolates were *E. coli* O157:H7 and had only *stx2* genes. Two were *E. coli* O157:NM; both were positive for *stx1* genes, and one also had *stx2* genes.

Characteristics of *E. coli* O157 from deer and cattle are found in Table 2. Genomic DNA of all *E. coli* O157 isolates from wild deer and cattle was evaluated by PFGE (Fig. 3). Two deer isolates had indistinguishable PFGE patterns; the third isolate was genetically distinct. Bovine isolates comprised four PFGE patterns: one pattern was found in one beef and six dairy cattle, a second PFGE pattern was found in four beef animals, and the two *E. coli* O157:NM isolates each had distinct patterns. The PFGE patterns of bovine isolates did not match patterns of deer isolates.

DISCUSSION

The clinical response of inoculated deer is consistent with the subclinical fecal shedding of *E. coli* O157:H7 by naturally

or experimentally exposed domestic cattle and sheep (1, 2, 4, 8, 13, 14). Although two inoculated deer had mild nonhemorrhagic diarrhea during a portion of the trial, it is unknown if the loose feces was caused by *E. coli* O157:H7 or by other variables. The lack of attaching and effacing lesions or adherent bacteria in any deer and the subclinical shedding by five other deer suggest that *E. coli* O157:H7 is mildly pathogenic or nonpathogenic in deer of this age.

Patterns of fecal shedding of *E. coli* O157:H7 and necropsy findings among inoculated deer were quite similar to those of inoculated cattle and sheep. Although individual variation has been observed, inoculated adult cattle, steers, calves, and sheep transiently shed high numbers of *E. coli* O157:H7 bacteria shortly after inoculation, with fecal shedding decreasing dramatically by 2 weeks and often becoming intermittently detectable thereafter (1, 2, 4, 13, 14). Similarities of deer fecal shedding patterns to those of domestic ruminants suggest that shedding by deer may be transient, as it is in cattle and sheep. However, additional studies are necessary to evaluate persistence and long-term shedding of *E. coli* O157:H7 in deer.

Necropsy results suggest that *E. coli* O157:H7 may be found in contents at nearly all levels of the GI tract in deer recently exposed to the bacterium, but the large intestine may be the site where the bacteria persist for longer periods. These sites are similar, but not identical, to those of inoculated calves in which the rumen and colon were the primary sites of long-term localization of *E. coli* O157:H7 (2). The presence of higher populations of *E. coli* O157:H7 bacteria in GI contents than in corresponding mucosa and the lack of adherent *E. coli* O157:H7 suggest that bacterial localization and proliferation in deer occurred within the contents of the GI tract in the absence of mucosal colonization as hypothesized for calves (2).

The failure to detect antibodies against *E. coli* O157:H7 in exposed deer most likely was due to minimal interaction between the bacteria and the host as evidenced by the lack of adherent bacteria or mucosal lesions in GI sections of the animals. Antibody responses to *E. coli* O157:H7 lipopolysaccharide were prompt and sustained in cattle inoculated with 10^{10} CFU of *E. coli* O157:H7, but inoculation of steers with 10^7 CFU resulted in little or no change in preinoculation titers (4). Thus, antibodies against *E. coli* O157:H7 may have been detectable if deer had received a greater inoculum or if ELISA

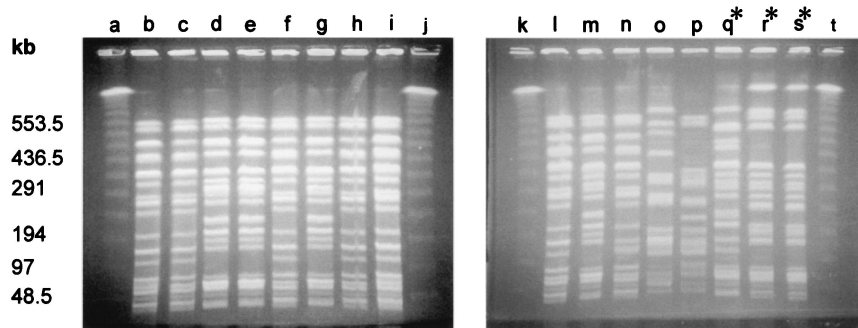


FIG. 3. PFGE of *Xba*I digests of enterohemorrhagic *E. coli* O157 isolates from cattle and free-ranging white-tailed deer. Isolates are *E. coli* O157:H7 except where noted. Lanes: a, j, k, and t, DNA pulse marker; b, cattle 547; c, cattle 541; d, cattle 57; e, cattle 197; f, cattle 472; g, cattle 117; h, cattle 302; i, cattle 128; l, cattle 538; m, cattle 317; n, cattle 249; o, cattle 486 (*E. coli* O157:NM); p, cattle 186 (*E. coli* O157:NM); q*, deer 602; r*, deer 594; s*, deer 569.

was performed using highly purified lipopolysaccharide, rather than whole cells.

Five strains were used in the inoculum because *E. coli* O157:H7 strains may differ in their virulence attributes and in their ability to colonize an animal species (2). Eight *E. coli* O157:H7 isolates obtained from two deer at completion of the contact transmission trial were genetically indistinguishable and matched the inoculum strain from venison jerky associated with human infections (12). Although evaluation of isolates from only two animals provides limited information, findings suggest that *E. coli* O157:H7 from a cervine source may persist longer and/or spread more readily in deer than other strains derived from cattle or humans. However, additional inoculation trials using several *E. coli* O157:H7 isolates from deer, domestic ruminants, humans, and other sources are necessary to confirm this preliminary observation.

Our field study represents the first investigation of the prevalence of *E. coli* O157:H7 in sympatric free-ranging deer and domestic cattle. Previous surveys of deer for *E. coli* O157:H7 involved environmental samples of deer feces, and the actual number of deer sampled was unknown (17, 19). The results of the current study suggest that the prevalence of *E. coli* O157:H7 is low among wild deer in the southeastern United States; however, there may be scattered locations where deer sporadically harbor the bacterium.

The role of wild deer in the epidemiology of *E. coli* O157:H7 in cattle is undetermined. Genetically indistinguishable isolates of *E. coli* O157:H7 were obtained from deer and cattle feces on the same Texas ranch, and deer have been suggested as reservoirs or disseminators of the bacterium (17, 19). However, the design of surveys to date has precluded determining the direction of the spread of this organism between wild and domestic animals. Deer isolates from the current study differed genetically from cattle isolates of this study as well as from 40 banked isolates of *E. coli* O157:H7 at the University of Georgia's Center for Food Safety and Quality Enhancement (T. Zhao and M. P. Doyle, unpublished data). The apparent lack of maintenance of *E. coli* O157:H7 in the wild deer from one year to the next and the disparity between the strains found in deer and cattle in the current study suggest that deer were not serving as a reservoir of *E. coli* O157:H7 for cattle at this site.

The factors that lead to *E. coli* O157:H7 carriage by deer at specific locations are unknown. The presence of cattle, the primary reservoir, may be a necessary or contributing factor. A single strain of *E. coli* O157:H7 disseminated through cattle via contaminated animal drinking water, and the strain also was isolated from feed, flies, and a wild pigeon on a Wisconsin farm (20), which suggests that a similar scenario could result in *E. coli* O157:H7 exposure of wild deer frequenting cattle farms. Cattle were present at the site where positive deer were found in the current study; however, wild deer and domestic cattle shared range at other sites in our study, and *E. coli* O157:H7 was not recovered from those deer populations. Cattle at those sites were not surveyed for *E. coli* O157:H7.

E. coli O157:H7 was not detectable in uncooked meat from the positive deer identified in this study. However, because *E. coli* O157:H7 has been detected in deer feces, the bacteria have been recovered from multiple levels of the GI tract of inoculated deer, and contaminated meat from deer has been confirmed as the source of human infections, deer carcasses

and venison should be handled with the same precautions recommended for beef, pork, and poultry.

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