Concentrations of airborne endotoxin and microorganisms at a 10,000-cow open-freestall dairy[1]

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ABSTRACT: Confined animal production systems produce increased bioaerosol concentrations, which are a potential respiratory health risk to individuals on site and downwind. In this longitudinal study, airborne endotoxin and microorganisms were collected during the spring, summer, and fall at a large, open-freestall dairy in southern Idaho. Compared with the background ambient atmosphere, both endotoxin and culturable heterotrophic bacteria concentrations were up to several-hundred-fold greater 50 m downwind from the facility, then decreased to near background concentrations at 200 m. However, downwind fungi concentrations were not increased above background concentrations. At 50 m downwind, the average inhalable endotoxin concentration ranged from 5 to 4,243 endotoxin units per m^{-3}, whereas bacteria concentrations ranged from 10^2 to 10^4 cfu per m^{-3} of air. Although the bioaerosol concentrations did not follow a seasonal trend, they did significantly correlate with meteorological factors. Increasing temperature was found to be positively correlated with increasing bacteria ($r = 0.15$, $P < 0.05$), fungi ($r = 0.14$, $P < 0.05$), and inhalable endotoxin ($r = 0.32$, $P < 0.001$) concentrations, whereas an inverse relationship occurred between the concentration and solar radiation. The airborne concentrations at 50 m were also found to be greatest at night, which can likely be attributed to changes in animal activity and wind speed and reduced exposure of the airborne microorganisms to UV radiation.

Key words: airborne, bioaerosol, concentrated animal-feeding operation, dairy, endotoxin, manure

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INTRODUCTION

Bioaerosols are airborne viable and nonviable biological particles (e.g., bacteria, fungi, virus) and their by-products and fragments (e.g., endotoxin). Bioaerosols generated at concentrated animal-feeding operations (**CAFO**) may cause adverse health effects such as allergy and toxicosis in animals, workers, and residents in nearby communities (Dungan, 2010). To date, most CAFO studies have investigated bioaerosols within animal housing units, near mechanical ventilation systems, downwind of the facility, or all 3 (Chang et al., 2001; Wilson et al., 2002; Schulze et al., 2006; Chinivasagam et al., 2009). The general trend is that bioaerosol concentrations are typically the greatest indoors, less near the exhaust of the ventilation systems, and decrease with distance to background concentrations within a few hundred meters of the animal production facilities.

Data are limited that describe the diurnal and seasonal effects on CAFO bioaerosol emissions. In addition to meteorological effects, the management of animals, housing, and manure at CAFO can have an impact on bioaerosol emissions. In the western United States, the predominant dairy systems are open-lot, where cattle are housed in large pens, or open-freestall, where the cows are housed indoors for much of the time. Because these management systems are very different, there is potential for large differences in bioaerosol emissions at these 2 types of production facilities (Dungan and Leytem, 2011).

The objective of this longitudinal study was to measure airborne endotoxins and culturable microorganisms over 3 seasons (i.e., fall, spring, and summer) at a large open-freestall dairy. Bioaerosol samples were collected at upwind and downwind sites (outdoors only) and at select times during the day and night to assess diurnal effects upon emissions. This study compliments a previous study by the authors, who quantified bio-

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aerosols at an open-lot dairy of similar size (Dungan et al., 2010a,b).

MATERIALS AND METHODS

This study monitored air quality from a commercial dairy and no animals were directly involved in the study; therefore, no Animal Care and Use Committee approval was necessary.

Open-Freestall Dairy

The open-freestall dairy investigated in this study was located in southern Idaho and contained 10,000 Holstein cows. A schematic of the dairy is presented in Figure 1. There were 6 barns at the facility, 4 of which were approximately 670 m long, and the remaining 2 barns were about one-half of the size of the longest barns. The barns were oriented lengthwise in an east-west direction. The barns contained side curtains that were maintained in the closed position during colder months and open during the summer. Between each set of barns was an exercise yard, which was generally available to the cows when it was warmer outside. The yards between the barns were harrowed on a regular basis when in use by the cows. Manure in the alleys was flushed daily and then sent to a solids separator. The liquid waste was used in an anaerobic digester system for biogas production; digester effluent was stored in lagoons, which were located to the north of the facility. During the growing season, the facility used wastewater from the lagoons to irrigate crops via center pivot sprinklers. The facility was surrounded by irrigated crop land on all 4 sides.

Sampling Sites

Three sites at the dairy were used for sampling: an upwind site (approximately 200 m upwind from facility), 50 m downwind from the centerline of a barn

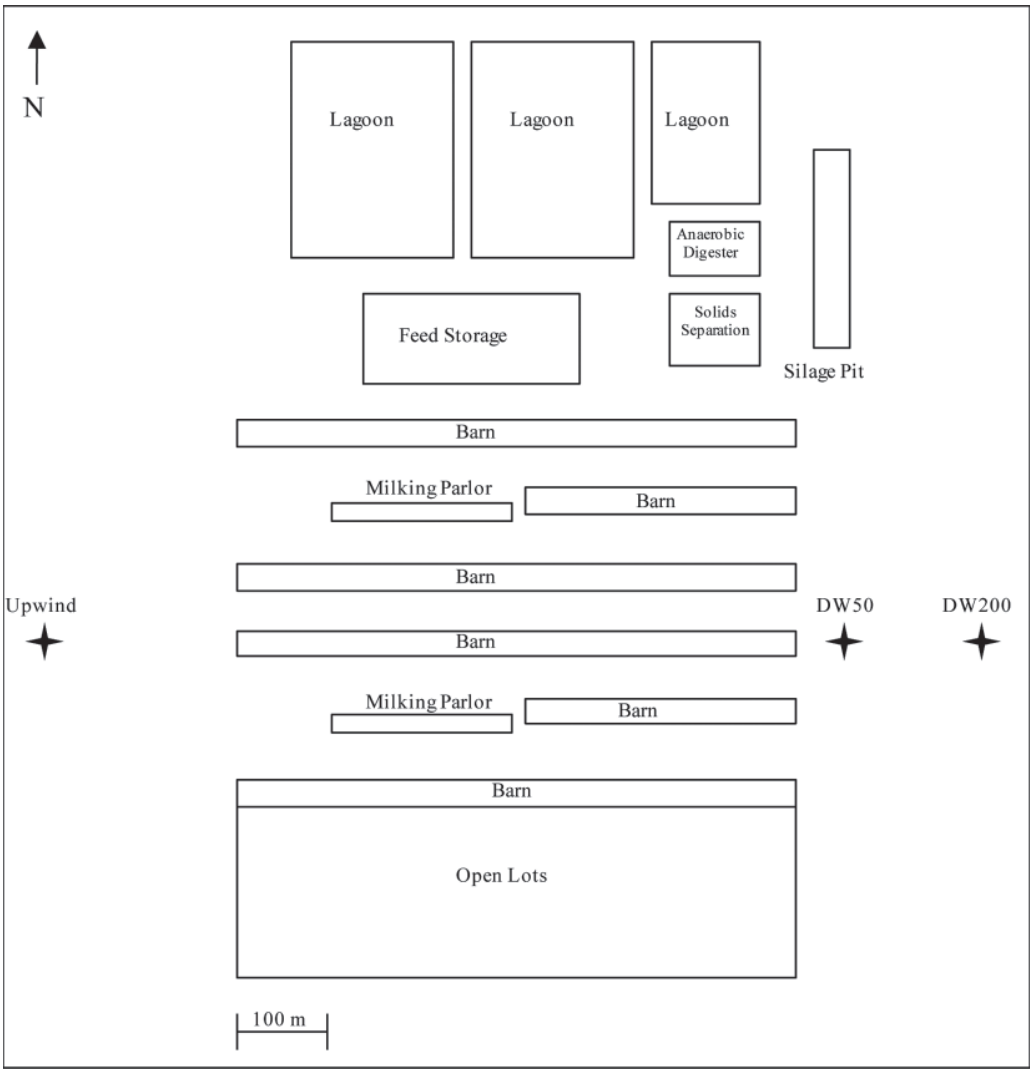

Figure 1. Schematic of the open-freestall dairy with upwind and downwind sampling sites. DW50 and DW200 = 50 and 200 m downwind from the centerline of a barn, respectively.

(**DW50**), and 200 m downwind from the centerline of a barn (**DW200**). The downwind sites were generally located on the east side of the dairy; the prevailing wind was from the west (Figure 1). Bioaerosol samples were collected during the fall (October and November of 2009), spring (April, May, and June of 2010), and summer (August and September of 2010). During each season, samples were collected on at least 8 separate days (4 d of endotoxins and 4 d of airborne microorganisms). Bioaerosol samples were not collected during late spring and summer at DW200 because of the presence of silage corn.

Meteorological data, including air temperature, relative humidity, wind speed, wind direction, and solar radiation, were collected using a Campbell Scientific (Logan, UT) model 21X data logger. Data were recorded every 1 min, then averaged into 15-min increments.

Airborne Endotoxin

Endotoxins associated with airborne dust were collected on 25-mm, 1.0 μm-pore-size polycarbonate track-etched filters (Whatman, Florham Park, NJ), which were housed in 25-mm button aerosol samplers (SKC Inc., Eighty Four, PA) or Delrin open-face filter holders (Pall Corporation, East Hills, NY). The button sampler in particular was designed to improve the collection characteristics of inhalable dust (Aizenberg et al., 2000). The endotoxin samplers were mounted 1.5 m above ground level and exposed to ambient conditions for approximately 180 min at an air flow rate of 4 $L \cdot min^{-1}$ using a Vac-U-Go sampling pump (SKC Inc.). Endotoxins were collected 3 times each day; 0800 to 1200 h (morning), 1300 to 1700 h (afternoon), and 1800 to 2300 h (night). The polycarbonate filters were processed and analyzed using kinetic *Limulus* lysate assay as described by Dungan and Leytem (2009).

Airborne Bacteria and Coliphage

Glass impingers (SKC Inc.) were utilized to capture airborne bacteria (i.e., heterotrophs, *Escherichia coli*, total coliforms) and coliphage. To account for diurnal fluctuations, impinger samples were collected 4 times each day; 0900 to 1100 h (morning), 1200 to 1400 h (noon), 1500 to 1700 h (afternoon), and 1800 to 2300 h (night). Before their use, each impinger was sterilized, and then filled with 30 mL of sterile impingement solution (0.1% peptone, 0.1% antifoam B emulsion, pH 6.8). The impingers were mounted 1.5 m above ground level and operated for approximately 90 min at a flow rate of 8.5 $L \cdot min^{-1}$ using a Vac-U-Go sampling pump (SKC Inc.). After sampling, the collection vessels were stored in a cooler with ice packs, then transported to our laboratory. Upon receipt, the samples were stored at 5°C for no longer than 18 h before being processed. Complete details on the setup and operation of the impingers and cultivation of the microorganisms can be found in Dungan et al. (2010b).

Airborne Fungi

Airborne fungi were collected directly on potato dextrose agar (**PDA**) through the use of single-stage cascade impactors (BioStage 200, SKC Inc.). The PDA plates were treated with 200 mg of streptomycin $sulfate \cdot L^{-1}$ to inhibit bacterial growth. A QuickTake 15 sampling pump (SKC Inc.) was used to pull vacuum at a flow rate of 14 $L \cdot min^{-1}$. The impactors were mounted 1.3 m above ground level at each of the sampling sites. Because only 1 impactor was available for each site, 3 samples were collected in succession for a total of 2 or 5 min for each PDA plate. Impactor samples were collected during each of the impinger sampling events. Fungal colonies were enumerated after 5 d of growth at 25°C.

Statistical Analyses

Bioaerosol concentration data were tested for normality using the Shapiro-Wilk test with the CAPABILITY procedure (SAS Inst. Inc., Cary, NC). The data from this longitudinal study were analyzed using the MIXED procedure of SAS with date as the repeated measure and time of day and sampling site as the fixed effects. Means separation was carried out using the difference of the least squares means with the Tukey-Kramer adjustment and α-level of 0.05. Pearson correlation coefficients (r) were calculated for bioaerosol concentration (endotoxin, culturable bacteria, and fungi), air temperature, wind speed, relative humidity, and solar radiation using the CORR procedure of SAS. Statements of statistical significance were based on a $P < 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Meteorological Data

Ambient weather data at the open-freestall dairy are presented in Table 1 and represent conditions during the time at which bioaerosol samples were being collected. Minimum air temperatures ranged from −3.6 to 25.0°C, whereas maximum air temperatures ranged from 4.3 to 32.8°C. August was the warmest month, and impinger samples were not collected from December to March because this equipment cannot be operated at temperatures near or below freezing. The average relative humidity ranged from 12 to 85%, and the average high for solar radiation was 772 $W \cdot m^{-2}$. Bioaerosol samples were mainly collected when the wind was from the west (average of 251°), except on a few occasions when the wind was from the east or southeast (average of 115°). The average wind speed ranged from 1.5 to 7.0 $m \cdot s^{-1}$.

Airborne Endotoxin

Upwind (background) and downwind airborne endotoxin concentrations at the open-freestall dairy are presented in Table 2. Regardless of collection method

Table 1. Ambient weather data at the open-freestall dairy during the time of bioaerosol collection only[1]

Item	Day-month	AT min, °C	AT max, °C	RH, %	WS, $m \cdot s^{-1}$	WD, °	SR, $W \cdot m^{-2}$
Fall	26-Oct	3.7	17.1	32.9	3.7	127	221
	28-Oct	−1.8	4.8	57.6	6.2	251	232
	29-Oct	0.2	4.3	50.3	6.3	257	296
	2-Nov	2.3	13.0	56.7	1.5	95	286
	4-Nov	−0.1	18.4	54.6	2.6	99	241
	5-Nov	4.5	21.5	26.0	2.8	197	147
	9-Nov	−3.6	15.6	38.3	3.1	135	243
	12-Nov	−0.8	4.5	71.1	4.7	246	171
Spring	6-Apr	0.6	6.8	51.3	4.1	246	353
	7-Apr	4.0	11.2	43.1	2.4	253	586
	9-Apr	−1.9	7.0	34.4	2.5	244	417
	13-Apr	4.5	10.8	56.9	6.0	265	324
	15-Apr	2.5	21.2	33.5	3.4	129	623
	22-Apr	5.7	8.0	84.8	3.8	247	42
	10-May	4.2	13.7	63.2	4.1	268	275
	19-May	11.7	20.4	43.6	3.2	251	416
	2-Jun	17.0	21.3	48.4	2.1	228	393
	9-Jun[2]	16.4	19.4	58.5	1.7	241	9
	10-Jun[2]	10.1	10.7	57.6	6.3	252	1
Summer	3-Aug	23.0	31.3	23.7	3.7	302	772
	5-Aug	20.3	32.6	39.1	2.9	96	355
	12-Aug	14.4	22.5	42.3	3.3	276	476
	18-Aug	25.0	32.8	12.4	7.0	270	—[3]
	1-Sep[2]	16.1	17.3	54.6	2.3	234	0
	2-Sep[2]	11.0	15.6	44.5	1.5	121	0

[1]AT, air temperature; RH, relative humidity; WS, wind speed; WD, wind direction; SR, solar radiation. Average values for RH, WS, WD, and SR.

[2]Night sampling event only.

[3]Data not available.

(i.e., button vs. open-face), the average upwind concentration was <33 endotoxin units (**EU**)$\cdot m^{-3}$ of air. The upwind endotoxin concentrations were similar to values reported by other researchers in various background environments (Mueller-Anneling et al., 2004; Madsen, 2006). At 50 m downwind from the dairy, the average inhalable endotoxin (i.e., button samples) concentration ranged from 5 to 4,243 EU$\cdot m^{-3}$, whereas the open-face airborne endotoxins ranged from 20 to 2,621 EU$\cdot m^{-3}$. When 200 m downwind from the facility, airborne endotoxin concentrations were substantially less with average concentrations ranging from 15 to 159 and 15 to 207 EU$\cdot m^{-3}$, for the button and open-face aerosol samplers, respectively. Overall, the average inhalable airborne endotoxin concentrations at the upwind site and 50 and 200 m downwind were 5, 426, and 56 EU$\cdot m^{-3}$, respectively (Figure 2). The respective open-face concentrations were 5, 283, and 68 EU$\cdot m^{-3}$. These average endotoxin concentrations are similar to those reported at a large open-lot dairy, where concentrations at upwind and downwind (5 and 200 m) sites were 28, 169, and 71 EU$\cdot m^{-3}$, respectively (Dungan et al., 2010b).

At the open-freestall dairy, the effect of sampling site was determined to be significant ($P < 0.0001$) and followed the trend of DW50 > DW200 > upwind (inhalable endotoxin only). Although the same trend occurred with the open-face samples, there was only a significant difference ($P = 0.05$) between the upwind and DW50 sites (Figure 2). However, there was no significant difference between the average button and open-face endotoxin concentrations at each of the sampling sites ($P > 0.29$). These results suggest that the button and open-face samplers both collected a similar size fraction of the airborne particulate matter (i.e., aerodynamic diameter <100 μm).

Figure 3 presents the button and open-face airborne endotoxin concentrations at the upwind, DW50, and DW200 sites in the morning, afternoon, and night. At the upwind and DW200 sites, there was no significant effect of time on the inhalable endotoxin concentration ($P > 0.21$). At DW50 there was an effect of time; the concentration at night was significantly greater than in the morning ($P = 0.04$), but it was similar to the afternoon ($P = 0.42$). With respect to the open-face samples, there was no significant effect of time ($P > 0.31$), although at DW50 the trend was for an increase in airborne endotoxin concentration from morning to night. These results are similar to those obtained at an open-lot dairy, where airborne endotoxin concentrations increased from the morning to the evening (Dungan and Leytem, 2009). The increase in airborne endotoxin concentration toward the evening and night can be attributed to increased animal activity and slower wind speed. Whereas airborne endotoxin concentrations are generally increased in the immediate downwind environment at dairies, both predicted and measured concentrations have been shown to decrease with distance and reach background concentrations within 500 to 2,000 m (Dungan and Leytem, 2011).

Table 2. Average daily airborne endotoxin concentrations (endotoxin units·m^{-3} of air) upwind and downwind of the open-freestall dairy[1]

Item	Day-month	Upwind	Downwind (50 m)	Downwind (200 m)
Button[2]				
2009	28-Oct	2.8	29.4	69.4
	4-Nov	11.1	28.6	19.6
	9-Nov	1.7	15.0	15.2
	12-Nov	0.3	4.6	29.2
2010	6-Apr	—[3]	—	—
	9-Apr	1.1	31.7	22.8
	15-Apr	—		—
	10-May	0.5	550	159
	10-Jun	0.4	183	—
	3-Aug	5.1	72.1	—
	5-Aug	11.4	445	123
	12-Aug	0.9	654	—
	18-Aug	1.9	1,127	—
	1-Sep	2.7	981	—
	2-Sep	32.9	4,243	—
Open face[4]				
2009	28-Oct	2.7	24.3	62.4
	4-Nov	8.1	28.2	30.3
	9-Nov	3.0	19.6	15.2
	12-Nov	1.7	38.8	47.6
2010	6-Apr	2.6	140	70.1
	9-Apr	2.1	58.4	39.1
	15-Apr	4.4	49.6	15.5
	10-May	1.7	741	207
	10-Jun	0.2	57.4	—
	3-Aug	24.0	22.3	—
	5-Aug	5.5	362	191
	12-Aug	1.5	430	—
	18-Aug	—	—	—
	1-Sep	1.0	1,273	—
	2-Sep	23.7	2,621	—

[1]Average of morning, noon, and night data (n = 9).
[2]Button aerosol samplers were used to collect inhalable dust particles.
[3]Data not available.
[4]Open-face samplers were used to collect a wide range of unspecified dust particle sizes.

Whereas the particle-size collection characteristics of the open-face samplers are unknown, the button aerosol samplers were used to enhance the collection of particles with an aerodynamic diameter of <100 μm. Particulate matter with a diameter <100 μm is particularly hazardous when deposited anywhere in the respiratory tract because it can cause respiratory disease, discomfort, and increased mortality (Cambra-López et al., 2010). Endotoxins are derived from the outer membrane of gram-negative bacteria and are a potent inducer of inflammatory reactions in the respiratory tract when inhaled (Portengen et al., 2005). Exposure to increased concentrations of airborne endotoxin is associated with decreased lung function, cough, chest tightness, and influenza-like symptoms (Castellan et al., 1987; Zock et al., 1998; Rylander, 2006). In contrast, some occupational studies suggest that exposure to endotoxin may protect against atopic sensitization, asthma, and cancer (Lange, 2000; Holla et al., 2002; Portengen et al., 2005). Mastrangelo et al. (1996) found that dairy farmers had a reduced risk for lung cancer when compared with crop/orchard farmers, concluding that it was because they were exposed to greater airborne endotoxin concentrations.

Airborne Bacteria and Fungi

The airborne heterotrophic bacteria and filamentous fungi concentrations at the open-freestall dairy are presented in Table 3. At the upwind site, the average bacteria concentration ranged from 5.8×10^2 to 1.0×10^4 cfu·m^{-3} of air. When measured 50 m downwind, the average bacteria concentration increased dramatically and ranged from 3.1×10^3 to 3.7×10^5 cfu·m^{-3}, then decreased at 200 m downwind to a range of 1.2×10^3 to 1.6×10^4 cfu·m^{-3}. The average airborne fungi concentrations at the upwind site ranged from 56 to 1,484 cfu·m^{-3}, whereas at 50 and 200 m downwind they ranged from 60 to 1,004 and 83 to 1,587 cfu·m^{-3}, respectively. The upwind and downwind concentrations at the open-freestall dairy fell within ranges reported at an open-lot dairy in southern Idaho containing a similar number of cows (Dungan et al., 2010b). Figures 4a and 4b present the average airborne bacteria and

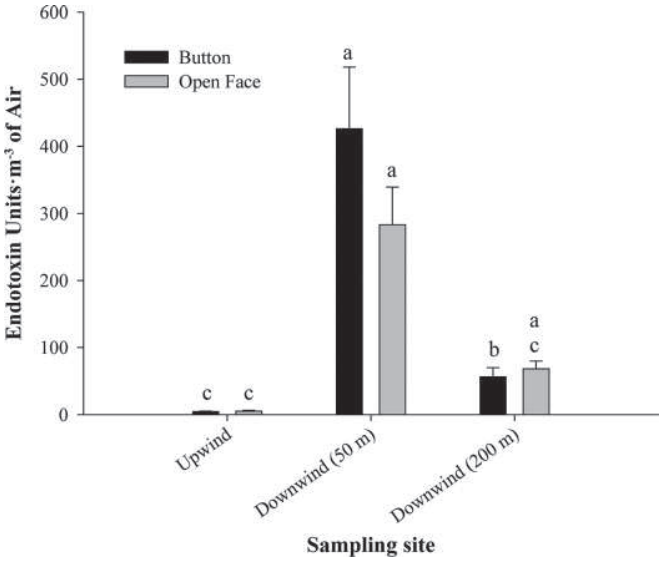

Figure 2. Average airborne endotoxin concentrations at the upwind and downwind sites at the open-freestall dairy. Endotoxin samples were collected using either button aerosol samplers (SKC Inc., Eighty Four, PA) or open-face filter holders (Pall Corporation, East Hills, NY). Letters (a–c) above the columns indicate significant differences between the sample sites ($P < 0.05$).

fungi concentrations during the course of the study, respectively. The average bacteria concentrations at the upwind, DW50, and DW200 sites were 2.8×10^3, 8.4×10^4, and 7.9×10^3 cfu·m^{-3}, respectively. Sampling site tended to affect bacteria concentrations (DW50 > upwind, $P < 0.10$). Respective fungi concentrations at upwind, DW50, and DW200 were 620, 515, and 493 cfu·m^{-3}, indicating that the downwind concentrations were less than background and that the facility appears to be a sink for the fungal spores.

A variety of aerosolized bacteria and fungi can cause respiratory ailments, infection, and toxicosis when inhaled by humans (Stetzenbach, 2007). Within animal housing units, there is an increase in the overall microbial load (Lange et al., 1997; Adhikari et al., 2004), leading to concerns about occupational exposures and offsite transport of microbial by-products and pathogens (Donham et al., 1989; Cole et al., 2000). In a study at a swine confinement operation, Green et al. (2006) found that airborne bacteria (*Staphylococcus aureus* and total coliforms) concentrations were about 300-fold greater indoors than upwind of the facility. Outdoor bacteria concentrations steadily decreased with distance from the facility, reaching background concentrations at about 150 m. At a ventilated broiler shed, outdoor concentrations of *Staphylococcus* and *Corynebacterium* were detected at 10^6 cfu·m^{-3} when 20 m downwind of an exhaust fan, but dropped off to background concentrations at 400 m (Chinivasagam et al., 2010). Downwind of an open-lot dairy, the average airborne bacteria concentration was approximately 16-fold less at 200 m than at the edge of the cattle pens; however, the airborne fungal concentration did not decrease at 200 m (Dungan et al., 2010b). Matković et al. (2009) found that airborne fungal concentrations approached background concentrations at downwind distances as close as 5 to 50 m from a dairy barn.

During the study, aerosolized *E. coli* was also detected in the downwind environment on 6 separate days (data not presented). The average concentration ranged from 1 to 133 cfu·m^{-3} of air, with most days having nondetectable concentrations. Although a variety of gram-negative bacteria have been recovered from within animal houses (Zucker et al., 2000; Chang et al., 2001; Chinivasagam et al., 2009), some researchers have indicated difficulty in cultivating these organisms at

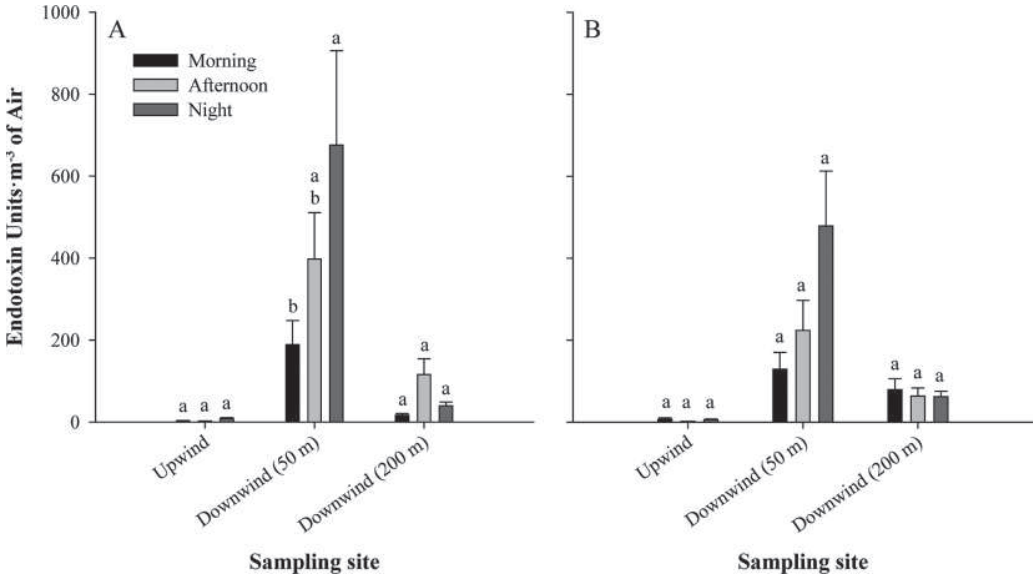

Figure 3. Average airborne endotoxin concentrations during morning, afternoon, and night sampling events at the open-freestall dairy. Panel A, button aerosol samplers (SKC Inc., Eighty Four, PA) and panel B, open-face filter holders (Pall Corporation, East Hills, NY). Letters (a,b) above the columns indicate significant differences between the sample times at each site ($P < 0.05$).

Table 3. Average daily airborne heterotrophic bacteria and filamentous fungi concentrations (cfu·m^{-3} of air) upwind and downwind of the open-freestall dairy[1]

Item	Day-month	Upwind	Downwind (50 m)	Downwind (200 m)
Bacteria				
2009	26-Oct	1,668	374,448	1,201
	29-Oct	3,876	9,777	14,830
	2-Nov	592	2,471	2,871
	5-Nov	10,370	24,405	5,797
2010	7-Apr	1,564	4,274	5,614
	13-Apr	1,736	17,454	12,105
	22-Apr	2,389	3,131	2,279
	19-May	2,174	66,609	15,707
	2-Jun	4,008	5,526	6,457
	9-Jun	577	10,233	—[2]
	3-Aug	1,553	117,209	—
	5-Aug	4,271	154,556	—
	12-Aug	1,314	128,649	—
	18-Aug	1,513	73,327	—
	1-Sep	1,304	119,295	—
	2-Sep	2,022	252,387	—
Fungi				
2009	26-Oct	1,443	586	494
	29-Oct	266	813	375
	2-Nov	601	637	548
	5-Nov	726	327	521
2010	7-Apr	56	60	83
	13-Apr	156	67	158
	22-Apr	467	633	186
	19-May	387	274	386
	2-Jun	1,016	921	1,587
	9-Jun	—	—	—
	3-Aug	1,484	790	—
	5-Aug	948	1,004	—
	12-Aug	352	495	—
	18-Aug	260	240	—
	1-Sep	362	571	—
	2-Sep	552	638	—

[1]Average of morning, noon, afternoon, and night data (n = 12).
[2]Data not available.

open feedlots (Wilson et al., 2002). The limited detection of airborne *E. coli* downwind of the freestall dairy could be caused by environmental conditions (e.g., solar radiation) that reduce overall numbers or the transformation of airborne organisms into a viable but nonculturable state. Airborne total coliform and coliphage (fecal pollution indicators) were not detected downwind of the freestall dairy (data not presented), and similar environmental and viability factors may have been responsible.

Figure 5a presents the average airborne bacteria concentrations during the morning, noon, afternoon, and night sampling events. At the upwind site, there was a slight but significant effect of time between the noon and afternoon or night events ($P < 0.04$). The effect of time was more pronounced at DW50; the night concentration was significantly greater than both morning and afternoon events ($P < 0.03$). At DW200, there was no significant difference between the time events ($P > 0.15$). Figure 5b presents the average airborne fungi concentrations during morning to night sampling events. Although there do not appear to be clear trends among the upwind samples, the fungi concentration in the afternoon was significantly greater than at noon ($P = 0.002$), but it was similar to the morning and night events ($P > 0.50$). At DW50, the fungi concentration was greatest at night and significantly different than the morning and noon events ($P < 0.009$). As with the airborne heterotrophic bacteria, there was no significant difference between the sampling events for fungi at DW200 ($P > 0.10$). The fact that bacteria and fungi concentrations were greatest in the night samples (DW50 only) may be related to several factors, such as changes in animal activity and wind speed, but it could also be a result of reduced exposure to UV radiation, thus increasing overall microbial survivability.

Effect of Ambient Weather on Bioaerosol Concentrations

Correlation analyses between the bioaerosol concentrations at the downwind sites and meteorological factors are presented in Table 4. Both button and open-face endotoxin samples were positively correlated with

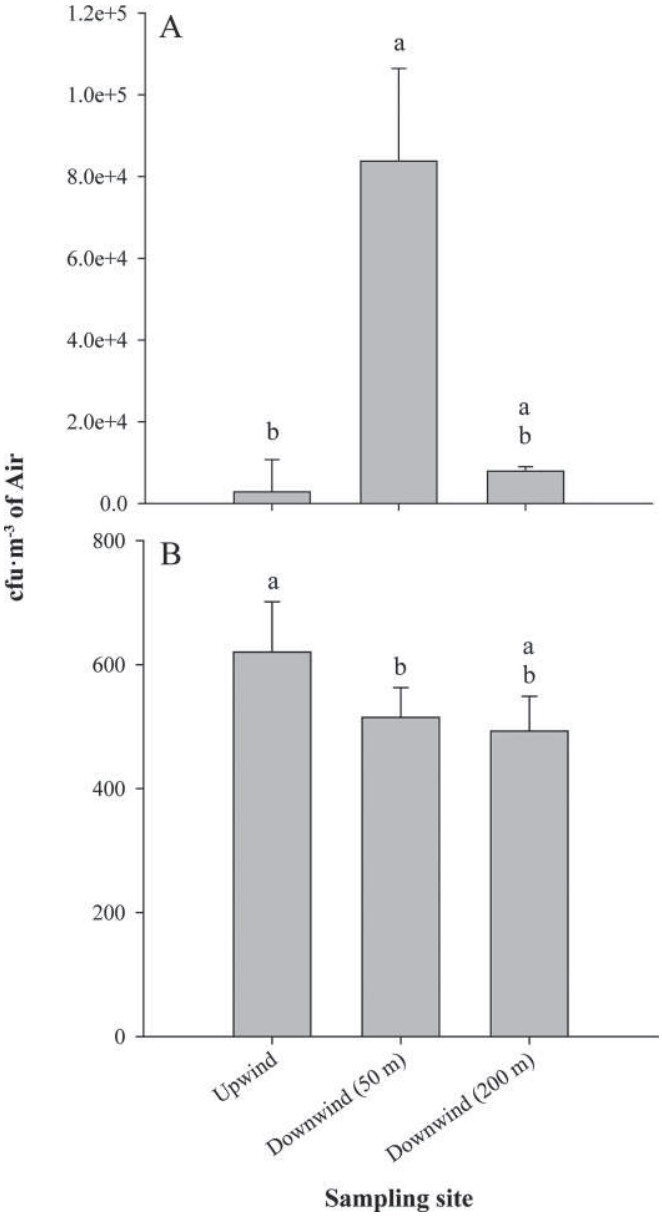

Figure 4. Average airborne heterotrophic bacteria (panel A) and filamentous fungi (panel B) concentrations at the open-freestall dairy. Letters (a,b) above the columns indicate significant differences between the sample sites ($P < 0.10$).

air temperature and negatively correlated with solar radiation. In the former case, the positive relationship could be related to increased growth of gram-negative bacteria with increasing temperatures. In the latter case, increased solar intensity and exposure of the endotoxin to UV radiation could be affecting the integrity of the lipopolysaccharide (**LPS**) molecule. The concentrations of endotoxin in open-face samples were also negatively correlated with relative humidity and wind speed. Although one would not expect increases in relative humidity to affect the structural integrity of the LPS, the negative relationship is more likely a result of temperature because humidity was generally decreased during the summer months. Interestingly, the negative correlation between endotoxin concentration and wind speed contradict results obtained by the authors at an open-lot dairy (Dungan et al., 2010a).

Airborne bacteria concentrations were positively correlated with air temperature and wind speed, but negatively correlated with relative humidity and solar radiation (Table 4). Although the wind speed and solar radiation correlations are representative of results published in the literature, the temperature and relative humidity correlations were opposite of what we expected. Similar results though, were obtained in a study of airborne bacteria at an agricultural location, where total and culturable concentrations increased with increasing temperature and decreasing relative humidity (Tong and Lighthart, 2000). Results from outdoor studies may indeed differ from those obtained in controlled laboratory studies because temperature and relative

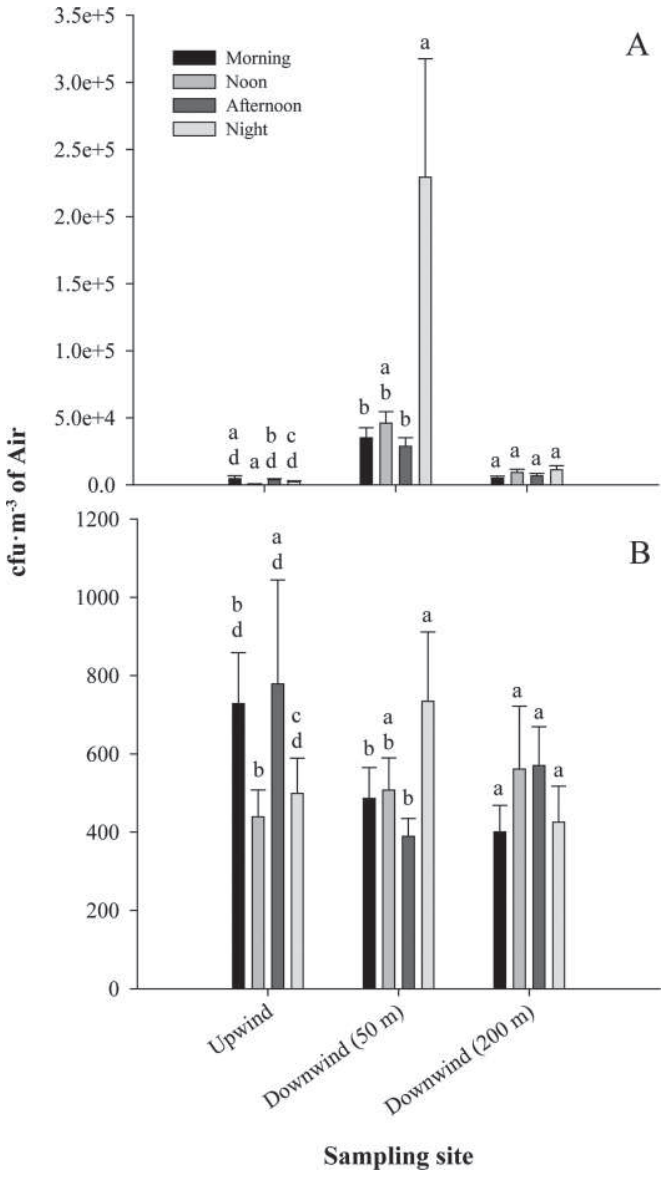

Figure 5. Average airborne heterotrophic bacteria (panel A) and filamentous fungi (panel B) concentrations during morning, noon, afternoon, and night sampling events at the open-freestall dairy. Letters (a–d) above the columns indicate significant differences between the sample times at each site ($P < 0.05$).

Table 4. Pearson correlation coefficients (r) between bioaerosol concentrations and meteorological factors at the downwind sampling sites[1]

Item	AT, °C	RH, %	WS, $m \cdot s^{-1}$	SR, $W \cdot m^{-2}$
Button,[2] $EU \cdot m^{-3}$	0.318***	−0.150	0.074	−0.178*
Open face	0.232***	−0.023***	−0.155*	−0.181*
Bacteria, $cfu \cdot m^{-3}$	0.149*	−0.166**	0.165**	−0.137*
Fungi	0.138*	0.117	−0.246***	−0.117

[1]AT, air temperature; RH, relative humidity; WS, wind speed; SR, solar radiation.

[2]EU, endotoxin units.

*, **, ***Indicate significant difference within a row at the 0.05, 0.01, and 0.001 probability levels, respectively.

humidity not only affect viability and growth, but also release mechanisms (Jones and Harrison, 2004). In addition, the effect of any meteorological factor on its airborne concentration may differ from year to year because of extremes in other variables. Airborne fungi concentrations at the open-freestall dairy were positively correlated with air temperature and negatively correlated with wind speed. Whereas most fungal studies have shown increased airborne concentrations with increasing temperature and decreasing relative humidity, increased turbulence and wind speed enhance spore detachment, resulting in greater concentrations (Latham, 1982; Savery, 1986; Bock et al., 1997).

Many studies have shown that the viability of airborne bacteria generally decreases with increases in temperature and solar radiation and decreases in relative humidity (Ehrlich et al., 1970; Marthi et al., 1990; Ko et al., 2000; Nehme et al., 2008). However, because of the interrelationship between temperature and relative humidity, it is often difficult to separate their effects (Mohr, 2007). At temperatures of 20 to 50°C, the death rate for aerosolized suspensions of *E. coli* was reduced about 2-fold when humidity was increased from 20 to 80% (Poon, 1966). Using a bench-scale aerosol reactor, Paez-Rubio and Peccia (2005) found that *Mycobacterium parafortuitum* was more susceptible to solar inactivation under moderate relative humidity (50 to 60%) than under high humidity (85 to 95%). In contrast, *E. coli* was more susceptible to solar inactivation under high relative humidity (85 to 95%). Ulevičius et al. (2000) found that outdoor airborne fungal propagules collected in the early morning and late evening were more sensitive to solar radiation than propagules collected in the afternoon. The lethal effect on propagules was likely caused by the elimination of radiation-sensitive fungi that were more abundant during hours of low solar intensity. Similar results were shown to occur with outdoor airborne bacteria (Tong and Lighthart, 1997). In another outdoor study, Chi and Li (2007) found a significant negative correlation between airborne bacteria concentrations and temperature or UV intensity, whereas no significant correlations between airborne fungi and these meteorological factors were identified. Targets of temperature- and radiation-induced inactivation of airborne microorganisms are membrane phospholipids, proteins, and nucleic acids (Cox, 1995).

In summary, the open-freestall dairy produced increased bioaerosol concentrations that were up to several-hundred-fold greater than at upwind sites. Culturable bacteria and endotoxin concentrations were found to be the greatest 50 m downwind and then decreased to near background concentrations at 200 m from the facility. The trend of decreasing concentration with distance suggests that the risk of exposure to bioaerosols also decreases with distance. With respect to culturable airborne fungi, the downwind concentrations were found to be less on average than background. Although the bioaerosol concentrations did not follow a seasonal trend, they did significantly correlate to meteorological factors. Increasing temperature was found to be positively correlated with increasing endotoxin, bacteria, and fungi concentrations, whereas an inverse relationship occurred between the concentration and solar radiation. Meteorological factors or viable nonculturable status of specific bacterial populations may have been responsible for the lack of detection of total coliform and coliphage. This, however, does not suggest the absence of these and other fecal-related organisms in the ambient atmosphere of the dairy. Even though most of the cows, manure, and feed were contained within the barns at the freestall dairy, the bioaerosol concentrations were within ranges determined at a similar size open-lot dairy in the same region of Idaho (Dungan et al., 2010a,b). Therefore, differences in animal housing and manure management between the 2 dairies do not appear to greatly affect the bioaerosol concentrations. Because only 2 dairies have been investigated by our group to date, it should be cautioned that the observed spatial trends and bioaerosol concentrations may not be representative of the whole dairy industry.

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