Ambient Endotoxin Concentrations and Assessment of Offsite Transport at Open-Lot and Open-Freestall Dairies

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Endotoxins are derived from gram-negative bacteria and are a potent inducer of inflammatory reactions in the respiratory tract when inhaled. To assess daily fluctuations of airborne endotoxin and their potential for transport from dairies, endotoxin concentrations were monitored over an 8-h period at upwind (background) and downwind (5 m from edge of dairy) locations on three separate days at two dairies. The dairies consisted of an open-lot or an open-freestall production system, both of which were stocked with 10,000 milking cows. Upwind concentrations were stable throughout the sampling period, averaging between 1.2 and 36.8 endotoxin units (EU) m^{-3}, whereas downwind concentration averages ranged from 179 to 989 EU^{-3}. Downwind endotoxin concentrations increased with wind speed, animal activity, and lot management practices, resulting in concentrations up to 136-fold higher than upwind concentrations. An area-source model was used to predict downwind ground-level endotoxin concentrations at distances up to 2000 m from the production facilities. Predicted concentrations decreased with distance and reached background levels within 500 to 2000 m, depending on the source emission rate and meteorological conditions.

Over the last few decades, the intensification of animal production in industrialized nations has led to the creation of larger concentrated animal feeding operations (CAFOs), some of which house over 100,000 animals at one facility (Centner, 2003). As a result, CAFOs produce vast amounts of feces and urine, most of which is temporarily stored on site and eventually applied to agricultural lands. The manures are a source of pathogens and microbial byproducts, which can become aerosolized during their removal and land application (Millner, 2009). Manure management and storage practices can also produce air pollutants such as particulate matter and volatile compounds, as well as a variety of bioaerosols, that may have adverse health effects on animals, farm workers, and individuals in nearby residences (Cole et al., 2008; Mitloehner and Calvo, 2008).

Inhalation of bioaerosols and dusts from animal feeding operations has been associated with increased levels of allergy, asthma, and infectious disease (Cole et al., 2000; Von Essen and Auvermann, 2005). Airborne endotoxins, in particular, have received much attention because they are a potent inducer of inflammatory reactions in the respiratory tract when inhaled (Portengen et al., 2005). Endotoxins are heat-stable lipopolysaccharide molecules from the cell wall of gram-negative bacteria, with the lipid A portion of the molecule causing most of the toxicity (Liebers et al., 2008). Acute exposures to airborne endotoxin concentrations as low as 50 endotoxin units (EU) m^{-3} have been linked to health impairments such as nose and throat irritation, shortness of breath, chest tightness, cough, decreased lung function, and fever (Milton et al., 1996; Zock et al., 1998; Smit et al., 2005; Rylander, 2006). Endotoxins are ubiquitous, being found indoors (Rao et al., 2007) and in rural and urban ambient environments (Carty et al., 2003; Heinrich et al., 2003). Elevated endotoxin concentrations have been reported inside closed and open-style animal housing units (Seedorf et al., 1998; Chang et al., 2001; Schierl et al., 2007).

Although many animal-based studies have focused on indoor air quality and occupational exposures, very little attention has been given to endotoxins in the areas surrounding animal housing units. Investigations into the management practices at CAFOs that affect endotoxin emissions and their offsite transport are of particular interest because they could present a health risk to downwind individuals. In Idaho, the dairy industry has grown by over 61% during the last decade, and Idaho is now the third largest milk-producing state, with

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Abbreviations: CAFO, concentrated animal feeding operation; EU, endotoxin units; PFW, pyrogen-free water.

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559,000 milking cows (USDA National Agricultural Statistical Service, 2010). Some of the largest dairies contain as many as 10,000 milking cows; this has raised concerns regarding air quality, especially in southern Idaho, where about 70% of the cows are located. To gain an understanding of the conditions that influence the release of endotoxin from these large dairy operations, downwind airborne endotoxin concentrations were measured over an 8-h period at two 10,000-cow dairies. Because airborne endotoxin is a potential offsite hazard, the data were also used in an area-source model to predict ground-level concentrations at downwind distances of up to 2000 m from the dairy operations.

Materials and Methods

Dairies and Sample Sites

Two commercial dairies in southern Idaho, each with 10,000 milking cows, were investigated in this study. Both dairies were stocked with Holstein cows, with a small percentage of the herd consisting of Jersey cows. The first dairy was an open-lot production system, where manure from the lots was scraped and piled in the pens or vacuumed from feed alleys daily and placed into a manure storage area. The lots were harrowed daily when dry. The second dairy was an open-freestall production system, where animals were housed in a freestall barn but had access to open-lot areas between the barns. Manure was flushed from the barns daily and conveyed to a manure storage facility, and the open-lots were harrowed daily when dry. During the warmer months, the cattle spent a large portion of the day outside.

Total airborne endotoxin samples were collected in the summer of 2009 from the open-lot dairy on 18, 22, and 26 June and from the open-freestall dairy on 12 and 21 August and 21 September. Samples were collected at upwind (background) and downwind sites during low to medium wind events (1–6 m s^{-1}), with three replicate samples simultaneously collected at each site. The upwind site was located about 200 m from the dairy, and the downwind site was 5 m from the edge of the dairy. Samples were collected hourly (in triplicate) at each location starting at approximately 1000 h and continuing over an 8-h period for a total of 24 samples collected at each site on each sampling day. The samples were collected on days when the winds were from a direction that ensured that the upwind site did not have any potential transport from the dairy and the downwind site captured wind only coming from the production facility.

Endotoxin Sampling

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 a filter holder) were placed at each site and set at a height and separation distance of 1.5 m. The tripods were oriented so that they were perpendicular to the prevailing wind direction. Vacuum was applied to the filter holders using a Vac-U-Go sampling pump (SKC, Eighty Four, PA) at a rate of 2 L min^{-1}. Forty-eight samples were collected during each 8-h event for a total of 288 samples during the study. When not being used, the filter holders were stored in pyrogen-free tins. Filters were then transported to the laboratory in a cooler with ice packs and stored in 2-mL pyrogen-free polypropylene tubes at −20°C until processed. Except for the filter holders, all materials were depyrogenated by heating at 250°C for 30 min or purchased pyrogen-free. The filter holders were depyrogenated by cleaning with soap and water, then soaking in 70% ethanol for 10 min followed by a rinse with pyrogen-free water (PFW). The holders were autoclaved at 121°C and 1.23 atm for 1 h.

Meteorological data, including air temperature, wind speed, wind direction, relative humidity, and solar radiation, were collected with a portable weather station and a data logger (model 21X; Campbell Scientific, Logan, UT). The data logger was programmed to average meteorological data in 15-min increments.

Endotoxin Extraction and Analysis

To extract the endotoxins from the polycarbonate filters, 1.5 mL of PFW containing 0.05% Tween 20 (v/v) was added to the 2-mL tubes. The filters were then sonicated at room temperature for 20 min. Immediately afterward, the filters were removed from the Tween 20 solution using depyrogenated forceps. The samples were frozen at −20°C until analysis. Previous research at our laboratory with endotoxin extracts has shown that multiple freeze-thaw events do not cause a substantial loss of airborne endotoxin (Dungan and Leytem, 2009).

The extracts were analyzed for endotoxin using the *Limulus* amebocyte lysate Kinetic-QCL test kit (Lonza, Walkersville, MD) as recommended by the manufacturer. The sample extracts were defrosted and vortexed for 1 min at high speed, and then 50-μL aliquots were dispensed into a pyrogen-free, 96-well microplate (Corning Inc., Corning, NY). Afterward, 50-μL aliquots of β-glucan blocker (Lonza) were added to each well, and the microplate was shaken at 400 rev min^{-1} for 1 min. The microplate was then incubated for 15 min at 37°C. After incubation, a 96-channel pipette (Transtar-96; Corning, Inc.) was used to 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. Quality control operations included the analysis of trip blanks, negative controls, and duplicate samples. Matrix spikes were not used because inhibition and enhancement did not occur in endotoxin extracts from these dairies (Dungan, 2011). Endotoxin standards were made with lyophilized *Escherichia coli* O55:B5 and prepared in PFW containing Tween 20. An eight-point calibration curve ranging from 0.005 to 50 EU mL^{-1} was used ($r^2 \geq 0.98$).

Area-Source Model

To predict downwind concentrations of airborne endotoxin, an area-source dispersion model first described by Parker et al. (1977) was used. This model was later used by Dowd et al. (2000) to predict downwind concentrations of pathogens released during the land application of biosolids. The airborne endotoxin concentration (EU m^{-3}) at distance χ from the downwind edge of the dairies was calculated from the following formula (Eq. [1]):

$$= \left\{ \frac{Q}{\sqrt{2\pi}\,\bar{u}\,\sigma_z\{\chi\}\,\gamma_o} \right\} \times \left\{ 1 + 2\sum_{n=1}^{\infty} \left(\exp\left[-0.5 \left(\frac{2nH_m}{\sigma_z\{\chi\}} \right)^2 \right] \right) \right\} \times \left\{ \operatorname{erf}\left[\frac{\gamma_o/2+\gamma}{\sqrt{2}\,\sigma_y\{\chi\}} \right] + \operatorname{erf}\left[\frac{\gamma_o/2-\gamma}{\sqrt{2}\,\sigma_y\{\chi\}} \right] \right\} \qquad [1]$$

where Q is the area source emission rate (EU s^{-1}), $\bar{u}$ is the mean wind speed (m s^{-1}), H_m is the depth of the mixing layer (m), γ_o is the crosswind dimension (m) of the area source, γ is the crosswind distance (m) from the centerline of the area source, and $\sigma_z\{\chi\}$ and $\sigma_y\{\chi\}$ are the vertical and lateral diffusion coefficients, respectively. The vertical diffusion coefficient $\sigma_z\{\chi\}$ is given as (Eq. [2]):

$$= \frac{\sigma E' \chi_o}{\ln\left[\frac{\sigma E'(\chi + \chi_o) + \sigma_{zo}}{\sigma E'(\chi) + \sigma_{zo}}\right]} \qquad [2]$$

where χ_o is the wind dimension (m) of the area source, σ_{zo} is the vertical source dimension (m), and $\sigma A'$ is the standard deviation of the wind elevation angle in radians. The lateral diffusion coefficient $\sigma_y\{\chi\}$ is given as (Eq. [3]):

$$= \sigma A' \ (\chi + \chi_o/2) \qquad [3]$$

where $\sigma A'$ is the standard deviation of the azimuth wind angle in radians. The meteorological input parameters for H_m, $\sigma E'$, and $\sigma A'$ were obtained from Parker et al. (1977). The dimensions for the open-lot and freestall dairies were 1130 × 650 m and 472 × 666 m, respectively. Although airborne endotoxins from the dairy originate from the lot surface, the vertical source dimension ($\sigma_{zo} = h/2.15$) was computed using an h value of 1.5 m because that was the height at which the samples were collected at the edge of the lots. In addition, centerline concentrations from the dairies were only considered; therefore, γ was set at zero.

To predict downwind endotoxin concentrations using the area-source model, an endotoxin emission rate from the source had to be calculated. This was accomplished by backward calculating Q using Eq. [1] and average downwind data from the open-face filters. Once Q was determined, it was used along with the average wind speed from the 8-h events to forward calculate endotoxin concentrations at downwind distances up to 2000 m (i.e., $\chi < 3\chi_o$). For the purposes of this model, it was assumed that (i) endotoxin decay and deposition were negligible, (ii) wind direction and velocity were constant over the modeled time and distance, (iii) particle and wind velocity were equal, (iv) atmospheric stability class was unstable based on weather conditions during the sampling periods, (v) terrain was flat with no obstructions, and (vi) no additional endotoxin sources were present downwind of the dairies.

Statistical Analyses

All data were analyzed using SAS statistical software version 9.2 (SAS Institute, 2008). Endotoxin concentrations were tested for normality using the UNIVARIATE procedure. Significant differences between the upwind and downwind concentrations were determined using the two-sample paired t test. To determine the relationship between ambient meteorological conditions and endotoxin concentrations, Pearson correlation coefficients were calculated. Statements of statistical significance were based on $P < 0.05$ unless stated otherwise.

Results and Discussion

The dairies investigated for this study were located in a high desert region of southern Idaho, which experiences hot and dry conditions during the summer months. The average ambient weather conditions at the dairies during the time of sampling are listed in Table 1. The average ambient temperature ranged from 16.4 to 31.2°C, with a relative humidity of 26 to 44%. The average wind speed ranged from 1.8 to 4.9 m s^{-1}, and samples were collected when the wind was predominantly from the west (270 ± 14°) or east (96 ± 19°). Solar radiation was generally high at ≥599 W m^{-2} because high-pressure systems with minimal cloud cover dominated at the time. Only 2.5 cm of precipitation occurred during the summer, and no precipitation events occurred during sample collection.

Figures 1 and 2 show the airborne endotoxin concentrations during each 8-h sampling period at the open-lot and open-freestall dairies, respectively. Overall, the upwind concentrations were very stable throughout the sampling period, and average concentrations ranged from 1.2 and 36.8 EU m^{-3} (Table 2). In a study of particulate matter with an aerodynamic diameter of 2.5 and 10 μm ($PM_{2.5}$ and PM_{10}, respectively) in small towns with animal operations, ambient endotoxin concentrations during a 6-mo period were reported to be <0.3 EU m^{-3} (Heinrich et al., 2003). Ambient endotoxin concentrations in PM_{10} from multiple locations throughout southern California were all <5.5 EU m^{-3} (Mueller-Anneling et al., 2004). Madsen (2006) found that within towns and upwind of industrial areas, inhalable (i.e., particles with an aerodynamic diameter <100 μm) endotoxin concentrations were <10 EU m^{-3} throughout the year. In a recent study conducted in Beijing, China, ground-level endotoxin concentrations were reported to range from 3 to 54 EU m^{-3} over a 2-d period (Li et al., 2010). Overall, our background concentrations fall very close to values reported by these researchers.

Downwind of the open-lot dairy, the concentrations generally peaked at least once per 8-h event (Fig. 1). On 18 June, the endotoxin concentration peaked at 4987 EU m^{-3} (4 h after sample initiation), which was the highest concentration recorded during the three sampling events at the open-lot dairy. Although the remainder of the downwind concentrations was ≤813 EU m^{-3} on this day, the lot in front of the sample site was harrowed at this time, which explains the concentration

Table 1. Average ambient weather conditions during the 8-h airborne endotoxin sampling events.

Dairy	Date	AT†	RH	WS	WD	SR
		°C	%	m s^{-1}	degrees	W m^{-2}
Open-lot	18 June	22.3 (1.3)‡	34.2 (8.8)	4.9 (0.48)	270 (7)	838 (135)
	22 June	17.0 (1.8)	41.7 (6.1)	2.6 (0.33)	265 (21)	851 (131)
	26 June	22.5 (3.3)	44.0 (11.6)	3.1 (1.2)	256 (15)	785 (200)
Freestall	12 Aug.	27.5 (2.5)	29.4 (6.5)	3.3 (0.74)	290 (11)	727 (163)
	21 Aug.	31.2 (4.3)	31.3 (12.3)	1.8 (0.36)	109 (82)	697 (138)
	21 Sept.	16.4 (2.2)	26.1 (5.2)	2.9 (0.83)	83 (50)	599 (163)

† AT, air temperature; RH, relative humidity; SR, solar radiation; WD, wind direction; WS, wind speed.

‡ Value in parentheses are SD ($n = 33$).

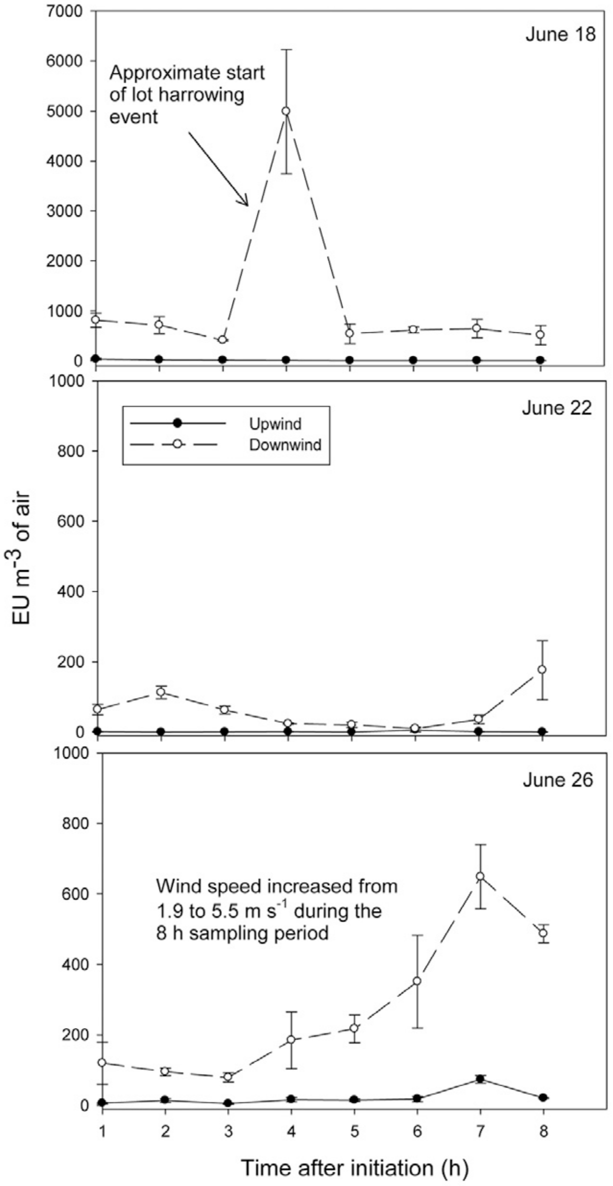

Fig. 1. Airborne endotoxin concentrations at the open-lot dairy during 8-h sampling events on 18, 22, and 26 June. Error bars represent the SEM (*n* = 3).

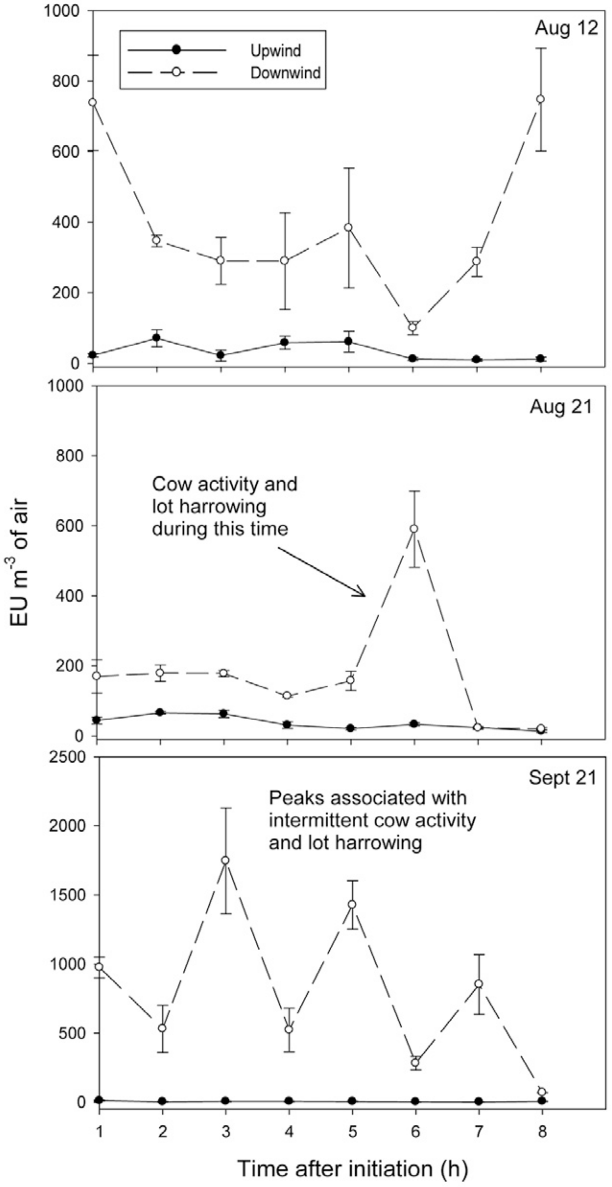

Fig. 2. Airborne endotoxin concentrations at the open-freestall dairy during 8-h sampling events on 12 and 21 August and 21 September. Error bars represent the SEM (*n* = 3).

peak. Harrowing is a management practice commonly used to distribute the manure on the soil surface to enhance drying and avoid the buildup of manure in concentrated sections of the lots; however, it also increases fugitive dust emissions. As a result of the high wind speed (average of 4.9 m s^{-1}) during the 8-h sampling event, the dust and associated manure generated during lot harrowing was readily carried downwind, thus leading to the high airborne endotoxin concentration recorded at 4 h. On 22 June, the average wind speed was 2.6 m s^{-1}, and downwind concentrations were relatively stable at ≤176 EU m^{-3} (average 8-h concentration of 63.2 EU m^{-3}). On 26 June, the downwind endotoxin concentration after 1 h of sampling was 120 EU m^{-3}, and this steadily increased to 648 EU m^{-3} after 7 h. During this same time, the wind speed increased from 1.9 to 5.5 m s^{-1}.

At the downwind site from the open-freestall dairy, the airborne endotoxin concentrations also fluctuated throughout the sampling period. On 12 August, the concentrations peaked at about 740 EU m^{-3} at 1 and 8 h but were generally <400 EU m^{-3} during the remainder of the sampling period. On 21 August, the endotoxin concentrations were <180 EU m^{-3} except for one peak at 6 h, which reached a concentration of 590 EU m^{-3}. As on 18 June at the open-lot dairy, the high concentration at 6 h was a result of the increased dust load caused by lot harrowing and cow activity noted at the time of sampling. On 21 September, the downwind airborne endotoxin concentrations fluctuated dramatically throughout the 8-h sampling event. During the early portion of the sampling event (i.e., 1 and 2 h), lots were being harrowed; afterward, cows were intermittently using the lot areas between the freestall barns. The minimum and maximum endotoxin concentrations were 70 and 1747 EU m^{-3}, respectively, with an average concentration of 801 EU m^{-3}.

When a correlation analysis of the airborne endotoxin concentrations and ambient weather data from the open-lot and freestall dairies was conducted, there was a significant effect of wind speed ($r = 0.37$; $P < 0.0001$). There were no significant effects of temperature, solar radiation, and relative humidity ($P > 0.08$). Unlike viable airborne microorganisms, nonviable biological fragments such as endotoxins are highly resistant to radiation and temperature.

Except in a few instances, the downwind endotoxin concentrations were higher than at the upwind site, irrespective of dairy (Fig. 1 and 2). The average downwind concentrations during the 8-h events were 5- to 136-fold higher than the upwind concentrations (Table 2). Although airborne endotoxins are naturally present in background ambient environments, as wind moves across the dairy lots and barns it picks up additional endotoxin, causing increases at the downwind sites. Animal activity and lot harrowing also contribute to the suspension of particulate matter. Although there is little literature available related to off-site transport of endotoxin from animal operations, it has been shown that land application of biosolids produced increased downwind endotoxin concentrations (Brooks et al., 2006; Paez-Rubio et al., 2007). The land application of materials such as biosolids generates suspended particulate matter in the same way that lot harrowing or animal activity does, allowing greater amounts of endotoxin to be transported downwind.

The endotoxins, which are derived from the cell wall of gram-negative bacteria, may be associated with mineral and organic particles or are free units in the air. Increases in the gram-negative bacterial populations at the dairies can be attributed to the high fecal load within the lots and barns. The increased bacterial load could also be associated with the animal feed and bedding material (i.e., straw), but samples from these materials were not collected and analyzed for confirmation. Because only total airborne endotoxins were collected in this study, there is no information on the percentage of endotoxin associated with inhalable, thoracic, and respirable mass fractions. Endotoxins associated with particles having a 50% cutoff-point at an aerodynamic diameter of ≤100 μm are particularly deleterious when deposited in the respiratory tract and in the gas-exchange regions because they are known to cause respiratory discomfort and disease (Jacobs, 1989).

Table 2. Average airborne endotoxin concentrations at the upwind and downwind sampling sites.

Dairy	Date	Upwind	Downwind
		EU† m^{-3}	
Open-lot	18 June	11.7a‡ (2.7)§	989b (279)
	22 June	1.2a (0.7)	63.2b (14.4)
	26 June	21.3a (4.8)	273b (45)
Freestall	12 Aug.	34.2a (6.9)	398b (55)
	21 Aug.	36.8a (4.2)	179b (37)
	21 Sept.	5.9a (0.9)	801b (124)

† Endotoxin units.

‡ Row means followed by different letters indicate a significant difference between the upwind and downwind sites ($P < 0.001$).

§ Values in parentheses are SEM ($n = 24$).

The calculated Q values for the dairies ranged from 2.8 × 10^{6} to 1.2 × 10^{8} EU s^{-1} (Table 3). When these calculated Q values were used in the area-source model along with the average wind speed, the downwind concentration was predicted to decrease with increasing distance from the dairy operation (Table 4). For example, on 18 June, the average endotoxin concentration at the downwind edge of the open-lot dairy was 989 EU m^{-3}, but at 100, 1000, and 2000 m downwind the predicted concentrations were 456, 89, and 33 EU m^{-3}, respectively. Dungan et al. (2010) conducted a year-long study at an open-lot dairy and found that airborne endotoxin concentrations at 200 m downwind were lower than those measured at the edge of the cattle lots. When data from that study (i.e., data from the downwind edge of the lots) were used in the area-source model to predict endotoxin concentrations at 200 m from the dairy, there was a very good agreement ($r^2 = 0.61$) between modeled and field concentrations (Fig. 3). The average ratio of modeled to field concentrations at 200 m downwind was 1.0, based on data from 43 model runs under varying environmental conditions. This suggests that the model is a potentially useful tool in predicting downwind airborne endotoxin concentrations. However, additional model validation studies that take into account distances from the source, particulate matter, and meteorological conditions are necessary. In general, the model predicted that the endotoxin concentrations in the downwind environment of these dairies will be close to background concentrations at distances between 500 and 2000 m.

Conclusions

Although many animal-based studies have focused on indoor air quality issues, few have focused on outdoor endotoxin concentra-

Table 3. Calculated emission rates of endotoxin from the dairies during 8-h sampling events.

Dairy	Date	*Q*†
		EU‡ s^{-1}
Open-lot	18 June	1.17 × 10^{8}
	22 June	3.38 × 10^{6}
	26 June	1.84 × 10^{7}
Freestall	12 Aug.	1.15 × 10^{7}
	21 Aug.	2.76 × 10^{6}
	21 Sept.	2.00 × 10^{7}

† *Q*, area source emission rate. Values are averages ($n = 8$).

‡ Endotoxin units.

Table 4. Predicted ground-level airborne endotoxin concentrations downwind of the dairies.

Dairy	Date	Distance downwind								
		5 m	50 m	100 m	250 m	500 m	750 m	1000 m	1500 m	2000 m
						EU† m^{-3}				
Open-lot	18 June	989	591	456	291	181	124	89	52	33
	22 June	63	38	29	19	12	8	6	3	2
	26 June	283	169	131	83	52	36	26	15	10
Freestall	12 Aug.	398	231	171	94	47	29	19	10	6
	21 Aug.	179	104	77	42	21	13	9	5	3
	21 Sept.	801	465	343	189	95	58	38	21	13

† Endotoxin units.

tions and downwind transport. In this study, open-lot and open-freestall dairies in southern Idaho were found to be a source of airborne endotoxin with concentrations ranging from 5- to 136-fold higher than ambient background concentrations. Although these dairies differ with respect to animal housing and management, the average airborne endotoxin concentrations in the downwind environment were similar between the two operations. The main factors affecting airborne endotoxin emissions were wind speed, lot management, and animal activity. As wind speed increased, there was enhanced suspension and transport of endotoxins from the animal housing areas. Likewise, as animal activity increased or lot management practices that generate dust occurred, there was an increase in the emission rate of airborne endotoxins. The use of an area-source model to predict downwind ground-level endotoxin concentrations suggests that they can be transported up to 2000 m from the production facility, depending on the source emission rate and meteorological conditions. In all but one instance, concentrations were predicted to be <50 EU m^{-3} within 1000 m of the facility, a level that has been shown to cause acute respiratory effects. Even though we did not attempt to determine the actual risk of exposure, the area-source model may have future applications in helping to predict if downwind individuals are being exposed to endotoxin concentrations that could cause health complications. Although there is no imminent regulatory precedent to do so, these dairies could reduce endotoxin emissions and potential onsite and offsite exposures by implementing dust mitigation strategies.

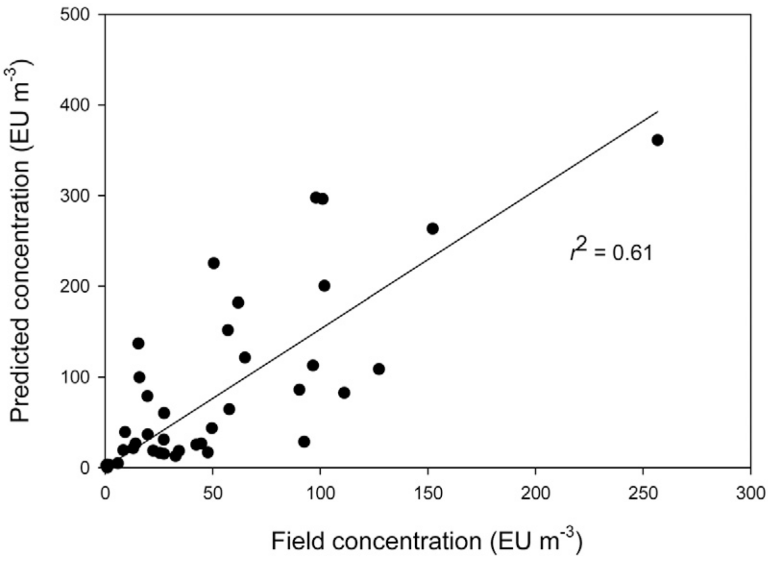

Fig. 3. Comparison of predicted ground-level endotoxin concentrations from the area-source model versus field data collected 200 m downwind from an open-lot dairy.

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