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Factors Associated with the Presence of Coliforms in the Feed and Water of Feedlot Cattle

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The objective of this study was to investigate coliform counts in feedlot cattle water and feed rations and their associations with management, climate, fecal material, and water *Escherichia coli* O157 using a cross-sectional study design. Coliform counts were performed on feed samples from 671 pens on 70 feedlots and on water samples from 702 pens on 72 feedlots in four U.S. states collected between May and August 2001. Management and climate factors were obtained by survey and observation. Month of sampling (higher in May and June), presence of corn silage in the ration (negative association), temperature of the feed 1 in. (ca. 2.5 cm) below the surface at the time of sampling (negative association), and wind velocity at the time of sampling (positive association) were significantly associated with $\log_{10}$ coliform levels in feed. Month of sampling (lower in May versus June July and August), water pH (negative association), and water total solids (positive association) were significantly associated with $\log_{10}$ water coliform levels. Coliform counts in feed and water were not associated with prevalence of *E. coli* O157 in cattle feces or water. Management risk factors must be interpreted with caution but the results reported here do not support the use of coliform counts as a marker for *E. coli* O157 contamination of feed or water.

Coliform bacteria, including *Escherichia* spp., *Klebsiella* spp., and *Enterobacter* spp., are considered to be an indicator of fecal contamination in feed and water. As such they may be an indicator of contamination of feed and water with fecally transmitted food-borne disease agents such as *E. coli* O157 and *Salmonella* spp. The epidemiology and ecology of *Salmonella* spp. and *E. coli* O157 suggest fecal contamination of feed or water may be a possible source of exposure to cattle on farm (5, 6, 29). On-farm control efforts have received considerable attention and disease modeling suggests that decreased shedding in feces could decrease beef contamination with *E. coli* O157 (14). If feed and water are a significant source of exposure for cattle to potential food-borne pathogens, on-farm control efforts to decrease fecal prevalence will need to account for feed and water contamination through a feed and water safety and security program.

Salmonellae have been commonly found in feed, but until recently *Escherichia coli* O157 had only been found rarely in cattle feed (5, 9, 11, 12, 25). *E. coli* O157 has been shown experimentally to survive and even replicate in moistened feed at room temperature, and replication of generic fecal *E. coli* has also been demonstrated in livestock feeds (17). *Escherichia coli* O157 have recently been detected in significant numbers of feed samples in a study to assess the effects of culture techniques on isolation of *E. coli* O157 from feed (6). *E. coli* O157 has been commonly found in water sources, including tanks and ponds, and free-flowing streams (7, 11, 16, 21). In an experimental water microcosm model, *E. coli* O157 survived for at least 245 days (15).

We hypothesized that if feed or water is a source of *E. coli* O157, feed and water coliform levels might be a marker for *E. coli* O157 contamination and subsequent cattle exposure. If so, coliform levels might provide a simple method of monitoring feed and water quality and safety in the feedlot. The objective of the analysis reported here was to investigate coliform counts in feedlot feed and water samples, and their association with water and fecal *E. coli* O157, as well as management and climate factors in midwestern U.S. feedlots.

MATERIALS AND METHODS

Feedlot selection and sample collection. The study population was comprised of commercial feedlots in four states in the United States (Kansas, Nebraska, Oklahoma, and Texas). The data for this analysis are based on methods previously described (23). Feedlots reported on in this study are a subset of those reported previously including only those where feed and water was collected and analyzed for coliforms. Briefly, the authors selected feedlots based on previous contact, geographic location, and willingness to participate. Each feedlot was visited once between May and August of 2001 and up to 10 pens per feedlot were included in the study. Selected pens contained cattle receiving their final feedlot ration and were within 1 month of anticipated market date. If more than 10 pens on a feedlot met the inclusion criteria, the 10 pens closest to the market date were selected. If less than 10 pens met the inclusion criteria, then all pens that did so were selected.

A single feed sample of approximately 1 kg was collected by combining 10 grab samples from multiple areas in the feedbunk. The feed samples were collected from the bunk without regard to whether cattle had already accessed the feed. Within each sampled pen, 15 cattle were observed to defecate and the fresh fecal

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samples were collected off the pen floor. Three water samples and two water tank sediment samples were obtained from a single water tank in each pen for *E. coli* O157 culture. A single water sample was taken from each tank for coliform and total solids analysis at a private lab (SDK Labs, Hutchinson, Kansas). Samples were identified by feedlot, pen and sample type at the time of collection. Collected samples were stored on ice and shipped overnight to the laboratory at Kansas State University. Sample processing began within 24 h of collection.

Standard coliform counts. Total coliforms (*Escherichia* spp., *Klebsiella* spp., and *Enterobacter* spp.) were quantified in water by most probable number quantification using protocol 9223 from Standard Methods for the Examination of Water and Wastewater (1) and recorded as the quantity per ml of sample. Serial dilutions used allowed a maximum count of 1.21×10^4 CFU/ml to be quantified. Counts higher than this maximum were recorded as the maximum value. Total suspended solids were recorded as the quantity per ml and determined using protocol 2540D from Standard Methods for the Examination of Water and Wastewater (1).

Coliform counts were performed on feed samples collected from individual pens in each feedlot. After feed samples arrived at the laboratory each sample was thoroughly mixed and ten grams of feed was placed in a sterile plastic bag containing 90 ml of distilled water. This sample was mixed for 30 sec, and serially diluted to provide 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions. From each dilution, 100 μl was spread plated onto MacConkey agar and incubated at 37°C for 18 h. Following incubation the number of colonies on each plate was counted, and a coliform count for the sample was calculated.

Identification of *E. coli* O157 in feed, feces, and water. Feed was collected and cultured as part of a cross-sectional survey on management associations with *E. coli* O157 in feedlots (22, 23, 24), and the sensitivity of detection of *E. coli* O157 in feed samples (6). Feed *E. coli* O157 culture results were only obtained on a subsample of the data reported here (504 pens, 54 feedlots). Briefly, for the feed samples two enrichment methods were used in parallel. For each method 10 g of feed was added to 90 ml of enrichment medium and incubated for 6 h at 37°C (6). Fecal samples were cultured by the addition of 1 gram of feces to 9 ml of gram-negative broth containing 0.05 μg/ml cefixime, 10 μg/ml cefsulodin, and 8 μg/ml vancomycin, and samples were incubated at 37°C, for 6 h. Water and sediment samples were vortexed and 5 ml of water or sediment was added to 5 ml double-strength tryptic soy broth (Difco, Detroit, MI) and incubated for 24 h at 44°C.

Following incubation, the identification protocol for fecal feed and water samples was the same. Samples were vortexed and 1 ml of the enrichment broth was added to 20 μl Dynabeads (Dynal, Inc., Lake Success, NY) for immunomagnetic separation. After immunomagnetic separation, 50 μl of the sample was spread plated on Sorbitol MacConkey agar plates supplemented with cefixime (0.05 μg/ml) and tellurite (2.5 μg/ml) and incubated overnight at 37°C. Following incubation up to six sorbitol-negative colonies with typical *E. coli* O157 morphology were picked onto blood agar plates using sterile toothpicks. The blood agar plates were incubated overnight at 37°C and an indole test was performed on each colony. Colonies with a positive indole reaction were checked for the O157 antigen with a latex agglutination assay (Oxoid, Basingstoke, Hampshire, United Kingdom). Agglutination-positive colonies were confirmed as *E. coli* by Rapid A.P.I. tests (bioMerieux, Hazelwood, MO).

Collection of feedlot data. Feedlot management data were collected using a personally administered questionnaire. One of six field-sampling personnel on the project interviewed the feedlot manager at each sampled feedlot. Additional feedlot data were accumulated by observation at the feedlot by the field-samplers. The questionnaire collected data on management and climate factors to assess their association with coliform counts in cattle feed as well as *E. coli* O157 in feed, feces, and water (25, 27). A hand-held pH meter (pHep3, Hanna Instruments, Woonsocket, RI) was used to test water pH in each of the sampled water tanks. A hand-held weather meter (Kestrel 3000, Nielsen-Kellerman, Chester, PA) was used to calculate temperature, humidity, and heat index at the start of sampling at each feedlot. The same instrument was used to measure the average wind speed over a 30-second period in the feed bunk area of each sampled pen. Following the visit total amount of precipitation during the previous week and date of the last precipitation prior to the sampling date were obtained from a web-based information source (http://www.wunderground.com) using five-digit ZIP codes.

A copy of the complete survey is available on request from M. W. Sanderson. A description of the survey development and pretesting and management question categories and climate variables is available elsewhere (23).

Statistical analysis. All statistical analysis was performed in STATA (STATA, version 8, College Station, Texas). The analyses for both the feed and water models were at the pen level. Since the coliform count data were not normally distributed the outcome variable for all analysis was the $\log_{10}$ of the feed (or water) coliform count. Due to the presence of 0 counts, we added 1 to all coliform counts before transforming with the $\log_{10}$ function. Fecal *E. coli* O157 culture results at the individual-sample level were collapsed to yield a single estimate of percent positive for fecal *E. coli* O157 shedding for each pen of cattle. Water tank *E. coli* O157 culture results for water and sediment in each individual tank were collapsed to categorize water tanks as positive or negative. Water tanks with one or more positive cultures for *E. coli* O157 were categorized as positive. Separate statistical models were developed for water coliform and feed coliform counts.

In the initial development of the models, the fecal, feed, and water *E. coli* O157 results from each pen and biologically plausible feedlot and pen management and climate factors were individually tested for univariate association with the $\log_{10}$ coliform count of the feed or the $\log_{10}$ coliform count of the water in a linear model controlling for feedlot effects as a random variable (xtreg, STATA 8.0). Variables significantly associated with $\log_{10}$ coliform count ($P \leq 0.2$) in these screening models were entered into a multivariable, linear model controlling for feedlot as a random effect (xtreg, STATA 8.0). Factors were removed by order of the largest P value (Wald Chi-square) until all factors remaining in the model were significant at $P \leq 0.05$. Excluded variables were then offered back into the model one by one and retained if they were significantly associated with $\log_{10}$ coliform count in feed or water ($P \leq 0.05$). Biologically plausible 2-way interactions were tested for variables included in the model. Variables screened and offered to the models are presented in Tables 1 and 2. Goodness of fit was assessed using R^2 values and visual assessment of residual distribution.

Since the measure of total solids in water includes coliforms, an additional model was developed using the same techniques but without including total solids to determine if the inclusion of total solids prevented other variables from entering the model.

RESULTS

Feed coliform model. Samples were collected from May to August 2001. Feed samples were collected from 671 feedbunks from 71 feedlots. Due to missing data, feed samples and complete data were available for the final feed coliform model from 642 feedbunks in 70 feedlots in the states of Kansas ($n = 29$), Nebraska ($n = 20$), Oklahoma ($n = 9$), and Texas ($n = 12$). Feedlot size varied considerably. Number of cattle placed on feed in the previous 12 months ranged from 7,500 to 273,062, with a median of 41,000. Number of cattle on feed the day of the visit varied from 2,882 to 102,000, with a median of 20,000. The number of cattle in individual pens included in the study ranged from 22 to 414, with a median of 113. Feed coliform counts ranged from 0 to 1.24×10^7 with a median of 6,800 CFU per gram. Coliform counts were 0 in 8.8% of feed samples, less than 54,000 CFU/g in 75% of feed samples and less than 1.96×10^5 in 90% of feed samples. Prevalence of *E. coli* O157 in individual fecal samples collected from cattle in the feed coliform study population was 10.3% (980/9,522), and prevalence of *E. coli* O157 in pen water tanks was 12.3% (78/634).

Month of sampling (higher in May and June), presence of corn silage in the ration (negative association), temperature of the feed 1 in. (ca. 2.6 cm) below the surface at the time of sampling (negative association), and wind velocity at the time of sampling (positive association) were significantly associated with $\log_{10}$ coliform counts in feed (Table 3). There were no differences in feed $\log_{10}$ coliform count between Kansas, Nebraska, Oklahoma, or Texas feedlots. There was no association between $\log_{10}$ coliform count in feed and presence of *E. coli* O157 in feed, or pen prevalence of *E. coli* O157 in feces. Both the residual error and variance of this model were approximately equal to one. The feedlot variance parameter estimate was significant and indicated approximately 56% of the variance was at the feedlot level. The R^2 for the model was 0.239

TABLE 1. Feedlot level and climate variables tested for univariate association with the $\log_{10}$ coliform count in cattle feed bunks and water tanks in feedlots in four states between May and August 2001[a]

Factor	Feed model	Water model
Feedlot level		
Feedlot demographics		
Month of sampling	Y*	Y*
State (KS, NE, OK, TX)	Y	Y
No. on-feed past 12 months	Y*	Y
Same holding pens for receiving and shipping (Y/N)	Y	Y
No. of pens on site	Y	Y
% of pens occupied on day of visit	Y*	Y
No. of cattle on site on day of visit	Y	Y
Acreage of feedlot	Y	Y
Water management		
Water tanks routinely cleaned? (Y/N)	Y	Y
Frequency of water tank cleaning	Y	Y
Method of water tank cleaning (chlorine, empty and refill, scrubbed while full, scrubbed while empty)	Y	Y
Feed storage		
Mineral supplement, protein supplement, fat supplement, feed additives, energy concentrate, roughage; for each feed, producers were asked to choose bags, sealed containers, uncovered piles, covered piles, or does not apply	Y	N
Wildlife		
Method of fly control (Y/N for each method listed): manure removal, predatory insects, insecticide ear tags, environmental sprays, animal sprays/powder/pour-on, larvacide feed additives, fly traps, fly bait, other	Y*	Y*
Frequency of other animals seen in pens/alleys: dogs/foxes/coyotes, stray cats, deer/elk, rodents, small mammals, birds (for each species group, producers were asked to choose: at least daily, at least weekly, at least monthly, occasionally, or never)	Y*	Y*
Aggressiveness of control measures in pens, alleys: dogs/foxes/coyotes, stray cats, deer/elk, rodents, small mammals, birds (for each species group, producers were asked to choose: aggressive, moderate, minimal, no control program, or not considered a problem)	Y*	Y
Frequency of other animals seen in feed storage areas: dogs/foxes/coyotes, stray cats, deer/elk, rodents, small mammals, birds (for each species group, producers were asked to choose: at least daily, at least weekly, at least monthly, occasionally, or never)	Y*	N
Aggressiveness of control measures in feed storage areas: dogs/foxes/coyotes, stray cats, deer/elk, rodents, small mammals, birds (for each species group, producers were asked to choose: aggressive, moderate, minimal, no control program, or not considered a problem)	Y*	N
Environmental management		
Use of permanent sprinklers for dust control (Y/N)	Y*	Y
Use of mobile sprinklers for dust control (Y/N)	Y	Y
Use of mechanical scrapers for dust control (Y/N)	Y	Y
Use of increased cattle density for dust control (Y/N)	Y	Y*
Frequency of removal of manure during feeding period	Y*	Y
Manure stored on feedlot premises (Y/N)	Y	Y
Same machinery used to feed and clean pens (Y/N)	Y	
Climate variables		
Temperature at start of sampling (°C)	Y*	Y*
Humidity at start of sampling (%)	Y	Y
Heat index at start of sampling (°C)	Y*	Y*
Weather at start of sampling (sunny, partly cloudy, mostly cloudy, light rain, or heavy rain)	Y	Y
Amount of rainfall in previous 7 days (inches)	Y*	Y
Days since last rainfall	Y	Y

[a] *, variables that passed screening ($P \leq 0.2$) and were offered to the final model.

and visual observation of the residual distribution did not indicate the model was misspecified.

Water coliform model. Water samples were collected for coliform counts from 702 water tanks in 72 feedlots. Due to missing data, water samples and complete data were available for the final water coliform model from 661 watertanks in 68 feedlots in the states of Kansas ($n = 30$), Nebraska ($n = 19$), Oklahoma ($n = 7$), and Texas ($n = 12$). Feedlot size varied considerably but was very similar to the lots included in the feed coliform model. Only the median number of cattle in individual pens included in the study differed from the feedlots in the feed coliform model (113 versus 112 head). Water coliform counts ranged from 0 to 1.21×10^4/ml with a median of 525 CFU per ml. Coliform counts were 0 in only one sample, were less than 1,950 CFU/ml in 75% of water samples, and less than 7070 CFU/ml in 90% of water samples.

TABLE 2. Pen-level factors tested for univariate association with $\log_{10}$ coliform count in cattle feed bunks and water tanks in feedlots in four states between May and August 2001[a]

Factor	Feed model	Water model
Pen level		
Demographic information		
New additions during production (Y/N)	Y	Y
Water management		
Tank water chlorinated (Y/N)	Y	Y
Days since water tank cleaned	Y	Y
Water temperature (°C)	Y	Y
Water pH	Y	Y*
Water total solids	Y	Y*
Water clarity	Y	Y*
Feed		
Percent dry matter in ration	Y	N
Time since feed last delivered to pen	Y	N
Feedstuffs in ration		
Specific components in ration (Y/N) reported for corn, milo, canola, whole cottonseed, cottonseed meal, urea, soybean meal, liquid protein, alfalfa hay, alfalfa silage, corn silage, cottonseed hulls, beet pulp, corn gluten, potato, tallow, wheat midds, dried brewers grains, probiotics	Y*	N
Feed antibiotics		
Antibiotics ever included in ration (Y/N)	Y	N
Antibiotics currently in ration (Y/N)	Y	N
Specific antibiotics currently in ration (Y/N): chlortetracycline, chlortetracycline/sulfamethazine, oxytetracycline, sulfamethazine, tetracycline, tylosin	Y*	N
Number of days antibiotics included in the ration for: chlortetracycline, chlortetracycline/sulfamethazine, oxytetracycline, sulfamethazine, tetracycline, tylosin	Y	N
Ionophores included in the ration (Y/N)	Y*	N
Pen and cattle characteristics		
Cattle density (ft.^{2}/head)	Y	Y
No. of cattle in pen	Y	Y
% of cattle fecal samples positive for *E. coli* O157	Y	Y
Presence of *E. coli* O157 in pen feedbunk (Y/N)	Y*	N
Presence of *E. coli* O157 in pen water tank (Y/N)	N	Y
Wetness of pen: cattle dry, mud/manure below fetlock of cattle, mud/manure above fetlock of cattle	Y	Y
Windbreaks provided (Y/N)	Y	Y
Shade provided (Y/N)	Y	Y
Sprinklers/misters provided (Y/N)	Y	Y
Mounds provided (Y/N)	Y	Y
Wind velocity, feedbunk area (ft./min)	Y*	Y*

[a] *, variables that passed screening ($P \leq 0.2$) and were offered to the final model.

Month of sampling (lower in May versus June July and August), water pH (negative association), and water total solids (positive association) were significantly associated with $\log_{10}$ water coliform levels (Table 4). There were no differences in feed $\log_{10}$ water coliform count between Kansas, Nebraska, Oklahoma, or Texas feedlots. There was no association between $\log_{10}$ coliform count in water and pen prevalence of *E. coli* O157 in feces, or whether water tanks were positive for *E. coli* O157. The residual error and variance estimate at the feedlot level for this model were 0.75 and 0.5. The feedlot variance parameter estimate was significant indicated approximately 31% of the variance was at the feedlot

TABLE 3. Associations between management and climate factors and feed coliform counts, with the feedlot effect controlled, in cattle feed samples in 635 pens on 68 feedlots sampled between May and August 2001

Variable	β	SE	P^a	95% confidence interval
Intercept	6.3	0.58		
Mo of sampling				
May or June	Referent			
July or August	−1.008	0.29	0.001	−0.43, −1.59
Corn silage in ration				
Yes	−1.14	0.29	0.000	−1.71, −0.56
No	Referent			
Feed temp (°C)	−0.04	0.009	0.000	−0.05, −0.018
Wind velocity (ft/min)	0.0004	0.0002	0.049	1.4×10^{-6}, 0.0007

[a] Wald chi-square statistics: overall R^2, 0.239. Variance parameter estimates: feedlot, 1.14; residual, 1.006; fraction due to feedlot, 0.56.

TABLE 4. Associations between management and climate factors and water coliform counts, with the feedlot effect controlled, in cattle feed samples in 661 pens on 68 feedlots sampled between May and August 2001

Variable	β	SE	P^a	95% confidence interval
Intercept	6.1	0.89		
Mo of sampling				
May	Referent			
June, July, or August	0.56	0.17	0.001	0.23, 0.9
Water pH	−0.26	0.12	0.03	−0.49, −0.03
Water total solids	0.0009	0.0002	0.000	0.0004, 0.001

[a] Wald chi-square statistics: overall R^2, 0.085. Variance parameter estimates: feedlot, 0.5; residual, 0.75; fraction due to feedlot, 0.31.

level. The R^2 for the model was 0.085 and visual observation of the residual distribution did not indicate the model was misspecified. The exclusion of total solids from the modeling process did not allow any additional variables to come into the model (data not shown).

DISCUSSION

Coliform bacteria are lactose fermenting bacteria belonging to the family Enterobacteriaceae including *Escherichia coli* and *Klebsiella* and *Enterobacter* species (4). They are considered a marker for fecal contamination. Coliforms are easily enumerated in feed and water samples and as such could serve as valuable indicator organisms for contamination of feed and water by *E. coli* O157 and other food-borne organisms transmitted in feces. Generic *E. coli* is commonly found and may replicate in cattle feeds (17), but procedures to enumerate them are more involved than for coliforms.

The seasonal nature of *E. coli* O157 shedding in cattle (10, 18, 28) and the finding of genetically indistinguishable isolates in herds separated by long distances (20) have led to the suggestion that feed may be an environmental reservoir and route of regional distribution (12). Experimentally, very low doses of *E. coli* O157 may result in colonization in some calves. Besser et al. (2) showed colonization in 2 of 17 calves exposed orally to <300 CFU of *E. coli* O157. Once some calves are colonized, they may amplify *E. coli* O157 and transmit it to calves in contact. Therefore, relatively small amounts of contamination in feed or water could result in widespread shedding.

With the recent finding of a relatively high prevalence of *E. coli* O157 in feed (2) and the common presence of *E. coli* O157 in water sources (7, 11, 16, 19, 21, 24), we hypothesized that management factors associated with coliform counts in feed and water would also be associated with presence of *E. coli* O157 in feedstuffs and water and serve as an indicator organism to guide management decisions in an overall feed safety and security program

In the feed coliform model, corn silage in the feedlot ration was negatively associated with the $\log_{10}$ coliform count in feed. Little work has been reported on coliform levels in feed, however, Lynn et al. (17) reported that generic *E coli* are commonly present and even replicate in silage based dairy rations. Changes in *E coli* concentrations in these dairy rations were negatively correlated with concentrations of acetate and propionate in the feed (17). Subsequent studies in the same lab have found little evidence of *E. coli* replication in dairy feeds, rather, they report that silage is inhibitory to growth of *E. coli* O157 (12). The fermentation process of ensiling may be valuable in decreasing coliforms, generic *E. coli*, and *E. coli* O157. In contrast, enterobacteria and *E. coli* O157 proliferate in aerobically spoiled (poorly fermented) grass silage (8). In properly ensiled grass, general enteric bacteria and inoculated *E. coli* O157 were not detectable past 19 days into the ensiling procedure, coinciding with a drop in pH and elevation of organic acid levels (3). Our data are consistent with previous work indicating proper fermentation of forage feedstuffs may be an effective way to control general enterobacteria and *E. coli* O157 and that organic acid presence in silage may be effective in decreasing post ensiling replication of *E. coli* O157.

Coliform counts in feed were higher in the months of May and June than in July and August. Environmental variables temporally associated with month could affect contamination probability, coliform survival, or replication and subsequent counts.

Coliform counts in feed were negatively associated with feed temperature in the bunk 1 in. below the surface at the time of collection; as feed temperature increased feed coliform counts decreased. Average feed temperature increased over the course of the study from May to August (May, 20°C; June, 26°C; July, 30°C; and August, 33°C). Generic *E. coli* has been shown to replicate faster in cattle feed stored at 21°C than at 37°C (17) and this may account for this association.

The measured velocity of the wind was positively associated with feed coliform counts. Increased wind increases dust and potentially contamination of feed by coliforms carried in the air. If feed coliform contamination is related to dusty conditions and resulting aerosolization of coliforms in dried manure then use of sprinklers may decrease contamination. The use of permanent sprinklers on the feedlot for dust control was not included in the final model but was the last variable to be excluded from the model ($P = 0.12$). We did not collect information on the frequency of use of permanent sprinklers or the time since they had last been used.

Water coliform counts were higher in this study than in a previous study involving water on dairy farms (16) but similar to a previous study in feedlot water tanks (27). In the water coliform model, coliform counts were lower in the month of May compared to the months of June, July, and August. Average water temperature at the time of collection increased from May to August, most dramatically from May to June (May, 15°C; June, 24°C; July, 27°C; and August, 28°C) but water temperature at the time of collection was not related to water coliform levels. Water coliform levels may be more related to long term water temperatures than to daily variability. LeChevallier et al. (14) noted water coliform bacteria were significantly higher in treated human water supplies when water temperatures were above 15°C. If this is true, water tanks may not warm adequately to support increased coliform levels until later in the summer. As such feed may be a more significant source of coliform exposure in the early summer and water more significant later. Increased coliform levels in water later in the summer could also be related to the amount of time cattle spend at the tanks as the ambient temperature increases,

resulting in increased consumption of water and opportunities for contamination.

Water coliform counts were negatively associated with water pH; as pH increased water coliform counts decreased. The range of water pH observed in the study was from 6.6 to 9.7 (mean, 7.37, and median, 7.4). Decreased coliform levels in alkaline water samples suggest that maintaining a higher pH may help control coliform levels, but no relationship has been identified between water pH and the presence of *E. coli* O157 in water or cattle feces (22, 26).

Water coliform counts were positively associated with water total solids in the tank. Total solids are a measure of dissolved and suspended solids in the water. Dissolved solids include calcium, chlorides, nitrate, phosphorus, iron, sulfur, and other ions. Suspended solids include silt and clay as well as plankton, algae, bacterial small organic debris, and other small particulate matter. We do not have any estimate of the proportion of dissolved and suspended solids in the water samples. Dissolved solids would be related to feedlot-specific water source quality issues. Suspended solids may be related to contamination issues either at the water source or in the water tank, including general tank cleanliness.

There was no relationship between water coliforms and days since the water tank had been cleaned, which is consistent with previous studies that have failed to show an effect of tank cleaning (27).

We found no relationship between feed coliform levels and feed *E. coli* O157 presence or water coliform levels and water *E. coli* O157. Further, risks identified for presence of *E. coli* O157 in feed in a subset of these feedlots (24) do not parallel risks for elevated coliform counts identified in this study. Neither do risks identified for the presence of *E. coli* O157 in water (24) parallel the risks associated with water coliform levels in this study. We also found no significant relationship between feed or water coliform count and fecal prevalence of *E. coli* O157 within the pen. As such, this study provides no support for the use of feed or water coliform counts as a measure of *E. coli* O157 exposure. The cross-sectional nature of this study does not rule out a possible association involving temporal variables.

Not all contamination of feed or water with bovine feces will result in *E. coli* O157 contamination. In this study only 10% of cattle were shedding *E. coli* O157 in their feces, so most fecal contamination would result in elevated coliform levels but not necessarily result in *E. coli* O157 contamination. Further, the cross-sectional, one-time sampling design of the present study does not allow the evaluation of any issues related to the persistence or sequence of exposure to feed or water contaminated with high levels of coliforms and subsequent fecal shedding. Temporal issues may be important in assessing any potential relationship between feed coliforms or *E. coli* O157 and *E. coli* O157 presence in cattle feces.

This study was unable to assess whether feed or water coliform levels were consistent within each feedlot or exhibited substantial variability over time. As such, risk factors for persistently high coliform levels could be relevant. Feedlots that maintain persistently elevated coliform counts could be more at risk for feed *E. coli* O157 contamination and subsequent cattle exposure. Such a temporally related association would not have been captured by this study design. Coliforms and *E. coli* O157 persist in water tanks (15), but feed is turned over daily in feedbunks. As such, the temporal relationship of feed contamination may be more important in assessing the relationship between feed coliforms or *E. coli* O157 and fecal *E. coli* O157. If fecal contamination and high coliform levels are a sporadic event, then fecal *E. coli* O157 shedding would likely occur several days after the contamination, and any association would only be detected by a longitudinal study. In previous work, Sargeant et al. (24) found no relationship between feed *E. coli* O157 and fecal *E. coli* O157, perhaps due to temporal issues. A longitudinal study of feedlots and the temporal appearance of coliforms and *E. coli* O157 in feed, water, and feces may be more appropriate to assess this relationship.

Alternatively, the source of coliform bacteria in the feed and water in this study may be other than from cattle. Wildlife contact with feed may be a source of coliform contamination but not commonly result in *E. coli* O157 contamination. Finally, feed coliforms may be too general a measure to identify a relationship with *E. coli* O157. Enumeration of generic *E. coli* in feed may be more useful in identifying any relationship between fecal contamination and *E. coli* O157 presence. As with all cross-sectional studies, the management risk factors identified here must be interpreted with caution due to the possibility of residual confounding resulting in spurious associations.

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