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Preventive Veterinary Medicine 35 (1998) 11–19

PREVENTIVE VETERINARY MEDICINE

Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the Northwestern USA

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Accepted 2 December 1997

Abstract

Samples from cattle, other domestic and wild animals, flies, feeds, and water-troughs were collected from 12 cattle farms and tested for *Escherichia coli* O157. *E. coli* O157 was isolated from bovine fecal samples on all 12 farms with a within herd prevalence ranging from 1.1% to 6.1%. *E. coli* O157 was also found in 1 of 90 (1.1%) equine fecal samples, 2 of 65 (3.1%) canine fecal samples, 1 of 200 pooled bird samples (0.5%), 2 of 60 pooled fly samples (3.3%), and 10 of 320 (3.1%) water-trough sample sets (biofilm and water). No *E. coli* O157 were isolated from 300 rodents, 33 cats, 34 assorted wildlife, or 335 cattle feed samples. Indistinguishable pulsed-field gel electrophoresis patterns of *Xba*I digested chromosomal DNA and Shiga toxin types were observed for bovine and water-trough isolates from two farms and for one equine and two bovine isolates from one farm. © 1998 Elsevier Science B.V.

Keywords: Cattle—microbiological diseases; *Escherichia coli*; USA

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PII S0167-5877(98)00050-6

1. Introduction

Although *Escherichia coli* O157 exists in most cattle operations, the prevalence with which it exists is highly variable among herds (Hancock et al., 1994, 1997a,b,c). Furthermore, the shedding of *E. coli* O157 appears to be strongly clustered temporally such that longitudinal sampling within a herd may reveal few or no *E. coli* O157-positive samples for months followed by a burst of isolations from cattle (Hancock et al., 1997a,b). These findings suggest the possibility of a reservoir for *E. coli* O157 external to cattle. Naturally occurring *E. coli* O157 has been reported in non-bovine species (Rice et al., 1995; Kudva et al., 1996) but the relative importance of other species in the ecology of this organism remains unknown. It is also possible that environmental sources (especially feed and water) may play a role in transmission of *E. coli* O157 within and between farms.

The purpose of the present study is to investigate the non-bovine sources of *E. coli* O157 on cattle farms, specifically: (1) whether *E. coli* O157 can be found in feces of non-bovine species on cattle farms (2) whether *E. coli* O157 strains isolated from non-bovine species are of indistinguishable subtypes as those found in bovines on the same farm, and (3) whether cattle feed and water contain *E. coli* O157.

2. Materials and methods

2.1. Sampling design

Four cattle farms were selected in each of the states of Idaho, Oregon, and Washington for a total of 12 farms. Two of the farms from each state were cattle feedlots and two were dairies. Farms were selected primarily on the basis of their willingness to cooperate in the study, however, all farms were typical for this region of the USA. The six study feedlots ranged in size from 250 to 14 750 total cattle with a median of 1550. The six dairies ranged in size from 290 to 1700 total cattle (all ages) with a median of 904. Domesticated species other than cattle were present on all of the farms, and all farms reported that a variety of birds were frequently present.

Sampling was scheduled to occur on three occasions separated by approximately one month. The first sampling trips were made to each farm in July and August and all sampling was completed by mid-November. Additional sampling trips were necessary (beyond the scheduled three) in order to collect sufficient numbers of non-bovine samples. These were interspersed with scheduled visits.

2.2. Sampling methods

2.2.1. Bovine fecal samples

On each of the three sampling visits to each feedlot, 10 fresh fecal pat samples were collected from each of six pens: the three pens containing those cattle which had been present in the feedlot for the shortest period of time and the three pens containing those cattle which had been present for the longest period of time. On each of the three

sampling visits to each dairy, 60 fresh fecal pat samples, equally distributed among pens containing heifers aged 6–24 months, were collected. Fresh fecal pats were sampled by insertion of a cotton-tipped swab, removal of excess feces, and placement into a tube containing tryptic soy broth (Difco, Detroit, MI) containing 40 μg/ml vancomycin (Abbott Laboratories, Chicago, IL) and 50 μg/ml cefixime (Wyeth-Ayerst Research, Pearl River, NY) (TSBcv).

2.2.2. Other livestock fecal samples

Fecal samples from each non-bovine livestock animal on all farms were sampled by fecal pat swabs or by collecting single fecal pellets (depending on the form of the stool) which were then placed into culture tubes containing TSBcv.

2.2.3. Non-domesticated animal fecal samples

At least 5 h were expended per farm during the course of the study in search of wildlife fecal samples. Pelleted feces were collected as single pellets; soft feces were sampled using swabs, and then placed into tubes containing TSBcv.

2.2.4. Canine and feline fecal samples

Swabs of apparently fresh feces or rectal swabs were collected and placed into tubes containing TSBcv. All available fecal piles and animals were sampled.

2.2.5. Bird fecal samples

On each farm pooled samples of bird feces were collected from fences and roosting areas using a sterile wooden applicator and placed into a sterile container. Approximately 3–10 g were collected on each visit. A limited number of dead birds were found on some farms. For these birds the intestinal contents were sampled using cotton tipped swabs as described for cattle fecal samples.

2.2.6. Rodent samples

Twelve live traps (Victor Tin Cat, Woodstream, Lititz, PA) were set per farm in feed-storage areas and other areas suspected to be frequented by rodents. Additional rodents were captured directly from nesting areas. Captured rodents were euthanized using carbon dioxide gas and placed into sterile bags.

2.2.7. Fly samples

The contents of two or more fly traps (Victor Flying Insect Trap, Woodstream, Lititz, PA) were collected per farm per sampling visit. A small jar of autoclaved ground beef was used as an attractant. Traps were left hanging for at least 5 days, after which the captured flies were killed using a pyrethrin-based insecticide, and the flies transferred to a sterile container.

2.2.8. Water-trough and biofilm-trough samples

On each scheduled farm visit all water-troughs (maximum of 10) were sampled. Where more than 10 water-troughs were present, those located in pens containing sampled cattle were preferentially sampled. From each selected water-trough, both the

water and the biofilm on the sides and bottom of the trough were sampled. At least 50 ml of water were collected from each trough by placing the mouth of a sterile plastic sampling jar at the water surface. Biofilm samples were collected using a sterile wooden tongue depressor and glove.

One to three well-water samples (500 ml) were collected from nine of 11 farms that used well water for cattle water-troughs.

2.2.9. Cattle feed samples

All component feeds (prior to further mixing beyond the form in which they were received by the farm) from each farm were sampled (50 g).

2.3. Shipment and handling of samples

All samples were shipped to the laboratory by overnight delivery. Feed samples were shipped in an insulated container without ice, and all other samples were shipped on ice.

2.4. Laboratory methods

2.4.1. Fecal pat and rectal swab samples from all species

All samples were cultured by incubating in TSBcv at 37°C for 16–24 h, followed by plating on a modified Sorbitol MacConkey agar plate. *E. coli* O157 isolation from fecal samples was based on lack of sorbitol fermentation and beta-glucuronidase activity and possession of both O157 antigen and Shiga toxin genes. The detailed methods for isolating *E. coli* O157 are described elsewhere (Sanderson et al., 1995).

2.4.2. Composite bird fecal samples

At the laboratory, 10 ml of TSBcv were added to each sample and then cultured as for fecal samples.

2.4.3. Rodent fecal samples

At the laboratory, up to 10 fecal pellets from each euthanized rodent were extracted, added to 3 ml TSBcv, and cultured as for fecal samples.

2.4.4. Fly trap samples

To each fly-trap composite sample, 10 to 50 ml of TSBcv (dependent upon the number of flies) were added to each bag and then homogenized (Stomacher 80, Seward Medical, London, UK). The samples were then cultured as described for fecal samples.

2.4.5. Water-trough samples

For each sample, 30 ml of trough water were transferred into a sterile specimen cup and combined with 30 ml of a 2 × concentrate of TSBcv, mixed, and incubated overnight at 44°C. The samples were then cultured as described for fecal samples. The use of 44°C rather than 37°C for an incubation temperature was based on pilot studies that found bacterial overgrowth to be common for water-trough samples incubated at 37°C. Also, 100 *E. coli* O157 isolates from numerous sources grew as well at 44°C as at 37°C in enrichment broth and on the modified Sorbitol MacConkey agar plates.

2.4.6. Trough biofilm samples

For each sample, 20 to 50 ml of a 2× concentrate of TSBcv (dependent on the volume of the sample) were added after which the suspension was briefly shaken and then incubated overnight at 44°C. The samples were then cultured as described for fecal samples.

2.4.7. Well water samples

Well water samples were analyzed by filtration through a 0.45 μm pore size membrane filter. The filter was placed into 50 ml of TSBcv and then cultured in the same manner as fecal samples.

2.4.8. Cattle feed samples

For each sample 15 g of feed were placed into a sterile specimen cup and then mixed with 60 ml of TSBcv. Samples were incubated overnight at 44°C (see justification for use of 44°C vs. 37°C in methods for water-trough samples). The samples were then cultured as described for fecal samples.

2.4.9. Subtyping

Isolates were typed by comparison of both pulsed field gel electrophoresis (PFGE) patterns of *Xba*I digested chromosomal DNA, and by Shiga toxin type. Chromosomal DNA was extracted (Barrett et al., 1994), and PFGE performed (CHEF DR-II electrophoresis unit, BioRad Laboratories, Hercules, CA) at 6 V/cm of gel for 25 h using a linear ramp of 5 to 50 s. Shiga toxin typing was performed using either a polymerase

Table 1
Results of *E. coli* O157 cultures from cattle feces, feces of other animals, and environmental sources in 12 Pacific Northwest cattle farms

Source of sample	Number tested		Samples/farm		Number positive		Number positive/farm	
	Samples	Farms	Min.	Max.	Samples (%)	Farms	Min.	Max.
Cattle—total	2143	12	132	196	63 (2.9)	12		
Feedlot	1046	6	132	180	38 (3.6)	6	3	11
Dairy	1097	6	179	196	25 (2.3)	6	2	7
Horse	90	7	2	23	1 (1.1)	1	0	1
Dog	65	8	1	25	2 (3.1)	1	0	2
Cat	33	3	4	24	0 (0.0)	0	0	0
Rodent	300	11	3	105	0 (0.0)	0	0	0
Bird (pooled)	200	12	9	32	1 (0.5)	1	0	1
Fly (pooled)	60	11	2	10	2 (3.3)	2	0	1
Wildlife	34	6	1	14	0 (0.0)	0	0	0
Cattle feed	335	12	10	42	0 (0.0)	0	0	0
Trough water	327	12	14	30	4 (1.2)	3	0	2
Trough biofilm	320	12	13	30	6 (1.9)	5	0	2
Trough (either water or biofilm)	320	12	13	30	10 (3.1)	5	0	4
Source water	13	9	1	3	0 (0.0)	0	0	0

chain reaction technique (Begum et al., 1993) or DNA–DNA colony hybridization (Newland and Niell, 1988; Riely and Caffrey, 1990).

3. Results

E. coli O157 was isolated from bovine fecal samples on all farms (Table 1). The within-farm prevalence ranged from 1.1% to 6.1%. Among 1046 fecal samples collected in feedlots, 38 (3.6%) were positive. Among 1097 fecal samples collected in dairy farms, 25 (2.3%) were positive.

E. coli O157 was isolated from the feces of one horse and two dogs, from one pooled bird fecal sample, and from two composite fly samples and 10 water-trough samples (Table 1). The positive horse, one positive fly sample, and three positive water-trough samples were from dairy farms; the remaining positive samples from non-bovine sources were from feedlots. One or more non-bovine animals were *E. coli* O157-positive on

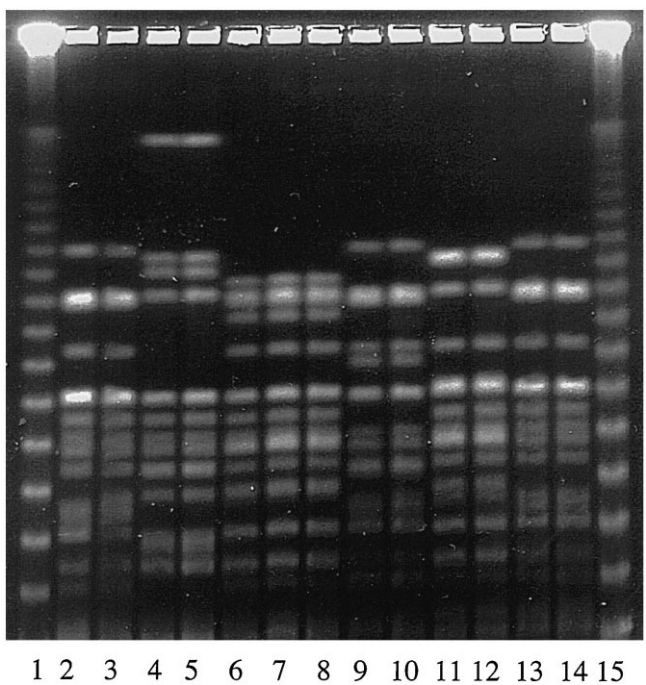

Fig. 1. *E. coli* O157 subtypes isolated from multiple sources on cattle farms. Classification of subtypes was based on pulsed-field gel electrophoresis of *Xba*I digested chromosomal DNA. Lanes one and 15 are λ DNA concatemer molecular wt standards, lanes two and three are cattle and water-trough-biofilm isolates from Farm A. Lanes 4 and 5 are cattle and water-trough-biofilm isolates from Farm B. Lanes six, seven and eight are cattle, and horse isolates from Farm C. Lanes nine and ten are dog and water-trough isolates from Farm A. Lanes 11 and 12 are water-trough and water-trough-biofilm isolates from Farm A. Lanes 13 and 14 are water-trough and water-trough-biofilm isolates from Farm D.

four farms (two feedlots and two dairies). *E. coli* O157 was not detected in samples from rodents ($n = 300$), cats ($n = 33$), wildlife ($n = 34$), or cattle feed samples ($n = 335$).

A total of 327 water-trough and 320 biofilm-trough samples were cultured, of which four (1.3%) water and six (2.0%) biofilm samples yielded *E. coli* O157 (Table 1). Among the 320 matched sets of water-trough and biofilm-trough sampled from the same troughs, none yielded *E. coli* O157 in both samples; thus, a total of 10 of the 320 (3.1%) troughs cultured by both methods were positive for *E. coli* O157. One or more water-troughs (water and/or biofilm) were *E. coli* O157-positive on five farms. Only one of the five farms from which *E. coli* O157 was recovered from water-troughs reported cleaning water-troughs during the study period. In contrast, trough cleaning during the study period was reported in five of the seven farms from which *E. coli* O157 was not recovered from water troughs ($p = 0.12$).

A comparison of *E. coli* O157 isolates by pulsed-field gel electrophoresis and Shiga toxin type demonstrated that on three farms, bovine isolates were indistinguishable from non-bovine isolates (Fig. 1). On two farms, bovine *E. coli* O157 isolates were indistinguishable from water-trough-biofilm isolates, and on one farm, two bovine isolates were indistinguishable from an equine isolate. On one farm, a canine isolate and a water-trough isolate were indistinguishable, and on two farms a water-trough isolate and a biofilm isolate (from separate sampling dates) were indistinguishable.

4. Discussion

At least one standard disease control text states that cattle are the reservoir for *E. coli* O157 (Bennenson, 1995). It has been clear for some time that beef products are a source of *E. coli* O157 in human food-borne disease outbreaks (Griffin and Tauxe, 1991; Barrett et al., 1994; Armstrong et al., 1996) and that live cattle are a source for meat contamination (Chapman et al., 1993; Hancock et al., 1994, 1997a,b,c; Rice et al., 1997). Yet, for purposes of control, it is important to distinguish the concept of the reservoir—the species or habitat in which the organism is sustained over the long term —from that of an incidental host which merely serves as a vehicle for transmission. Based on the evidence from the present study as well as from previous ones (Kudva et al., 1996; Rice et al., 1995), *E. coli* O157 can, at least transiently, colonize a number of species other than cattle which clearly raises questions about cattle being the reservoir species. Furthermore, sufficient data do not exist to unequivocally designate cattle as a reservoir species.

Beyond cattle, the ecology of the agent has not been investigated with extensive longitudinal studies in natural populations. In cattle the agent seems to colonize only transiently, and long term carriers have not been found (Besser et al., 1997). The shedding of *E. coli* O157 in herds of cattle is intermittent to the degree that the agent cannot be detected on a majority of sampling visits (Hancock et al., 1997a,b). These findings do not exclude cattle being a reservoir for *E. coli* O157 since latent infections could account for the pattern. Yet, it would be inappropriate to assume in the absence of data that latent infections necessarily account for the intermittent shedding and lack of

detectable carriers. The pattern of detectable shedding in cattle seems completely compatible with that expected in an incidental host. From this perspective, the hypothesis that cattle are the reservoir species for *E. coli* O157 has been prematurely accepted.

At least three models could account for the data in the present study and those in the published literature. First, *E. coli* O157 may have multiple reservoir species including, or not including, cattle. Second, *E. coli* O157 may be able to transiently colonize many species—but one or more species serve as the reservoir. Third, as suggested by the relatively high prevalence of *E. coli* O157 from water-troughs, the agent could possibly have an environmental reservoir, such as the sedimentary layer of water-troughs. The hypothesis of an environmental reservoir is in conflict with the common view that *E. coli* are obligate gut parasites which can survive transiently in the environment but not multiply. Yet, various strains of *E. coli*, notably including *E. coli* O157, are able to multiply prolifically in the environment if provided with moisture and a nutrient source (Lynn et al., 1998). Marine sediments have been found to provide an environment in which *E. coli* have sufficient nutrients to survive and multiply (Davies et al., 1995).

5. Conclusion

The means by which *E. coli* O157 is sustained and transmitted on farms is unknown, but the present study rules out those models which require strict host-specificity of the organism for cattle. It also raises the possibility that a reservoir other than cattle might exist.

Acknowledgements

This research project was funded by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services and USDA NRICGP Grant No. 94-37201-1217. The authors thank the many field personnel—both state and federal— who provided an invaluable service to this research project.

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