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Airborne Microbial Flora in a Cattle Feedlot

S. C. Wilson,[1]* J. Morrow-Tesch,[2] D. C. Straus,[1] J. D. Cooley,[1] W. C. Wong,[1] F. M. Mitlöhner,[3] and J. J. McGlone[3]

Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, Texas 79430,[1] *and USDA-ARS Livestock Issues Research Unit,*[2] *and Department of Animal Science & Food Technology, Texas Tech University,*[3] *Lubbock, Texas 79409-2141*

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A total of 1,408 cattle held in eight commercial feedlot pens were used to examine the quantity and diversity of microorganisms in cattle feedlot air. The effect of two feeding patterns on the generation of airborne dust and the total numbers of microorganisms was also examined (four feedlot pens/treatment). Microbial samples were collected, and dust particles that were 2.5 μm or less in diameter were measured with a Dustrak monitor during the evening dust peak for 4 days at sites both upwind and downwind of the feedlot pens. An Andersen biological cascade sampler was employed with different medium and incubation combinations for the capture and identification of bacteria and fungi. The results showed that when bacteria were considered, only nonpathogenic gram-positive organisms were recovered. However, gram-negative bacteria may have been present in a viable but nonculturable state. Fungi were recovered in smaller numbers than bacteria, and none of the fungi were pathogenic. The Dustrak results showed that one feeding pattern resulted in cattle behavior that generated levels of downwind dust lower ($P = 0.04$) than the levels generated by the behavior resulting from the other feeding pattern. However, the Andersen sampler results showed that there were no differences between feeding patterns with regard to the total number or diversity of microorganisms. The disparity may have been due to the different operating principles of the two systems. The overall numbers of microorganisms recovered were lower than those reported in studies of intensively housed farm animals in which similar recovery techniques were used.

High dust production is a feature of many cattle feedlots (20). MacVean et al. (13) found that a broad temperature range and an increase in dust particles in the 2.0- to 3.3-μm size range in feedlots were associated with an increased incidence of cattle pneumonia. This was attributed to the dust load stressing the respiratory system and predisposing cattle to infection by bacterial and viral pathogens. The numbers and types of microorganisms that are either bound to feedlot dust or individually airborne may also play a contributing role. However, there have been no previous studies that have examined the microbial composition of cattle feedlot air.

Dry, warm conditions and active cattle behavior have been found to be the principal contributors to dust production in cattle feedlots. This production peaks during the evening hours (F. W. Mitlöhner, J. L. Morrow, J. W. Dailey, and J. J. McGlone, unpublished data). This is an active period for cattle, characterized by increased numbers of agonistic interactions and walking and running behaviors (8). A standard feeding practice in some West Texas feedlots is to feed at sunrise, 1000 h, and 1200 h. This protocol results in cattle that have digested their food by the evening, which coincides with the active, dust-generating period. It was hypothesized that feeding cattle at sunrise, noon, and sunset would replace the evening active period with a period of eating and ruminating, thus reducing cattle activity and dust generation. However, it is not known if reducing dust levels would correspondingly reduce the numbers of airborne bacteria and/or fungi and the diversity of these organisms.

The objectives of this study were (i) to identify and measure the concentrations of members of the bacterial and fungal flora in cattle feedlot air by using Andersen biological cascade samplers and agar plates incubated at a range of temperatures and in a range of atmospheres, (ii) to compare the effects of two feeding regimens and subsequent dust generation (as measured with a Dustrak monitor) on overall airborne microbial numbers and species diversity, and (iii) to determine if there is any correlation between dust levels measured with a Dustrak sampler and airborne microbial numbers determined with an Andersen sampler.

MATERIALS AND METHODS

The experiment was conducted at a commercial feedlot in the panhandle region of Texas. A total of eight pens holding an average of 176 animals each were used. The study was conducted over 4 days in May 2000. Upwind and downwind samples were collected from two pens each day. The protocol was reviewed and approved by the Texas Tech University Animal Care Committee prior to initiation of the study.

Animals. The cattle in the feedlot pens were a mixture of *Bos taurus* and *Bos indicus* breeds, predominantly *B. taurus*. The average initial weight of the animals was 354 kg (standard error, 8.4 kg). The stocking density was 15.2 m^2/head. The average pen size was 1,350 m^2. The pens were filled in pairs across feeding patterns as cattle arrived at the feedlot until all pens were occupied. The cattle had approximately a 1-week acclimation period to adjust to the feeding times. Tylan and Rumensin (Elanco Animal Health, Indianapolis, Ind.) were added to the feed. The pens were orientated east-west and were located at the southeast end of the feedlot.

Microbial and dust sampling protocol. Samples were taken when the prevailing winds were from the south or southeast, towards the feedlot pens. The samplers were placed at a height of 1 m immediately upwind and downwind of the pens. The upwind samplers collected air flowing from an opposite open field that was separated from the feedlot pen by an unsurfaced road, a wire fence, and the unsurfaced feedlot alleyway. Sampling was conducted during the major evening dust peak (1700 to 2300 h).

* Corresponding author. Mailing address: Department of Microbiology and Immunology, TTUHSC, 3601 4th St., Lubbock, TX 79430. Phone: (806) 743-2466. Fax: (806) 743-2334. E-mail: stewilso@ttacs.ttu.edu.

TABLE 1. Means and standard deviations for airborne microbial flora recovered immediately upwind of cattle feedlot pens under two feeding regimens (four pens per treatment)

Organism	Mean CFU/m^3 of air[a]							
	Control feeding treatment				Test feeding treatment			
	BA, 22°C	BA, 37°C	BA Ano	SAB	BA, 22°C	BA, 37°C	BA Ano	SAB
Bacillus cereus	95 (94.0)[b]	28 (9.8)	28[c]		25 (24.8)	107 (29.2)	39 (14.8)	
Bacillus coagulans		49[c]	28[c]		14[c]	28[c]	11 (5.0)	
Bacillus licheniformis					14[c]			
Bacillus megaterium		63[c]						
Bacillus subtilis	492 (365.4)	179 (76.2)	252[c]		375 (290.0)	238 (217.8)	277 (153.4)	
Corynebacterium aquaticum	140[c]	46 (5.0)	7[c]		105 (59.4)	49[c]	49[c]	
Corynebacterium bovis		56[c]						
Corynebacterium genitalium	42[c]				91[c]			
Corynebacterium pseudotuberculosis	35[c]	98[c]			46 (54.4)			
Corynebacterium renale group	158 (74.2)	151 (14.8)			203 (69.2)	224 (59.4)		
Helocococcus kunzei	14[c]	56 (19.8)	91[c]		21[c]	81 (44.6)	133[c]	
Micrococcus luteus	28[c]	7[c]	98[c]		42 (19.8)	112 (69.3)	130 (5.0)	
Paenibacillus macerans		7[c]				39 (24.8)		
Yersinia pseudotuberculosis						7[c]		
Alternaria sp.				14[c]				63 (49.4)
Aspergillus sp.								7[c]
Bipolaris sp.				28[c]				7[c]
Chrysosporium sp.				39 (14.8)				60 (34.6)
Cladosporium sp.				56 (30.6)				75 (19.3)
Penicillium sp.				107 (91.0)				21 (12.1)

[a] BA, blood agar; BA Ano, blood agar plates incubated anaerobically at 37°C; SAB, Sabouraud's agar incubated at 25°C.
[b] The values in parentheses are standard deviations.
[c] Only one count for the organism was obtained; therefore, there is no standard deviation.

Assays of airborne microbial flora. Microbes were identified and quantified on agar plates that were placed in Andersen biological cascade impactors (Andersen Sampler, Inc., Atlanta, Ga.). The samplers were run for 5 min with calibrated vacuum pumps (28.3 liters/min). Stages 1, 5, and 6 of the Andersen samplers were used. Glass petri dishes filled to the two-thirds level with paraffin wax were placed in the remaining stages (stages 2, 3, and 4). Stage 1 collected particles that were 7 μm in diameter. For humans this stage represents particles that can enter the nose (nonrespirable). Stage 5 collected particles that were 2.1 to 1.1 μm in diameter. These particles can enter the terminal bronchi (respirable). Stage 6 collected particles that were 0.65 to 1.1 μm in diameter. These particles can enter the lung alveoli (respirable).

Agar plates were sampled for aerobic bacteria that grew at 22°C on sheep blood agar, aerobic bacteria that grew at 37°C on sheep blood agar, anaerobic bacteria that grew at 37°C on sheep blood agar (GasPak BBL; Becton Dickinson Micro Systems, Sparks, Md.), and fungi and yeasts that grew at 24°C on Sabouraud's dextrose agar (2) (Fisher Scientific, Pittsburgh, Pa.). Fungi were identified by examination of colony morphology, followed by microscopic examination of spores and hyphae (18, 19). Bacteria were identified by Gram staining and then by using bacterial identification kits (Becton Dickinson Micro Systems).

Dust level measurement. Dustrak monitors (model 8520; TSI Inc., Shoreview, Minn.) were situated in the same locations as the Andersen samplers. Using a photometric measuring system, the monitors recorded particles that were less than 2.5 μm in diameter. Dust levels were measured every 10 min during the main evening dust peak (1700 to 2300 h) on each day of sampling.

Feeding treatments. There were four treatment pens and four control pens. The control pens represented the standard feeding pattern of the feedlot. The animals in these pens were fed 33, 33, and 34% of their daily ration at 0710, 1000, and 1200 h, respectively. The animals in the test pens were fed 30, 20, and 50% of their daily ration at 0700, 1000, and 1830 h, respectively. The feed composition and amount were the same for both treatments.

Weather data. The samples were collected between 1700 and 2300 h. Temperature and humidity were recorded at 1800 h on all 4 days of sampling.

Experimental design and statistical analysis. The experimental design was a completely randomized design with the feedlot pen as the experimental unit. The two feeding regimens were the treatments. Data were transformed by using a $\log_{10}$ transformation where conditions of normality and equal variance were not met. Data were analyzed by using the General Linear Model in SAS (SAS Institute Inc., Cary, N.C.). The model included treatment and day effects.

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RESULTS

All of the airborne bacteria recovered were gram-positive organisms. None were considered to be pathogenic to the bovine respiratory system. All fungi recovered were also considered to be nonpathogenic. Tables 1 and 2 show the species and the means and standard deviations for all microorganisms recovered immediately upwind and downwind of the feedlot pens. For brevity, the stages of the Andersen samplers are combined.

Tables 1 and 2 also show that regardless of treatment, the sites downwind of the feedlot pens had higher numbers of bacteria and fungi than the sites upwind of the feedlot pens. The upwind dust levels were similar for both feeding treatments; the control and test pens had 0.039 ± 0.005 and 0.038 ± 0.005 mg of dust/m^3 of air, respectively. However, the downwind levels of dust were significantly different ($P = 0.04$); the control feeding treatment dust level was 0.177 ± 0.002 mg of dust/m^3 of air, and the test feeding dust level was 0.097 ± 0.016 mg of dust/m^3 of air. With regard to the overall numbers of microorganisms, there were no significant differences between treatments at the $P = 0.05$ level for both the upwind and downwind sites for all medium and incubation combinations for all stages of the Andersen sampler. Table 3 shows least-\squares means and standard errors for the downwind counts. Table 4 shows the temperatures and humidities on the days on which the experiment was performed.

TABLE 2. Means and standard deviations for airborne microbial flora recovered immediately downwind of cattle feedlot pens under two feeding regimens (four pens per treatment)

Organism	Mean CFU/m^3 of air[a]							
	Control feeding treatment				Test feeding treatment			
	BA, 22°C	BA, 37°C	BA Ano	SAB	BA, 22°C	BA, 37°C	BA Ano	SAB
Bacillus cereus	648 (232.6)[b]	305 (101.5)	81[c]		550 (767.2)	378 (465.2)	49 (49.4)	
Bacillus coagulans	42[c]	56[c]	35[c]		32[c]	42[c]	133[c]	
Bacillus megaterium						84[c]		
Bacillus subtilis	2,363 (2,731.4)	320 (68.3)	347[c]		1,491 (1,652.6)	380 (387.6)	266 (118.8)	
Chrysobacterium meningosepticum		14[c]				350[c]		
Corynebacterium aquaticum	4,256[c]	119 (69.3)			1,712 (1420.6)	249 (311.8)	161[c]	
Corynebacterium bovis		287[c]				189[c]		
Corynebacterium genitalium	238[c]							
Corynebacterium pseudotuberculosis	7[c]				7[c]			
Corynebacterium renale group	819 (729.2)	413 (297.0)			1,087 (1,399.6)	394 (313.6)		
Helocococcus kunzei	7[c]	63 (79.2)	168[c]		7[c]	189[c]	14[c]	
Micrococcus luteus	105[c]	1,106[c]	420[c]		49 (9.9)	616 (252.4)	298 (391.0)	
Paenibacillus macerans		46 (54.4)				53 (22.3)		
Alternaria sp.				42 (18.6)				56 (18.5)
Bipolaris sp.								28[c]
Chrysosporium sp.				35[c]				14 (9.6)
Cladosporium sp.				40 (22.6)				53 (32.6)
Penicillium sp.				12 (10.6)				42 (28.0)

[a] BA, blood agar; BA Ano, blood agar plates incubated anaerobically at 37°C; SAB, Sabouraud's agar incubated at 24°C.
[b] The values in parentheses are standard deviations.
[c] Only one count for the organism was obtained; therefore, there is no standard deviation.

DISCUSSION

Microorganisms in cattle feedlot air are subjected to high temperatures, desiccation, and UV light radiation. These factors affect microbial viability, particularly the viability of gram-negative bacteria (6, 14). In this study, only gram-positive bacteria were recovered. Table 4 shows that the weather at the time of collection was very warm and dry. The microbial recovery method may have also played a role in that gram-negative organisms, stressed by the environmental conditions, might have been in a viable but nonculturable state (11). Stewart et al. (17) found that these organisms can be damaged by the impact on the agar surface inside an Andersen sampler. On the other hand, Stewart et al. also found that although reduced in number, gram-negative colonies still formed. Also, other open-air studies performed with an Andersen sampler and agar media have revealed the presence of culturable gram-negative bacteria (5). The antibiotic Tylan was used as a feed additive in this study, and it was considered possible that this affected the results. However, Tylan is effective mainly against gram-positive bacteria. Specifically, it reduces the incidence of liver abscesses in feedlot cattle through its antibiotic activity on *Fusobacterium necrophorum* (15).

Gram-negative bacteria have been recovered from inside cattle houses (25), and the route of transmission for gram-negative respiratory pathogens, such as *Pasteurella multocida*, can be the air (22). Pathogenic gram-negative organisms, such as *Salmonella* sp. and *Escherichia coli*, have been

TABLE 3. Means and standard errors for airborne microbial flora recovered by using an Andersen sampler and a range of agar-incubation combinations from feedlot cattle pens under two feeding regimens (four pens/treatment)

Andersen stage	Incubation conditions	Mean CFU/m^3 of air[a]	
		Standard feeding treatment	Alternative feeding treatment
1	Blood agar, 22°C	1,533 (1,036.8)[b]	2,173 (1,294.3)
	Blood agar, 37°C	514 (381.1)	708 (412.7)
	Blood agar, anaerobic, 37°C	103 (101.2)	271 (186.6)
	Sabouraud's agar	61 (34.2)	57 (24.5)
5	Blood agar, 22°C	927 (474.4)	1,307 (941.6)
	Blood agar, 37°C	456 (66.1)	320 (240.6)
	Blood agar, anaerobic, 37°C	87 (50.8)	103 (63.3)
	Sabouraud's agar	22 (16.5)	24 (14.4)
6	Blood agar, 22°C	556 (512.8)	556 (435.3)
	Blood agar, 37°C	175 (91.1)	103 (91.8)
	Blood agar, anaerobic, 37°C	57 (53.2)	19 (11.2)
	Sabouraud's agar	8 (5.2)	0 (0)
All	Blood agar, 22°C	3,017 (1,420.9)	4,037 (2,649.4)
	Blood agar, 37°C	1,146 (425.9)	1,132 (562.9)
	Blood agar, anaerobic, 37°C	248 (194.6)	393 (241.5)
	Sabouraud's agar	92 (42.4)	82 (17.9)

[a] There were no significant differences between treatments at the $P = 0.05$ level.
[b] The values in parentheses are standard errors.

TABLE 4. Temperature and relative humidity values at a cattle feedlot at 1800 h for 4 May 2000, 5 May 2000, 28 May 2000, 30 May 2000

Date (mo/day/yr)	Temp (°C)	Relative humidity (%)
5/4/2000	28	55
5/5/2000	32	28
5/28/2000	33	19
5/30/2000	37	25

found in feedlot cattle fecal samples (7, 9), and interestingly, Wathes et al. (24) demonstrated experimentally that although *Salmonella enterica* serovar Typhimurium was viable for only a short period of time, airborne transmission could be a primary route of infection in calves for this organism. It is speculative as to whether the presence of dust in feedlot air may have had a protective effect for gram-negative organisms. However, Thorne et al. (23) stated that some airborne dusts support aggregates of microbes and that these aggregates might protect individual microorganisms. The dusts also can absorb gases and vapors. These data and results of previous studies suggest that it is likely that gram-negative organisms were present in the feedlot environment but were reduced in number by the environmental conditions and may have been in a viable but nonculturable state once they were airborne.

The presence of bacterial endotoxins (heat-stable biological compounds located in the cell envelopes of gram-negative organisms) may be a significant issue with regard to animal health. In humans, endotoxins affect humoral and cell-mediated immunity (3) and have the potential to affect lung function (10). Endotoxin is stable, and its toxic effects are known to persist for long periods of time in dust (26). High endotoxin levels have previously been found in cattle feedlot dust samples (16). Therefore, it is possible that the effects of endotoxin in conjunction with the allergic and irritant properties of the dust could predispose cattle to illness.

Thayer et al. (21) found organisms similar to those found in this study in a survey of different levels of a feedlot waste pile. Hubrant (12) also obtained similar results in a study in which dirt and runoff from cattle feedlot pens were examined. Only facultative anaerobic bacteria were recovered in this study. However, Chai et al. (4) recovered *Clostridium perfringens*, a strict anaerobe, in both indoor and outdoor air of calf stables.

The Dustrak results showed that there was a significant difference between the test and control feeding patterns as determined at the downwind sites. This was not reflected by the Andersen sampler results at any of the stages. This may have been due to the microbial selectivity of the Andersen sampler protocol and to the operating principle of the Dustrak system. In this system a photometer measures the aggregate signal from a cloud of particles in a certain size range. This does not allow detection of individual particles. In this study, bacteria were recovered in a size range from 0.65 to 7.0 μm, indicating that it was possible for the bacteria to be airborne either on dust particles, in clumps, or individually.

The numbers of airborne organisms recovered in this study were lower than the numbers found in other related Andersen sampler studies of intensive farming systems. For example, bacterial numbers ranging from 3.3×10^5 to 1.44×10^6 CFU/m^3 of air have been found in intensive piggeries (5), and a level of 6.76×10^4 CFU/m^3 of air has been found in calf-housing operations (1). The probable reasons for the lower levels in this study are a high dispersion rate due to the dynamic nature of outside air, the low relative humidity, and the bactericidal effect of UV light.

In conclusion, under the conditions of this study, the culturable microbes in cattle feedlot air were mainly gram-positive bacteria. Gram-negative bacteria may have been present in a viable but nonculturable state. The dust levels measured with a Dustrak monitor showed reduced dust generation with one feeding pattern, but the microbial levels measured with an Andersen sampler did not reflect this change.

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