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Experimental Infection of Calves and Adult Cattle with *Escherichia coli* O157:H7

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Preweaned calves and adult cattle were inoculated with 10^{10} CFU of *Escherichia coli* O157:H7 strain 3081, a calf isolate which produces Shiga-like toxin, to define the magnitude and duration of fecal shedding of *E. coli* O157:H7 for each age group. Fecal samples of eight of eight, eight of eight, three of eight, and two of eight calves were positive at 2, 7, 14, and 20 weeks, respectively. In contrast, nine of nine, two of nine, and one of nine steers were positive at 2, 7, and 14 weeks, respectively. The magnitude of shedding (CFU per gram) by individual animals at any one time postinoculation varied widely within each age group but was greater for calves as a group. The differences in shedding patterns between adults and calves were statistically significant. After inoculation, 25 of 29 animals remained healthy and 4 of 17 calves had transient diarrhea. Histologic sections of the brain, kidney, jejunum, ileum, cecum, and colon taken at necropsy from nine calves either 3, 14, or 18 days postinoculation or three adults either 2, 3, or 4 days postinoculation were normal. *E. coli* O157:H7 was recovered from the alimentary tracts of all of the animals necropsied, and there was no evidence of spread to the liver, spleen, or kidneys. Four calves that had ceased shedding were reinfected when inoculated again with the same strain. *E. coli* O157:H7 was recovered from none of five and two of five adults inoculated with 10^4 and 10^7 CFU, respectively. If one assumes that the *E. coli* strain and cattle used in this study are representative of the larger populations encountered in the field, then these observations suggest the following conclusions. (i) Fecal shedding of toxigenic *E. coli* O157:H7 varies widely among animals of the same age group but persists longer in calves than in adults. (ii) *E. coli* O157:H7 does not spread from the alimentary tract to other organs. (iii) Previous infection does not prevent reinfection by the same strain of *E. coli* O157:H7. (iv) The infectious dose of in vitro-grown *E. coli* O157:H7 for normal adult cattle is high ($>10^4$ and probably $\geq 10^7$ CFU). (v) Most cattle infected with *E. coli* O157:H7 remain clinically normal.

Escherichia coli O157:H7 was first identified as a human pathogen following two geographically separate outbreaks of hemorrhagic colitis in the United States in 1982 (37). An epidemiological investigation of those outbreaks established an association between illness and eating of hamburgers purchased at a fast food chain. Furthermore, *E. coli* O157:H7 was recovered from a raw hamburger patty obtained from the same lot of hamburger used by the suspect fast food chain during the time of the outbreak. Person-to-person transmission of the infection also occurs (14). In several hospital laboratory surveys of stool samples submitted for culture, *E. coli* O157:H7 was one of the most common enteric pathogens isolated (7, 13, 22). Although some persons infected with *E. coli* O157:H7 may be asymptomatic, symptoms may include nonbloody diarrhea, bloody diarrhea, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura (14).

Between 1982 and 1993, there were 32 U.S. outbreaks of *E. coli* O157:H7 illness among humans. Of the 24 outbreaks classified as food borne, 17 were attributed to consumption of undercooked or unpasteurized bovine products (1). Although *E. coli* O157:H7 has been isolated from retail cuts of pork, lamb, and poultry (11), there are no reports of recovery from live animals other than bovines and humans. The epidemiological link between human disease and consumption of bovine products has been supported by the isolation of *E. coli* O157:H7 from calf or adult bovine feces collected from farms or feedlots in the United States, Canada, the United Kingdom, Germany, and Spain (5, 6, 8, 23, 24). *E. coli* O157:H7 was recovered from 0.3 to 2.2% of cattle feces collected from surveyed farms and feedlots in the United States and Canada (1, 9, 38).

Little is known of either the duration and magnitude of *E. coli* O157:H7 shedding in cattle feces or the effect of infection on animal health. Wells et al. (38) found that fecal shedding lasted from 8 to at least 46 days in some animals. Although the first bovine *E. coli* O157:H7 isolate was recovered from a calf with colibacillosis (28), in other studies *E. coli* O157:H7 was recovered from healthy animals (6, 9, 23, 38). We addressed these issues by experimentally infecting calves and adult cattle with *E. coli* O157:H7. Our primary objective was to define the pattern (magnitude and duration) of fecal excretion of *E. coli* O157:H7 (shedding) by experimentally inoculated cattle. Our secondary objectives were to determine if the pattern of shedding by cattle changes with age and to identify the tissue localization of the organism in cattle during periods of high- and low-intensity shedding.

(A preliminary report of this work was presented at the 94th General Meeting of the American Society for Microbiology.)

MATERIALS AND METHODS

Inoculation strain. *E. coli* O157:H7 strain 3081 was isolated from a preweaned calf by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Veterinary Services Laboratories, Diagnostic Bacteriology Laboratory, during the U.S. Department of Agriculture's National Animal Health Monitoring System National Dairy Heifer Evaluation Project (1991 and 1992) (36). The strain was resistant to kanamycin and ampicillin and produced Shiga-like toxins (SLT) I and II (17, 27). The strain hybridized to probes derived

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from CVD419 (21) and *eae* (16), virulence attributes associated with human *E. coli* O157:H7 strains.

Animals. Calves and adults were housed in accordance with the guidelines of the American Association for Laboratory Animal Care in climate-controlled BL-2 containment barns. Pens had individual floor drains and were cleaned twice daily. Preweaned 3- to 14-week-old Jersey and Holstein bull calves ($n = 17$) were inoculated in several groups (1 to 4 per group) at different times. Calves were housed individually, but within a group some calves could have nose-to-nose contact. The calves were fed in the morning and the afternoon and maintained on milk replacer (without antibiotics) for at least 2 weeks postinoculation (p.i.). Calves were fed a diet appropriate for their age throughout the experiment. Adults ($n = 22$) included 1-year-old Jersey heifers previously infected with *Leptospira* sp. inoculated as one group, as two groups of 1-year-old Jersey steers, and as one group of 3-year-old Holstein and Jersey steers fed brains from sheep infected with scrapie when they were 1 month old. All adults and calves were clinically normal at the time of inoculation. The adult groups were housed in separate barns, with each animal in an individual pen, an arrangement which prevented nose-to-nose contact.

Culture and animal inoculation. A Trypticase soy agar plate (BBL) was inoculated from a frozen stock of *E. coli* O157:H7 and then incubated for 18 h at 37°C. Colonies from this plate were used to inoculate mannitol-modified EC broth (2.0% tryptone [Difco], 0.15% no. 3 bile salts [Difco], 0.5% mannitol, 0.4% dibasic potassium phosphate, 0.15% monobasic potassium phosphate, 0.5% sodium chloride [pH 6.9]). Mannitol-modified EC broth was used in these studies because of its availability in our laboratory. The inoculated broth was incubated at 37°C with shaking (225 rpm) until the turbidity reached ~160 Klett units (~1.0×10^9 CFU/ml; green filter no. 54; Klett-Summerson). For calves, 10 ml of the log-phase culture (10^{10} CFU) was added to 500 ml of milk replacer, which was fed by nursing bottle. Steers were inoculated by gavage (with a stomach tube) with 10 ml of a log-phase culture (10^{10} CFU) or a dilution of the culture containing either 10^7 or 10^4 CFU added to 200 ml of Trypticase soy broth (TSB; BBL), followed by 300 ml of TSB. For rechallenge of four calves with 10^{10} CFU, the inoculum was prepared as described above and administered by stomach tube as described for steers. Two calves were reinoculated at 21 weeks, and two were reinoculated at 33 weeks after primary inoculation.

Testing for *E. coli* O157:H7. To test for the presence of *E. coli* O157:H7 in animals prior to inoculation, 10 g of feces from each animal was added to 100 ml of TSB containing 0.15% bile salts (Difco) and incubated for 18 h at 37°C. The culture was serially diluted in phosphate-buffered saline (PBS; 15 mM KH_2PO_4, 8 mM Na_2HPO_4, 137 mM NaCl, 2.6 mM KCl [pH 7.4]). A 0.1-ml volume was then spread on sorbitol MacConkey agar (Oxoid) and incubated for 18 h at 37°C. Ten sorbitol-negative colonies from each sample were tested for O157 antigen by latex bead agglutination assay (Oxoid). All animals were culture negative prior to inoculation. After inoculation, feces were collected from the pen floor on selected days, except at necropsy, when they were collected directly from the animal. Feces were tested within 5 h of defecation. For direct plating, 1.0 g of feces was added to 4.0 ml of PBS in a capped tube (17 by 100 mm), dispersed with a Vortex Genie (setting 8, 1 min), and serially diluted in PBS. A 0.1-ml volume was then spread on KASMAC (sorbitol MacConkey agar containing kanamycin [100 μg/ml; Sigma] and ampicillin [100 μg/ml; Sigma]). After incubation for 12 to 16 h at 37°C, selected sorbitol-negative colonies typical of *E. coli* O157:H7 strain 3081 were tested for O157 antigen by latex bead agglutination. For enrichment, 10 g of feces was added to 100 ml of TSB–0.15% bile salts. Cultures were incubated for 16 h at 37°C, diluted in PBS, and then plated on KASMAC. Bile, abomasum and reticulum contents, tissue rinses, and tissue homogenates were directly plated, or 1.0-ml samples were inoculated into 10.0 ml of TSB–0.15% bile salts and then incubated, diluted, and plated as described above. Occasionally, TSB–0.15% bile salts and KASMAC were supplemented with tellurite (2.5 μg/ml; Sigma) to reduce background fecal microbial flora (39).

Tissue collection and examination. Animals were euthanized with sodium pentobarbital. Tissues were collected aseptically from calves at 3 ($n = 3$), 14 ($n = 4$), or 18 ($n = 2$) days p.i. and from three heifers at 2 ($n = 1$), 3 ($n = 1$), or 4 ($n = 1$) days p.i. Histologic sections of the brain, kidneys, jejunum (~1 m distal to the anterior root of the mesentery), ileum (~1 m proximal to the ileocecal valve), and cecum and the apex of the spiral colon were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (19). Samples of the tonsils, liver, bile, spleen, kidneys, mesenteric lymph nodes (from a position close to the anterior root of the mesentery, the middle of the small intestine, and the ileal cecal colic node); the abomasum and reticulum contents, and the rumen, jejunum, ileum, cecum, and colon and their contents were collected from the locations described above for bacteriologic testing. Approximately 10-g samples of the tonsils, liver, spleen, and kidneys were weighed and added to a measured volume of PBS (usually equal to the weight of the sample) in a Whirl-Pak 8736 puncture-proof bag. Tissues were pummelled in a Stomacher 80 homogenizer for 2 min. Rumen, jejunum, ileum, cecum, and colon samples were weighed and then rinsed of their contents in 50 ml of PBS before Stomacher processing. The PBS rinse and the corresponding tissue homogenate were both tested for *E. coli* O157:H7, and the number of CFU isolated from each was added.

Statistical analysis. A comparison of CFU per gram of feces among adults, calves, and reinoculated calves for days 1 to 13 and between adults and calves for days 14 to 100 was done by analysis of variance (SAS Institute) on a repeated-measures split-plot design (33) with CFU per gram of feces from animals in a group as the main experimental unit for the plot and days p.i. as a subplot. Fecal samples from which *E. coli* O157:H7 was not recovered were assigned a value of 0; samples positive by enrichment only (<50 CFU/g) were assigned a random number between 0 and 50. To estimate daily (days 1 to 14) fecal shedding of adults and calves, the relationship between observed fecal CFU per gram and days p.i. was fitted as a polynomial of time for each group. The CFU per gram for days with no observation was predicted by the polynomial. To calculate geometric means (see Table 1), samples positive by enrichment only were assigned a random number between 0 and the sensitivity of the assay (<20 or <50 CFU/g).

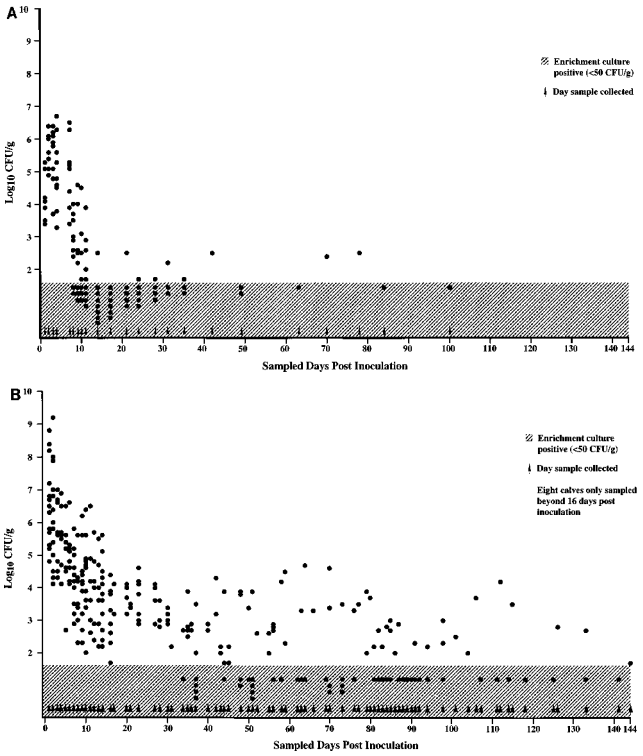

FIG. 1. Time course of fecal shedding of *E. coli* O157:H7. Animals were inoculated with 10^{10} CFU of *E. coli* O157:H7 strain 3081 on day 0. A, adult cattle; B, calves.

RESULTS

Animal health. Following inoculation with 10^{10} CFU of *E. coli* O157:H7 strain 3081, 25 of 29 animals remained healthy. Four calves had transient (<24 h) episodes of nonbloody diarrhea within 24 h of inoculation. The cause of this diarrhea was not determined. Histologic sections of the brain stem, kidneys, jejunum, ileum, cecum, and colon taken from euthanized and necropsied calves and adults had no significant lesions. No histologic changes consistent with attaching-and-effacing lesions (16, 26, 29) or adherent bacterial layers (4) were seen in sections of intestine.

Duration and magnitude of *E. coli* O157:H7 shedding in feces. Feces from all animals inoculated with 10^{10} CFU of *E. coli* O157:H7 strain 3081 were culture positive. There was wide variation among individuals (within both age groups) in the magnitude and duration of detectable shedding. Despite the wide variation among individual animals, the shedding patterns of adults (Fig. 1A) and calves (Fig. 1B) were significantly different for days 1 to 13 ($P = 0.0001$) and days 14 to 100 ($P = 0.04$). Calves shed greater numbers of detectable organisms (CFU per gram) and shed them for a longer period of time. Eight calves (two groups of four) and nine steers were sampled

TABLE 1. Recovery of *E. coli* O157:H7 from cattle inoculated with the organism 2 to 18 days previously

Sample source	Recovery from adults necropsied at 2–4 days p.i.			Recovery from calves:					
				Necropsied at day 3 p.i.			Necropsied at days 14–18 p.i.		
	No. positive/ total	Mean[a]	Range[b]	No. positive/ total	Mean[a]	Range[b]	No. positive/ total	Mean[a]	Range[b]
Tonsil	0/3			3/3	4.3×10^2	$<2.0 \times 10^1$–1.2×10^4	1/6	$<2.0 \times 10^1$	
Liver	0/3			0/3			0/3		
Bile	0/3			0/3			0/3		
Spleen	0/3			0/3			0/3		
Kidney	0/3			0/3			0/3		
Rumen	3/3	2.9×10^1	$<2.0 \times 10^1$–8.0×10^1	3/3	9.4×10^2	4.5×10^2–2.5×10^3	3/4	$<2.0 \times 10^1$	2.0×10^1–1.5×10^2
Reticulum	1/3	$<2.0 \times 10^1$		3/3	1.7×10^3	6.4×10^2–7.5×10^3	1/4	$<2.0 \times 10^1$	
Abomasum	2/3	$<2.0 \times 10^1$	$<2.0 \times 10^1$–3.2×10^2	2/3	5.3×10^1	2.5×10^2–6.2×10^2	0/4		
LN-1[c]	0/3			0/3			0/6		
LN-2[d]	0/3			1/3	$<2.0 \times 10^1$		0/6		
LN-3[e]	0/3			2/3	$<2.0 \times 10^1$	$<2.0 \times 10^1$–3.5×10^1	1/6	$<2.0 \times 10^1$	
Jejunum	0/3			0/3			0/6		
Ileum	0/3			3/3	2.1×10^2	$<5.0 \times 10^1$–1.0×10^4	1/6	$<2.0 \times 10^1$	
Cecum	3/3	5.8×10^1	3.6×10^1–1.2×10^2	3/3	5.0×10^5	2.0×10^5–2.4×10^6	5/6	1.7×10^2	9.0×10^1–6.9×10^4
Colon	2/3	2.5×10^1	$<5.0 \times 10^1$–4.0×10^2	3/3	1.2×10^5	5.3×10^4–2.2×10^5	5/6	5.2×10^1	$<5.0 \times 10^1$–5.4×10^3
Feces	3/3	1.0×10^5	1.5×10^2–1.3×10^7	3/3	6.3×10^6	5.0×10^6–1.0×10^7	6/6	6.0×10^2	5.0×10^1–2.0×10^4

[a] Geometric mean recovery (CFU per gram).
[b] Range of recovery (CFU per gram).
[c] LN-1, mesenteric lymph node associated with anterior small intestine.
[d] LN-2, mesenteric lymph node associated with middle small intestine.
[e] LN-3, mesenteric lymph node associated with posterior small intestine.

at intervals beyond 2 weeks p.i. The two groups of calves were sometimes sampled on different days p.i. Calves were fecally positive for 2 (eight of eight calves), 7 (eight of eight), 14 (three of eight), and 20 (two of eight) weeks p.i. At the time this was written, one calf was still shedding at 27 weeks p.i. In contrast, steers were positive for 2 (nine of nine adults), 7 (two of nine), and 14 (one of nine) weeks p.i. Among calves, the longest detectable shedding was by a Holstein; in adults, it was by a Jersey steer. Total feces excreted in 48 h (data not shown) from four calves and two steers were collected and weighed to estimate the average daily fecal output of calves and adults. By using this fecal weight estimate and the CFU per gram predicted by the polynomials describing the group shedding patterns (days 1 to 13), we estimated that the total number of CFU shed by calves during this time period was greater than that of adults, even though the adults produced more feces. In contrast to the case for the adults inoculated with 10^{10} CFU, *E. coli* O157:H7 was recovered from none of the five adults inoculated with 10^4 CFU and from only two of the five adults inoculated with 10^7 CFU. The magnitude of shedding by the two infected animals was low ($<5.0 \times 10^1$ CFU/g).

To assess whether previous infection with *E. coli* O157:H7 would prevent reinfection by the homologous strain, four calves were reinoculated (10^{10} CFU, 13 to 14 weeks after the last positive fecal sample was collected). The calves were reinoculated at an age (8 to 10 months) when they were eating an adult diet and their rumen function status was assumed to be more like that of adults than that of milk-fed calves. Feces from the reinoculated calves were collected and cultured for 2 to 4 weeks p.i. (data not shown). All shed *E. coli* O157:H7 for at least 2 weeks following reinoculation. The day 1 to 13 shedding patterns of reinoculated calves were not significantly different ($P = 0.9$) from those of adults. When the shedding patterns of reinoculated calves and calves inoculated once were compared, the differences were significant ($P = 0.005$). The calves inoculated once shed significantly more CFU per gram. Two calves which were fecally positive for 14 and 20 weeks after primary inoculation were positive for 4 and 3 weeks, respectively, following reinoculation.

Distribution of *E. coli* O157:H7 at necropsy. *E. coli* O157:H7 was recovered from all of the animals necropsied (Table 1). The greatest recovery (CFU per gram) of *E. coli* O157:H7 was from the gastrointestinal tracts of calves necropsied at 3 days p.i. The tonsils, mesenteric lymph nodes, and ilea of calves were occasionally culture positive, but those of adults were not. *E. coli* O157:H7 was not recovered from the liver, bile, spleen, kidneys, or jejunum of either calves or adults.

DISCUSSION

Fecal shedding of *E. coli* O157:H7 (Fig. 1A and B) was significantly greater in calves (as a group) than in adults, a result which is consistent with those of farm surveys in which rates of isolation of *E. coli* O157:H7 from immature animals exceeded those of adults (1, 38). Adults have a fully developed forestomach compartment, the rumen, where the combination of a high volatile fatty acid concentration and a low pH inhibits the growth of *E. coli* O157:H7 (30). The shedding pattern difference between age groups was probably, at least in part, due to age-related differences in rumen function. There was no significant difference between the shedding patterns of the reinoculated calves and adults. The significant shedding pattern difference between calves inoculated once and reinoculated calves and between calves inoculated once and adults could be due to differences in gastrointestinal physiology due to age, diet, inoculation route (nursing bottle versus stomach tube), or an immune response. The shedding of *E. coli* O157:H7 by the reinoculated calves is consistent with maintenance of the organism within a herd.

Occasionally, a positive fecal sample was obtained from a previously culture-negative animal. Intermittent excretion probably reflects the sensitivity of the assay rather than spontaneous reinfection by other animals, since positive adults ($<10^3$ CFU/g) were housed adjacent to others which remained

consistently negative. Further, one calf tested positive ($<10^2$ CFU/g) for 6 weeks longer than a calf with which it had nose-to-nose contact.

Recovery of *E. coli* O157:H7 from tissues varied among animals of the same age group (Table 1). Numbers of recovered bacteria were usually greater in calves than in adults. At necropsy, the organism was isolated from the tonsils of several calves but not from those of adults. It is not known if *E. coli* O157:H7 was adhering to the tissue or if this was a transient association due to the route of inoculation, ingestion of fecal material, or regurgitation of stomach contents. Tonsillar isolates of SLT-producing *E. coli* from healthy cattle have been reported (12). Occasionally, low numbers of *E. coli* O157:H7 bacteria were recovered from calf mesenteric lymph nodes. Since *E. coli* O157:H7 is not considered to be invasive (20), the organism was probably translocated from the bowel to the lymph nodes (3). Because there was no recovery from the liver, bile, spleen, or kidneys, the *E. coli* O157:H7 bacteria in mesenteric lymph nodes were probably destroyed in the reticuloendothelial system and probably did not spread to other organs.

The most (CFU per gram) *E. coli* O157:H7 bacteria were recovered from the large bowels of both calves and adults. The trend of increasing numbers from the cecum to feces in adults could be due to multiplication during passage through the large bowel and concentration of digesta as it travelled to the anus.

Although there are reports of isolation from calves with diarrhea (5, 28), the organism is usually recovered from healthy cattle (6, 9, 23, 38); *E. coli* O157:H7 strain 3081 does not appear to be a pathogen of cattle. After inoculation, all of the adults and most of the calves remained healthy. Four calves had transient nonbloody diarrhea. These calves were not necropsied, nor were their feces examined for other possible pathogens. The cause of the diarrhea is unknown. Spontaneous, transient diarrhea occurs occasionally in otherwise healthy experimental calves (25).

E. coli O157:H7 strain 3081 produces attaching-and-effacing lesions in gnotobiotic pigs (10) and elaborates SLT. These are virulence attributes of *E. coli* O157 strains which cause disease in humans and some SLT-producing *E. coli* strains isolated from diarrheal calves (15, 26, 29, 32). The histologic sections of intestines from animals in this study were normal. The lack of evidence of adherent bacteria or attaching-and-effacing lesions in the large or small bowel may be due to tissue *E. coli* O157:H7 counts below 10^6 CFU/g, the threshold for recognition of adherent layers of bacteria in histologic sections (4). Subclinical infection of pigs with a pig isolate of SLT-producing *E. coli* frequently results in histologically detectable areas of vascular necrosis (19). No such lesions were detected here in cattle subclinically infected with SLT-producing *E. coli* O157:H7, nor have they been reported in cattle naturally infected with *E. coli* O157:H7.

The inoculation dose of farm-acquired *E. coli* O157:H7 necessary to establish shedding calves or adults is unknown. Our data indicate that 10^7 CFU of in vitro-grown bacteria are sufficient to infect some, but not all, adult cattle (an inoculum of $>10^7$ CFU was also required to generate an increase in serum antibody to O157 lipopolysaccharide [18]). Therefore, we chose an inoculum of 10^{10} CFU to establish experimental infections in our standard model. Most (16 of 17) calves shed $\geq 10^6$ CFU/g of feces, and some adults approached that peak magnitude of shedding for several days during the first week p.i. If the shedding patterns reported here are similar to those that occur on farms, then heavy contamination (≥ 10 g) by feces excreted during the high-shedding period may be required to propagate the infection within a herd. Alternatively, there may be highly susceptible individuals among which the infection is propagated or in vivo-grown organisms may be more highly infectious than those used here.

Because outbreaks of human illness have been attributed to consumption of cattle products and because of the widespread distribution of the organism in the cattle population, cattle have been considered by some as reservoir hosts of *E. coli* O157:H7 (6, 9, 38). Reservoir status could occur as the chance result of widespread exposure of cattle to the organism. It could also occur if cattle (in comparison with other animals) are biologically more susceptible to infection and prone to the carrier, shedder state. Our results do not support the latter hypothesis in that 1- to 3-day-old chickens are susceptible to infection by lower doses of *E. coli* O157:H7 and shed the organism in feces at higher numbers and for a longer duration (2, 31, 34, 35) than did the cattle and calves in the current study. These apparent differences between cattle and chickens could reflect real species differences in susceptibility. Alternatively, they could reflect differences in the innate colonizing abilities of the *E. coli* O157:H7 strains used in the cattle and chicken experiments or the fact that the cattle used here were older than the chickens used by others (2, 31, 34, 35).

The magnitude and duration of *E. coli* O157:H7 excretion by calves in this study suggest that interventions to reduce on-farm spread of the organism in this population are warranted. However, since most ground beef is derived from adults, reduction of *E. coli* O157:H7 in this population is critical. Research on strategies to reduce exposure and eliminate long-term shedding by both calves and adults is needed. The bovine model of infection described here can be useful in that research.

This study demonstrated that calves and adult cattle experimentally infected with *E. coli* O157:H7 usually remained healthy. A wide variation in the magnitude and duration of fecal excretion of *E. coli* O157:H7 by animals of similar ages was observed. As a group, calves shed *E. coli* O157:H7 in greater numbers and for a longer duration than did adults. However, shedding by individual animals from both groups persisted for months. *E. coli* O157:H7 was recovered from the alimentary tracts of all of the animals necropsied; however, there was no evidence of spread to the liver, spleen, or kidneys. A focus of infection was not identified, and all histologic sections of tissues taken at necropsy were normal. Calves no longer excreting *E. coli* O157:H7 shed the organism after reinoculation with the same strain.

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