

# Effects of aerosolized feedyard dust that contains natural endotoxins on adult sheep

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**Objective**—To determine the clinical, clinicopathologic, and histologic effects of aerosolized feedyard dust that contains natural endotoxins on adult sheep.

**Animals**—Eighteen 3-year-old Saint Croix sheep.

**Procedure**—A prospective randomized controlled study was conducted. There were 2 treatment groups (dust-endotoxin group,  $n = 9$ ; control group, 9). Aerosolized feedyard dust was provided continuously during a 4-hour period for each application (once in week 1, 3 times in week 2, and 7 times in week 3) to sheep in a semiairtight tent. All sheep were euthanized and necropsied 8 hours after the treatment group received the last dust treatment. Variables measured before and after each dust treatment were rectal temperature, total WBC count, and concentrations of fibrinogen and haptoglobin.

**Results**—Mean amount of dust administered during each treatment was 451 g/4 h. Filter collection indicated 51 mg of dust/m<sup>3</sup> and 7,423 ng of endotoxin. Mean rectal temperature at 8 hours (40.4 C) and mean WBC counts 12 and 24 hours after dust treatment were significantly higher for the treated group than the means of the respective variables for the control group. Similar responses were observed with repeated dust-endotoxin treatments; however, with each subsequent treatment, there was a diminished response. Sheep in the treatment group had generalized alveolar septal thickening and hypercellularity.

**Conclusions and Clinical Relevance**—Feedyard dust induced a temporary febrile response and leukocytosis in sheep in the treatment group. Exposure to dust that contains endotoxins may be a stressor preceding acute infectious respiratory tract disease of marketed sheep. (*Am J Vet Res* 2002;63:28–35)

Air quality in urban and rural communities has become a pressing public health issue during the past 2 decades. In rural communities, agriculture practices are believed to be responsible for much of the air pollution.<sup>1-3</sup>

Agricultural industries such as large integrated poultry farms<sup>4</sup> in the southeastern United States, large

swine farms<sup>5,6</sup> in the Oklahoma and Texas panhandle area, and large feeder calf operations in the Southern High Plains produce large amounts of manure. For example, 1 feedyard may stock 100,000 calves/y, which results in 100,000 tons of dry manure. Manure has a large content of gram-negative bacteria, and when manure becomes dry, it produces dust particles<sup>7</sup> that contain endotoxins<sup>8</sup> originating from the gram-negative bacteria. A substantial portion of environmental dust-borne endotoxin is in the form of microvesicles.<sup>8</sup>

All gram-negative bacteria produce endotoxins. The outer membrane of gram-negative bacteria is composed of lipopolysaccharide molecules (ie, endotoxin). The most toxic part of endotoxin is the lipid-A portion.<sup>9</sup> Endotoxins are a relatively heat-stable biologically active material that profoundly affects humoral and cell-mediated immunity when injected parenterally.<sup>10,11</sup> Complement and the coagulation systems also are affected by endotoxins, and endotoxins interact with many cell types.<sup>12</sup>

Effects of aerosolized administration of environmental endotoxin or organic dust to ruminants have not been reported. Little literature exists on the parenteral use of purified endotoxin in ruminants,<sup>13-16</sup> although endotoxin has been used to induce mastitis in cows.<sup>17</sup> The influence of inhaled endotoxins has seldom been investigated under conditions similar to those encountered in field conditions.<sup>18</sup> In 1 report,<sup>19</sup> investigators described the use of dust-borne endotoxins and endotoxin-contaminated feed in hogs.

We examined the literature concerning inhalation of endotoxins in humans, because there is a paucity of reports on that topic in ruminants. Most of the aerosol experiments in humans involve the use of purified endotoxin.<sup>11</sup> However, an enormous amount of information has been compiled on occupational endotoxin exposures.<sup>2,3</sup> Results of 1 study<sup>20</sup> indicate that endotoxin contained in organic dust is the most reactive part of the dust particle when inhaled by humans. In another study,<sup>b</sup> it was indicated that the endotoxin part of feedyard organic dust was the active component when inhaled by ruminants.

Clinically, endotoxin (doses of 20 to 300  $\mu$ g) administered via the respiratory route in humans commonly induces tightness of the chest, irritation of the airways, and fever 6 to 8 hours after exposure. Less common signs include headache, pain in the joints and muscles, nausea, and tiredness.<sup>21</sup> Cough, dyspnea, and sputum production were reported after a dose of 0.9  $\mu$ g of endotoxin/ml.<sup>22</sup> An increase in peripheral total leukocyte counts and neutrophils was observed in humans 6 hours after inhalation of a solution containing 30 to 60  $\mu$ g of endotoxin.<sup>23</sup>

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The objectives of the study reported here were to determine the clinical, clinicopathologic, and histologic effects of aerosolized feedyard dust that contains natural endotoxins on 9 adult sheep treated in a semiairtight tent,<sup>c</sup> compared with 9 control sheep. Also, the responses of the sheep to dust-endotoxin were compared with those reported in humans.

## Materials and Methods

**Animals**—Twenty 3-year-old female Saint Croix sheep were housed in a barn. There were 3 sheep/pen; each pen was 7,420 cm<sup>2</sup>. Sheep were treated for internal helminth parasites<sup>d</sup> and coccidia.<sup>e</sup> Sheep were limit fed a commercial pelleted ration (44% grain concentrate, 20% alfalfa hay, 30% cottonseed hulls and meal, and 5% molasses, supplemented with vitamins A and E and trace minerals), and water was available ad libitum. Sheep were randomly allotted to control and treatment groups (9 sheep/group) and allowed to adjust to their environment for 2 weeks. The remaining 2 sheep were used as carbon black control sheep. Protocols for all animal experimentation were approved by the Regional Animal Care and Use Committee.

**Preparation of feedyard dust**—Dried manure was removed from an active feedyard and stored in plastic barrels for 1 to 6 months. The dried manure was pulverized by passing it through 2-mm sieve roller drums.<sup>f</sup> The particles then were passed through a series of sieves,<sup>g</sup> and the particles were further reduced in size, using an automated soil grinder.<sup>h</sup> The dust (5.96% moisture) was stored in covered buckets until used. Three 10-g samples of dust from each of 3 buckets were collected and analyzed for endotoxin content.

Microbial activity (bacterial and fungal) of the dust was determined, using standard microbial dilution techniques. Thermophilic bacteria were cultured on **brain-heart infusion (BHI)** medium at 55 C and quantified 24 hours later, and mesophilic bacteria were cultured on BHI medium at 28 C and quantified 24 hours later. Anaerobic bacteria also were cultured on BHI medium at 28 C and quantified 48 hours later. Plates were placed in anaerobic jars<sup>i</sup> fitted with gas-generator envelopes<sup>k</sup> for production of an anaerobic atmosphere, and indicator strips<sup>l</sup> were used to verify that the atmosphere was anaerobic. Thermophilic fungi were cultured on malt agar<sup>m</sup> at 50 C and quantified 6 days later, whereas mesophilic fungi were cultured on malt agar and **Littman oxgall agar (LOA)**<sup>n</sup> medium at 28 C. Colonies on malt agar were quantified at 24 hours, and colonies on LOA were quantified at 96 hours. The LOA medium contained inhibitors to prevent overgrowth by rapidly growing fungi. Antibacterial inhibitors (chlortetracycline<sup>o</sup> [5 mg/L] and streptomycin sulfate<sup>p</sup> [100 mg/L]) were incorporated into malt agar to prevent bacterial growth, and an antifungal inhibitor (cycloheximide<sup>q</sup> [100 mg/L]) was incorporated into the BHI media to prevent fungal growth.

Feedyard dust also was analyzed by use of the multiple-tube method for gram-negative microbes (total coliforms [9221B], total fecal coliforms [9221E], and gram-positive *Enterobacter faecalis* index organism [9230B]).<sup>24</sup> Isolation of *Salmonella* spp was accomplished by use of the following technique. An aliquot (10 g) of dust was placed in a tube and diluted by addition of 90 ml of 2X peptone broth<sup>r</sup> containing 25 µg of novobiocin/ml. Tubes were incubated at 37 C for 24 hours. Ten milliliters of the solution was then diluted by addition of 90 ml of tetrathionate broth<sup>s</sup> containing 2% iodine, and the tubes were incubated at 37 C for 24 hours. Plates containing brilliant green agar<sup>t</sup> were streaked with the solution and incubated for 24 hours at 37 C. *Salmonella*-like colonies that grew on the plates were inoculated into urease agar,<sup>v</sup> triple-sugar-iron agar,<sup>w</sup> lysine-iron agar,<sup>x</sup> and xylose-lysine-deoxycholate agar.<sup>y</sup> *Salmonella*-like colonies also were

streaked onto tryptose agar<sup>z</sup> with 5% bovine blood. These assays were repeated 3 times.

Aerosolized dust also was analyzed for endotoxin content by collecting dust for 30 minutes into three 2-stage microbial-cascade impactors.<sup>aa</sup> Two glass Petri dishes, each containing 20 ml of reverse-osmosis water,<sup>bb</sup> were carefully placed in the top (stage 1) and bottom (stage 6) of a 6-stage microbial cascade impactor.<sup>cc</sup> In addition, a series of 3 glass bottles (traps), each of which contained 20 ml of reverse-osmosis water, were inserted into the impactor to assay for endotoxin passing through the impactor that was lost to the environment. Samples collected into the 3 traps were combined for analysis. Samples analyzed for endotoxin concentration included water from stage 1 (nonrespirable particles), stage 6 (respirable particles), and the traps (respirable particles). For reporting, stage 1 was designated 0, and stage 6 was designated 00.

**Determination of basic respiratory values**—After an acclimation period of 2 weeks, basic respiratory values were determined for the sheep. Tidal air is defined as the volume of air inspired or expired in 1 respiration. Tidal air for a 40-kg sheep is 296 ml, as determined by use of the following equation<sup>25</sup>: tidal air = weight of animal (in g) × 0.0074. Ventilation rate per minute is the tidal air volume times the number of inspirations per minute, which typically is 20 for sheep and goats. Thus, 20 × 296 ml = 5,920 ml of air inspired/min. In 4 hours, each sheep inspired (5,920 ml/min × 240 min)/1,000 ml = 1,420.8 L of air.

**Administration of aerosolized dust to sheep**—On each dust treatment day, sheep were moved into a semiairtight dust tent (183 × 244 × 213 cm) made of 8-oz vinyl-coated polyester fabric that was supported on a pipe frame. The tent had zippered doors at each end that provided access. The tent was divided into 3 stalls, each of which accommodated 3 sheep. Box fans (50 cm in diameter) were placed inside each of the tents to circulate air and cool the sheep. A polyvinyl chloride (PVC) pipe (3.75 cm in diameter) was used to supply the dust. The pipe entered the tent and was affixed to the interior of the tent wall. It emptied onto a set of baffles placed near the ceiling in the center of the tent. A second tent of similar design was used to contain the nondust (control) sheep.

An aliquot (1,500 g) of prepared feedyard dust was placed in a hopper<sup>dd</sup> that had a 0.6-cm auger in the bottom. The auger speed was set to uniformly deliver 950 g of dust during the 4-hour application. The dust was augered into a metal funnel that led to a jet mill<sup>ee</sup> (138 KPa) that further pulverized it and separated larger particles, which were lifted into a stainless-steel trap.<sup>ff</sup> An air vibrator (run with 69 KPa of air) was attached to the outside of the hopper and touched the funnel; the vibrations prevented build-up of dust in the funnel. Smaller particles from the jet mill were blown through the PVC pipe and out onto baffles located near the ceiling of the tent. Dust dissemination by the jet mill was augmented with air produced by a gasoline-powered portable air compressor<sup>gg</sup> and with air from a large blower motor.<sup>hh</sup>

The treatment group was exposed to aerosolized dust for 4 hours on 1 day during week 1, 3 days during week 2, and 7 days during week 3. During week 4, sheep in the treatment group were allowed to rest for 4 days after the seventh dust treatment. They then were again exposed to dust application for 4 hours. Eight hours after completion of this dust treatment, the sheep were euthanatized and necropsied.

Control sheep were handled in the same manner as the treatment group, except they were not exposed to aerosolized dust. Samples and measurements were obtained at the same time as those of the treatment sheep.

**Collection of data and samples**—Prior to each 4-hour dust application, blood samples were collected from a jugu-

lar vein, and rectal temperatures were obtained (baseline data). Clinicopathologic variables (total WBC count, absolute neutrophil and lymphocyte counts, and haptoglobin and fibrinogen concentrations) and rectal temperatures were measured 4, 8, 12, and 24 hours after the completion of the dust treatment during week 1. Rectal temperatures also were recorded at 95 hours after completion of the dust treatment during week 1.

During week 2, rectal temperatures were obtained prior to the first dust treatment and 4, 8, and 12 hours after completion of each of the 3 dust treatments. Additionally, rectal temperature was measured 24 hours after completion of the third dust treatment. Total WBC counts were obtained prior to the first dust treatment and 4, 8, 12, and 24 hours after completion of the third dust treatment.

During week 3, rectal temperatures were obtained prior to the first dust treatment and 4, 8, and 12 hours after the completion of each dust treatment. Additionally, rectal temperature was measured 24 hours after completion of the seventh dust treatment. Total WBC counts were obtained prior to the first dust treatment, 4, 8, and 12 hours after completion of the first and seventh dust treatments, and again 24 hours after completion of the seventh dust treatment.

**Exposure to carbon black**—During week 4, carbon black dust treatments were completed in 2 sheep to determine whether aerosol particles were more discernible in the lungs than dust particles from manure. The 2 sheep were exposed to an aerosol containing 200 g of carbon black<sup>ii</sup> mixed with 1,000 g of dust for 4 hours on each of 4 days, similar to the regimen used for the treatment group. Rectal temperatures were measured 4, 8, 12, and 24 hours after completion of the first carbon black exposure; 4, 8, and 24 hours after completion of the second carbon black exposure; 4, 8, 12, and 17 hours after completion of the third carbon black exposure; and 4, 6, and 8 hours after completion of the fourth carbon black exposure. Sheep then were euthanatized and necropsied.

**Necropsy**—All necropsies were performed by a board-certified veterinary pathologist. Gross and histologic examinations of the tissues were performed by the pathologist, who was not aware of the group status of each sheep. Tissue samples from all 7 lung lobes of 9 control sheep, 8 treatment sheep, and 2 sheep treated with aerosolized carbon black were harvested. Tissues from the 7 lung lobes were immediately placed in buffered 10% formalin. The tissues were processed, sectioned, and stained with H&E, and the sections were examined microscopically, using a double-blind procedure.

**Clinicopathologic analysis**—The WBC counts, serum concentration of haptoglobin,<sup>26,27</sup> and plasma concentration of fibrinogen<sup>28</sup> were determined by personnel at another laboratory.<sup>ii</sup>

**Analysis of dust-endotoxin**—The kinetic chromogenic semiquantitative limulus amoebocyte lysate assay was used; this assay is nonreactive to glucans.<sup>kk</sup> Aliquots of extracts were serially diluted in 10-fold increments, using pyrogen-free water. A 100- $\mu$ l aliquot of each dilution was mixed with 100  $\mu$ l of freshly prepared limulus amoebocyte lysate containing chromogenic substrate in a pyrogen-free microtiter plate<sup>ll</sup> that had been stored at 37 C. Color development was monitored every 15 seconds, using a microtiter plate-reading spectrophotometer.<sup>mm</sup> This instrument determines the time interval required to reach an absorbency value of 0.03; this interval then was compared with values for an endotoxin standard curve developed for the range of 5 ng/ml to 0.5  $\mu$ g/ml. Values for standards were linear over a range of 5 logarithms on a log-log plot. Unknown samples were calculated by the software, using linear interpolation. All dilutions were assayed in duplicate, and a parallel dilution was spiked with 50  $\mu$ g of endotoxin to assess enhance-

ment or inhibition of activity by any of the extracted samples. Only those dilutions that did not have evidence of enhancement or inhibition and that were parallel to the standard curve were used. Mean values for 3 replicates were calculated. Mean coefficient of variation for these assays was 7.6%. To convert endotoxin activity to equivalent mass units of the EC6 reference standards,<sup>nn</sup> a factor of 10 equivalent units/ng of endotoxin was used. Analysis of endotoxin was conducted by personnel at another laboratory.<sup>oo</sup>

**Quantification of dust and determination of particle size**—A 5-stage cyclone device<sup>pp</sup> was used to quantify small particles of dust (< 0.32  $\mu$ m) that were collected on filter paper after stage 5. Stages 1 through 5 measured particles of 5.4, 2.1, 1.4, 0.65, and 0.32  $\mu$ m, respectively.

Eight 47-mm filter holders<sup>qq</sup> were equipped with 0.45- $\mu$ m filters.<sup>rr</sup> These filters were used to determine the amount of dust in the air of the tent. Vacuum pumps were used to collect 128.3 L of air/min. All determinations were performed in duplicate. Filters were weighed, placed in the holders, and weighed again after 30 minutes in the tent to determine the quantity of dust in the tent during the dust treatment. Mean values were calculated for duplicate samples.

Five open Petri dishes were placed on a board that supported the cyclone device. These dishes were allowed to collect dust particles that settled out of the air during the 4-hour period. Dishes were weighed before and after collection of dust particles. Collection of larger particles was determined for the entire area of the tent.

A particle size analyzer<sup>ss</sup> with optical bench (dual 466-nm blue light-emitting diode and 2 mW 633-nm helium-neon laser-light source) was used to determine the proportion of various-sized particles in the feedyard dust. This analysis was performed in duplicate.

**Statistical analysis**—Mean values for measured variables were compared by use of an ANOVA.<sup>29</sup> Mean values for rectal temperature, total WBC counts, neutrophil and lymphocyte counts, and fibrinogen and haptoglobin concentrations were compared between treatment and control groups throughout the study and within collection periods for specific sample days. Significant differences between mean values also were determined by the use of the Bonferroni and Dunnett adjusted paired *t*-test ( $P \leq 0.05$ ), which allowed pairwise comparisons of means for the 2 groups within any day of sample collection. Fifty percent endpoints were calculated, using the method of Reed and Muench.<sup>30</sup>

## Results

**Feedyard dust**—Feedyard dust contained 146  $\mu$ g of endotoxin/g of dust. Mean  $\pm$  SEM number of viable microbial colonies per gram of dust was  $6.3 \times 10^7 \pm 2.4 \times 10^7$  for anaerobic bacteria,  $1.5 \times 10^8 \pm 1.9 \times 10^7$  for mesophilic bacteria,  $6.3 \times 10^6 \pm 3.3 \times 10^6$  for thermophilic bacteria,  $1.4 \times 10^4 \pm 1.0 \times 10^4$  for mesophilic fungi, and  $1.3 \times 10^3 \pm 4.5 \times 10^2$  for thermophilic fungi.

Mean number of coliforms per gram of dust was  $1.7 \times 10^5 \pm 1.4 \times 10^5$ . Mean number of fecal coliforms was  $3.1 \times 10^4 \pm 0.0/g$  of dust, and mean number of *Enterococcus faecalis* was  $1.8 \times 10^5 \pm 1.4 \times 10^5/g$  of dust. *Salmonella* spp were not isolated from feedyard dust.

Dry feedyard dust was analyzed by passing two 1-gram samples through the analyzer. The histograms were extremely similar. Particle size ranged from 0.5 to 275  $\mu$ m (mean, 85.36  $\mu$ m).

**Analysis of feedyard dust during dust application**—Mean  $\pm$  SEM values for the amount of dust dur-

Table 1—Mean (SEM) amount of aerosolized feedyard dust applied during each 4-hour application period

Week*	Dust left in hopper (g)†	Dust in traps (g)	Dust in tent		Cyclone stage					Cyclone filter (g)	Dust in 5 Petri dishes		Tent area (cm <sup>2</sup> )	Passive dust (g)
			g	(g/m <sup>3</sup> )/4 h	1	2	3	4	5		g	cm <sup>2</sup>		
1	604.7	507.8	387.5	40.7	0.1207	0.0736	0.1007	0.0269	0.0329	0.0003	0.7086	392.7	44,594	18.044
2	502.8 (7.1)	536.8 (24.4)	460.4 (31.5)	48.4 (31.5)	0.2260 (0.0211)	0.0699 (0.0156)	0.0985 (0.0115)	0.0248 (0.0041)	0.0277 (0.0016)	0.0079 (0.0013)	0.9600 (0.0553)	ND	44,594	24.447 (1.409)
3	509.0 (65.4)	524.1 (53.9)	466.9 (25.2)	49.1 (2.6)	0.2305 (0.0285)	0.0905 (0.0101)	0.1444 (0.0074)	0.0491 (0.0036)	0.0631 (0.0154)	0.0110 (0.0015)	1.1159 (0.0987)	ND	44,594	28.417 (2.491)
4	507.9 (28.4)	399.3 (6.1)	292.9 (34.5)	30.8 (3.6)	0.0191 (0.0049)	0.0088 (0.0007)	0.0163 (0.0013)	0.0138 (0.0028)	0.0681 (0.0181)	0.1299 (0.0052)	0.6102 (0.0048)	ND	44,594	15.537 (0.124)

\*There was 1 dust application in week 1, 3 dust applications in week 2, 7 dust applications in week 3, and 1 dust application in week 4. †The hopper was initially filled with 1,500 g of dust for each dust application.  
ND = Not determined.

ing the 4-hour application periods was calculated (Table 1). Mean  $\pm$  SEM amount of dust collected from the tent for each 30-minute period during the 4-hour application periods was determined. Values were as follows: 0 to 0.5 hours,  $49.63 \pm 4.92$  mg/m<sup>3</sup>; 0.5 to 1.0 hours,  $46.87 \pm 3.90$  mg/m<sup>3</sup>; 1.0 to 1.5 hours,  $33.48 \pm 2.86$  mg/m<sup>3</sup>; 1.5 to 2.0 hours,  $47.88 \pm 3.45$  mg/m<sup>3</sup>; 2.0 to 2.5 hours,  $50.04 \pm 4.36$  mg/m<sup>3</sup>; 2.5 to 3.0 hours,  $58.68 \pm 7.34$  mg/m<sup>3</sup>; 3.0 to 3.5 hours,  $47.22 \pm 1.91$  mg/m<sup>3</sup>; and 3.5 to 4.0 hours,  $70.96 \pm 10.16$  mg/m<sup>3</sup>. Overall mean was  $50.59 \pm 10.74$  mg/m<sup>3</sup>.

**Clinical effects**—Anxiety or restlessness was not observed in sheep in the treatment group during any of the 4-hour dust applications. A few sheep coughed during the dust application. In general, coughing seemed to increase for 1 to 2 hours after the treated sheep were returned to their pens. When treated sheep were removed from the dust tent, their wool was extremely dark and coated with particulate matter from the dust. Food consumption of treated sheep decreased by a third on the morning after the first dust application, compared with consumption for the control sheep. Clinical signs of adverse health were not observed in the control sheep.

Mean rectal temperatures of the treated sheep for week 1 (ie, 1 dust treatment) were significantly ( $P < 0.001$ ) higher, compared with values for control sheep. Mean rectal temperatures of the treatment group were significantly higher 4, 8, 12, and 24 hours after the first dust treatment, compared with corresponding mean rectal temperatures of the control sheep (Fig 1). The amplitude of difference in mean rectal temperatures appeared to decrease between the 2 groups over time after a series of dust applications; therefore, a state of tolerance apparently developed after repeated dust treatments.

**Clinicopathologic effects**—Total WBC counts increased significantly in treated sheep following the first dust application during week 1. Mean absolute total WBC counts of treated sheep 12 and 24 hours after the first dust application were significantly higher, compared with corresponding mean counts for control sheep (Fig 2).

At 4 hours after the first dust treatment during week 1, treated sheep had a significant increase in absolute mean number of neutrophils and a significant

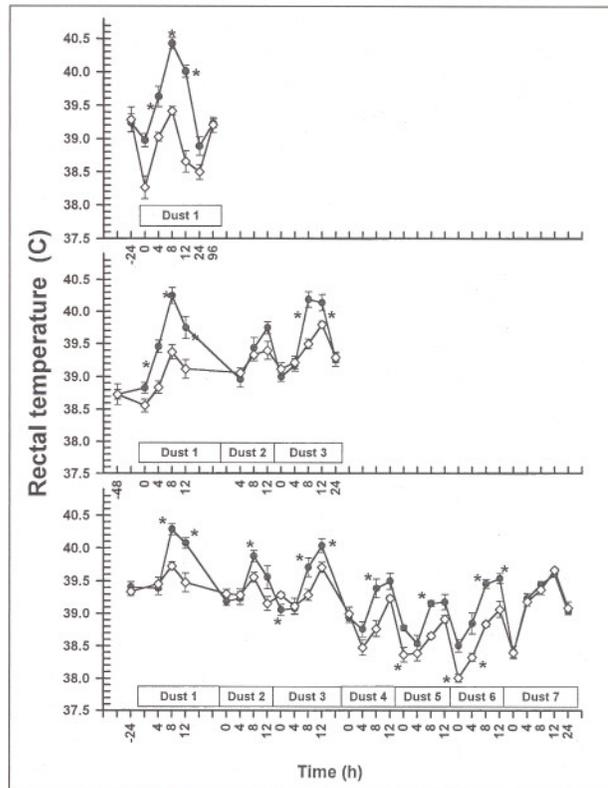


Figure 1—Mean ( $\pm$  SEM) rectal temperatures in 9 sheep exposed to aerosolized feedyard dust for 4-hour periods (solid circle) and 9 control sheep (open circle). Dust treatments were administered once during week 1 (top panel), on 3 days (dust 1, 2, and 3) during week 2 (middle), and daily for 7 days (dust 1 through 7) during week 3 (bottom). \*For a specific time point, values differ significantly ( $P \leq 0.05$ ) between the 2 groups. Time 0 = Start of 4-hour dust application period.

decrease in absolute mean number of lymphocytes, compared with values for the control sheep (Fig 3 and 4). Following the third dust treatment during week 2, there was not a significant difference in mean absolute number of neutrophils between the treated and control groups. After the first dust treatment during week 3, mean absolute number of neutrophils was significantly increased in the treated sheep at 8 and 12 hours, compared with values for the control sheep. By 12 hours, mean number of neutrophils had increased to  $5,540 \pm 556$  neutrophils/ $\mu$ l for the treated sheep. However, by the seventh dust treatment during week 3, mean

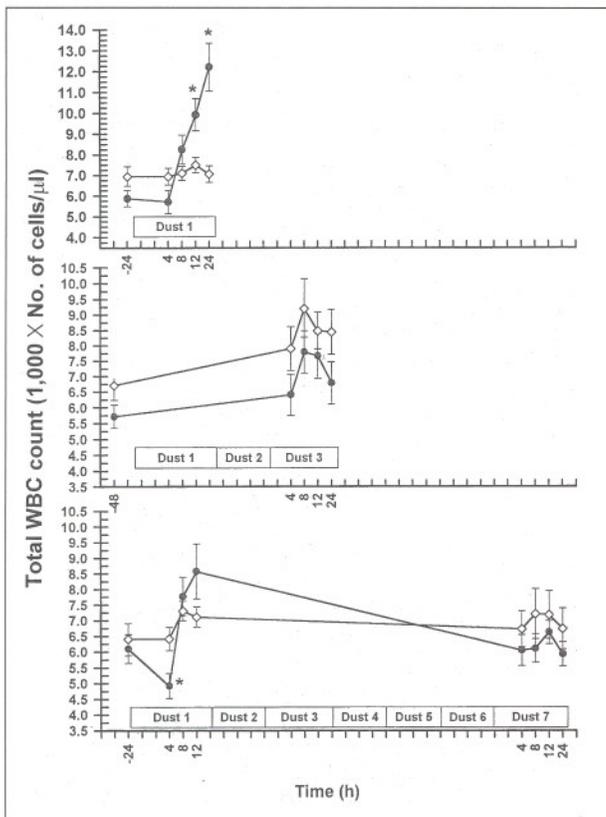


Figure 2—Mean ( $\pm$  SEM) total WBC counts for treated and control sheep. See Figure 1 for key.

absolute number of neutrophils in the treated group was less than in the control group.

Mean absolute number of lymphocytes 8 hours after the first dust treatment in the treated group was significantly lower, compared with the value for the control sheep. However, the lymphocytes were nonresponsive after the seventh dust treatment during week 3, when the absolute mean number of lymphocytes for control and treated sheep was almost identical.

Mean haptoglobin concentrations were not significantly different between the treated and control sheep following the first dust treatment. Haptoglobin concentrations were significantly higher in treated sheep after the third dust treatment during week 2, compared with concentrations in control sheep. Mean haptoglobin concentration of the treated sheep 48 hours prior to the third dust application in week 2 was 0. However, mean concentration was  $35.9 \pm 14.3$ ,  $23.6 \pm 9.7$ ,  $20.5 \pm 10.5$ , and  $43.9 \pm 20.0$  mg/dl 4, 8, 12, and 20 hours after the third dust application during week 2. Values for treated sheep were significantly higher at all time points, except for values obtained at 12 hours, compared with values for the control sheep (0 mg/dl). Mean haptoglobin concentrations were not significantly different between treated and control sheep following 7 dust treatments during week 3.

Mean fibrinogen concentrations were not significantly different between the groups after 1 dust treatment during week 1 and 3 dust treatments during week 2. Significant differences were detected 8 hours after the seventh dust treatment during week 3. At that

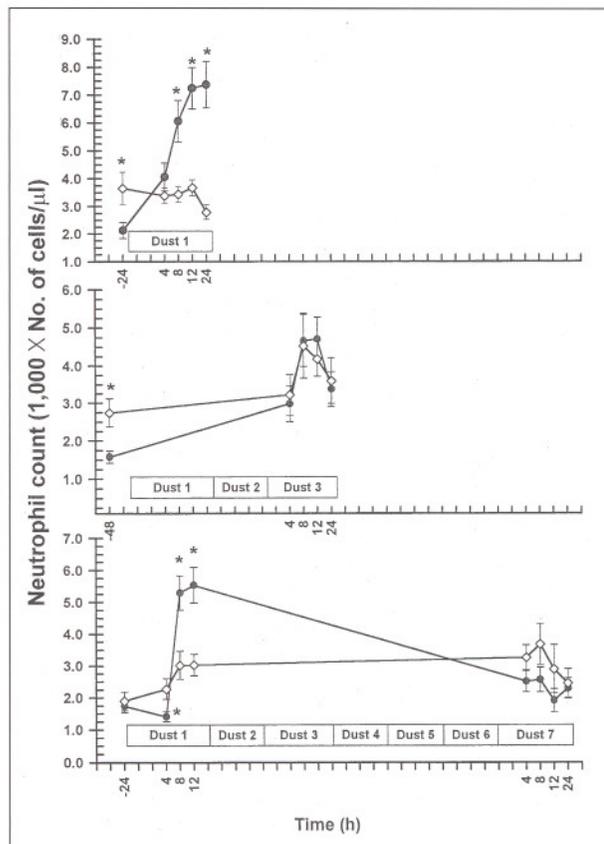


Figure 3—Mean ( $\pm$  SEM) total neutrophil counts for treated and control sheep. See Figure 1 for key.

time, mean fibrinogen concentration for treated sheep increased to  $481 \pm 45.3$  mg/dl.

**Necropsy**—During necropsy, we did not detect grossly observable differences between the respiratory tracts of control and treated sheep. Furthermore, substantial microscopic lesions were not observed in the control sheep. One sheep from the treated group had caseous lymphadenitis in an enlarged thoracic lymph node. This sheep had erratic temperatures and WBC counts during the study; therefore, data for this sheep were not included in the statistical analysis.

Histologic changes were observed in all treated sheep. Generalized alveolar septal thickening and hypercellularity as a result of infiltration of macrophages, lymphocytes, and plasmacytes; a few eosinophils was evident. Minimal widely scattered bronchiolar and terminal airway exudation of neutrophils and eosinophils; increased numbers of perivascular macrophages, lymphocytes, plasmacytes, and a few eosinophils; and increased numbers of alveolar and airway macrophages filled with fine gray-black dust-like particulate material and silica crystals were reported. One sheep in the treatment group had a focal area of chronic fibrosing exudative pneumonia localized to the ventral aspect of the right apical lobe. Mediastinal lymph nodes did not have discernible or had only a minimal number of macrophages filled with gray-black dust-like particulate material and silica crystals. In general, mild sub-

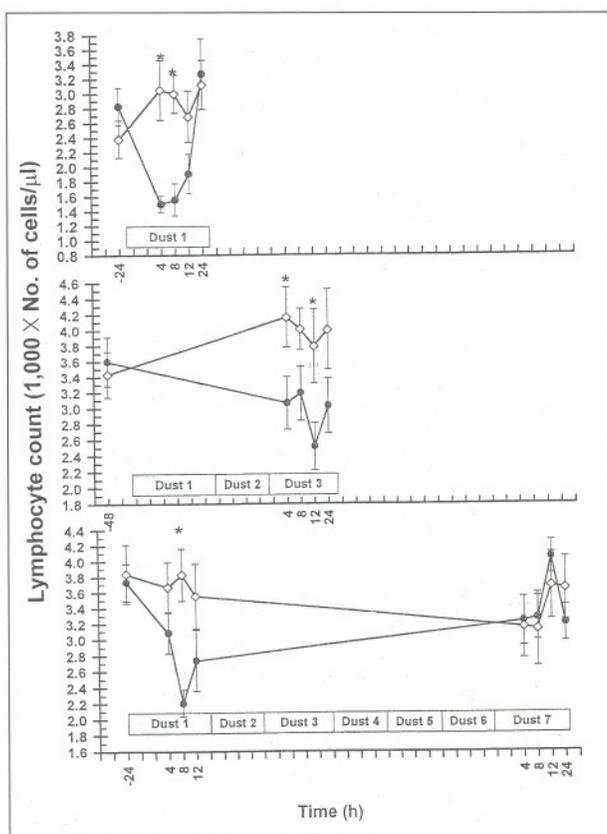


Figure 4—Mean ( $\pm$  SEM) absolute lymphocyte counts for treated and control sheep. See Figure 1 for key.

acute interstitial pneumonia was observed in the treated sheep.

Sheep exposed to aerosolized carbon black had discernible findings. Lymph nodes in the respiratory tract of these sheep were black, and some had a black ring on the cut surface. All lobes of the lungs had a uniform speckled appearance under the pneumopleural lining. Carbon black also was clearly visible on the cut surface of the lungs. Microscopically, there was generalized alveolar septal and airway macrophage accumulation of carbon black particles with alveolar septal thickening and hypercellularity as a result of infiltration of macrophages, lymphocytes, plasmacytes, and a few eosinophils. There was minimal widely scattered bronchiolar and terminal airway exudation of neutrophils and eosinophils. Macrophages in the mediastinal lymph nodes often were filled with carbon black particles.

## Discussion

Examination of the consequences of inhaling dust that contains endotoxins is important for ensuring the health of humans and domestic animals, especially in geographic areas of the country known for their amount of aerosolized dust. Organic dust is an important health concern in rural communities,<sup>1-3</sup> in regions where the soil is cultivated for agricultural purposes,<sup>31</sup> and in areas where harvesting of vegetable fibers<sup>32-36</sup> and processing of grains produces dust.<sup>37,38</sup> Dust also is a problem in operations that involve feeding of a large number of animals.<sup>7,39,40,a</sup> It is important that animal waste be prevented from polluting the water, land, or atmosphere.<sup>41</sup>

Dust is 1 of the largest nuisance problems in rural areas,<sup>42</sup> and endotoxin is the most active component of organic dust.<sup>3</sup> In another study,<sup>b</sup> we determined that it was the endotoxin portion and not the microbial component of feedyard organic dust that causes fever and leukocytosis. Few studies have been conducted to determine the effects that dust-endotoxin has on domestic animals. The response to endotoxin is rapid, and the effects appear to be transitory. Repeated episodes of endotoxin exposures or continuous exposure to endotoxins may ameliorate negative reactions when body systems cannot respond adequately to the repeated stimulation. In fact, tolerance to lipopolysaccharide in human blood monocytes has been explained on the cellular level.<sup>43</sup> It appears that when monocytes are stimulated with lipopolysaccharide, they efficiently produce cytokine; however, during secondary stimulation, this response is only minimal.

It is recognized that CD14 on phagocytes is an important receptor site for endotoxins<sup>44,45</sup> and that lipopolysaccharide-binding protein forms a complex and interacts with CD14, leading to cellular activation.<sup>46</sup> Also, soluble CD14 may act as a negative regulator of T-cell activation and function in humans.<sup>47</sup>

In 1 report<sup>18</sup> in pigs, investigators used exposure to flour dust (1 to 15 mg/m<sup>3</sup>) and dust-borne endotoxin (50 to 2,500 ng/m<sup>3</sup>) for 6 days. In that study, dust-borne endotoxin in pigs did not have effects that were attributable to endotoxin alone. However, the dust-borne endotoxin febrile effect, similar to the effect described in the sheep reported here, was detected in those pigs at 8 hours, and leukocytosis was evident between 12 and 24 hours. This may have been related to time of sample collection rather than the inability of the pigs to respond in a timely manner.

Large amounts of information can be found on the effects of purified endotoxin in humans and other animals. However, this does not solve the agricultural dust-endotoxin problem that exists for humans and domestic animals, especially in areas where concentrations of cattle are clustered in large feedyards and dusty conditions prevail.

High concentrations of endotoxin were found in the material on the surface of feedyards; therefore, collecting the surface scrapings was an appropriate source of natural endotoxins. In reviewing the literature, variables and time periods were selected to determine the optimum effect that aerosol exposure to dust-endotoxin may induce in sheep. It was suspected that ruminants may be quite resistant to dust-endotoxin because of their natural environment. Therefore, a maximum dose of dust-endotoxin was selected on the basis of maximum output of the equipment used. The amount of dust used in this study appeared to be less than that which can be detected in the Southern High Plains. The dust concentration in the tent was determined as mean total amount of dust introduced into the tent (ie, 447 g/240 min = 1.87 g/min). This dose was on the high side, because some dust would cling to the inside of the tent, and some particles were so large that they would fall to the floor. Dust particles > 20  $\mu$ m will settle to the floor. This was represented as passive dust fall-out (Table 1). Dose of dust determined by use of the filter-collection method for

each 30-minute period during a 4-hour application more closely approximated the actual amount of dust to which the sheep were exposed. Total distribution of particle sizes of the dust was analyzed. However, the larger particles emitted from the jet mill were removed by a trap before they entered the tent.

To determine the amount of dust that the sheep inspired, it was necessary to determine the amount of air a sheep will inspire during a 4-hour period. It was found that sheep each inspired 1.42 m<sup>3</sup> of air during that time. Using the 6-stage simulation of the respiratory tract, the distribution and amount of dust particles can be determined for specific sites in the respiratory system. Obviously, the dust application was adequate to trigger a biological reaction to the endotoxin contained in the feedyard dust. Sheep were exposed to 51 mg of dust and 7,423 ng of endotoxin/m<sup>3</sup> of tent air at any time during the 4-hour treatment period.

The 5-stage cyclone device collects only dust particles < 5.4 µm, which are sufficiently small that they can proceed deep into the respiratory tract of sheep. The apparatus collected dust particles that ranged from 5.4 to 0.32 µm by aspirating air from the tent at a rate of 38.3 L/min (6,792 L/4-hour treatment period). Mean total respirable dust that all stages of the cyclone apparatus were capable of collecting was 0.4758 g/4 h. Respirable dust particles (ie, those that reach the alveoli) are considered to be the most toxic. A goat typically inspires 1,420.8 L of air/4 h. This represents 21% of the air passing through the 5-stage cyclone apparatus. Therefore, the calculated dose of respirable dust particles that a goat inspires in 4 hours is 0.1 g, which represents 14.6 µg of endotoxin that could be inhaled into the alveoli. This calculation does not include the nonrespirable dust particles (10 to 6 µm) that contact the mucous membranes of the respiratory tract extending from the nasal mucosa to the smallest bronchioles. Soluble endotoxin located on the nonrespirable dust particles also has the ability to trigger CD14 endotoxin receptor sites on the mucous membranes.

After multiple dust treatments, the sheep apparently became refractory (tolerant), but they again became reactive after they were rested for several days from dust-endotoxin exposure. Therefore, cellular systems in sheep appear incapable of responding normally to endotoxin after repeated daily dust-endotoxin exposure. The reactions observed in the sheep following 1 dust treatment were similar to those observed in humans following exposure to purified endotoxin.<sup>21,23</sup>

The sheep were euthanatized and necropsied 8 hours after the last dust-endotoxin treatment, which theoretically was an optimum time as determined on the basis of the febrile and leukocyte responses; however, gross differences were not observed between the lungs of treated and control sheep. Histologic lesions were observed 8 hours after the last dust treatment in treated sheep but not in control sheep. Histologic examination revealed mild subacute interstitial pneumonia in the lungs of sheep in the treatment group. Dust granules could only be seen microscopically in phagocytic cells.

In sheep exposed to carbon black, the size of the carbon black granules was approximately the size of the prepared feedyard dust; however, during gross

examination, the carbon black granules were easily observed under the pleural surface of the lungs in all lobes as well as on the cut surface of the lungs. These particles were easily observed histologically in many phagocytes. Carbon black particles also were easily observed grossly in the mediastinal lymph nodes.

Adult sheep were susceptible to dust-endotoxin exposure during a 4-hour period, as determined on the basis of increased rectal temperature, leukocytosis, an increase in absolute number of neutrophils, and a decrease in absolute number of lymphocytes following 1 dust treatment. These reactions were similar to those in humans exposed to purified endotoxins. Additional daily exposure of sheep to feedyard dust resulted in a decrease in the response of the reactions, thus indicating a temporary tolerant state. It appears that feedyard organic dust may serve as another stressor involved in pneumonia of marketed sheep.<sup>39</sup>

<sup>a</sup>Purdy CW, Straus DC, Parker DP, et al. Feedyard comparisons of endotoxin in the air (abstr), in *Proceedings. 78th Annu Meet Conf Res Workers Anim Dis* 1997;76.

<sup>b</sup>Purdy CW, Straus DC, Hoover MD. Treatment of feedyard dust and its effect on weanling goats (abstr), in *Proceedings. Annu Meet Conf Res Workers Anim Dis* 2000;52.

<sup>c</sup>Custom fabricated canvas tent, Wolfe Canvas, Amarillo, Tex.

<sup>d</sup>Ivomec, MSD AGVET, Merck & Co Inc, Rahway, NJ.

<sup>e</sup>Amprolium, MSD AGVET, Merck & Co Inc, Rahway, NJ.

<sup>f</sup>Custom fabricated roller drums, Production Research Lab, Bushland, Tex.

<sup>g</sup>US standard sieves (ASTM specifications), EH Sargent & Co, Chicago, Ill.

<sup>h</sup>Automated mortar-and-pestle type Asplin soil grinder, Nasco, Fort Atkinson, Wis.

<sup>i</sup>BHI media, Difco Laboratories, Detroit, Mich.

<sup>j</sup>Anaerobic jars, Becton Dickinson Co, Cockeysville, Md.

<sup>k</sup>BBL Gas Pak Plus envelopes dry anaerobic indicator strips, Becton Dickinson and Co, Cockeysville, Md.

<sup>l</sup>BBL dry anaerobic indicator strips, Becton Dickinson Co, Cockeysville, Md.

<sup>m</sup>Malt agar, Difco Laboratories, Detroit, Mich.

<sup>n</sup>LOA agar, Difco Laboratories, Detroit, Mich.

<sup>o</sup>Chlortetracycline, Sigma Chemical Co, St Louis, Mo.

<sup>p</sup>Streptomycin sulfate, Sigma Chemical Co, St Louis, Mo.

<sup>q</sup>Cycloheximide, Sigma Chemical Co, St Louis, Mo.

<sup>r</sup>Peptone broth, Difco Laboratories, Detroit, Mich.

<sup>s</sup>Novobiocin, Sigma Chemical Co, St Louis, Mo.

<sup>t</sup>Tetrathionate broth, Difco Laboratories, Detroit, Mich.

<sup>u</sup>Brilliant green agar, Difco Laboratories, Detroit, Mich.

<sup>v</sup>Urease agar, Difco Laboratories, Detroit, Mich.

<sup>w</sup>Triple-sugar-iron agar, Difco Laboratories, Detroit, Mich.

<sup>x</sup>Lysine-iron agar, Difco Laboratories, Detroit, Mich.

<sup>y</sup>XLD agar, Difco Laboratories, Detroit, Mich.

<sup>z</sup>Tryptose agar, Difco Laboratories, Detroit, Mich.

<sup>aa</sup>2-stage microbial cascade impactor, Andersen Sampler Inc, Atlanta, Ga.

<sup>ab</sup>Millipore reverse-osmosis water system (3216), Continental Water Systems, Lubbock, Tex.

<sup>ac</sup>6-stage microbial cascade impactor, Andersen Sampler Inc, Atlanta, Ga.

<sup>ad</sup>AccuRate dry material feeder, Hopper, Whitewater, Wis.

<sup>ae</sup>Jet-O-Mizer, Fluid Energy Processing & Equipment Co, Hatfield, Pa.

<sup>af</sup>Custom fabricated stainless-steel trap, In-Tox Products, Albuquerque, NM.

<sup>ag</sup>Gasoline-powered portable air compressor, Stewart-Warner, Johnson City, Tenn.

<sup>ah</sup>Portable blower and vacuum motor (3 horse power), Grainger, Amarillo, Tex.

<sup>ai</sup>Activated charcoal powder, Spectrum Chemical, Gardena, Calif.

<sup>aj</sup>Texas A&M University, Veterinary Medical Diagnostic Laboratory, Amarillo, Tex.

- <sup>kk</sup>Glucans, BioWhittaker Inc, Wakersville, Md.  
<sup>ll</sup>Microtiter plates, Dynatech Corp, Chantilly, Va.  
<sup>mm</sup>MR5000 microplate reading spectrophotometer, Dynatech Corp, Chantilly, Va.  
<sup>nn</sup>EC6 reference standards, US Pharmacopeia, Washington, DC.  
<sup>oo</sup>IBT Reference Lab, Lenexa, Kan.  
<sup>pp</sup>Five-stage cyclone device, In-Tox Products, Albuquerque, NM.  
<sup>qq</sup>Millipore swinnex filter holders (47 mm), Millipore Corp, Bedford, Mass.  
<sup>rr</sup>Filters (47 mm, 4.5 micron), Millipore Corp, Bedford, Mass.  
<sup>ss</sup>Mastersizer 2000 particle size analyzer, Malvern Instruments Inc, Southborough, Me.

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