Airborne Endotoxin Concentrations at a Large Open-Lot Dairy in Southern Idaho

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Endotoxins are derived from gram-negative bacteria and are a potential respiratory health risk for animals and humans. To determine the potential for endotoxin transport from a large open-lot dairy, total airborne endotoxin concentrations were determined at an upwind location (background) and five downwind locations on three separate days. The downwind locations were situated at of the edge of the lot, 200 and 1390 m downwind from the lot, and downwind from a manure composting area and wastewater holding pond. When the wind was predominantly from the west, the average endotoxin concentration at the upwind location was 24 endotoxin units (EU) m^{-3}, whereas at the edge of the lot on the downwind side it was 259 EU m^{-3}. At 200 and 1390 m downwind from the edge of the lot, the average endotoxin concentrations were 168 and 49 EU m^{-3}, respectively. Average airborne endotoxin concentrations downwind from the composting site (36 EU m^{-3}) and wastewater holding pond (89 EU m^{-3}) and 1390 m from the edge of the lot were not significantly different from the upwind location. There were no significant correlations between ambient weather data collected and endotoxin concentrations over the experimental period. The downwind data show that the airborne endotoxin concentrations decreased exponentially with distance from the lot edge. Decreasing an individual's proximity to the dairy should lower their risk of airborne endotoxin exposure and associated health effects.

In southern Idaho, there are 750 dairies, with a total of 549,000 milking cows (USDA National Agricultural Statistics Service, 2008). Idaho is the third largest milk-producing state in the USA, behind Wisconsin and California. Although 46% of the production facilities contain <200 cows, the trend is increasing toward larger, more concentrated production facilities. As of 2006, 10% of the dairy operations contained >2000 cows, with some of the largest facilities containing up to 10,000 animals. With an overall increase of approximately 80% in the number of milk cows over the last decade, concerns have been raised over the growing number of concentrated dairy production facilities and their environmental impact in southern Idaho.

The inhalation of airborne microorganisms and their constituents (also called bioaerosols) can be detrimental to health through infection, allergy, or toxicosis (Crook and Sherwood-Higham, 1997). Due to the high stocking densities at concentrated dairy production facilities, bioaerosols may be at sufficiently high levels to cause adverse health effects in animals and workers (Roeder et al., 1989; Thorne et al., 1992; Cullor and Smith, 1996; Schulze et al., 2006; Schierl et al., 2007). Endotoxins, which are cell wall components of gram-negative bacteria, have received much attention due to their ability to induce acute inflammatory reactions in the respiratory tract when inhaled (Rylander, 2007; Liebers et al., 2008). Clinical manifestations are cough, airway irritation, and decreased lung function. At high levels of exposure, flue-like symptoms may develop. Lipopolysaccharides are responsible for most of the biological properties characteristic of bacterial endotoxins (Michel, 2003), which can be found in animal feces and plant matter (Radon et al., 2002; Spann et al., 2006). Although humans are exposed to trace amounts of endotoxin in settled dusts every day, airborne endotoxin is of greatest concern because inhalation is the primary route of exposure.

Although ambient air concentrations are generally <10 endotoxin units (EU) m^{-3} (Heinrich et al., 2003; Mueller-Anneling et al., 2004; Madsen, 2006), studies have shown that exposure to endotoxin concentrations around 50 EU m^{-3} can cause acute lung function changes (Milton et al., 1996; Zock et al., 1998). In contrast, a possible protective effect from endotoxin exposure on atopic sensitization and lung cancer has been shown (Enterline et al., 1985; Mastrangelo et al., 1996; Holla et al., 2002; Eduard et al., 2004; Portengen et al., 2005). At animal operations, indoor airborne endotoxin concentrations measured via the *Limulus* amebocyte lysate (LAL) assay have ranged from 3 to 800 EU m^{-3}

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Abbreviations: DW, downwind; EU, endotoxin units; LAL, *Limulus* amebocyte lysate.

in dairy barns, 2 to 3200 EU m^{-3} in swine houses, and 3 to 12,800 EU m^{-3} in poultry houses (Zucker and Müller, 1998; Chang et al., 2001; Bakutis et al., 2004; Portengen et al., 2005; Schierl et al., 2007). The offsite transport of endotoxins during the handling of organic byproducts has been addressed by Madsen (2006) and Brooks et al. (2005). Few studies have monitored airborne endotoxin concentrations within, and at, the surrounding perimeter of open animal feedlots (Purdy et al., 2004). This is of particular interest because offsite transport of endotoxins to nearby residences and communities could present a respiratory health risk.

The objective of this study was to determine airborne endotoxin concentrations at several locations within a large-scale, open-lot dairy and assess their potential for offsite transport. Samples were collected at six locations at the dairy and upwind and downwind of the dairy during the morning, afternoon, and evening to monitor diurnal effects.

Materials and Methods

Dairy and Sample Locations

Endotoxin samples were collected from a 10,000 milking cow dairy in southern Idaho on 24 and 26 June and 9 July in 2008. The samples were collected in the morning (0600–0900), afternoon (1200–1500), and evening (1800–2100) from six locations at the dairy (Fig. 1). The six locations consisted of an upwind site (i.e., background control) and sites at the downwind edge of the open lots (DW1), 200 m downwind of the edge of the open lots (DW2), 1390 m downwind of the edge of the open lots (DW3), downwind of the composting area (Compost), and downwind of the wastewater holding pond (Lagoon). The prevailing wind direction is from the west/southwest.

Endotoxin Sampling

Total airborne endotoxins were collected on 25-mm, 1.0-μm pore size polycarbonate track-etch filters (Whatman, Florham Park, NJ), which were housed in 25-mm, open-face Delrin filter holders (Pall Corporation, East Hills, NY). Three tripods, each mounted with an open-face filter, were placed at each location and set at a height of 1.5 m with a distance of 1.5 m between each tripod. The tripods were oriented perpendicular to the wind direction. Vacuum was applied to the open-face filters using an SKC Vac-U-Go sampling pump (SKC, Eighty Four, PA). The samples were collected for 75 min at a rate of 2 L min^{-1}. A total of 162 samples were collected during the study. When not being used, the open-face filter holders were stored in pyrogen-free tins. Filters were then transported to the laboratory in a cooler with ice packs and stored immediately at −20°C. Except for the open-face filters, all materials were depyrogenated by heating at 250°C for 30 min or purchased pyrogen free. The open-face filter holders were depyrogenated by rinsing with 70% ethanol and autoclaving for 1 h at 1.23 atm and 121°C.

Meteorological data, including air temperature, wind speed, wind direction, relative humidity, and solar radiation (Table 1), were collected throughout the sampling periods using a Campbell Scientific (Logan, UT) model 21X data logger.

Endotoxin Extraction and Analysis

Within 24 h of collection, the polycarbonate filters were transferred to 2-mL, pyrogen-free polypropylene tubes and stored dry at −20°C until processed. To extract the endotoxins from the polycarbonate filters, 1.5 mL of pyrogen-free water containing 0.05% Tween 20 (v/v) was added to the 2-mL tubes. The filters were then sonicated at room temperature for 30 min. Immediately afterward, the filters were removed from the Tween 20 solution using depyrogenated forceps. The samples were then frozen at −20°C for future analysis.

The extracts were analyzed for endotoxin using the LAL Kinetic-QCL test kit (Lonza, Walkersville, MD) as recommended by the manufacturer. Endotoxin standards (lyophilized *Escherichia coli* O55:B5) were prepared in pyrogen-free water containing 0.025% Tween 20. An eight-point calibration curve ranging from 0.005 to 50 EU mL^{-1} was used, and correlation coefficients (r) were ≥0.98. In general, 10 EU is approximately equal to 1 ng of endotoxin. The sample extracts were defrosted, vortexed for 1 min at high speed, and diluted twofold in β-glucan blocker (Lonza, Walkersville, MD). The β-glucan blocker reduces the interference of β-1,3-glucans, which have been shown to inhibit and enhance the LAL reaction (Morita et al., 1981; Roslansky and Novitsky, 1991; Milton et al., 1997). One hundred–microliter aliquots of the diluted sample were then added to a pyrogen-free, 96-well microplate (Corning Inc., Corning, NY) and incubated for 15 min at 37°C. After incubation, a 96-channel pipette (Transtar-96, Corning, Inc.) was used to rapidly dispense 100 μL of the Kinetic-QCL reagent to each of the wells. The microplate was then immediately placed into an ELx808 absorbance microplate reader (BioTek Instruments, Inc., Winooski, VT) to initiate the test. The pH of the sample when combined with the Kinetic QCL-reagent was 7.5. To ensure quality control and assurance, trip blanks, dilution blanks, and duplicate samples were run regularly.

Statistical Analysis

Endotoxin concentrations were tested for normality using the Shapiro-Wilk test with the PROC CAPABILITY procedure of SAS (SAS Institute, 2004). The data were analyzed using the Mixed Models procedure of SAS with location as a fixed effect and date, time, and their interaction as random effects. Means separation was performed using the difference of the least squares means with Tukey-Kramer adjustment and an α level of 0.05. To determine the relationship between ambient weather conditions and endotoxin concentrations, Pearson correlation coefficients were calculated. Statements of statistical significance were based on $P < 0.05$ unless otherwise stated.

Results

Ambient weather conditions at the dairy over the three sampling dates are shown in Table 1. In general, air temperature in the morning (13.5–19.3°C) was slightly cooler than the afternoon (24.5–30.7°C) and evening (26.0–31.9°C) sampling periods. Relative humidity decreased throughout the day, averaging 56% in the morning, 28% in the afternoon, and 21%

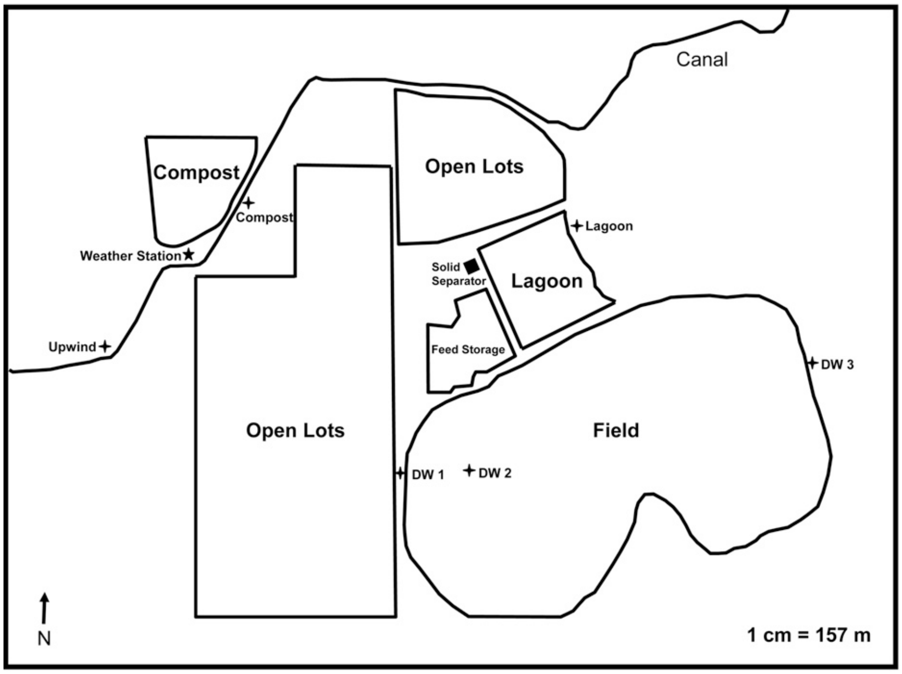

Fig. 1. Site map of the sampling locations on the open-lot dairy.

in the evening for the three sampling dates. The wind speed ranged from 1.3 to 1.5 m s^{-1} in the morning, increasing to 3.0 to 5.5 m s^{-1} in the afternoon and then remaining somewhat consistent, with ranges from 2.5 to 6.3 m s^{-1} in the evening. Wind direction was generally from the south/southwest in the morning (147–215°), changing to west in the afternoon and evening (254–290°). Solar radiation was lowest in the morning (average 184 W m^{-2}) and peaked in the afternoon (average 911 W m^{-2}) and decreased again by the evening (average 355 W m^{-2}). No precipitation events occurred during the study.

In general, there was a great deal of variation in airborne endotoxin concentrations and therefore high standard errors associated with measurements at each time (Table 2). At the upwind location, concentrations ranged from 0.71 to 144 EU m^{-3}, with the majority of the concentrations being <18 EU m^{-3}. The highest concentration of endotoxins measured at the upwind location was during the morning of 9 July, when winds were from the south/southeast. At the DW1 location, concentrations ranged from 20 to 895 EU m^{-3}, with the majority of concentrations being >132 EU m^{-3}. At the DW1 location, airborne endotoxin concentrations generally increased from the morning to the evening, with the exception of 24 June, when the afternoon concentrations were very low. Endotoxin concentrations ranged from 16 to 358 EU m^{-3} at the DW2 location and were generally less than the DW1 concentrations. At the DW3 location, airborne endotoxin concentrations generally decreased further (compared with DW1 and DW2) and ranged from 20 to 97 EU m^{-3}. Endotoxin concentrations at DW3 tended to be more consistent than at the other two downwind locations. The airborne endotoxin concentrations measured at the lagoon ranged from 25 to 115 EU m^{-3} and were generally consistent with all but one measurement, being >82 EU m^{-3}. At the compost location, endotoxin concentrations were greatest in the morning (100–145 EU m^{-3}) when winds were

Table 1. Ambient weather data measured over the experimental period.

Sampling date/ time	Air temperature	RH†	WS	WD	Solar radiation
	°C	%	m s^{-1}	degrees	W m^{-2}
June 24					
Morning	13.5	63	1.3	208	103
Afternoon	24.5	33	3.6	255	922
Evening	26.1	28	2.5	280	325
June 26					
Morning	15.7	60	1.5	215	159
Afternoon	25.1	30	5.5	268	908
Evening	26.0	22	6.3	290	388
July 9					
Morning	19.3	44	1.4	147	289
Afternoon	30.7	21	3.0	254	902
Evening	31.9	14	3.3	286	352

† RH, relative humidity; WD, wind direction; WS, wind speed.

out of the southwest and decreased to between 5 and 88 EU m^{-3} during the afternoon and evening sampling periods.

An ANOVA was performed on the data to determine the effect of location on airborne endotoxin concentration. Because wind direction varied from southeast to west, only data where the wind was predominantly from the west (248–292°) were included in the analysis as these were the only times that the sampling stations were truly downwind of the different locations. The effect of location was significant ($P > 0.0001$) and followed the trend DW1 > DW2 > DW3 = upwind = lagoon = compost (Fig. 2). Airborne endotoxin concentrations decreased exponentially ($r^2 = 0.99$) from the edge of the lot (259 EU m^{-3}) to 1390 m downwind (49 EU m^{-3}), reaching a concentration at DW3 that was not significantly different from the upwind location (24 EU m^{-3}). The lagoon and compost locations were also not significantly different from the upwind location, with averages of 89 and 36 EU m^{-3}, respectively.

Table 2. Airborne endotoxin concentrations measured over 3 d at the large open-lot dairy.

Sampling date/time	Endotoxin concentrations by location					
	Upwind	DW1	DW2	DW3	Lagoon	Compost
	EU m^{-3}					
June 24						
Morning	117 (41.0)†	393 (57.3)	15.8 (7.4)	46.3 (21.0)	82.2 (10.7)	100 (3.5)
Afternoon	0.71 (0.38)	19.8 (18.7)	46.8 (5.2)	40.5 (7.9)	103 (10.8)	6.3 (2.7)
Evening	112 (41.1)	533 (271)	114 (36.1)	70.9 (11.4)	24.6 (5.9)	5.9 (4.6)
June 26						
Morning	0.10 (0.05)	96.6 (10.9)	56.0 (10.2)	19.6 (9.1)	115 (7.8)	145 (19.0)
Afternoon	17.3 (5.3)	186 (28.3)	206 (30.7)	97.4 (42.5)	106 (1.7)	69.7 (6.8)
Evening	3.0 (1.1)	895 (‡)	358 (66.4)	24.7 (8.1)	101 (1.1)	88.1 (42.8)
July 9						
Morning	144 (20.1)	61.9 (7.9)	51.5 (3.7)	25.2 (3.3)	99.4 (10.6)	116 (17.6)
Afternoon	8.2 (0.69)	132 (0.33)	94.4 (24.4)	32.3 (6.9)	110 (40.9)	38.3 (11.3)
Evening	5.1 (1.5)	260 (50.9)	189 (13.6)	30.5 (7.6)	88.0 (34.3)	4.8 (2.0)

† Standard error of the mean (n = 3).

‡ One replicate only.

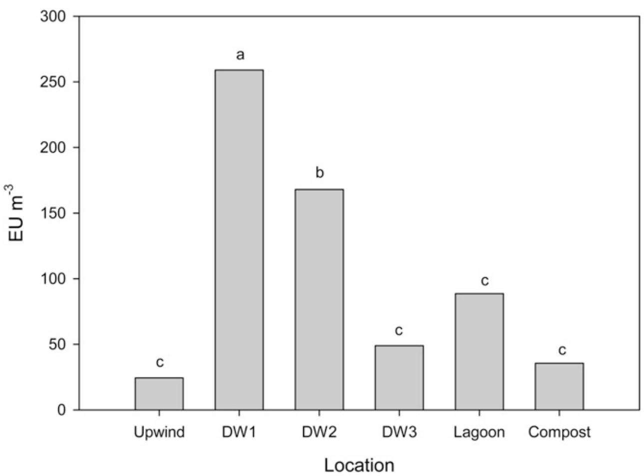

Fig. 2. Average airborne endotoxin concentrations measured at six locations on a large-scale, open-lot dairy. Letters above the columns indicate significant differences between the locations (*P* < 0.05). Data shown are when the wind was predominantly from the west (248–292°).

There were no significant correlations between ambient weather data collected and airborne endotoxin concentrations over the experimental period.

Discussion

The airborne endotoxin concentrations reported in the present study fall within the range of previously reported values for cattle production facilities of 0.3 to 3860 EU m^{-3} (Zucker and Müller, 1998; Spann et al., 2006; Schierl et al., 2007). There are few studies that have compared airborne endotoxin concentrations within animal production facilities at background or surrounding locations. Chang et al. (2001) reported average airborne endotoxin concentrations of 140 EU m^{-3} in swine buildings, which were approximately 15-fold greater than measurements made in surrounding areas. In the present study, airborne endotoxin concentrations measured at the edge of open lots were approximately 11-fold greater than those measured upwind of the production facility, which is similar to the increase seen by Chang et al. (2001).

At the lagoon location 600 m downwind of the edge of the open lots, endotoxin concentrations were not significantly greater than the upwind concentrations, even though there was potential for the airborne transport of endotoxins from the feed storage area as well as the solid separator house and the lagoon itself. Based on our limited data set, it appears that manure storage areas such as lagoons are not major sources of airborne endotoxins. The lack of a significant difference between concentrations measured downwind of the composting area and the upwind location suggests that the risk of airborne endotoxin generation from composting facilities may also be small. There is no published literature reporting airborne endotoxin concentrations at various locations on concentrated animal production facilities for comparison.

There was a great deal of variation in airborne endotoxin concentrations measured over any given day at the upwind, DW1, DW2, and compost locations, whereas concentrations at the lagoon and DW3 sites were more consistent. High concentrations of endotoxins in the morning of 24 June and 9 July at the upwind location are likely due to the wind direction, which was from the south/southwest. During these times, it is possible that there was transport of airborne endotoxins from the lot areas to the upwind sites. The upwind location on the evening of 24 June also had high airborne endotoxin concentrations; at this time, the irrigation pivot in the field to the northwest was operating and could have contributed to the high airborne endotoxin concentrations because these pivots pump canal water, which can have high bacterial loads. The high concentrations reported each morning at the compost location at all sampling dates are also likely to be due to wind direction. Because the wind was predominantly from the south/southwest at these times and the lot area was directly south of the compost location, there is a high probability that airborne endotoxins were transported from the lots to this location. On 26 June, the compost rows were being re-piled and turned, which could have resulted in higher airborne endotoxin concentrations on that day.

At the DW1 and DW2 locations, the concentrations of airborne endotoxins tended to increase from morning to evening, with the exception of DW1 on 24 June, when the afternoon concentrations were low. Typically, the cows were at the feed bunkers and milked early in the morning. Because the cows were not in the immediate vicinity of the sampling location at this time, endotoxin concentrations tended to be lower. In the afternoon, and especially in the evening, there was more cow activity throughout the lots, which likely generated more airborne particulate matter and led to the higher airborne endotoxin concentrations measured at these times. The increased drifting of dust particles in the lots during the afternoon and evening also appears to have enhanced endotoxin transport. The DW2 location, which is only 200 m from the edge of the lots, showed increased endotoxin concentrations during this high cow activity period. The high cow activity, however, did not appear to influence airborne endotoxin concentrations at the DW3 location, which was 1390 m from the edge of the lots.

Conclusions

In The Netherlands, the Dutch Expert Committee on Occupational Standards has recommended a health-based exposure limit of 50 EU m^{-3} for exposure to airborne endotoxins in the working environment over an 8-h period. The total airborne endotoxin concentrations measured at the edge of the open lot and at 200 m downwind of the open lot exceeded these thresholds, which could be a health concern for workers on the production facility. However, residents at greater distances from the dairy have a reduced risk for endotoxin exposure.

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