Aerobiologia

International Journal of Aerobiology

🦄 Springer

Assessment of bioaerosols at a concentrated dairy operation

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Abstract Increased bioaerosol loadings in downwind plumes from concentrated animal feeding operations (CAFOs) may increase the risk for allergy and infection in humans. In this study, we monitored airborne concentrations of culturable bacteria and fungi at upwind (background) and downwind sites at a 10,000 milking cow dairy over the course of a year. The average bacterial concentrations at the upwind site were 8.4×10^3 colony forming units (CFU) m^{-3} and increased to 9.9×10^5 CFU m^{-3} at the downwind edge of the cattle lots, decreasing to 6.3×10^4 CFU m^{-3} 200 m farther downwind. At the same sites, the average fungal concentrations were 515, 945, and 1,010 CFU m^{-3}, respectively. Significant correlations between the ambient weather conditions and airborne fungal and bacterial concentrations were identified. Sequence analysis of PCR-amplified DNA from bacterial clones and fungal isolates revealed genus and species level differences between upwind and downwind sites. Although we could not cultivate gram-negative bacteria, bacterial clones at downwind sites identified as being gram-negative matched with the following genera: *Acinetobacter*, *Bradyrhizobium*, *Escherichia*, *Idiomarina*, *Methylobacterium*, *Ralstonia*, and *Novosphingobium*. Fungal isolates from downwind matched with the following genera: *Acremonium*, *Alternaria*, *Ascomycte*, *Aspergillus*, *Basidiomycete*, *Cladosporium*, *Davidiella*, *Doratomyces*, *Emericella*, *Lewia*, *Onygenales*, *Penicillium*, *Rhizopus*, and *Ulocladium*. None of the bacterial and fungal sequence matches were affiliated with genera and species known to be pathogenic to humans. Overall, the data suggest that exposure to bioaerosols in the downwind environment decreases with increasing distance from the open-lot dairy.

1 Introduction

As populations increase and land resources decline, the intensification and industrialization of animal production facilities is projected to increase globally. In the United States of America, concentrated animal feeding operations (CAFOs) have been growing at a rapid pace since the 1960s (Centner 2003). Along with this growth, there is increasing concern regarding the effects of CAFOs on the health of workers, residents in nearby communities, livestock, and the environment. High animal densities are associated with the buildup of the overall microbial load in the

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production environment by virtue of increased volumes of animals, feed, manure, and wastewater (Millner 2009). As a result, there is a concurrent increase in the bioaerosol (e.g., bacteria, fungi, virus, endotoxin) and dust load released from CAFOs (Sweeten et al. 1988; Wilson et al. 2002; Rule et al. 2005; Dungan and Leytem 2009a).

In Idaho, there are approximately 750 dairies and as many as 549,000 milking cows (USDA, National Agricultural Statistics Service 2008). Over the last decade, the milking cow population has increased by 88% and the trend is increasing toward larger concentrated facilities. While 46% of the dairy operations in Idaho contain <200 cows, some of the largest operations have as many as 10,000 animals. Due to the increased bacterial loading at concentrated dairy and other animal operations, there is a potential for offsite transport of bioaerosols and subsequent adverse health effects in humans and livestock (Wilson et al. 2002; Green et al. 2006; Schulze et al. 2006; Heederik et al. 2007). The inhalation of airborne microorganisms and their fragments can be detrimental to health through infection, toxicosis, or allergy (Crook and Sherwood-Higham 1997).

A large open-lot dairy in southern Idaho was targeted for this study. The specific objectives were to (1) measure the concentration of airborne microorganisms at upwind (background) and downwind sites using all-glass impingers and single-stage cascade impactors; (2) determine statistical differences between airborne microorganism concentrations at each sample site and identify correlations with ambient weather data; (3) use a high-volume cyclonic sampler to capture airborne bacteria, then prepare clones from isolated DNA using PCR-amplified regions of 16S ribosomal DNA (rDNA) for sequence analysis and identification; and (4) PCR amplify the internal transcribed spacer (ITS) region of fungal isolates for sequence analysis and identification.

2 Materials and methods

2.1 Open-lot dairy

The dairy used in this study was a privately owned commercial dairy in southern Idaho, in a rural location, with 10,000 milking cows and a stocking density of approximately 55 m^2 cow^{-1} (Fig. 1). This dairy is similar in configuration to most open-lot production facilities in southern Idaho. The operation consists of 24 open-lot pens, two milking parlors, hospital barn, maternity barn, manure solid separator, lagoon (liquid storage pond), and compost yard. There are approximately 13,000 cows on the facility

Fig. 1 Map of the open-lot dairy with sampling sites
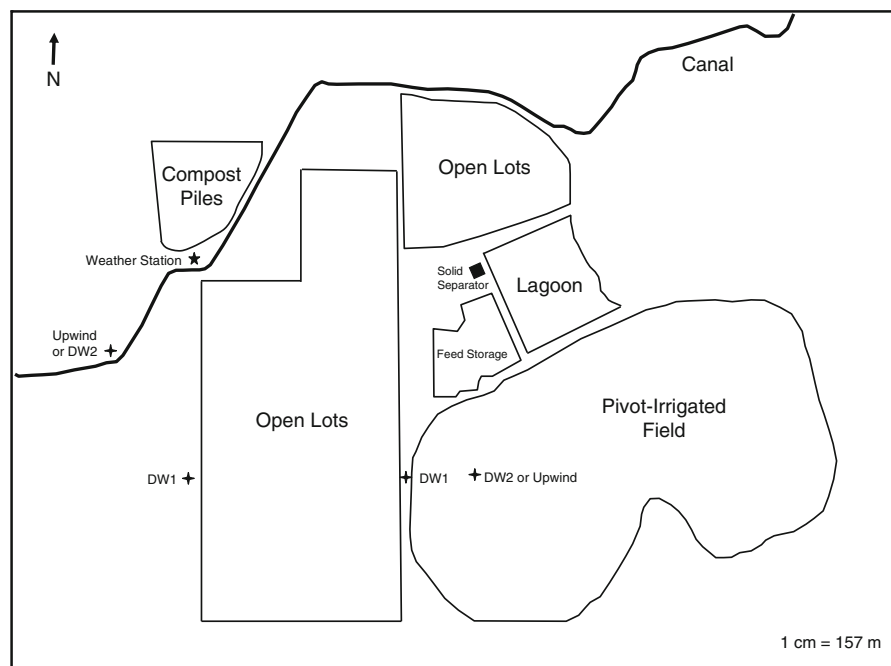
including milking cows, dry cows, and replacement heifers. Manure is scraped or vacuumed from feed alleys daily and placed into cells near the solid separator. The open-lot pens are harrowed daily when dry. The facility is surrounded by irrigated crop land on three sides and open range to the north. Three sites were used for sampling: an upwind site (approximately 200 m upwind from the open lots), 5 m downwind from the edge of lots (DW1), and 200 m downwind from the edge of the lots (DW2).

2.2 All-glass impingers

All-glass liquid impingers (SKC Inc., Eighty-Four, PA, USA) were utilized to capture airborne bacteria (i.e., heterotrophs, gram-negatives, *Escherichia coli*, and enterococci) and coliphage. Prior to their use, each impinger was autoclaved (30 min, 121°C, 1.23 atm) 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 on tripods at a height and spacing distance of 1.5 m. The flowrate through each of the impingers was set to 8.5 L min^{-1} using a Vac-U-Go sampling pump (SKC Inc.). While the manufacturer recommends a flowrate of 12.5 L min^{-1}, we conducted a field study to test the recovery of heterotrophic bacteria. Our results demonstrated that bacterial recoveries at 8.5 versus 12.5 L min^{-1} were, on average, 36% greater (unpublished data).

Bioaerosol samples were collected twice each day between the hours of 0800–1200 and 1300–1700. Three impingers were utilized at each sampling site and all samples were collected simultaneously. Samples were collected for 1 h and flowrate through the impingers was checked before and during operation with a rotameter. To reduce light penetration into the impingement solution, a plastic sleeve coated with aluminum foil was placed on the bottom portion of each impinger. After sampling, the collection vessels from the bottom of the impingers were aseptically stored and transported in a cooler with ice packs. Upon receipt at the laboratory, the samples were stored at 5°C for no longer than 18 h before being processed. The impingers were disinfected between sampling events by rinsing with 70% ethanol.

To account for any evaporation, the volume in each collection vessel was brought back up to 30 mL through additions of sterile impingement solution. The impingement solution was then serially diluted in phosphate-buffered saline and 0.1-mL aliquots of the dilutions were spread directly onto Petri plates containing tryptic soy agar (TSA) and MacConkey agar. Cyclohexamide at 50 mg L^{-1} was added to the TSA plates to inhibit the growth of fungi. The TSA and MacConkey agar were used for the enumeration of total heterotrophic and gram-negative bacteria, respectively. Through 47-mm, 0.45-µm pore-size membrane filters (Pall Corp., Ann Arbor, MI, USA), 100-mL aliquots of the dilutions were filtered, which were then placed onto MI agar and *m*Enterococcus plates. The MI agar (US Environmental Protection Agency 2002; method 1604) and *m*Enterococcus agar (Eaton et al. 2005, method 9230C) were used for the detection and enumeration of total coliforms and *E. coli* and fecal streptococci, respectively. The agar plates were incubated aerobically under the following conditions: TSA, 25°C for 5 days; MacConkey, 35°C for 5 days; MI, 35°C for 1 day; and *m*Enterococcus, 35°C for 2 days. Coliphage were enumerated using the pour plate method (9224B) as described by Eaton et al. (2005). To ensure quality control, blanks, trip blanks, and positive controls were utilized for all microbiological assays.

2.3 Single-stage cascade impactors

Airborne fungi were collected directly on potato dextrose agar (PDA) through the use of single-stage cascade impactors (BioStage 200, SKC Inc.). The cascade impactors are equipped with 200 holes (0.25 mm diameter), and a QuickTake 15 sampling pump (SKC Inc.) was used to pull vacuum at a flowrate of 14 L min^{-1}. The impactors were mounted 1.5 m off the ground at each of the sampling sites. Since only one impactor was available for each site, triplicate samples were collected in succession for a total of 2 or 5 min for each PDA plate. Fungal samples were collected twice each day, which occurred during the same times as the impinger samples were collected. The PDA plates were treated with 200 mg streptomycin sulfate L^{-1} to inhibit bacterial growth. After 5 days of growth at 25°C, fungal colonies were enumerated.

A microscopic examination of spores and hyphae and sequencing of the ITS region was used to identify fungal isolates. Fungal isolates from the PDA plates were cultured for up to 2 weeks in potato dextrose broth at room temperature with shaking (50 rev min^{-1}). The

isolates were then subsequently freeze dried and the DNA was extracted using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's recommendations. Polymerase chain reaction (PCR) of ITS region was performed using the ITS1, ITS4, and ITS5 primers (White et al. 1990; Gardes and Bruns 1993) at a final concentration of 0.3 μM. The PCR thermocycler conditions were 95°C for 10 min, then 30 cycles of 94°C for 30 s, 55°C for 40 s, and 72°C for 50 s, and single final extension at 72°C for 5 min. Products were visualized on 2.0% agarose gels by UV illumination and ethidium bromide staining.

2.4 High-volume sampler

Samples were collected at sites upwind and downwind of the lots, wastewater lagoon, and compost piles using a SASS 2300 (Smart Air Sampler System, Research International, Monroe, WA, USA). The SASS 2300 is a cyclonic system designed to collect particles with an aerodynamic diameter of 1–10 μm. Since only one SASS unit was available, samples at each site were collected at least one day apart. The system was operated at a flowrate of 325 L min^{-1} and sterile deionized (DI) water was used as the collection liquid as recommended by the manufacturer. The unit was placed approximately 1 m off the ground and operated for 8 h. Afterward, the concentrated liquid sample was transferred to a sterile 50-mL conical tube; the cyclone was subsequently rinsed twice with sterile DI (by temporarily operating the system for 1 min) and the rinsate was also added to the sterile tube. The SASS sample was transferred to the laboratory in a cooler and stored at −20°C until processed. To process the samples, they were initially thawed at room temperature and then passed through a 0.4-μm polycarbonate track-etch membrane. The membrane was added to a bead beating tube from an UltraClean soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), rapidly vortexed for 1 min, and then the membrane was aseptically removed using sterile forceps. The tubes were subjected to bead beating for 2 min and processed according to the manufacturer's protocol.

Nested PCR of the DNA was performed by first using the primer pair BA8F and UN1492R (Reysenback et al. 1994) with the following program: 95°C for 5 min, then 30 cycles of 94°C for 45 s, 46°C for 30 s, and 72°C for 1 min, and a single final extension

at 72°C for 5 min. Then, the V1–V3 region of the 16S rDNA was amplified with the primer pair 63F and BA518R (Muyzer et al. 1993; Marchesi et al. 1998) with the following program: 95°C for 5 min, then 30 cycles of 92°C for 1 min, 55°C for 30 s, and 72°C for 1 min, and a single final extension at 72°C for 5 min. The nested PCR product from primer pair 63F-BA518R was cloned into pGEM-T Easy Vector (Promega Corp., Madison, WI, USA). White colonies were randomly selected, grown overnight at 37°C in Luria–Bertani medium containing 0.1 g ampicillin L^{-1}. Plasmids from *E. coli* JM109 transformants were isolated using the UltraClean mini plasmid prep kit (MoBio Laboratories, Inc.).

2.5 Sequencing and identification

The plasmid inserts were amplified with the 63F primer and PCR product from the fungal isolates were sequenced by TACGen (Richmond, CA, USA) using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequence identification was performed using the BLAST database at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov). The 16S rDNA sequences and representative sequences from the BLAST database were aligned with the BioEdit Sequence Alignment Editor (Hall 1999), and the aligned sequences were classified using the Greengenes Web site (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi).

2.6 Statistical analysis

All data were tested for normality using the Shapiro–Wilk test with the PROC CAPABILITY procedure of SAS (SAS Institute 2004). Data that were not normally distributed was log transformed prior to analysis with untransformed numbers presented in the text. The data were analyzed using the Mixed Procedure of SAS with date as the repeated measure and site as the subject. Means separation was carried out using the difference of the least squares means with the Tukey–Kramer adjustment and α of 0.05. To determine the relationship between ambient weather data and airborne microorganism concentrations, Pearson correlation coefficients (r) were calculated. Statements of statistical significance were based upon $P < 0.05$ unless otherwise stated.

3 Results

The ambient weather conditions at the dairy are presented in Table 1. The weather data are averages from the periods during which bioaerosols were collected. The ambient temperature ranged from −6.2 to 30.2°C with an average of 14°C during the study. The relative humidity ranged from 18 to 90% with an average of 48%. Solar radiation was generally the highest in April–September and lowest in October–March with an average of 436 W m^{-2}. The wind speed ranged from 1.0 to 7.4 m s^{-1}, and the prevailing wind was from the west. Bioaerosols were only collected when the wind was predominantly from the west (avg. = 244°, SEM = 7.4°) or east (avg. = 115°, SEM = 13.6°). Bioaerosol sampling was not initiated during precipitation events; however, in two instances, light rain started once sampling had commenced. The upwind and downwind sampling sites at the open-lot dairy are shown in Fig. 1.

All-glass impinger samples were collected during all months, except December, January, and February,

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-April	6.4	29	712	2.8	Lots very wet with standing water in several lots along eastern edge, manure piles present
	28-April	17.1	25	616	3.5	
	19-May	24.2	34	702	4.2	All lots were cleaned out and manure piles removed, lots were dry
	22-May	10.7	57	344	2.4	
	16-June	24.8	27	695	2.2	Lots were dry, some buildup of manure piles
	18-June	20.9	38	679	3.2	
	28-July	24.5	43	652	2.1	Lots were dry, manure piles were present, new soil added to lots on eastern side
	30-July	20.3	26	na	3.7	
	18-August	30.2	18	611	3.5	Lots were dry, some buildup of manure piles
	20-August	25.0	27	587	1.0	
	22-September	13.3	60	458	4.1	Lots were dry, some buildup of manure piles
	25-September	19.6	28	288	1.0	
	27-October	16.0	19	302	2.1	Lots were wet and some areas very muddy, manure piles present
	29-October	11.2	47	279	1.6	
	17-November	10.5	48	228	2.7	Lots were wet and some areas very muddy, manure piles present
	19-November	5.3	69	175	2.7	
	15-December	−6.2	86	118	7.4	Lots were frozen over, manure piles present
2009	27-January	12.3	82	na	1.5	Lots were frozen over, manure piles present
	29-January	0.3	73	385	4.1	
	23-February	4.8	90	332	3.4	Lots were mostly frozen, some thawing with standing water in places, manure piles present
	25-February	5.6	71	250	2.4	
	16-March	12.0	55	136	3.8	Lots were very wet, with standing water and mud in places, manure piles present
	19-March	12.5	44	597	1.9	

na not available, *RH* relative humidity, *WS* wind speed

as the impingement solution was prone to freezing. Therefore, impinger samples were only collected on 17 of 24 possible days. No culturable gram-negatives and coliphage were recovered during the course of the study. While the samples were occasionally positive for *E. coli* and enterococci at the downwind edge of the lot, the concentrations were relatively low. *Escherichia coli* was recovered on 4 separate events at concentrations ranging from 8 to 67 CFU m^{-3}; enterococci was recovered on nine separate events at concentrations ranging from 2 to 21 CFU m^{-3} (data not shown). Airborne heterotroph concentrations were highly variable during the study, resulting in high standard errors at each measurement time (Table 2). At the upwind site, the heterotroph concentrations ranged from 9.1×10^2 to 3.7×10^4 CFU m^{-3}. At DW1, the heterotroph concentrations ranged from 1.5×10^4 to 1.4×10^7 CFU m^{-3} and were always greater than upwind concentrations. At DW2, the concentrations ranged from 4.4×10^3 to 5.3×10^5 CFU m^{-3} and were generally lower than DW1, but greater than upwind concentrations. Heterotroph concentrations tended to be the most consistent at the upwind site during July through October. An analysis of variance was performed on the data to determine the effect of sample site on the airborne heterotroph concentration. The average heterotroph concentrations were 8.4×10^3, 9.9×10^5,
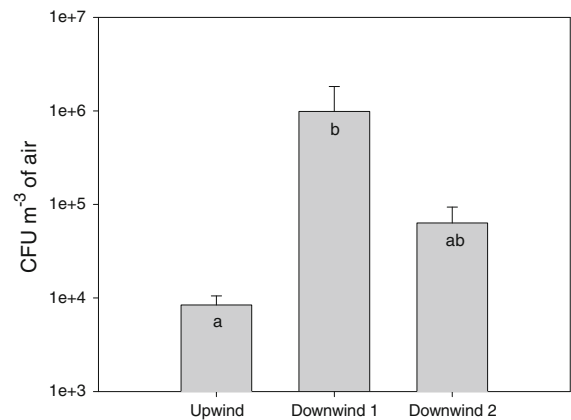

Fig. 2 Average airborne heterotroph concentrations measured at the upwind and downwind sites on the open-lot dairy. *Letters* within the columns indicate significant differences between the sample sites ($P < 0.05$). Downwind 1, 5 m from the edge of the lot; Downwind 2, 200 m from edge of lot

and 6.3×10^4 CFU m^{-3} for the upwind, DW1, and DW2 sites, respectively (Fig. 2). The effect of site was only significant between the upwind and DW1 sites ($P = 0.046$), but not between the two downwind sites or the upwind and DW2 site ($P > 0.08$). There were no statistically significant correlations between air temperature, relative humidity, or solar radiation and airborne heterotroph concentration at each sample site

Table 2 Average airborne concentrations of heterotrophs at the open-lot dairy

Year	Day-month	Upwind (CFU m^{-3})	Downwind 1 (CFU m^{-3})	Downwind 2 (CFU m^{-3})
2008	21-April	915 ± 621[a]	20232 ± 2350	6164 ± 3507
	28-April	5238 ± 3660	14593 ± 2824	6916 ± 1877
	19-May	9314 ± 5663	14349132 ± 14181077	124302 ± 93406
	16-June	9319 ± 2187	50629 ± 23314	13960 ± 3690
	18-June	37571 ± 19156	55963 ± 6943	19946 ± 4022
	28-July	1750 ± 700	100434 ± 37223	9115 ± 2029
	30-July	2184 ± 809	168856 ± 47350	54745 ± 9858
	18-August	16176 ± 8615	122475 ± 35954	29799 ± 7121
	20-August	3725 ± 661	24602 ± 7676	7534 ± 2336
	22-September	2190 ± 714	511118 ± 128192	529687 ± 314082
	25-September	5597 ± 1782	297077 ± 96683	118400 ± 17724
	27-October	8373 ± 3970	535564 ± 295209	44719 ± 11013
	29-October	9668 ± 3003	126224 ± 33178	25012 ± 6135
	17-November	11188 ± 5713	104021 ± 43227	32271 ± 4618
	19-November	4615 ± 2053	37573 ± 5599	19216 ± 7352
2009	16-March	3713 ± 1825	56111 ± 24479	30065 ± 10499
	19-March	11713 ± 4784	191806 ± 79870	4455 ± 1727

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

($P > 0.13$). However, there was a positive correlation between wind speed and heterotroph concentration at DW1 ($r = 0.23$, $P = 0.02$) and DW2 ($r = 0.30$, $P = 0.002$).

A more detailed analysis of the airborne bacterial community was conducted by collecting aerosol samples using a high-volume sampler. DNA extracts from the samples were subjected to PCR using

Table 3 Sequence matches of bacterial clones from upwind and downwind of various locations

Clone	Closest GenBank match	Source	% Match	Accession no.	Phylum
U-1	*Kocuria* sp. L5	Kartchner Caverns, Arizona	95	DQ192212.1	Actinobacteria
U-2	*Actinobacteria* clone DOK_DONFYM_clone811	Environmental sample	94	DQ829010.1	Actinobacteria
U-3	*Ralstonia pickettii* strain 149	Water Purifiers	99	EU730922.1	Proteobacteria
U-4	Uncultured *Microvirga* sp. clone AUVE_01B06	Cropland	99	EF650876.1	Proteobacteria
U-5	*Delftia* sp. CAT3-4-2	Wastewater treatment pond	99	FJ594443.1	Proteobacteria
U-6	*Nocardioidaceae bacterium* Ellin5426	Soil	98	AY673153.1	Actinobacteria
U-7	*Bradyrhizobium japonicum*	Ice core sample	96	AY169423.1	Proteobacteria
U-8	*Pelomonas aquatica* strain CCUG52631	Industrial water	98	AM501437.1	Proteobacteria
U-9	*Delftia* sp. CAT3-4-2	Wastewater treatment pond	99	FJ594443.1	Proteobacteria
U-10	*Roseomonas aquatica* type strain TR53T	Drinking water	97	AM231587.1	Proteobacteria
CL-1	*Ruminococcaceae bacterium* clone EMP_N10	Fecal sample	98	EU794265.1	Firmicutes
CL-2	*Bacteroidales bacterium* clone Cow183	Cow feces	99	AY859656.1	Bacteroidetes
CL-3	*Firmicutes bacterium* clone B11	Raw cow milk	99	EU029233.1	Firmicutes
CL-4	*Bacteroidetes bacterium* clone 912-C9		86	EU315425.1	Bacteroidetes
CL-5	*Firmicutes bacterium* clone B11	Raw cow milk	99	EU029233.1	Firmicutes
CL-6	*Acinetobacter* sp. TS11	Arsenic-contaminated environment	97	EU073077.1	Proteobacteria
CL-7	*Corynebacterium* sp. BBDP21	Swine effluent holding pit	94	DQ337522.1	Actinobacteria
CL-8	*Escherichia* sp. 4094	Swine	100	FJ405352.1	Proteobacteria
CL-9	Clostridales Family XIII bacterium clone EMP_F2	Fecal sample	94	EU794167.1	Firmicutes
L-1	*Atopostipes suicloacalis* strain PPC79	Swine feces	95	AF445248.2	Firmicutes
L-2	*Bradyrhizobium* sp. SEMIA 929	Ornithopus sativus	100	FJ390938.1	Proteobacteria
L-3	*Methylobacterium* sp. clone 1P-1-I17	Environmental	95	EU704833.1	Proteobacteria
L-4	*Cyanobacterium* clone 3	Environmental	97	FJ024312.1	Cyanobacteria
L-5	*Clostridium disporicum* strain NML 05A027	Clinical isolate	98	DQ855943.1	Firmicutes
L-6	*Idiomarina* sp. C4		97	EF554872.1	Proteobacteria
L-7	*Oscillatoria* sp. clone AV_4R-S-A21	Commercial aircraft cabin air	96	EU341185.1	Cyanobacteria
L-8	*Corynebacterium efficiens* YS-314		95	BA000035.2	Actinobacteria
L-9	*Clostridium* sp. clone MS159A1_E02	Human gastrointestinal	99	EF705259.1	Firmicutes
C-1	*Planococcus* sp. YIM C832	Haloalkaline soil	98	EU135681.1	Firmicutes
C-2	*Firimicutes bacterium* clone QEDP3DD01	Mesophilic anaerobic digester	99	CU924228.1	Firmicutes
C-3	*Ralstonia pickettii* strain 149	Water Purifiers	100	EU730922.1	Proteobacteria
C-4	*Flavobacteriaceae bacterium* YIM C421	Haloalkaline soil	93	EU135615.1	Bacteroidetes
C-5	*Clostridium lituseburense* strain H17	Anaerobic digester	99	EU887828.1	Firmicutes
C-6	*Clostridium* sp. clone M139	Raw cow milk	94	EU029303.1	Firmicutes
C-7	*Novosphingobium* sp. YC6720		98	EU707558.1	Proteobacteria
C-8	*Bacteriodales bacterium* clone CG2	Cow feces	93	EU573809.1	Bacteroidetes

U upwind, *CL* downwind of cattle lots, *L* downwind of lagoon, *C* downwind of compost piles

universal bacterial primers to amplify a section of 16S rDNA approximately 450 bp in length. The PCR products were used to produce representative clones that were subsequently used for sequence analysis and identification (Table 3). Data are presented for a total of 36 clones which were 86–100% homologous with known bacterial sequences. However, only 24 of 36 isolates could be identified to the genus level, with the remaining identified at the family level and higher. The clones from the upwind site and downwind of the cattle lots, lagoon, and compost piles were affiliated with the following phyla: *Actinobacteria*, *Bacteroidetes* (4 clones), *Firmicutes* (11 clones), and *Proteobacteria* (14 clones from the α-, β-, and γ-subdivision). Two bacterial clones were also identified as belonging to *Cyanobacteria*, but only downwind of the lagoon. At the upwind site, bacterial clones were affiliated with bacteria mostly from environmental origins and belonging to the following genera: *Bradyrhizobium*, *Delftia*, *Kocuria*, *Microvirga*, *Pelomonas*, *Rolstonia*, and *Roseomonas*. At the downwind sites, bacterial

clones were identified with the following genera: *Acinetobacter*, *Clostridium*, *Corynebacterium*, *Escherichia*, *Methylobacterium*, *Oscillatoria*, *Ralstonia*, and *Novosphingobium*. Of the 26 downwind clones, 10 matched sequences from isolates whose source was feces (cow, swine) and raw cow milk.

Total airborne fungal concentrations are presented in Table 4. At the upwind site, the fungal concentrations ranged from 36 to 1,289 CFU m^{-3}. At DW1 and DW2, the respective concentrations ranged from 181 to 2,314 CFU m^{-3} and 192 to 5,274 CFU m^{-3}. The average concentrations at the upwind, DW1, and DW2 sites were 515, 945, and 1,010 CFU m^{-3}, respectively (Fig. 3). To determine the effect of site on the airborne fungal concentrations, an analysis of variance was performed. The effect of site was determined to be significant between the upwind and downwind sites ($P < 0.0001$), but not between the two downwind sites ($P = 0.73$). At the upwind site, a correlation analysis between the airborne fungal concentration and ambient weather data confirmed that there was a significant

Table 4 Average airborne concentrations of fungi at the open-lot dairy

Year	Day-month	Upwind (CFU m^{-3})	Downwind 1 (CFU m^{-3})	Downwind 2 (CFU m^{-3})
2008	21-April	272 ± 78[a]	1229 ± 258	1148 ± 308
	28-April	156 ± 47	181 ± 66	192 ± 50
	19-May	240 ± 62	379 ± 69	629 ± 69
	22-May[b]	143 ± 48	140 ± 26	751 ± 327
	16-June	584 ± 145	408 ± 94	560 ± 115
	18-June	987 ± 303	521 ± 80	346 ± 52
	28-July	1289 ± 161	499 ± 95	864 ± 155
	30-July	927 ± 149	490 ± 86	573 ± 88
	18-August	949 ± 73	447 ± 42	501 ± 87
	20-August	517 ± 109	505 ± 77	312 ± 59
	22-September	836 ± 192	885 ± 215	511 ± 69
	25-September	871 ± 228	1458 ± 352	1710 ± 364
	27-October	436 ± 57	649 ± 89	1173 ± 74
	29-October	813 ± 59	1182 ± 159	854 ± 67
	17-November	352 ± 45	886 ± 120	1048 ± 303
	19-November	369 ± 57	1523 ± 190	749 ± 110
	15-December	679 ± 174	1576 ± 403	943 ± 128
2009	27-January	36 ± 0	1524 ± 180	5274 ± 1935
	29-January	37 ± 8	432 ± 89	310 ± 127
	23-February[b]	206 ± 31	2314 ± 649	1193 ± 287
	25-February	206 ± 49	2295 ± 250	1858 ± 374
	16-March	777 ± 95	1703 ± 183	904 ± 169
	19-March	157 ± 24	516 ± 115	829 ± 145

[a] Standard error of the mean ($n = 12$)

[b] Light rain event occurred during sampling
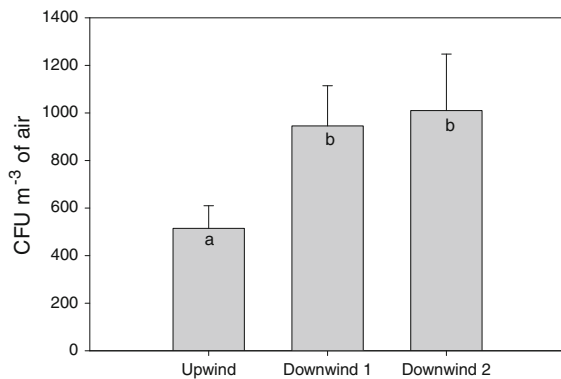
Author's personal copy

Fig. 3 Average airborne fungal concentrations measured at the upwind and downwind sites on the open-lot dairy. *Letters* within the columns indicate significant differences between the sample sites ($P < 0.05$). Downwind 1, 5 m from the edge of the lot; Downwind 2,200 m from edge of lot

effect of air temperature ($r = 0.23$, $P = 0.008$). At the DW1 site, there was a significant effect of air temperature ($r = -0.42$, $P < 0.0001$), relative humidity ($r = 0.47$, $P < 0.0001$), and solar radiation ($r = -0.45$, $P < 0.0001$), while at DW2 there was a significant effect of air temperature ($r = -0.20$, $P = 0.03$), relative humidity ($r = 0.31$, $P = 0.0005$), and solar radiation ($r = -0.32$, $P = 0.004$). Interestingly, at DW1, there was a slight negative correlation between wind speed and fungal concentration ($r = -0.23$, $P = 0.009$), which is in contrast to the positive correlation seen between wind speed and airborne heterotroph concentration.

To identify the predominant filamentous fungi present at the dairy, unique isolates were obtained from the impaction plates after each sampling event. A total of 13 isolates from upwind and 18 isolates from the downwind edge of the lots were obtained. The ITS region of the fungal isolates was PCR-amplified and the products were subjected to sequence analysis. The isolates were 78–100% homologous with known fungal sequences; all but one isolate could not be identified to the genus level (i.e., clone CL-7). Upwind isolates were identified as being from the following genera: *Actinomucor*, *Aspergillus*, *Aureobasidium*, *Epicoccus*, *Fusarium*, *Neosartorya*, *Penicillium*, *Phoma*, *Radulidium*, *Talaromyces*, and *Ulocladium* (Table 5). Downwind from the lots, the following genera were identified: *Acremonium*, *Alternaria*, *Ascomycte*, *Aspergillus*, *Basidiomycete*, *Cladosporium*, *Davidiella*,

Doratomyces, *Emericella*, *Lewia*, *Onygenales*, *Penicillium*, *Rhizopus*, and *Ulocladium* (Table 5).

4 Discussion

Bioaerosols are introduced into the atmosphere from various agricultural, municipal, industrial, and natural sources (Blanchard and Syzdek 1970; Brandi et al. 2000; Wilson et al. 2002; Brooks et al. 2005; Fracchia et al. 2006; Green et al. 2006; Spaan et al. 2006; Millner 2009). Airborne microorganisms or their components are typically associated with particulate matter or surrounded by a thin layer of water and have an aerodynamic diameter range of 0.5–100 μm (Lighthart 1994; Cox 1995a). Aerosol particles 1–5 μm are of most concern as they are readily inhaled or swallowed (Stetzenbach 2007). When bioaerosols are released from a source, they are transported short and long distances in air currents and are eventually deposited on surfaces (Brown and Hovmoller 2002; Jones and Harrison 2004). The transport and deposition of bioaerosols is affected by their physical properties and environmental conditions they encounter while airborne. Meteorological conditions that significantly affect the transport of bioaerosols are wind velocity, thermal gradients, and magnitude of air currents (Cox 1995a; Mohr 2007; Stetzenbach 2007). Important physical properties are size, density, and shape of the particles, while deposition of bioaerosols occurs through gravitational settling, wind impaction onto surfaces, adhesion, and other mechanisms (Wickman 1994). Factors that affect the viability of airborne microorganisms have been extensively studied; these include temperature, relative humidity, and solar radiation (Poon 1966; Marthi et al. 1990; Cox 1995b; Mohr 2007). In general, studies have shown decreased viability with decreases in relative humidity and increases in temperature and solar radiation. Oxygen concentration is also known to affect microbial survival, as it is involved in the inactivation of bioaerosols through the production of oxygen free radicals. While the above-mentioned atmospheric conditions influence viability, microbial factors such as the type, species, and strain of an organism also affect its airborne survival (Songer 1967; Ehrlich et al. 1970).

Compared to the upwind site, the airborne concentration of heterotrophic bacteria were statistically

Table 5 Sequence matches of fungal isolates from upwind and downwind of the cattle lots

Clone	Closest GenBank match	Source	% Match	Accession no.
U-1	*Epicoccum* sp. G7A	*Alliaria petiolata* (garlic mustard)	91	EF432273.1
U-2	*Phoma medicaginis* var. *medicaginis*	*Medicago sativa* (alfalfa)	99	DQ109960.1
U-3	*Fusarium tricinctum*	*Picea mariana* (black spruce)	99	DQ132835.1
U-4	*Fusarium equiseti*	Sorghum	98	EU595566.1
U-5	*Penicillium vinaceum* strain P11.5	Vampire bat guano	98	EU833227.1
U-6	*Penicillium commune* strain P4.2	Cave soil	75	EU833216.1
U-7	*Ulocladium* sp.	*Centaurea stoebe* (endophyte)	99	EF589899.1
U-8	*Neosartorya fischeri* isolate wb162	Nasal mucus	88	AF455541.1
U-9	*Aspergillus niger* strain MPVCT 344	Vineyard	99	EU440778.1
U-10	*Actinomucor elegans*	Potato pulp	99	AB113008.1
U-11	*Talaromyces flavus* isolate hn-50-1	Insect-associated isolate	99	EU287814.1
U-12	*Radulidium subulatum* strain CBS 287.84	Incubator for cell cultures	98	EU041789.1
U-13	*Aureobasidium pullulans* strain SUB 04-313		97	FJ744598.1
CL-1	*Rhizopus stolonifer* strain ATCC 6227b	ATCC isolate	78	AF543526.1
CL-2	*Aspergillus fumigatus* strain F3	Rhizospheric soil	99	FJ214371.1
CL-3	*Aspergillus niger* strain CH-A2010	Tequila industry waste	99	FJ668837.1
CL-4	*Lewia infectoria* isolate T3549	*Medicago lupulina* (black medick)	99	EF104194.1
CL-5	*Basidiomycete* sp. nasa47	Atacama desert, Chile	97	DQ683975.1
CL-6	*Arthopyreniaceae* IBL 03137	Coffea arabica	99	DQ682563.1
CL-7	*Cladosporium cladosporioides*	*Phragmites australis* (common reed)	98	AJ300334.1
CL-8	*Doratomyces* sp. HZ-10	Forest soil	99	EU301641.1
CL-9	*Alternaria* sp. FTJZZJ02	*Fritillaria thunbergii* Miq.	99	FJ196610.1
CL-10	*Penicillium soppii* strain IBT 19343		100	AM901674.1
CL-11	*Emericella rugulosa* strain SRRC 1173		99	EU289916.1
CL-12	*Onygenales* sp. BC8	Ross Sea region of Antarctica	99	DQ317338.1
CL-13	*Davidiella macrospore* strain CBS 107.20	Iris species	99	EF679369.1
CL-14	*Acremonium exuviarum* strain BMB4	Indoor isolate	100	AY882946.1
CL-15	*Penicillium janthinellum* strain P11.16	Vampire bat guano	98	EU833221.1
CL-16	*Ascomycete* sp. BC20	Ross Sea region of Antarctica	100	DQ317353.1
CL-17	*Ulocladium* sp.	Ross Sea region of Antarctica	99	DQ317352.1
CL-18	*Penicillium roqueforti* strain FSU 503		99	EU484267.1

U upwind, *CL* downwind of cattle lots

higher at the downwind edge of the lot (Fig. 2). The fact that the concentrations were higher at the downwind site supports the premise that animal confinement tends to increase the overall microbial load. As wind moves across the open lots, it aerosolizes particulate matter and fungal spores, leading to increases in the bioaerosol concentration in the downwind plume. While wind is a dominant factor, cow activity and lot harrowing are other mechanisms by which particulate matter are aerosolized in the lots. As the downwind distance from the source increases, one can expect bioaerosol concentrations to decrease as gravitational settling of particles occurs and other depositional mechanisms take place. Gravitational settling and impaction is an important transport mechanism by which suspended particles >5 μm in diameter are removed from the air (Nicholson 1995). For small particles (<0.1 μm in diameter), Brownian diffusion is the dominant transport mechanism, which increases with increasing temperature and decreasing particle size.

At 200 m from the edge of the lot (i.e., DW2), the heterotroph concentration was 16-fold lower than at 5 m from the lot edge (i.e., DW1), although not a

significant difference (Fig. 2). The lower concentration at DW2 could be a result of particles settling out, or a combination of other factors, such as loss of viability and/or dilution. In a study conducted by Green et al. (2006), total culturable bacteria were monitored up to 150 m downwind from a swine confinement operation. Inside the swine facility, the average airborne bacterial concentration was 1.8×10^3 CFU m^{-3}. The airborne bacterial concentration was shown to steadily decrease with distance from the swine operation, with concentrations at 150 m downwind being only 2.5-fold higher than at an upwind site at 82 CFU m^{-3}. In our study, the airborne heterotroph concentration was 8-fold higher at DW2 than the upwind site, but it was determined that these were statistically similar concentrations. Research on the land application of biosolids or at composting facilities has shown that bioaerosol concentrations decrease with increasing distance from the source (Brooks et al. 2004; Taha et al. 2005). Similarly, decreases in bioaerosol concentrations with increasing distance from wastewater treatment plants and land application of wastewater have been demonstrated (Brooks et al. 2004; Millner 2009). However, in the latter case, the microorganisms are associated with fine water droplets, but in our study they are likely bound to solid particles which certainly influence their viability. Desiccation of droplets is the main factor responsible for inactivation of bioaerosols in liquids (Mohr 2007).

A variety of airborne gram-negative bacteria, belonging to the families *Enterobacteriaceae*, *Pseudomonadaceae*, and *Neisseriaceae*, have been recovered in calf and other animal houses (Zucker et al. 2000; Millner 2009). In cow and calf stable, ≤6.5% of the viable airborne aerobic bacteria were identified as being gram-negative (Zucker and Müller 1998). A similar percentage of the airborne bacterial population was also determined to be gram-negative in swine houses (Chang et al. 2001; Kiekhaefer et al. 1995). While we did recover *E. coli* at relatively low concentrations at the downwind edge of the lot, we were unsuccessful in cultivating gram-negatives on selective agar. It is possible that the airborne gram-negative populations were not highly concentrated and, in combination with a 1 h sampling time with the all-glass impinger, we did not recover a high enough concentration for detection. Longer sampling times may also have been required, but due to

evaporation of the impingement solution this is often impossible, unless a non-evaporating fluid like mineral oil is used (Lin et al. 1999). In addition, it has been shown that bacteria can lose their ability to grow and form colonies on culturable plates due to physical stress of the sampling technique (Terzieva et al. 1996).

While PCR allows for the detection and identification of non-culturable airborne microorganisms, it does not allow one to distinguish between non-viable and viable microorganisms (Dungan and Leytem 2009b). Clones prepared from samples taken downwind from the cattle lots, lagoon, and compost pile were identified as being gram-negative and matched with the following genera: *Acinetobacter*, *Bradyrhizobium*, *Escherichia*, *Idiomarina*, *Methylobacterium*, *Ralstonia*, and *Novosphingobium* (Table 3). All of these genera were also identified in the upwind clones, except *Escherichia*, *Idiomarina*, and *Novosphingobium*. Downwind clones generally affiliated with gram-positive bacteria were identified as belonging to Firmicutes and Actinobacteria (Table 3). Immediately downwind from cattle feedlots, Wilson et al. (2002) found only non-pathogenic gram-positive bacteria such as *Bacillus*, *Chrysobacterium*, *Corynebacterium*, *Helococcus*, *Micrococcus*, and *Paenibacillus*. Since the majority of the clones in this study matched with sequences at the genus level or higher (not to species or strain level), knowledge of the pathogenicity of the airborne microorganisms could not be ascertained. In addition, due to limitations with DNA extraction and PCR amplification using universal primers, it should be noted that our survey is not comprehensive or even representative of all airborne bacterial genera and species. While many pathogenic strains of bacteria (e.g., *E. coli* and *Salmonella* spp.) are generally not considered a direct inhalation hazard, they are a potential infectious hazard when deposited in the throat and upper airway and swallowed (Pillai and Ricke 2002).

With respect to airborne coliphage as an indicator organism of viral contamination, the lack of positive recovery at the downwind sites could mean that the airborne concentrations were either too low and/or survivability of the coliphage in the aerosolized state is poor. In a study conducted by Carducci et al. (1999) at a sewage treatment plant, the airborne coliphage concentration decreased by 99% at 50 m, whereas reovirus and enterovirus only decreased by

15% at the same distance, suggesting that coliphage are not a good indicator of viral contamination. Results from the Carducci et al. (1999) study also found a significant correlation with total bacteria and fecal streptococci and aerosolized virus, possibly being more useful as an indicator of viral contamination than coliphage counts. The fact that we did not recover aerosolized coliphage in the downwind sites does not mean that virus, pathogenic or non-pathogenic, are not present. If total airborne bacterial counts could be used as an indicator of viral contamination, then one can speculate that the downwind samples contained virus as a result of elevated concentrations of airborne heterotrophs.

As with the heterotrophic bacteria, airborne fungal concentrations increased significantly at the downwind edge of the lot; however, the concentration did not decrease at 200 m downwind (Fig. 3). Compared to the upwind site with an average concentration of 515 CFU m^{-3}, at DW1 and DW2, the concentrations were on average 1.8- and 2-fold higher, respectively. Regardless of lot conditions (Table 1), the airborne concentrations were highly variable during the course of the study (Table 4), despite the fact that lots were frozen and/or covered with snow in December, January, and February. Snow has been shown to reduce airborne concentrations by removing spores from the air and by covering ground sources (Li and Kendrick 1995). Fungal spores can be aerosolized through natural ejection mechanisms, but wind is often necessary for aerosolization and carrying the spores downwind (Jones and Harrison 2004). Rain is also important, as spore concentrations are higher after a rain event, as it helps release spores that resist dislodgment by wind. Additionally, air temperature, relative humidity, solar radiation, and wind speed have been shown to be positively and negatively correlated with airborne concentrations of various species of fungi (Jones and Harrison 2004). In our study, the total airborne fungal concentrations were also positively and negatively correlated with the ambient weather data, but not consistently at all sites. For example, air temperature at the upwind site was positively correlated with the airborne fungal concentrations, while at DW1 and DW2, it was negatively correlated. This is likely related to the source of the fungal spores between the two sites, differences in vegetative cover (i.e., surrounding land is irrigated crop land and lots are bare soil), and/or the

fact that correlations were based on average ambient weather data for the sampling period and did not consider diurnal effects.

In a study of airborne fungi in an indoor dairy cattle shed, fungal species from the following genera were recovered: *Absidia*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Mucor*, *Rhizopus*, and *Syncephalastrum* (Adhikari et al. 2004). The concentration of culturable fungi at an outdoor station averaged about 500 CFU m^{-3} and in the cattle sheds it was reported to range from 165 to 2,225 m^{-3}, which is markedly similar to results obtained in our study at the upwind and downwind sites. While we did not determine the percent contribution of each fungal genera to the total culturable numbers, Adhikari et al. (2004) reported that more than 50% of the total fungal spores were contributed by *Aspergillus*, *Cladosporium*, *Nigrospora*, and *Penicillium*. In open-air swine houses, *Cladosporium* represented more than 90% of identified fungi (Chang et al. 2001). Other airborne fungal genera identified by Chang et al. (2001) were as follows: *Alternaria, Aspergillus (A. niger* and *flavus)*, *Aureobasidium*, *Botrytis*, *Candida*, *Curvularia*, *Drechslera*, *Fusarium, Geotrichum*, *Monilia*, *Oidium*, *Paecilomyces*, *Penicillium*, *Sclerotium*, *Stemphylium*, *Trichoderma*, and *Ulocladium*. While some species within the genera identified in our study (Table 5) are known plant pathogens and can cause disease in immuno-compromised individuals, none of the fungal isolates are considered to be highly pathogenic to otherwise healthy humans (St-Germain and Summerbell 1996). However, it should be made clear that our fungal survey was not comprehensive or even representative of all airborne fungal genera and species.

5 Conclusions

The airborne heterotrophic bacterial concentrations were shown to fluctuate throughout the year-long study, with no correlation to ambient weather conditions. While total concentrations were higher immediately at the downwind edge of the lot, concentrations declined 200 m further downwind. A decrease in the bioaerosol concentration with distance from the source is a trend commonly seen in other similar studies. Even though the heterotrophic bacteria were not necessarily pathogenic microorganisms, this trend of decreasing concentration with distance from the source

is indicative of the transport behavior of pathogenic and non-pathogenic bacteria alike. Based upon the survey of a small number of bacterial clones from downwind sites, none of the sequence matches appeared to be affiliated with genera that contain species known to be pathogenic to humans. Airborne fungal concentrations at the open-lot dairy were also shown to increase at the edge of the lot, but stayed elevated at 200 m downwind. This is likely due to the fact that fungal spores are not associated with particulate matter, therefore can be carried farther downwind as a result of differing physical properties from particulate-bound bioaerosols. While a comprehensive survey of airborne fungi was not conducted, none of the fungal species identified in this study are known human pathogens, except in cases where patients may be immuno-compromised. Based upon our results, it is evident that decreasing ones proximity to the downwind perimeter of a CAFO, such as an open-lot dairy, will decrease exposure to airborne microorganisms. Reduced exposure could certainly translate into decreased potential for infection if pathogenic microbes are present at elevated concentrations. However, since epidemiological studies were not simultaneously conducted, the actual risk of infection at each downwind site cannot be determined from our results.

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