Effects of using retention-pond water for dust abatement on performance of feedlot steers and carriage of *Escherichia coli* O157 and *Salmonella* spp

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RUMINANTS

Objective—To evaluate the effects of using retention-pond water for dust abatement on performance of feedlot steers and carriage of *Escherichia coli* O157 and *Salmonella* spp.

Design—Matched cohort studies.

Animals—2 groups of feedlot steers comprising 3,510 (pathogen carriage) and 3,737 (performance) animals housed in a large commercial feedlot in the Texas Panhandle.

Procedure—Steers were systematically allocated to treatment pens approximately 60 days after arrival (pathogen carriage) or at arrival (performance). For evaluation of pathogen carriage, feces and hide swab specimens were collected from 25 animals in each pen within 10 days of slaughter. Samples were submitted for bacterial culture for *E coli* O157 and were tested with a polymerase chain reaction-based assay for *Salmonella* spp. For evaluation of performance, pen weights of animals were obtained at arrival and slaughter and feed delivered to each pen was recorded. The exposure of interest for both studies was application of retention-pond water through fixed high-pressure sprinklers.

Results—Carriage of *E coli* O157 and *Salmonella* spp and animal performance were not adversely affected by exposure to retention-pond water. Prevalences of *E coli* O157 in feces, on hides, and either in feces or on hides for those exposed to retention-pond water were 8.3%, 8.9%, and 15.4%, respectively; prevalences for those unexposed to retention-pond water were 11.4%, 15.4%, and 22.6%, respectively.

Conclusions and Clinical Relevance—Results suggest that use of retention-pond water for dust abatement in feedlot pens does not adversely affect pathogen carriage or animal performance. (*J Am Vet Med Assoc* 2005;226: 1378–1383)

Feedlot dust may contain bacterial pathogens and reactive compounds such as endotoxin and, thus, may be a health hazard if respired or ingested.[1] Investigators have concluded on the basis of results of challenge studies[2,3] that animals exposed to feedlot dust may be more likely to suffer from adverse health outcomes than unexposed animals. In addition, airborne dust that escapes feedlots, otherwise known as fugitive dust, has considerable nuisance potential. Consequently, control of feedlot dust is an important consideration for feedlot managers, particularly during the summer. Effective dust abatement decreases animal and human exposure to respirable particles and aids in the reduction of fugitive dust.

Dust potential in feedlots is inversely associated with pen-surface moisture.[4] Application of water through fixed high-pressure sprinklers, therefore, is a common management strategy used to reduce dust potential on feedlots. In much of the High Plains region of the United States, however, access to sufficient quantities of fresh water for adequate dust control is limited. A potential source of water for use in dust abatement programs is storm-water retention ponds. Although regulations vary by state, these ponds are generally required to be of sufficient size to prevent escape of storm water from feedlots and frequently contain sufficient water to adequately meet dust control needs. However, others have shown that pathogens such as *Salmonella* spp, fecal coliforms, and endotoxin can consistently be isolated from feedlot retention ponds.[5,6] Thus, although retention ponds may be a convenient source of water for dust abatement, some authors have recommended that retention-pond water not be used for such purposes because it may pose a health hazard for cattle and may disseminate pathogens such as *Escherichia coli* O157 and *Salmonella* spp among feedlot cattle.[5,6]

Escherichia coli O157 and *Salmonella* spp are important causes of food-borne illness in the United States,[7] and beef is often implicated as a source of these bacteria. Pathogens in fresh beef are largely controlled by interventions implemented within commercial abattoirs under the oversight of the USDA's Food Safety and Inspection Service. These interventions substantially reduce the prevalence of *E coli* O157 and other bacteria on beef carcasses.[8-10] It is becoming increasingly apparent, however, that despite the effectiveness of these interventions, the prevalence of pathogens on cattle entering abattoirs is associated with the likelihood of pathogen recovery from carcasses.[8,9,a] Efforts are needed, therefore, to reduce the prevalence of *E coli* O157 and other pathogens in cattle entering the abattoir. Feedlot management practices that might influence preharvest prevalence of pathogens, such as dust abatement with retention-

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pond water, should be critically evaluated. The purpose of the study reported here was to evaluate the effects of using retention-pond water for dust abatement on carriage of *E coli* O157 and *Salmonella* spp by and performance of feedlot steers.

Materials and Methods

Animals—Cattle used in the study were housed in a commercial feedlot in the Texas Panhandle with a 1-time capacity of approximately 70,000 animals. Two groups of crossbred feedlot steers were used in the study. One consisted of 3,510 animals used to evaluate pathogen carriage. The other consisted of 3,737 animals used to evaluate performance. The 2 parts of the study were conducted 1 year apart.

Pathogen carriage—Steers enrolled in this part of the study were housed in 14 pens of approximately 251 animals each until 70 to 90 days prior to slaughter. Animals were not exposed to retention-pond water prior to entry into the study. Animals were systemically allocated to an exposed or unexposed cohort by alternately assigning 5 animals to 1 cohort and the next 5 animals to the other cohort as the animals moved through a handling facility. This process was repeated until each of the 14 groups of animals was divided into 2 pens of approximately 125 animals each. Ultimately, 28 pens (ie, 14 replicates comprising 2 pens/replicate) were included in the study. Pen configuration and area per animal were similar between cohorts.

Exposed animals were subject to dust abatement by application of retention-pond water to pen surfaces through the use of a computer-controlled, high-pressure sprinkler system.[b] The application rate per fixed-sprinkler head was approximately 1,400 L/min. Sprinklers with a 4-pen coverage were active for 6-minute cycles. Sprinklers with a 2-pen coverage were active for 3-minute cycles. The number of cycles used per day was determined at the discretion of the feedlot manager on the basis of recent rainfall and perceived dust potential, with the exception that animals in the exposed cohort were exposed to at least 1 cycle per day for 7 consecutive days prior to collection of samples for the study. Animals in the unexposed cohort were never exposed to retention-pond water.

All animals in each replicate were sent to slaughter on the same day. Within 10 days prior to slaughter, fecal samples and hide swab specimens were collected from a sample of animals in each pen in each replicate. Separate animal handling facilities were used for the exposed and unexposed cohorts to avoid cross-contamination. On the day of sample collection, approximately 90 animals/pen were moved into a sorting alley. As these animals moved along the alley, they were sorted such that 5 animals were moved to an animal processing facility and 10 animals were allowed to return to the pen. This process was repeated until 30 animals/pen were moved to the animal handling facility. Feces and a hide swab specimen were collected from the first 25 of the 30 animals from each pen to pass through the chute. For collection of hide swab specimens, two 600-cm^2 areas of the hide were swabbed with a single sterile, prehydrated sponge.[c] These 2 sites included the dorsal midline in the area of the withers and the perineum on the right side of the animal. Sponges were placed into Butterfield solution for transportation. Feces and sponges were stored on ice and transported on the day of collection for further processing. Data associated with each sample included date of collection, cohort (exposed or unexposed), replicate, and order in which animals arrived at the chute for sampling.

Samples were submitted for bacterial culture to isolate *E coli* O157.[11] In brief, 90 mL of gram-negative broth containing 8 mg of vancomycin/mL, 50 ng of cefixime/mL, and 10 mg of cefsulodin/mL was inoculated with 10 g of feces or with 10 mL of fluid from the swab specimens and incubated for 6 hours at 37°C. Immunomagnetic separation was used to capture *E coli* O157 cells. One milliliter of the broth culture was mixed with 20 mL of anti-O157 beads[d] for 30 minutes at room temperature. Beads were washed 3 times, and 50 mL of the bead-bacteria mixture was streaked on sorbitol-MacConkey agar plates containing 50 ng of cefixime/mL and 2.5 mg of tellurite/mL. Following a series of confirmatory steps,[11] suspect colonies were tested for the O157 antigen with a latex agglutination kit.[e] Isolates positive for agglutination were subject to additional confirmatory steps to confirm their identity and identify the presence of at least 1 gene encoding for production of shiga toxin. A commercially available **polymerase chain reaction (PCR)** system was used.[11,f]

Fecal and hide swab specimens were tested for *Salmonella* DNA by use of a commercial PCR assay[f] that incorporated probes specific for conserved sequences of bacterial DNA. Samples were processed according to the manufacturer's recommendations. Briefly, samples were enriched, and bacterial cells were then lysed. Results of the PCR assay (positive or negative) were electronically captured.

Samples from replicates 4 and 5 were also submitted for bacterial culture for *Salmonella* spp. Briefly, 25 g of feces was transferred into 225 mL of buffered peptone water, and the mixture was shaken for 1 minute. Next, 0.2 mL of the buffered peptone water sample was added to 10 mL of Rappaport-Vassiliadis medium and selenite cystine broth, which were incubated at 40°C for 24 hours. Typical colonies were subjected to an agglutination test for confirmation.[g] *Salmonella* isolates were shipped to the USDA National Veterinary Services Laboratory for serotyping.

Animal performance—The 3,737 steers used in the performance part of the study were allocated to exposed and unexposed cohorts at the time of feedlot arrival. Steers arrived at the feedlot between March 20 and July 21 and were routinely processed and allocated as described for steers in the pathogen carriage portion of the study, resulting in 24 pens (ie, 12 replicates comprising 2 pens/replicate) with approximately 156 steers in each pen. Pens of cattle were sent to slaughter between July 30 and January 14. Pen weights (cumulative weight of all animals within a pen) were obtained at the time of arrival and when animals were sent to slaughter. In addition, daily (as-fed) feed delivery per pen was recorded. Pen-level performance parameters were calculated as follows. Average body weight was calculated by dividing the pen weight by the number of animals in the pen. Mean daily weight gain was calculated by subtracting arrival weight from harvest weight; weight gain was then divided by the number of days the animals spent at the feedlot. As-fed feed delivery was adjusted to reflect dry matter delivered. Daily dry-matter intake per animal was calculated by dividing delivered dry matter by the number of animals in the pen. Feed efficiency was calculated by dividing mean daily weight gain by daily dry-matter consumption.

Cattle were observed daily by trained animal health personnel, and animals with signs of illness were moved to an animal handling facility and treated according to protocols developed by the consulting veterinarian. Animals that died were examined postmortem under the direct or indirect supervision of the consulting veterinarian, and cause of death was attributed to a body system. Morbidity and mortality rates were recorded for each pen. No ancillary diagnostic tests were performed on sick or dead animals.

Statistical analyses—Descriptive statistics were generated for both parts of the study. Data were analyzed with commercially available software.[h,i] Pen-level binomial response variables were generated for *Salmonella* and *E coli* O157 carriage. For each bacterium, a separate binomial

response variable was calculated for recovery from feces, hides, and either feces or hides. The denominator used was the number of animals from which samples were collected per pen. Exposure was the independent variable of interest. Mixed-model methodologies were used in which sample collection day and replicate were considered random variables to account for potential within-pen and within-sample-day clustering. The proportion of random variation attributable to each of these variables was calculated. Because the odds ratio becomes progressively poorer at approximating the relative risk as prevalence increases, relative risks were calculated. To calculate relative risks, model-predicted logits were computed for the exposed and unexposed cohorts and back-transformed to the normal scale. Model-predicted prevalence (risk) for each cohort was calculated from the back-transformed odds (ie, risk = odds/[1 + odds]).[12]

The order in which samples were collected in each pen was analyzed to evaluate the potential for animal-to-animal cross-contamination resulting from orderly passage through the animal handling facilities. A binomial response variable was established for each sample order (1 through 25) and considered the dependent variable. Sample collection order was the independent variable and considered continuous.

Performance parameters from the second part of the study were similarly analyzed, except that the dependent variables of interest were continuous. Replicate was considered a random variable.

Results

Pathogen carriage—Samples were collected from 700 animals from the 14 replicates on 6 sample collection days (June 9, June 23, July 1, July 7, July 15, and July 21). Samples were collected from 3 replicates on June 9 and July 7 and from 2 replicates on the other days. Sprinklers were activated for 1 or 2 cycles/d for the 7 days prior to sample collection (**Figure 1**).

Overall prevalences of *E coli* O157 in feces, on hides, and either in feces or on hides were 9.9%, 12.1%, and 19.0%, respectively. Estimated prevalences of *E coli* O157 did not vary significantly between the exposed and unexposed cohorts (**Figure 2**), and relative risks and odds ratios were not significantly different from 1 (**Table 1**). Recovery of *E coli* O157 from the hide swab specimens varied significantly ($P = 0.04$) with sample order. On average, the likelihood of recovering *E coli* O157 from hide swab specimens from successive animals decreased by 3% per animal (odds ratio, 0.97; 95% confidence limits, 0.94 to 1.0). Recovery of *E coli* O157 from feces or from either feces or hides did not vary significantly ($P > 0.30$) with sample order.

Day of sample collection and replicate accounted for a substantial amount of the random variation, indicating that prevalence varied significantly between days and replicates (**Figure 3**). Day of sample collection accounted for 18.3%, 14.6%, and 10.9% of the random variation in models of *E coli* O157 carriage in feces, on hides, and either in feces or on hides, respectively. *Escherichia coli* O157 was recovered from only 2.0% of fecal samples collected on June 23 but was recovered from 23.0% of fecal samples collected on July 15. Similar variation was observed for hide carriage. Replicate accounted for 29.3%, 14.1%, and 15.6% of the random variation in models of *E coli* O157 carriage in feces, on hides, and either in feces or on hides, respectively. The highest prevalence of *E coli* O157 in feces (40%) was observed in replicate 10 of the unexposed

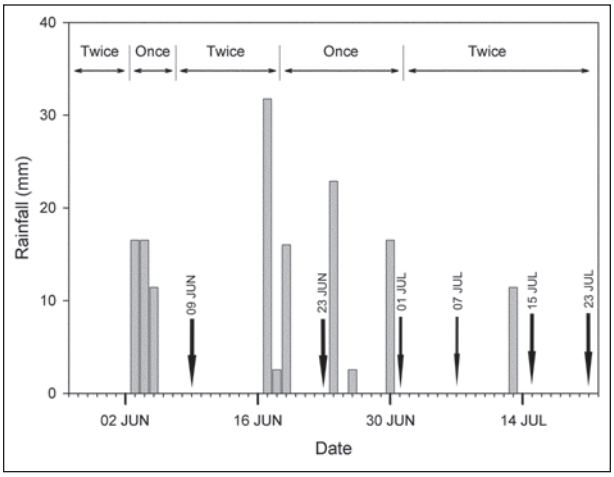

Figure 1—Amount of rainfall and number of cycles per day retention-pond water was used (horizontal arrows) in a study of the effects of using retention-pond water for dust abatement on *Escherichia coli* O157 and *Salmonella* spp carriage in feedlot steers. Vertical arrows indicate days on which fecal samples and hide swab specimens were collected. Samples were collected from 3 replicates on June 9 and July 7 and from 2 replicates on the remaining days.

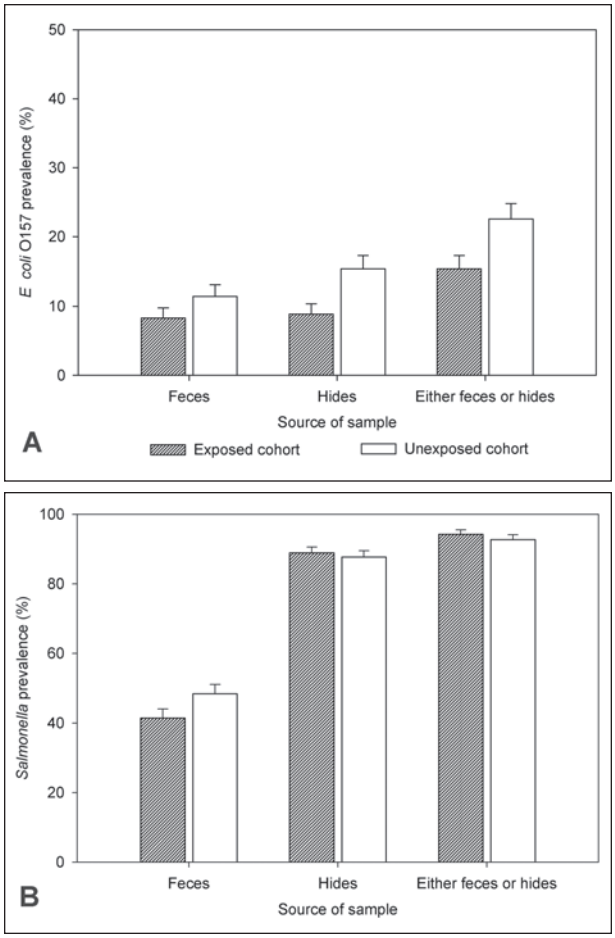

Figure 2—Crude estimates of the prevalence of *E coli* O157 (A) and *Salmonella* spp (B) in feces, on hides, and either in feces or on hides of feedlot steers that were (striped bars; n = 1,755) or were not (white bars; 1,755) exposed to retention-pond water used for dust abatement.

Table 1—Measures of effect associated with *Escherichia coli* O157 and *Salmonella* spp carriage in feces, on hides, and either in feces or on hides for feedlot steers that were (n = 1,755) exposed to retention-pond water used for dust abatement relative to feedlot steers that were unexposed (1,755).

Sample	RR	OR	95% CL	*P* value
E coli O157 carriage				
Feces	0.69	0.67	0.35–1.42	0.26
Hide	0.54	0.51	0.23–1.26	0.11
Feces or hide	0.64	0.59	0.28–1.32	0.18
Salmonella carriage				
Feces	0.77	0.72	0.28–1.32	0.42
Hide	1.01	1.12	0.43–1.54	0.82
Feces or hide	1.02	1.30	0.32–1.37	0.72

RR = Relative risk. OR = Odds ratio. CL = Confidence limits.

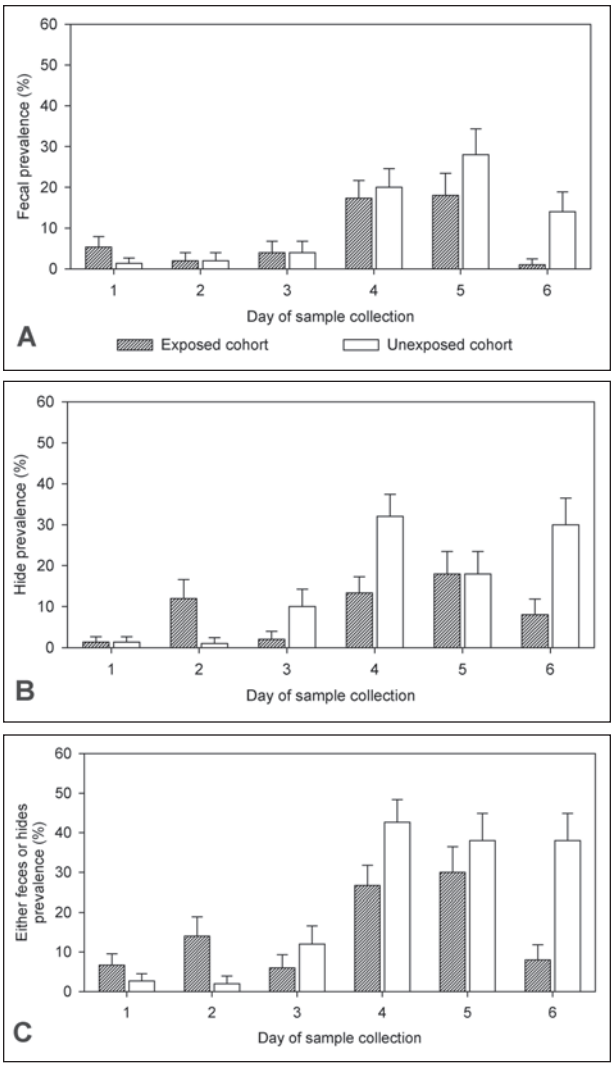

Figure 3—Prevalence of *E coli* O157 in feces (A), on hides (B), or either in feces or on hides (C) as a function of sample day for feedlot steers that were (striped bars; n = 1,755) or were not (white bars; 1,755) exposed to retention-pond water used for dust abatement.

cohort, and the lowest (0%) was observed in replicates 3, 4, 7, 13, and 14 of the exposed cohort and replicates 1, 3, 5, 7, and 14 of the unexposed cohort.

Estimated prevalences of *Salmonella* spp in feces, on hides, and either in feces or on hides were 44.9%, 88.4%, and 93.5%, respectively. Estimated prevalences of

Table 2—*Salmonella* serotypes recovered from the feces and hides of feedlot steers enrolled in a study to evaluate the effects of using retention-pond water for dust abatement on carriage of *Salmonella* spp.

	No. (%) of isolates	
Serotype	**Feces**	**Hide**
Anatum	25 (42)	36 (55)
Cerro	1 (2)	0 (0)
Kentucky	1 (2)	7 (11)
Mbandaka	18 (30)	17 (26)
Newington	12 (20)	3 (5)
Mixed serotypes	3 (5)	2 (3)
Total	**60 (100)**	**65 (100)**

Table 3—Number of cycles per day that retention-pond water was used in a study of the effects of using retention-pond water for dust abatement on performance of feedlot steers.

Date	No. of days	Cycles/d
March 20–April 3	15	0
April 4–April 27	24	1
April 28–June 11	45	2
June 12–July 8	27	3
July 9–July 25	17	2
July 26–July 31	6	1
August 1–September 1	32	0
September 2–September 9	8	1
September 10–September 21	12	0
September 22–September 30	9	1
October 1–January 14	105	0

Salmonella spp did not vary significantly between the exposed and unexposed cohorts (Figure 2), and relative risks and odds ratios were not significantly different from 1 (Table 1). Detection of *Salmonella* spp did not vary significantly ($P < 0.65$ for all models) with the order in which the animals moved through the handling facility.

Recovery of *Salmonella* spp did not vary to the same extent across days or replicates as did recovery of *E coli* O157. Day of sample collection accounted for only 13.8%, 11.3%, and 0.0% of the random variation in models of *Salmonella* carriage in feces, on hides, and either in feces or on hides. Replicate did not account for any of the random variation in any of the models of *Salmonella* carriage. *Salmonella* Anatum was the most commonly identified serotype and represented 48.8% of all isolates (**Table 2**). The 4 most commonly recovered serotypes were identified in 3 of the 4 pens. In 1 pen (replicate 4 of the unexposed cohort), only *Salmonella* Anatum was recovered.

Animal performance—Sprinkler usage varied from 0 to 3 cycles/d (**Table 3**). Mean arrival and har-

Table 4—Performance and health characteristics for feedlot steers that were (n = 1,877) or were not (1,860) exposed to retention-pond water used for dust abatement.

Variable	Exposed	Not exposed	*P* value
Mean daily weight gain (kg)	1.57	1.57	0.73
Daily dry-matter intake (kg)	9.23	9.19	0.51
Feed efficiency	0.17	0.17	0.41
Morbidity rate (%)	1.87	2.81	0.12
Mortality rate (%)	0.58	0.64	0.76
Death attributable to respiratory tract disease (%)	0.21	0.21	0.99

vest weight of steers were 346.6 and 591.4 kg (762.5 and 1,301.1 lb), respectively. Mean time in the feedlot was 155 days. For steers in the exposed cohort, mean number of days sprinklers were active was 76.2 days (range, 28 to 132 days), and mean exposure intensity (number of days exposed times number of cycles/d) was 141 cycles (range, 33 to 248 cycles). Overall morbidity and mortality rates were 2.3% and 0.64%, respectively. Overall rate of death attributable to respiratory tract disease was 0.21%. No significant differences in performance parameters were detected between exposed and unexposed cohorts (**Table 4**).

Discussion

Even though pathogens have routinely been isolated from feedlot retention ponds,[6] we did not detect an association between use of retention-pond water for dust abatement purposes and prevalence of *E coli* O157 or *Salmonella* spp in feedlot cattle in the present study. The fixed high-pressure sprinkler system, which is typical of systems used by large commercial feedlots, was designed for complete pen-surface coverage, so it was not possible for cattle to avoid contact with the water during application cycles. It is interesting, therefore, that an association between exposure to retention-pond water and hide carriage of either pathogen was not detected. This would seem to suggest that other factors, such as animal-to-animal transmission, are more important in determining prevalence than the use of retention-pond water.

We did not systematically evaluate the retention-pond water used in the present study for the presence of *E coli* O157 or *Salmonella* spp. While it is possible that these pathogens were not present in the retention-pond water, this seems improbable because the retention pond used in this study was similar to those described in previous studies[5,6]; these bacteria were found in the feces of animals in the pathogen carriage portion of the study; and storm-water runoff from pen surfaces, which included fecal material, constituted the majority of the retention-pond water.

We did not detect significant variations in animal performance or health outcomes between exposed and unexposed cattle in the second part of the present study. It is possible that the duration and intensity of exposure in some replicates were insufficient to produce an effect. However, mean exposure and intensity were 76.2 days and 141 cycles, respectively, and the intensity of water application used in the present study was typical of that used in feedlots in the High Plains for dust abatement.

Because pathogens and endotoxin are routinely recovered from feedlot retention ponds, other investigators have recommended that retention-pond water not be used for dust abatement purposes.[5,6] Their reasoning was in part based on concerns that the inevitable exposure of animals to retention-pond water during application would affect pathogen carriage[6] and animal health.[5] While these recommendations may ostensibly seem to be a logical extrapolation of their data,[5,6] the outcome variables about which their inferences were drawn were not measured. In the present study, however, we measured these outcome variables (ie, pathogen prevalence, morbidity rate, mortality rate, and performance) and did not detect an adverse effect of using water from a feedlot retention pond for routine dust control purposes.

Many factors are associated with prevalence of *E coli* O157. In particular, shedding demonstrates distinct temporal and spatial variations.[13] However, there appears to be little variation attributable to feedlot-level factors.[14-16] Day accounted for a substantial amount of the random variation in models of *E coli* O157 carriage in the present study. Day-to-day variation may be attributable to seasonal[17] or other factors, and pen-surface condition has been associated with shedding.[16] In the present study, the difference in dust potential of the pen surfaces between cohorts and, consequently, surface moisture was readily apparent, yet we did not observe variation in prevalence of *E coli* O157 carriage. It is possible that the difference in pen-surface conditions between the cohorts was not great enough to elicit an effect on *E coli* O157 prevalence as previously described.[16]

In the present study, we analyzed the data for an association with the order in which animals moved through the animal handling facilities so as to evaluate the potential for the facilities to act as a fomite for transmission of *E coli* O157 and *Salmonella* spp among animals. Not surprisingly, we did not detect any variations in prevalence of *E coli* O157 or *Salmonella* spp in feces. We did, however, detect variation in recovery of *E coli* O157 from hides. Interestingly, recovery was negatively associated with order and not, as one would expect, positively associated. The reason for this unexpected association is uncertain but may have been a spurious finding; this observation was not evident in carriage of *Salmonella* spp on hides.

Overall prevalence of *E coli* O157 in the present study was similar to that reported elsewhere.[13,14,16] *Salmonella* prevalence, however, was greater than previously reported.[18] We used a PCR-based assay to estimate prevalence, and it is possible that false-positive results (ie, poor specificity) may have contributed to the relatively higher prevalence in the present study. Nevertheless, these estimates were similar to data collected for another study[j] in which we used standard culture and isolation methods to recover *Salmonella* spp.[j]

Moreover, estimates of prevalence based on culture and isolation techniques for samples collected from replicates 4 and 5 were consistent with PCR assay-based estimates. *Salmonella* serotypes recovered from replicates 4 and 5 were in reasonable agreement with those reported previously,[18] yet were dissimilar from the isolates most commonly associated with human disease.[19]

Duration of infection with *E coli* O157 and shedding pattern may depend on prior exposure, although it is possible to reinfect cattle with *E coli* O157 subsequent to an initial infection.[20] Furthermore, tremendous pen-to-pen variation in shedding dynamics and predominant strains within feedlot pens have been reported.[13,21] These results indicate that within-pen transmission dynamics may be an important consideration when evaluating *E coli* O157 prevalence in commercial feedlots. We designed the first part of our study, therefore, so that animals within each replicate would have a similar exposure history prior to allocation to treatment pens. To achieve this, animals were commingled in pens of approximately 251 animals (a replicate) for approximately 60 to 120 days. Presumably, this pre-allotment commingling should have afforded sufficient within-replicate dissemination of wildtype *E coli* O157 and *Salmonella* spp, if present. Replicate accounted for 14% to 30% of the random variation in models of *E coli* O157 carriage, indicating that the study design was adequate and, where appropriate, ought to be considered for future studies of *E coli* O157 in feedlot cattle housed in commercial production settings. Replicate, however, did not account for any random variation in the models of *Salmonella* carriage.

Recent work has indicated that *E coli* O157 strains may vary in pathogenicity and that, consequently, some strains may be more likely to produce disease in people than others. For example, strains containing a specific allele produce greater quantities of shiga toxin, and isolates from countries that experience a relatively greater human-disease burden are more likely to contain this allele.[22] It is theoretically plausible, therefore, that a minority of cattle isolates could be responsible for the majority of human disease. It is also conceivable that the presence of certain virulence factors may influence survivability in various environments such as retention ponds. Evaluation of a variety of virulence factors in addition to pathogen prevalence may be an important consideration for future studies, given our increasing understanding of this bacterium.

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