Aerobiologia (2010) 26:141–148
DOI 10.1007/s10453-009-9151-5

ORIGINAL PAPER

Year-long assessment of airborne endotoxin at a concentrated dairy operation

Robert S. Dungan · April B. Leytem ·
David B. Bjorneberg

Received: 27 August 2009 / Accepted: 14 December 2009 / Published online: 29 December 2009

Abstract In this study, we monitored total airborne endotoxins at upwind and downwind sites at a large open-lot dairy each month for a year. At the upwind site, the average airborne concentration was 28.5 endotoxin units (EU) m^{-3}, while at the downwind edge of the lot and 200 m from the lot edge, the average concentrations were 169 and 72 EU m^{-3}, respectively. At the downwind edge of the lot, there was a significant correlation between the airborne endotoxin concentration and wind speed or air temperature. A comparison between total and inhalable airborne endotoxin concentrations, near the end of the study, revealed no significant differences between the two endotoxin collection methods. Our data suggest that endotoxin exposure can be reduced as one increases their distance from the open-lot dairy.

Keywords Bioaerosol · CAFOs · Dairy · Endotoxin · Gram-negative bacteria · Lipopolysaccharide

1 Introduction

High stocking densities at animal feedlots have drastically changed modern agricultural practices. At concentrated animal feeding operations (CAFOs), large populations of single species are confined to increase production and reduce costs. Over the last decade, Idaho has grown to become the third largest milk-producing state and has approximately 550,000 milk cows (USDA, National Agricultural Statistics Service 2008). While half of the production facilities in Idaho have <200 cows, there is an increasing trend toward larger facilities, and some of the largest have as many as 10,000 animals. These trends are not just specific to Idaho; however, as other states and countries are experiencing increases in the number of CAFOs. As a result of high stocking densities at CAFOs, there is a concern over the release of bioaerosols, since they may cause adverse health effects in animals and workers (Lacey and Dutkiewicz 1994; Wilson et al. 2002; Rule et al. 2005; Heederik et al. 2007; Spaan et al. 2006).

Many gram-negative bacteria produce lipopolysaccharides (LPS) as part of the outer membrane of their cell wall. These potentially toxic LPS are called endotoxins and are released upon cell lysis. While LPS are composed of three covalently linked subunits (i.e. lipid A, core polysaccharide, and O-antigen or -polysaccharide), it is the lipid A portion that is responsible for toxicity (Bradley 1979). Exposure to airborne endotoxins can cause acute fever and inflammatory reactions in the respiratory tract, accompanied by cough, chest tightness, shortness of breath, and wheezing (Douwes and Heederik 1997; Zock et al. 1998; Rylander 2006). Chronic exposure

R. S. Dungan (✉) · A. B. Leytem · D. B. Bjorneberg
USDA-Agricultural Research Service, Northwest
Irrigation and Soils Research Laboratory, 3793 North
3600 East, Kimberly, ID 83341, USA
e-mail: robert.dungan@ars.usda.gov

to endotoxins in organic dusts from occupational settings can lead to decreased lung function, chronic bronchitis, and byssinosis (Castellan et al. 1987; Jacobs 1989; Smid et al. 1992; Mandryk et al. 2000).

In background ambient environments, inhalable, thoracic, and respirable endotoxin concentrations are generally <10 endotoxin units (EU) m^{-3} (Heinrich et al. 2003; Mueller-Anneling et al. 2004; Madsen 2006). However, exposure to relatively low ambient concentrations of 50–100 EU m^{-3} has been shown to cause respiratory effects (Castellan et al. 1987; Milton et al. 1996; Zock et al. 1998). A proposed health-based limit of 50 EU m^{-3} for an 8-h exposure period has been recommended by the Dutch Expert Committee on Occupational Standards (Heederik and Douwes 1997). At indoor animal facilities, total airborne endotoxin concentrations in dairy barns, swine houses, and poultry houses were as high as 800, 3,200, and 12,800 EU m^{-3}, respectively (Zucker and Müller 1998; Bakutis et al. 2004; Portengen et al. 2005; Schierl et al. 2007). In an open feedlot study conducted by Purdy et al. (2004), inhalable and respirable airborne endotoxins were collected at cattle feedlots ranging in size from 1,000 to 175,000 animals, with mean endotoxin concentrations of 84 EU m^{-3} in the winter and 26 EU m^{-3} in the summer. Investigations into the offsite transport of endotoxin from open feedlots are of particular interest, as they may present a respiratory health risk to individuals in nearby residences.

The purpose of this research project was to study total airborne endotoxin concentrations at upwind and downwind sites at a large open-lot dairy over the course of a year. In addition, during the last 2 months of the study, a comparison was made between total and inhalable airborne endotoxin concentrations. Correlations between ambient weather data and endotoxin concentrations were also determined.

2 Materials and methods

2.1 Dairy and sample sites

The open-lot dairy contained 10,000 milking cows and was located in southern Idaho at 1,070 m above sea level. The region is a high desert and generally receives <25 cm of precipitation a year. The dairy was adjacent to irrigated crop land on the west, south, and east and open range to the north. During the irrigation season (May through September), the east field was intermittently irrigated with blended dairy wastewater. Crops grown on the west, south, and east fields were alfalfa, potato, and silage corn, respectively. The nearest upwind animal production facility was ~2.7 km away.

The sampling sites consisted of an upwind site (UW), 5-m downwind of the edge of the cattle lots (DW1), and 200-m downwind of the edge of the lots (DW2). The sites changed depending if a westerly or easterly wind was present (Fig. 1). No air samples were collected when the irrigation system in the east field was utilizing dairy wastewater, and wind was from the east. Meteorological data, including air temperature, relative humidity, solar radiation, wind speed, and wind direction were collected using a Campbell Scientific (Logan, UT, USA) model 21X data logger.

2.2 Airborne endotoxin sampling

Airborne endotoxin sampling was carried out according to the method described by Dungan and Leytem (2009a). In brief, airborne endotoxins were collected on 25 mm 1.0 µm-pore-size polycarbonate track-etch filters (Whatman, Florham Park, NJ, USA), which were housed in 25-mm Delrin open-face filter holders (Pall Corporation, East Hills, NY, USA) or button aerosol samplers (SKC Inc., Eighty-Four, PA, USA). The button samplers are used to collect inhalable airborne endotoxins, which are associated with dust particles that have a median cut-point of 100 µm. Three tripods were placed at each site, which were set at a height of 1.5 m and distance of 1.5 m between each tripod. The tripods were oriented, so they were perpendicular to the wind direction, and vacuum was applied to the filters using a Vac-U-Go sampling pump (SKC Inc.). Each tripod was mounted with one open-face holder and/or button sampler.

Airborne endotoxins were collected 2 days out of each month from April 2008 to March 2009. Samples were collected twice each day, once in the morning (0800–1200) and afternoon (1300–1700). The endotoxin samples were collected up to 120 min at a rate of 2 l min^{-1}. Samples at all three sites (i.e. UW, DW1, and DW2) were collected simultaneously, and flow rates were checked regularly using a rotameter. When not being used, the open-face holders and

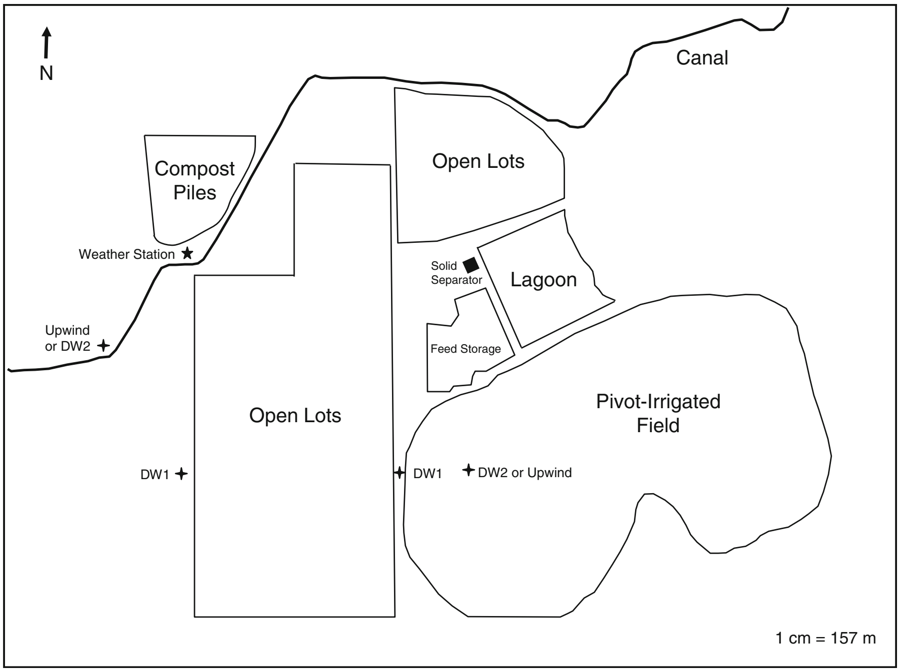

Fig. 1 Schematic of the open-lot dairy with sampling sites

button samplers were stored in pyrogen-free tins. Exposed filters were transported to the laboratory in a cooler, transferred to 2-ml pyrogen-free polypropylene tubes, and stored dry at −20°C until processed. Trip blanks were utilized to ensure the samples were not contaminated during preparation, transport, and storage.

Except for the open-face holders and button samplers, all materials were depyrogenated by heating at 250°C for 30 min or purchased pyrogen free. To depyrogenate the open-face holders and button samplers, they were first cleaned with soap and water, followed by successive rinses with pyrogen-free water and 70% ethanol, then autoclaved for 30 min (121°C, 1.23 atm).

2.3 Endotoxin extraction and analysis

The polycarbonate filters were processed as described by Dungan and Leytem (2009b). To extract the endotoxins from the polycarbonate filters, 1.5 ml of pyrogen-free water (PFW) 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 extracts were stored for no longer than 18 h at 5°C before being analyzed.

The extracts were analyzed for endotoxin using the LAL Kinetic-QCL test kit (Lonza Inc., Walkersville, MD, USA) as recommended by the manufacturer. All extracts were vortexed for 1 min at high speed, then 50 µl aliquots of sample and β-glucan blocker (Lonza Inc.) were added to each well of a pyrogen-free 96-well microplate (Corning Inc., Corning, NY, USA). Endotoxin standards (lyophilized *Escherichia coli* O55:B5) were prepared in PFW containing 0.025% Tween 20, since all extracts were diluted 2-fold using β-glucan blocker. The microplate was then shaken for 1 min at 400 rev min^{-1}, followed by incubation at 37°C for 15 min. After incubation, a 96-channel pipette (Transtar-96, Corning Inc.) was used to simultaneously dispense 100 µl of the Kinetic-QCL reagent into the wells. The microplate was then immediately placed into an ELx808 absorbance microplate reader (BioTek Instruments Inc., Winooski, VT, USA) to initiate the test. An 8-point calibration curve ranging from 0.005 to 50 EU ml^{-1} was used (10 EU $\approx$ 1 ng endotoxin), and r^2 values were $\geq$0.95. Quality control was checked regularly through the use of blanks and duplicate samples.

2.4 Statistical analysis

Endotoxin concentration data were log transformed and tested for normality using the Univariate Procedure of SAS (SAS Institute 2008), with untransformed numbers presented in the text. The transformed data were analyzed using the Mixed Procedure of SAS with date as the repeated measure and site or collection method (i.e. total and inhalable endotoxin) as the subject. Means separation was carried out using the difference in the least squares means with Tukey–Kramer adjustment and $\alpha = 0.05$. To determine the relationship between ambient weather data and endotoxin concentrations, Pearson correlation coefficients (r) were calculated. Statements of statistical significance were based upon $P < 0.05$ unless otherwise stated.

3 Results and discussion

Ambient weather conditions at the dairy during the year-long study are listed in Table 1. The weather data presented are an average of the morning and afternoon data from the times when air samples were collected only. The ambient temperature ranged from −7.8 to 32.8°C, with an average of 13.9°C. The relative humidity ranged from 16 to 93%, with an average of 47%. Solar radiation was generally the highest in April–September and lowest in October–March, with an average of 448 W m^{-2}. The wind speed ranged from 0.9 to 5.3 m s^{-1}, and the prevailing wind was from the west. Airborne endotoxin samples were only collected when the wind was predominantly from the west (avg. = 241°, SEM = 7°) or east (avg. = 94°, SEM = 15°). The

Table 1 Average ambient weather data and lot conditions during sample collection

Year	Day-month	Air temperature (°C)	RH (%)	Solar radiation (W m^{-2})	WS (m s^{-1})	Lot conditions
2008	21-Apr	3.9	38	502	2.2	Lots very wet with standing water in several lots along eastern edge, manure piles present
	28-Apr	20.4	20	728	2.8	
	19-May	25.5	31	728	4.7	All lots were cleaned out, and manure piles removed, lots were dry
	22-May	11.1	55	346	2.4	
	16-Jun	26.6	23	720	2.4	Lots were dry, some buildup of manure piles
	18-Jun	22.8	31	791	3.9	
	28-Jul	27.2	36	740	2.1	Lots were dry, manure piles were present, new soil added to lots on eastern side
	30-Jul	20.3	NA	NA	3.7	
	18-Aug	32.8	16	627	3.0	Lots were dry, some buildup of manure piles
	20-Aug	27.1	26	704	1.6	
	22-Sep	15.4	49	576	4.5	Lots were dry, some buildup of manure piles
	25-Sep	22.1	22	392	0.9	
	27-Oct	17.1	17	427	2.7	Lots were wet, and some areas very muddy, manure piles present
	29-Oct	13.9	41	298	1.4	
	17-Nov	9.2	52	194	2.9	Lots were wet, and some areas very muddy, manure piles present
	19-Nov	9.0	59	184	1.5	
	15-Dec	−7.8	76	243	5.3	Lots were frozen over, manure piles present
	17-Dec	−6.3	93	164	3.7	
2009	27-Jan	12.3	NA	NA	1.5	Lots were frozen over, manure piles present
	29-Jan	−0.2	74	363	3.8	
	23-Feb	4.9	90	251	3.1	Lots were mostly frozen, some thawing with standing water in places, manure piles present
	25-Feb	5.8	70	282	2.6	
	16-Mar	12.7	52	179	4.0	Lots were very wet, with standing water and mud in places, manure piles present
	19-Mar	8.8	58	426	2.9	

NA Not available, *RH* relative humidity, *WS* wind speed upwind and downwind sample sites are shown in Fig. 1. While every effort was made to collect the samples when precipitation events were not occurring, in only two instances, light rain events started once sampling had commenced.

The total airborne endotoxin concentrations at the upwind (i.e. background) and downwind sites are given in Table 2. As anticipated, the lowest endotoxin concentrations were found at the upwind site, where concentrations ranged from 0.8 to 140 EU m^{-3} (avg. = 28.5 EU m^{-3}). In studies of ambient endotoxin, Mueller-Anneling et al. (2004) and Heinrich et al. (2003) found that concentrations in Southern California and Germany, in particulate matter with an aerodynamic diameter of 10 µm (PM$_{10}$), were <5.5 EU m^{-3}. In Danish towns and upwind of industrial areas, inhalable (i.e. particles with aerodynamic diameter <100 µm) endotoxin concentrations were shown to be <10 EU m^{-3} throughout the year (Madsen 2006). While most of our upwind concentrations fall close to values reported by other researchers, it is possible that some of the elevated background concentrations are a result of assessing total airborne endotoxin instead of inhalable and respirable particles (discussion to follow). In addition, the dairy is located in an intensively managed agricultural region that contains numerous dairies (~750), cattle feedlots, and irrigated crop lands, which could be contributing to the elevated airborne endotoxin concentrations at the upwind site.

Table 2 Total airborne endotoxin concentrations at the open-lot dairy

Year	Day-month	EU m^{-3}		
		Upwind	Downwind 1	Downwind 2
2008	21-Apr	1.10 ± 0.38[a]	4.29 ± 0.65	1.59 ± 0.68
	28-Apr	0.93 ± 0.32	3.51 ± 0.44	1.94 ± 0.26
	19-May	2.35 ± 0.57	42.6 ± 9.67	27.9 ± 5.18
	22-May[b]	1.09 ± 0.38	13.4 ± 2.61	2.34 ± 1.08
	16-Jun	0.79 ± 0.17	2.64 ± 0.58	1.83 ± 0.32
	18-Jun	1.14 ± 0.14	3.01 ± 0.33	3.43 ± 0.81
	28-Jul	32.0 ± 15.9	55.0 ± 9.42	22.6 ± 4.03
	30-Jul	0.78 ± 0.17	72.3 ± 25.0	9.23 ± 2.03
	18-Aug	17.1 ± 3.78	199 ± 53.2	55.6 ± 20.4
	20-Aug	30.2 ± 12.7	382 ± 61.5	232 ± 67.2
	22-Sep	5.78 ± 1.11	472 ± 206	256 ± 67.8
	25-Sep	21.5 ± 6.39	79.4 ± 7.047	15.8 ± 1.88
	27-Oct	24.7 ± 9.31	96.8 ± 36.2	52.6 ± 16.3
	29-Oct	31.8 ± 10.6	94.3 ± 26.9	32.4 ± 6.94
	17-Nov	50.9 ± 7.61	215 ± 104	104 ± 15.7
	19-Nov	78.4 ± 22.4	138 ± 23.6	41.4 ± 11.3
	15-Dec	140 ± 23.1	849 ± 291	40.6 ± 9.93
	17-Dec	51.2 ± 5.68	165 ± 28.1	99.3 ± 45.3
2009	27-Jan	14.2 ± 3.08	254 ± 57.7	164 ± 60.8
	29-Jan	2.57 ± 1.25	64.6 ± 17.9	98.4 ± 21.7
	23-Feb[b]	1.30 ± 0.18	163 ± 64.2	21.3 ± 9.62
	25-Feb	1.32 ± 0.55	186 ± 47.9	71.7 ± 23.6
	16-Mar	88.4 ± 35.3	228 ± 34.2	261 ± 21.0
	19-Mar	83.4 ± 14.1	265 ± 37.0	102 ± 14.7

[a] Standard error of the mean ($n = 6$)

[b] Light rain event occurred during sampling

At the DW1 site (i.e. downwind edge of the open-lot), airborne endotoxin concentrations ranged from 2.6 to 849 EU m^{-3} (avg. = 169 EU m^{-3}). Compared to the upwind site, the concentrations at DW1 were less consistent and are likely related to the many variables that occur on a daily basis at the dairy (e.g. lot harrowing, equipment movement, and cow activity). In dairy barns, total airborne endotoxin concentrations were reported to be as high as 800 EU m^{-3} and up to 16-fold higher in pig and poultry houses (Zucker and Müller 1998; Bakutis et al. 2004). Schierl et al. (2007) reported maximum respirable and inhalable endotoxin concentrations of 61 and 66 EU m^{-3} in dairy barns, respectively. The results from Schierl et al. (2007) were also similar to those obtained by Seedorf et al. (1998), who investigated inhalable and respirable endotoxins in cow and cattle housing facilities. To our knowledge, aside from the work conducted by Purdy et al. (2004) on cattle feedlots (concentration range of 8.2–93 EU m^{-3}), we are unaware of any other studies that address endotoxin emissions from open-lot animal production facilities.

In this study, the highest concentration of 849 EU m^{-3} at DW1 occurred on a day when the average wind speed (i.e. 5.3 m s^{-1}) was the highest during the year-long study. Interestingly, the highest concentration at DW2 (i.e. 200 m from the edge of the lot) did not occur on the same day but occurred on March 16 when the wind speed was slightly lower at 4.0 m s^{-1} (Table 1). A correlation analysis of wind speed versus endotoxin concentration at DW1 ($r = 0.33$, $P < 0.0001$) and DW2 ($r = 0.17$, $P = 0.04$) confirmed that there was a significant effect of wind speed. At the upwind site, there was also a significant effect of wind speed on the airborne endotoxin concentration ($r = 0.21$, $P = 0.01$). With respect to air temperature, there was a negative correlation with concentration at the upwind ($r = -0.20$, $P = 0.02$) and DW1 ($r = -0.35$, $P < 0.0001$) sites. There was no correlation with relative humidity at the downwind sites ($P > 0.09$), but there was at the upwind site ($r = 0.26$, $P = 0.002$). With respect to solar radiation, there was a significant negative correlation with concentration at the upwind site ($r = -0.40$, $P < 0.0001$), but not at the downwind sites ($P > 0.21$). As solar radiation increases, one could speculate that UV radiation affects the integrity of the LPS molecule.

At DW2, the total airborne endotoxin concentrations ranged from 1.6 to 261 EU m^{-3} (avg. = 72 EU m^{-3}) and were generally lower than DW1 and higher than the upwind site. Figure 2 shows the average endotoxin concentration at each of the sampling sites over the year-long study. The average endotoxin concentration at DW2 was 2.4-fold lower than DW1 and approximately the same amount higher than the upwind site. An analysis of variance was performed on the data to determine the effect of site on airborne endotoxin concentration. The effect of site was significant ($P < 0.0001$) and followed the trend DW1 > DW2 > upwind. These data reinforce the fact that endotoxins are associated with particles (e.g. dust, water droplets) that settle out as distance from the lot increases. In an intensive study conducted by Dungan and Leytem (2009a), it was shown that the airborne endotoxin concentrations were 2- and 7-fold lower at 200 and 1,390 m from the edge of an open-lot dairy, respectively. At 1,390 m from the lot edge, the concentration was not significantly different from the upwind site.

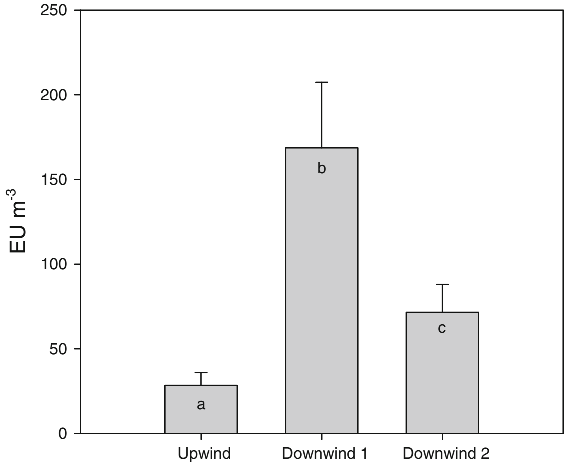

Fig. 2 Average total airborne endotoxin concentrations, over the course of a year, at the *upwind* and *downwind* sites at the open-lot dairy. *Errors bars* represent the standard error of the mean ($n = 24$). *Letters* above the *columns* indicate significant differences between the sites ($P < 0.0001$)

Figure 3 shows a comparison between the total and inhalable airborne endotoxin concentrations at all three sampling sites at the open-lot dairy in February and March. The open-face holders we utilized to collect total airborne endotoxin are not selective for particle size, while the button samplers screen particles with an aerodynamic diameter <100 µm. Based upon this fact, one might expect inhalable endotoxin concentrations to be lower than the total airborne concentrations. When the data were analyzed using the Mixed Procedure of SAS (SAS Institute 2008) with date as the repeated measure and collection method as the subject, there was no significant effect of collection method on the airborne endotoxin concentration ($P > 0.07$). However, at DW2 in February, there was a significant difference between the total and inhalable endotoxin concentrations ($P = 0.01$). At this site, the average total endotoxin concentration was 35 EU m^{-3}, while the average inhalable concentration was 10 EU m^{-3}. Our data indicate that the total endotoxin samples were likely comprised of particles with an aerodynamic diameter <100 µm. Although this observation may be specific to our study, it is important to consider endotoxin associated with inhalable and respirable particle sizes. Inhalable and respirable endotoxin particles are particularly hazardous when deposited in the respiratory tract and in the gas-exchange regions, as they are known to cause respiratory discomfort and disease (Jacobs 1989; Rylander 2006).

4 Conclusion

It is well established that airborne endotoxin concentrations as low as 50 EU m^{-3} can cause acute respiratory effects. In this study, total airborne endotoxin concentrations often exceeded this value

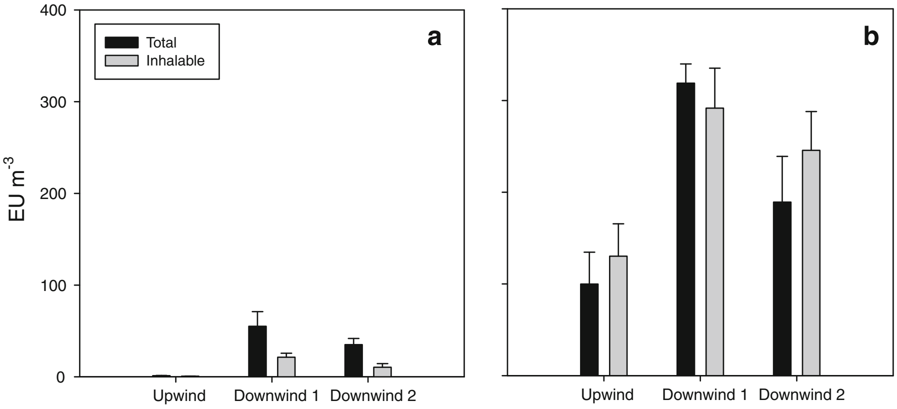

Fig. 3 Average total and inhalable airborne endotoxin concentrations at the *upwind* and *downwind* sites collected on (**a**) February 23 and 25 and (**b**) March 16 and 19. *Errors bars* represent the standard error of the mean ($n = 6$)

at the upwind and downwind sites throughout the year. However, at the upwind site, the average concentration was well below this value. The airborne endotoxin concentration was the greatest at the edge of the open-lot, as the wind moving across the lot picked up particles containing endotoxin. At the downwind edge of the lot, there was a significant and direct relationship between wind speed or air temperature and airborne endotoxin concentration, but not relative humidity and solar radiation. At 200-m downwind from the lot edge, the risk of exposure to endotoxin is decreased as suspended particulate matter settles out, resulting in lower airborne endotoxin concentrations. Overall, our data suggest that endotoxin exposure can be reduced as one increases their distance from the facility.

Acknowledgments The authors are grateful for the assistance from Sheryl Verwey, Susie Hansen, Myles Miller, Ann Pool, and Patsy Heinemann.

References

Bakutis, B., Monstviliene, E., & Januskeviciene, G. (2004). Analyses of airborne contamination with bacteria, endotoxins and dust in livestock barns and poultry houses. *Acta Veterinari Brno, 73*, 283–289.

Bradley, S. G. (1979). Cellular and molecular mechanisms of action of bacterial endotoxins. *Annual Review of Microbiology, 33*, 67–94.

Castellan, R. M., Olenchock, S. A., Kinsley, K. B., & Hankinson, J. L. (1987). Inhaled endotoxin and decreased spirometric values. *New England Journal of Medicine, 317*, 605–610.

Douwes, J., & Heederik, D. (1997). Epidemiologic investigations of endotoxins. *International Journal of Occupational and Environmental Health, 3*, S26–S31.

Dungan, R. S., & Leytem, A. B. (2009a). Airborne endotoxin concentrations at a large open lot dairy in southern Idaho. *Journal of Environmental Quality, 38*, 1919–1923.

Dungan, R. S., & Leytem, A. B. (2009b). The effect of extraction, storage, and analysis techniques on the measurement of airborne endotoxins from a large dairy. *Aerobiologia., 25*, 265–273.

Heederik, D., & Douwes, J. (1997). Towards and occupational exposure limit for endotoxins? *Annals of Agricultural and Environmental Medicine, 4*, 17–19.

Heederik, D., Sigsgaard, T., Thorne, P. S., Kline, J. N., Avery, R., Bønløkke, J. H., et al. (2007). Heath effects of airborne exposures from concentrated animal feeding operations. *Environmental Health Perspectives, 115*, 298–302.

Heinrich, J., Pitz, M., Bischof, W., Krug, N., & Borm, P. J. A. (2003). Endotoxin in fine (PM2.5) and coarse (PM2.5–10) particle mass of ambient aerosols. A temporo-spatial analysis. *Atmospheric Environment, 37*, 3659–3667.

Jacobs, R. R. (1989). Airborne endotoxins: An association with occupational lung disease. *Applied Industrial Hygiene, 4*, 50–56.

Lacey, J., & Dutkiewicz, J. (1994). Bioaerosols and occupational lung disease. *Journal of Aerosol Science, 25*, 1371–1404.

Madsen, A. M. (2006). Airborne endotoxin in different background environments and seasons. *Annals of Agricultural and Environmental Medicine, 13*, 81–86.

Mandryk, J., Alwis, K. U., & Hocking, A. D. (2000). Effects of personal exposures on pulmonary function and work-related symptoms among sawmill workers. *The Annal of Occupational Hygiene, 44*, 281–289.

Milton, D. K., Wypij, D., Kriebel, D., Walters, M. D., Hammond, S. K., & Evans, J. S. (1996). Endotoxin exposure-response in a fiberglass manufacturing facility. *American Journal of Industrial Medicine, 29*, 3–13.

Mueller-Anneling, L., Avol, E., Peters, J. M., & Thorne, P. S. (2004). Ambient endotoxin concentrations in PM_{10} from southern California. *Environmental Health Perspectives, 112*, 583–588.

Portengen, L., Preller, L., Tielen, M., Doekes, G., & Heederik, D. (2005). Endotoxin exposure and atopic sensitization in adult pig farmers. *Journal of Allergy and Clinical Immunology, 115*, 797–802.

Purdy, C. W., Straus, D. C., Parker, D. B., Wilson, S. C., & Clark, R. N. (2004). Comparison of the type and number of microorganisms and concentration of endotoxin in the air of feedyards in the southern high plains. *American Journal of Veterinary Research, 65*, 45–52.

Rule, A. M., Chapin, A. R., McCarthy, S. A., Gibson, K. E., Schwab, K. J., & Buckley, T. J. (2005). Assessment of an aerosol treatment to improve air quality in a swine concentrated animal feeding operation. *Environmental Science and Technology, 39*, 9649–9655.

Rylander, R. (2006). Endotoxin and occupational airway disease. *Current Opinion in Clinical Immunology, 6*, 62–66.

SAS Institute. (2008). *SAS/STAT user's guide. Release 9.2*. Cary, NC: SAS Institute.

Schierl, R., Heise, A., Egger, U., Schneider, F., Eichelser, R., Neser, S., et al. (2007). Endotoxin concentration in modern animal houses in southern Bavaria. *Annals of Agricultural and Environmental Medicine, 14*, 129–136.

Seedorf, J., Hartung, J., der Schrö, M., Linkert, K. H., Phillips, V. R., Holden, M. R., et al. (1998). Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. *Journal of Agricultural Engineering Research, 70*, 97–109.

Smid, T., Heederik, D., Houba, R., & Quanjer, P. H. (1992). Dust- and endotoxin-related respiratory effects in the animal feed industry. *American Review of Respiratory Disease, 146*, 1474–1479.

Spaan, S., Wouters, I. M., Oosting, I., Doekes, G., & Heederik, D. (2006). Exposure to inhalable dust and endotoxins in agricultural industries. *Journal of Environmental Monitoring, 8*, 63–72.

USDA, National Agricultural Statistics Service. (2008). [online]. Available at http://www.nass.usda.gov (verified on 2 Jun 2008).

Wilson, S. C., Morrow-Tesch, J., Straus, D. C., Cooley, J. D., Wong, W. C., Mitlöhner, F. M., et al. (2002). Airborne microbial flora in a cattle feedlot. *Applied and Environmental Microbiology, 68*, 3238–3242.

Zock, J.-P., Hollander, A., Heederik, D., & Douwes, J. (1998). Acute lung function changes and low endotoxin exposures in the potato processing industry. *American Journal of Industrial Medicine, 33*, 384–391.

Zucker, B. A., & Müller, W. (1998). Concentrations of airborne endotoxin in cow and calf stables. *Journal of Aerosol Science, 29*, 217–221.