Effects of Farm Manure-Handling Practices on Escherichia coli O157 Prevalence in Cattle

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ABSTRACT

Thirty-six dairy herds in Idaho, Oregon, and Washington were selected on the basis of cattle housing and manure-handling practices. Approximately 60 fecal samples from heifers were collected monthly in each herd for 6 months and cultured for Escherichia coli O157. One hundred seventy-nine of 12,664 (1.41%) individual fecal samples from 27 of the 36 herds (75%) were culture positive for E. coli O157. Within-herd prevalence ranged from 0% to 5.5% with a strong clustering toward the lower end of this range. A tendency was observed for herds to maintain either a relatively low or high prevalence of E. coli O157. Prevalence of E. coli O157 was similar in herds which housed heifers in dry lots and on pasture with and without application of manure. Also, application of manure to cattle forage crops was not associated with the prevalence of E. coli O157 in dairy herds.

Key words: E. coli O157, cattle, manure, epidemiology, grazing, forage crops; pasture

Since it was first identified in 1982, Escherichia coli O157 has become recognized as an important agent of food-borne disease with worldwide distribution (6, 12–15). Although cattle have been demonstrated to be a major source for human exposure, other possible sources have not been ruled out, and it has not been demonstrated whether cattle are a reservoir or an incidental host (3, 5, 7, 8, 19). Diversity in the prevalence of E. coli in cattle has been demonstrated and variables that affect this diversity have been tentatively identified (10, 11). If farm management variables that affect E. coli O157 prevalence in cattle can be confirmed, then these variables might be exploited to reduce its prevalence in cattle, leading to a potential increase in the safety of food products of bovine origin. Since E. coli O157 does not cause disease in cattle, the justification for changes in management practices used by cattle producers must arise from the economics of food-safety considerations alone.

In initial studies on the epidemiology of E. coli O157 in cattle the organism was found in a minority of herds (<10%) and at a very low prevalence (<0.3%) (6, 7). However, these studies were not optimally designed to provide reliable estimates of herd prevalence or the degree to which within-herd prevalence varies.

The present study was designed to estimate the prevalence of E. coli O157 in 36 Pacific Northwest (PNW) dairy herds and to determine if an association exists between manure-handling practices and the prevalence of E. coli O157.

MATERIALS AND METHODS

Study herds

Thirty-six herds, 12 each in the states of Idaho, Oregon, and Washington were selected on the basis of (i) owner willingness to allow access to their dairy herd, (ii) the availability of at least 60 post-weaned heifers for sampling throughout the 6-month study period, and (iii) the use of specific housing practices for heifer cattle. The 12 herds in each state were allocated into three groups on the basis of the following heifer management categories: heifers housed in dry lots (group D), heifers housed on pastures on to which manure was applied (group M), and heifers housed on pasture which had not had any manure applied to grazing land in the current growing season (group P). After allocation of herds in this manner the owner of one P herd in Washington began applying manure to pasture where the heifers were housed, so that herd was reclassified as an M herd.

Sample collection

Each herd was sampled monthly for 6 months (July to December 1994) with the exception of two herds which were sampled five times due to lack of access to the herd on the final sampling date. Approximately 60 fresh fecal samples were collected from post-weaning heifers in each herd as follows: Fresh fecal pats were sampled by evenly coating a cotton-tipped swab with feces and then placing the swab into a culture tube containing 3 ml of tryptic soy broth (Difco Laboratories, Detroit, MI) to which had been added 50 ng of cefixime (Wyeth-Ayerst Research, Pearl River, NY) and 40 μg of vancomycin (Abbott Laboratories, Chicago, IL) per ml (TSBcy). Also collected were two 50-ml samples of manure from the manure storage area on each of
two consecutive monthly visits to each of the 12 herds classified as M herds. All fecal and manure samples were maintained under refrigeration and shipped on ice for overnight delivery to the laboratory.

Bacteriological methods
After arrival at the laboratory, fecal samples were briefly agitated and then incubated for 18 to 24 h at 37°C. Detection of E. coli O157 in fecal samples was performed as described elsewhere (16), and was based on a lack of sorbitol fermentation, the ability to ferment lactose, a lack of beta-glucuronidase activity, and agglutination with O157 antisera. All E. coli O157 isolates were assayed for Shiga-like toxin-coding genes using a polymerase chain reaction (PCR) assay utilizing primers specific for SLT-I and SLT-II (2). Fecal coliform counts for each manure sample was estimated using a membrane filter technique following standard methods (4).

Dairy herd management questionnaire
On the first visit to each dairy, information regarding weaning methods for calves, feeds and feeding methods for heifers and cows, and use of antibiotics and ionophores was collected. On each sampling visit a brief questionnaire was completed to verify that herds remained properly classified for manure-handling practices and to determine if changes in the way the herd was managed had been implemented.

Data analysis
The Kruskal-Wallis nonparametric analysis of variance was used to compute index P values between the distribution of E. coli O157 prevalence in different heifer cattle groups and manure-handling practices (1).

RESULTS

A total of 12,664 fecal samples were collected from heifers during 214 visits to the 36 study farms. Of these, 179 (1.41%) were culture positive for E. coli O157, and all isolates possessed genes coding for Shiga-like toxin types I, II, or both I and II. The organism was detected in 27 of the 36 herds (75%). Within-herd prevalence ranged from 0% to 5.5% with a strong clustering toward the lower end of this range (Figure 1). The highest E. coli O157 prevalence noted in any herd on any single sampling date was 26.7%. One hundred and forty-seven of the 214 (68.9%) sampling visits yielded no positive samples. A moderate correlation was noted of within-herd prevalence with successive visits, indicating a tendency for herds to maintain relatively low or high prevalence status (Table 1).

Thirteen of the study herds housed heifers that were exposed to manure-applied pasture (M) at some time during the study. These herds had a distribution of E. coli O157 prevalence very similar to that of herds in which heifers were grazed on pasture to which manure had not been applied during the current growing season (P) and herds in which heifers were maintained in dry lots (D). A slightly higher proportion of M herds (85%) did have at least one positive sample when compared to D (82%) or P (66%) herds. Yet, median within-herd prevalence of E. coli O157 was highest for D herds (1.66%) compared to 0.42% for P herds and 0.83% for M herds (P = .49) (Figure 2). In seven herds in which a <15-day waiting period was observed after manure application prior to grazing, the median prevalence of E. coli O157 was 0.83%, compared to 1.09% in other herds. In five herds where manure was applied to heifer pasture and there was less than 30 days of manure storage, the median prevalence of E. coli O157 was 0.83%, compared to 1.09% in other herds.

When E. coli O157 prevalences were computed on the basis of heifer environment at the time samples were collected, heifers in dry lots tended to have a higher prevalence throughout the sampling period than did pastured heifers, although this difference was not statistically significant. The prevalence of E. coli O157 in all herds declined sharply during the final sampling period (28 November to 20 December). A total of 1,057 samples were collected from animals grazing pasture to which manure had been applied in the last 30 days. Seventeen of these samples were positive for E. coli O157 (1.6%), compared to 127 of 9,478 (1.3%) in samples collected from heifers not grazing on land to which manure had been applied during the last 30 days (P = .48).

Among 17 herds where manure was applied to grain and/or truck crops (such as potatoes, peas, and asparagus) the median prevalence of E. coli O157 was 0.55% compared to 1.66% in other herds. Among 26 herds where manure was applied to forage crops the median prevalence of E. coli O157 was 1.37% compared to 0.06% among other herds.

In this study the herd prevalence (percentage of herds in which the agent occurs) of E. coli O157 in Pacific Northwest dairy herds was 75%. It seems likely that additional sampling would have resulted in the detection of E. coli O157 on at least some of the nine farms where it was not found during the six sampling visits. These findings are in contrast to herd prevalence estimates of <10% made in previous studies (7, 8). The differences between prevalence found in the current study and those in previous studies are most likely associated with the longitudinal sampling design of the present study and the use of a more sensitive culture method to detect E. coli O157. The current study detected E. coli O157 in several herds in which the organism was not detected in previous studies utilizing a single-time-point sampling with fewer animals sampled. Furthermore, one-time-point sampling of herds will reduce the power to detect E. coli O157 if shedding is temporally clustered. The present study provides strong evidence of temporal clustering, as demonstrated by the fact that 68.9% of sampling visits resulted in no positive samples even though 75% of the herds were eventually found to be positive.

The present study could be criticized in that only a relatively small number of herds (n = 36) were sampled and that herds were not selected in a truly random manner. However, the intensive sampling employed in each herd could not have been extended to a larger number of herds without substantially increasing the resources involved in collecting and culturing the samples. Although herd selection was not random, there was no a priori reason to believe that selected herds would have a greater or lesser E. coli O157 prevalence than other Pacific Northwest herds.

The main implication of the nearly ubiquitous distribution of E. coli O157 in dairy herds relates to the potential efficacy of trace-back programs using E. coli O157 positives identified at slaughter plants or through human disease investigations. Presumably, a trace-back program would work only if a very small percentage of herds represented the source for human food-borne exposure to E. coli O157.

One competing alternative to trace-back programs as a preharvest strategy involves the identification of farm-management factors which modulate the prevalence of E. coli O157 in cattle herds. If such factors exist, they could be exploited in an effort to reduce the overall prevalence of the agent in cattle populations. The wide variation of within-herd prevalence observed in the present study is solid evidence for differences in E. coli O157 ecology among herds and, by inference, for the existence of modulating factors associated with these differences. The correlations among herd prevalence observed during the six sampling periods indicate that the tendency for herds to have either a low or high prevalence is a relatively stable phenomenon, indicating that there may indeed be management variables that modulate the ecology of E. coli O157.

The suspected modulating factor of central interest in the present study was the application of manure to heifer grazing pastures and cattle forage crops. An earlier study had shown a tentative relationship between application of manure to grazing land and prevalence of E. coli O157 in a herd (8). The hypothesis was tested in the present study by selecting equal numbers of herds in which heifers were maintained on pasture with manure applied, on pasture to which manure had not been applied, and in dry lots. The failure to see a higher prevalence in herds in which heifers were grazed on pasture with manure applied is strong evidence that manure application to pasture land is not an important risk for E. coli O157 transmission in dairy heifers.

An alternative to spreading manure on grazing land is to spread it on land where cattle forage crops are grown. The present study provides some evidence of an association between application of manure to roughage crops and E. coli O157 prevalence. Yet, when the subset of herds where manure was applied to roughage crops within 30 days of harvest was compared to herds where manure was not applied, no hint of a higher prevalence of E. coli O157 was observed. Given the reportedly short survival time of E. coli after application of manure onto pasture or crops (9), the effect of manure application to forage crops on E. coli O157 prevalence would be expected to be the strongest in herds which had a short period between application of manure and harvest. Thus, the destination and handling practices of stored manure appear to have little or no impact on the ecology of E. coli O157.
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REFERENCES