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DETERMINANTS OF CULTURABLE BIOAEROSOL CONCENTRATIONS IN DAIRY BARNS

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Abstract: The concentration of bioaerosols to which dairy farmers are exposed is potentially related to environmental factors, such as climatic conditions and individual management practices. An unprecedented heavy rainfall that was 250% of normal during the growing season of feed and bedding materials provided an unique opportunity for study. Individual dairy management practices differ as to barn construction, type of ventilation system, storage moisture of feed rations, quality of bedding materials, and animal density. The aim of this study was to identify the environmental factors affecting the concentrations of culturable bioaerosols in dairy barns. In this cross-sectional study of 48 dairy barns, area samples were collected using all-glass impingers. Culturable bioaerosols were analyzed to determine airborne concentrations of yeasts, molds, mesophilic bacteria, and thermophilic bacteria. The time-weighted geometric mean concentrations of these bioaerosols collected over the work-shift were 1.8×10^4 cfu/m^3 for yeasts, 0.8×10^4 cfu/m^3 for molds, 81.1×10^4 cfu/m^3 for mesophilic bacteria, and 0.4×10^4 cfu/m^3 for thermophilic bacteria. These concentrations ranged from two to three orders of magnitude among the different barns. Bioaerosol concentrations did not differ between barns that used feed and bedding grown during extremely high rainfall and barns that used feed and bedding grown during normal rainfall. Multiple regression analyses were used to describe which environmental factors exhibited the strongest correlation with the concentration of bioaerosols. From these analyses, we conclude that efforts to reduce exposure to bioaerosols in dairy barns should focus on ventilation and storage moisture of feed rations.

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INTRODUCTION

High concentrations of bioaerosols are present in dairy barns [25]. The environment within a dairy barn provides many nourishing substrates for the growth of fungi, bacteria, and arthropods. These substrates include cattle epithelium, manure, feed rations, and bedding materials. Microorganisms colonizing these substrates may become airborne during dairy management activities of feeding, bedding, milking, and cleaning. The inhalation exposure to these bioaerosols may put farmers at risk for respiratory diseases such as: organic dust toxic syndrome, hypersensitivity pneumonitis, asthma, and bronchitis [13].

The concentration of bioaerosol exposure in an agricultural building may be related to characteristics of individual farm management practices [1]. Individual

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dairy management practices differ as to barn construction, type of ventilation system, storage moisture of feed rations, quality of bedding materials, and animal density. Dairy farmers spend a majority of their time at work in the structure where cows are milked. There are two different barn set-ups in the Midwest United States, stanchion barns and milking parlors. Stanchion barns are the traditional and most common type. These barns have a separate stall for each cow to which the cow is tethered to a post for 6 to 24 hours a day. Hence, many of the activities associated with dairying, such as milking, feeding, bedding, and manure removal are completed in the stanchion barn. The farmers are present in the barn during all these activities. Many large-scale farmers have chosen to utilize milking parlors instead of stanchion barns. Milking parlors are specialized rooms that hold from 6 to 10 cows, for simultaneous milking. Cows are in the milking parlors only when they are milked, as their housing and feeding is in an adjacent building or feedlot. Activities such as feeding and manure removal are often automated and the farmer is not directly exposed to these activities. A unique exposure in the milking parlors is the frequent use of a pressurized water hose to keep the parlor clean during milking.

Ventilation is essential to maintain health and production of a dairy herd. Proper ventilation is needed to remove moisture and manure gases year-round, and excess animal heat during warm weather. Additionally, ventilation may also reduce the concentration of bioaerosols in an agricultural building [18]. Different types of ventilation systems used in stanchion barns are mixing, exhaust, and supply systems. In a mixing fan system, a series of axial fans is suspended above each row of cows. The airflow above two rows of cows in a barn is in opposite directions, hence the air is continuously redistributed, rather than exchanged. There are two types of exhaust ventilation: tunnel ventilation and wall exhaust fans. Tunnel ventilation involves exhausting all of the air through one end of the barn using a bank of fans and drawing the air into the building on the opposite end through a large door or window. This type of ventilation system pulls air through the barn at a sustained rate of 1 to 2 m/s. Due to the cooling effect of the breeze generated within the barn, tunnel ventilation systems cannot be operated in winter. A barn with a well-designed system of wall exhaust fans will exchange as much air as a tunnel ventilation system. However, in most cases there is not any perceivable rate of airflow throughout the barn. A supply system uses a fan and duct to force air into a building, hence creating positive pressure.

Feed stuffs must supply energy, protein, fat, minerals, and vitamins to the dairy cow. The feed stuffs can be categorized as bulk feeds, which provide roughage, and grain-based feeds, which meet the specialized nutritional requirements. Bulk feeds are hays (grasses, clover, and alfalfa) and silages (grasses, cornstalks, and oat chaff). Hays are stored at approximately 10% moisture and are dry to the touch. In contrast, silages are stored at approximately 55% moisture and are moist to the touch. Corn is the predominant component of grain-based feeds. Depending on the individual farmer, the corn may either be harvested and stored at approximately a level of 25% moisture, which is moist to the touch; or artificially dried below a moisture level of 15% before storage, which is dry to the touch.

Bedding materials for cows provide insulation from the floor and a degree of absorbency to keep cows dry. Straw, sawdust, paper, and cornstalks are bedding materials used in stanchion barns. Distribution of these materials creates a high exposure to organic dusts [16]. Further exposure to these materials is created when the barn is cleaned. Barn cleaning typically involves the removal of manure and old bedding by scraping and with an automatic gutter system. A final step of barn cleaning is the application of lime. Lime is commonly used to absorb moisture and disinfect barn floors.

A wet growing season may make these feed stuffs and bedding materials used in dairying more susceptible to colonization with microorganisms. For example, the concentration of Gram-negative bacteria in cotton dust is lower in cotton grown in arid California than cotton grown from the humid southeastern USA [6]. In another study, the population of *Pseudomonas aeruginosa* in lettuce fields was proportional to amount of rainfall [7]. Additionally, the condition of fodder at storage has been shown to be related to the exposure of thermophilic actinomycetes in dairy barns [3]. Dalphin *et al*. found that hay in dairy barns which was dried mechanically before storage released a lower concentration of thermophilic actinomycetes by approximately one order of magnitude than hay that was not artificially dried. Ultimately, a wet growing season may increase the incidence of hypersensitivity pneumonitis among dairy farmers [23].

The goals of this study were twofold: 1) identify sources and activities within dairy barns that are related to concentration of bioaerosols, and 2) investigate the association of ambient rainfall during the growing season with concentration of bioaerosols released from those crops when they are used for feed and bedding.

MATERIALS AND METHODS

Subjects. The concentrations of bioaerosols were originally measured at 23 dairy barns that used crops from the growing season of 1991, which was of average rainfall (25.5 cm) [9]. These barns, *dry farms*, are located in Marathon, Clark, and Wood Counties in Wisconsin. Following the heavy rainfall (63.0 cm) and flooding of 1993 at barns in the same climatic region, *wet farms*, the bioaerosol concentrations were measured using the same methodology. These farms were arbitrarily chosen from Fayette County, Iowa. Typically, the temperature, rainfall, and length of the growing season are similar for the farms located in all four counties [27]. But in this study, the rainfall was 250% of normal during the growing season for *wet farms*, see Table 1 [14, 15]. With data already

Table 1. Climatological conditions at dairy farms during June, July, and August (actual and average 30 year).

Location	N	Study year	rainfall (cm)		temperature (°C)	
			actual	average	actual	average
Dry farms	23	1991	25.5	30.4	20.0	19.4
Wet farms	24	1993	63.0	33.1	19.4	21.2

collected for the 23 *dry farms*, the number of *wet farms* to sample was pre-selected to have sufficient statistical power in order to detect a minimum difference of 1/2 order of magnitude (log_{10}) in the number of colony forming units per cubic meter (cfu/m^3) between the two groups. An assumption of three for the geometric standard deviation in the number of cfu/m^3 among dairy barns of a similar environment was based on our earlier study [9]. Using a conventional sample size equation [28], an estimated sample size of 25 *wet farms* was calculated to be needed in order to have 90% statistical power to detect a minimum difference of 1/2 order of magnitude in the number of cfu/m^3 between *wet farms* and *dry farms*.

Environmental Analytes. Area sampling was completed in the spring, at which time feed and bedding were being used from the growing seasons in Table 1. Air sampling instruments were hung in a basket approximately 1.5 m above the floor in the center aisle of each barn to represent the height of farmer's breathing zone. Time-weighted average concentrations during the morning chores (median 3 hours) were measured for culturable microorganisms, total dust, and CO_2. An all-glass impinger (AGI-30, ACE Glassworks, Vineland, NJ) was used to collect air samples for the enumeration of cfu/m^3 of yeasts, molds, mesophilic bacteria, and thermophilic bacteria, as previously reported [25]. Total dust samples were collected at 18 l/min in open face cassettes with a copolymer filter (37 mm, 0.8 μm pore size, DM-800, Gelman Sci., Ann Arbor, MI). Following gravimetric analysis (MT-5 ultramicrobalance, Mettler Instrument Corp., Hightstown, NJ) corrected for systematic changes in field blanks, the concentration of dust was reported as mg/m^3. The concentration of CO_2 in ppm was measured with the use of passive diffusion tubes (500/a-D, National Draeger Inc., Pittsburgh, PA). A dry bulb thermometer measured temperature during the sampling period. Relative humidity during sampling was measured with use of a digital psychrometer or a sling psychrometer. The two devices were not calibrated to one another; thus, relative humidity was treated as a dichotomous variable as > 70% or < 70%.

Observations of Farm Management Practices. Observations of individual farm management practices were made during sampling by the authors. The type of ventilation being utilized in each barn during sampling was categorized as either: mixing (series of axial fans that redistributed air inside the barn), tunnel (large exhaust fans at one end of barn), supply (a fan and duct that forces air into the barn), or passive (doors, windows, and wall exhaust fans). Barns using wall exhaust fans were included in the category of passive since no barns in this study utilized a balanced system of wall exhaust fans, but had only one or two operating. The type of bulk feed at each barn was categorized into hay (grasses dry to the touch) or silage (grasses moist to the touch). The grain-based feeds were categorized into dry grain (corn dry to the feel) or moist grain (corn moist to the touch). Straw, sawdust, newspaper, and cornstalks were the types of bedding used in the stanchion barns. The straw bedding materials were not always distributed everyday, so the activity of distribution was not necessarily sampled at each barn. As a result, the exposure to bedding materials was categorized into three variables: fresh straw (the distribution of straw bedding on day of sampling), day old straw (the use of straw bedding that was not distributed on day of sampling), and no bedding (the use of no bedding at all). Distribution of lime was dichotomized into use or no use. Animal density was calculated as cows per cubic meter of barn.

Statistical Analyses. Environmental parameters commonly lack a normal distribution [11]. Therefore, all data of a continuous distribution were log transformed. Following transformation, the Shapiro and Wilk's W statistic did not reject the null hypothesis that the data were drawn from a normal distribution [22]. SAS PROC UNIVARIATE (SAS Institute, Cary, NC) was used to calculate the Shapiro and Wilk's W statistic, geometric mean, and geometric standard deviation for each variable.

A single factor ANOVA was used to test equivalence of means. Homogeneity of variances among means was evaluated with Bartlett's test [2]. Null hypotheses were rejected at a 5% level of significance with 2-sided p-values. If ANOVA led to rejecting a null hypothesis of equal means, the Newman-Keuls multiple range test located the difference among group means at a 5% level of significance [28]. Pearson's linear correlation coefficient was used to evaluate the extent of relationships among environmental analytes.

Forward stepwise regression analyses were used to describe which environmental factors exhibited the strongest correlation with concentration of bioaerosols. The independent variables of a continuous distribution were animal density, barn volume, and temperature during sampling. The other independent variables were ambient rainfall (wet farms or dry farms), type of ventilation (mixing, tunnel, supply, or passive), type of bulk feed (hay, silage, or neither), type of nutritional feed (dry corn, moist corn, or neither), type of bedding (straw distributed during sampling, straw not distributed during sampling, or use of no bedding), number of cows in barn, relative humidity during sampling. All independent variables plus an interaction term of independent variables with ambient rainfall or use of tunnel ventilation

Table 2. Bioaerosol exposure by type of barn set-up.

Analyte	Milking parlor n = 6	Stanchion n = 41
	Geometric Mean ± Geometric Standard Deviation (Range below)	
Yeasts ($cfu/m^3 \times 10^4$)*	0.6 ± 2.3 (0.3 - 1.5)	2.0 ± 4.2 (0.2 - 27.0)
Molds ($cfu/m^3 \times 10^4$)	0.6 ± 2.4 (0.2 - 24.5)	0.8 ± 5.7 (0.04 - 36.3)
Mesophilic bacteria ($cfu/m^3 \times 10^4$)	52.4 ± 1.8 (26.3 - 131.8)	85.3 ± 3.9 (7.6 - 575.4)
Thermophilic bacteria ($cfu/m^3 \times 10^4$)	0.3 ± 2.0 (0.1 - 0.7)	0.4 ± 3.3 (0.04 - 2.9)
CO_2 (ppm)	1400 ± 1.5 (930 - 2360)	1100 ± 1.5 (330 - 2360)
Total dust (mg/m^3)	0.3 ± 3.0 (0.06 - 0.8)	0.5 ± 3.6 (0.09 - 5.4)

* $p<0.10$ that mean concentrations in that row are not equal by ANOVA.

Table 3. Bioaerosol exposure by amount of ambient rainfall during the growing season.

Analyte	Dry Farms n = 22	Wet Farms n = 19
	Geometric Mean ± Geometric Standard Deviation (Range below)	
Yeasts ($cfu/m^3 \times 10^4$)	1.9 ± 3.5 (0.2 - 17.8)	2.3 ± 5.1 (0.7 - 27.0)
Molds ($cfu/m^3 \times 10^4$)	1.0 ± 5.7 (0.04 - 36.3)	0.7 ± 5.9 (0.05 - 13.5)
Mesophilic bacteria ($cfu/m^3 \times 10^4$)	81.7 ± 2.8 (7.6 - 371.5)	89.8 ± 5.3 (8.9 - 575.4)
Thermophilic bacteria ($cfu/m^3 \times 10^4$)	0.2 ± 3.2 (0.04 - 1.9)	0.5 ± 3.0 (0.1 - 2.9)
CO_2 (ppm)	1120 ± 1.6 (330 - 2360)	1070 ± 1.4 (600 - 2120)
Total dust (mg/m^3)	0.8 ± 2.7 (0.09 - 5.4)	0.3 ± 3.3 (0.03 - 2.3)

were candidate variables considered for entry into the regression model. Variables entered at the $p < 0.05$ level comprised the final regression model. The coefficient of determination, R^2, reported the percent of total variability in the concentration of an environmental analyte attributable to the regression model. The partial R^2 reported the percent of total variability in the concentration of an analyte attributable to an individual variable removed from the effect of other variables in the model. These analyses were performed using the REG procedure of SAS software.

RESULTS

Summary Data. An exposure assessment to bioaerosols was completed at 48 dairy operations. Six of the dairy operations used milking parlors and 42 were traditional stanchion barns. A summary of the bioaerosol concentrations for the two types of barn set-ups is presented in Table 2. Of the 42 stanchion barns sampled, one barn had a much greater number of yeasts (600×10^4 cfu/m^3), mesophilic bacteria (5000×10^4 cfu/m^3), and thermophilic bacteria (6×10^4 cfu/m^3) than the other 41 barns. This barn of recent construction had the air intake for a supply ventilation system six feet over a manure pit. This outlier was dropped from subsequent analysis. A single factor ANOVA tested if the mean bioaerosol concentrations were equal in both barn set-ups, see Table 2. For each of the environmental analytes, there was no significant difference between values in the stanchion barns and milking parlors. The six milking parlors were dropped from subsequent analyses, since they did not include the farm management practice variables of ventilation, feeding, and bedding that subsequent analyses evaluated.

Ambient Rainfall. A single factor ANOVA tested the null hypothesis that the amount of ambient rainfall received by the crops was not related to the concentration of bioaerosols released from those crops during their distribution. The mean concentrations of the six environmental analytes at 22 *dry farms* and 19 *wet farms* are compared in Table 3. For each of the environmental analytes, there was no significant difference in the mean concentration between *wet farms* and *dry farms*.

Ventilation. The type of ventilation used in the stanchion barns during sampling was categorized into passive, mixing, supply, or tunnel. For each of the six environmental analytes, a single factor ANOVA tested if the mean bioaerosol concentrations were equal among the four types of ventilation, see Table 4. We rejected ($p < 0.01$) the null hypothesis of equal thermophilic bacteria concentrations among the four types of ventilation. Specifically, the barns with mixing ventilation had a concentration of thermophilic bacteria that was from three to eight-fold higher than the other barns. The null hypothesis that the concentration of CO_2 in the barns was equal for the four types of ventilation was also rejected ($p < 0.05$). Barns with tunnel ventilation had a concentration of CO_2 that was approximately one-half of all other barns. Although not necessarily statistically significant, the barns with tunnel ventilation were associated with the lowest concentration of molds, mesophilic bacteria, thermophilic bacteria, CO_2, and total dust.

Feed Rations. Generally, dairy producers feed their cows a source of bulk feed and a source of feed to meet specialized nutritional requirements. Bulk feeds distributed in the barn were either hay or silage. Some farmers fed these bulk feeds outside and hence there was no

Table 4. Bioaerosol exposure by type of ventilation.

Analyte	Passive n = 22	Mixing n = 12	Supply n = 3	Tunnel n = 4
	Geometric Mean ± Geometric Standard Deviation (Range below)			
Yeasts ($cfu/m^3 \times 10^4$)	1.6 ± 5.7 (0.07 - 26.9)	1.2 ± 2.3 (0.3 - 5.0)	4.5 ± 1.8 (3.0 - 6.7)	5.4 ± 1.7 (3.2 - 9.1)
Molds ($cfu/m^3 \times 10^4$)	1.0 ± 5.1 (0.05 - 34.7)	1.5 ± 6.1 (0.09 - 36.8)	0.5 ± 3.0 (0.2 - 1.1)	0.4 ± 3.1 (0.1 - 0.8)
Mesophilic bacteria ($cfu/m^3 \times 10^4$)	81.1 ± 3.4 (8.9 - 478.6)	88.2 ± 3.0 (13.8 - 575.4)	113.8 ± 1.4 (87.9 - 147.2)	22.8 ± 3.8 (7.6 - 100.0)
Thermophilic bacteria ($cfu/m^3 \times 10^4$)***	0.3 ± 2.0 (0.04 - 2.0)	0.8 ± 2.6 (0.2 - 2.9)	0.1 ± 1.8 (0.08 - 1.8)	0.1 ± 1.5 (0.08 - 0.2)
CO_2 (ppm)**	1110 ± 1.5 (430 - 2000)	1220 ± 1.3 (870 - 2120)	1730 ± 1.5 (1270 - 2360)	650 ± 1.6 (330 - 870)
Total dust (mg/m^3)	0.5 ± 3.8 (0.04 - 5.4)	0.4 ± 4.1 (0.03 - 4.5)	1.1 ± 1.2 (1.0 - 1.3)	0.3 ± 2.8 (0.09 - 1.1)

*** $p < 0.01$ that mean concentrations in that row are not equal by ANOVA; ** $p < 0.05$ that mean concentrations in that row are not equal by ANOVA; Boxes enclose a mean not equal to other means in that row by Newman-Keuls.

bioaerosol generation within the barns. For each of the six environmental analytes, the values representing three different feeding practices are compared in Table 5. The null hypotheses of equal means for concentration of yeasts, mesophilic bacteria, thermophilic bacteria, CO_2, or total dust were not rejected by ANOVA. However, the concentrations of molds were not equivalent ($p < 0.05$) between the three different feeding practices. The barns using hay as a bulk feed were associated with a concentration of molds that was at least five-fold larger than that found in barns utilizing silage or no bulk feed at all. Similarly, although not reaching statistical significance, the concentration of thermophilic bacteria was three-fold larger. Nutritional feeds distributed in the barns were either dry grain or moist grain. The bioaerosol concentrations associated with farmers using either grain type are in Table 6. In barns that used dry grain, the concentration of molds was four-fold larger ($p < 0.05$) and the concentration of total dust was three-fold larger ($p < 0.05$) than in barns that used moist grain.

Bedding Materials and Barn Cleaning. Bedding materials for cows provide insulation from the floor and a degree of absorbency to keep cows dry. Depending on

Table 5. Bioaerosol exposure by type of bulk feed.

Analyte	Hay n = 9	Silage n = 14	Neither n = 18
	Geometric Mean ± Geometric Standard Deviation (Range below)		
Yeasts ($cfu/m^3 \times 10^4$)	2.0 ± 3.3 (0.1 - 5.6)	2.2 ± 4.2 (0.2 - 18.1)	1.0 ± 4.5 (0.07 - 26.9)
Molds ($cfu/m^3 \times 10^4$)**	3.9 ± 3.5 (1.0 - 36.8)	0.6 ± 3.9 (0.05 - 8.5)	0.7 ± 5.3 (0.07 - 34.7)
Mesophilic bacteria ($cfu/m^3 \times 10^4$)	99.6 ± 3.5 (13.8 - 574.4)	78.8 ± 3.1 (7.6 - 478.6)	61.9 ± 3.5 (8.9 - 371.5)
Thermophilic bacteria ($cfu/m^3 \times 10^4$)*	0.6 ± 3.0 (0.2 - 2.9)	0.2 ± 3.6 (0.04 - 1.6)	0.1 ± 1.8 (0.08 - 1.8)
CO_2 (ppm)	1320 ± 1.5 (670 - 2360)	1090 ± 1.6 (330 - 2120)	1010 ± 1.5 (430 - 2000)
Total dust (mg/m^3)	0.5 ± 4.0 (0.04 - 2.3)	0.7 ± 3.4 (0.09 - 5.4)	0.3 ± 3.7 (0.03 - 3.1)

*$p < 0.10$ that mean concentrations in that row are not equal by ANOVA; **$p < 0.05$ that mean concentrations in that row are not equal by ANOVA; Box encloses the mean not equal to other means in that row by Newman-Keuls.

Table 6. Bioaerosol exposure by type of nutritional feed.

Analyte	Dry Grain n = 30	Moist Grain n = 9
	Geometric Mean ± Geometric Standard Deviation (Range below)	
Yeasts ($cfu/m^3 \times 10^4$)	1.7 ± 3.9 (0.1 - 26.9)	1.4 ± 6.3 (0.07 - 17.8)
Molds ($cfu/m^3 \times 10^4$)**	1.4 ± 5.0 (0.08 - 36.8)	0.3 ± 3.9 (0.05 - 2.6)
Mesophilic bacteria ($cfu/m^3 \times 10^4$)	89.7 ± 3.0 (7.6 - 574.4)	42.8 ± 4.4 (8.9 - 478.6)
Thermophilic bacteria ($cfu/m^3 \times 10^4$)	0.4 ± 3.4 (0.04 - 2.9)	0.3 ± 1.9 (0.2 - 1.0)
CO_2 (ppm)	1130 ± 1.5 (330 - 2360)	1110 ± 1.4 (720 - 2120)
Total dust (mg/m^3)**	0.6 ± 3.4 (0.03 - 5.4)	0.2 ± 2.9 (0.06 - 1.7)

Note: Two farms that did not feed any nutritional feed during sampling are not included in analysis; **$p < 0.05$ that mean concentrations in that row are not equal by ANOVA.

Table 7. Bioaerosol exposure by type of bedding.

Analyte	Fresh Straw n = 19	Day Old Straw n = 9	No Bedding n = 8
	Geometric Mean ± Geometric Standard Deviation (Range below)		
Yeasts ($cfu/m^3 \times 10^4$)**	3.5 ± 2.6 (0.5 - 18.1)	0.9 ± 2.4 (0.2 - 4.3)	1.4 ± 4.9 (0.3 - 26.9)
Molds ($cfu/m^3 \times 10^4$)*	1.8 ± 5.3 (0.1 - 36.8)	0.6 ± 5.1 (0.09 - 27.9)	0.5 ± 2.5 (0.08 - 1.2)
Mesophilic bacteria ($cfu/m^3 \times 10^4$)	94.3 ± 3.0 (7.6 - 574.4)	50.5 ± 3.5 (8.9 - 371.5)	86.9 ± 3.4 (16.1 - 35.5)
Thermophilic bacteria ($cfu/m^3 \times 10^4$)	0.3 ± 3.0 (0.04 - 2.9)	0.3 ± 2.3 (0.06 - 1.0)	0.5 ± 2.8 (0.1 - 2.0)
CO_2 (ppm)	1040 ± 1.6 (330 - 1930)	1230 ± 1.4 (770 - 2120)	1040 ± 1.8 (430 - 2360)
Total dust (mg/m^3)*	0.7 ± 3.4 (0.04 - 5.4)	0.3 ± 3.5 (0.06 - 3.1)	0.2 ± 4.5 (0.04 - 1.4)

Note: Barns using cornstalks (n = 2), paper (n = 1), or sawdust (n = 1) are not included in analysis; * $p < 0.10$ that mean concentrations in that row are not equal by ANOVA; ** $p < 0.05$ that mean concentrations in that row are not equal by ANOVA; Box encloses the mean not equal to other means in that row by Newman-Keuls.

individual farmer and day of sampling, categories of bedding were fresh straw, day old straw, and no bedding. The bioaerosol concentrations for each category of bedding are compared in Table 7. Fresh straw had the highest mean concentration of yeasts, molds, mesophilic bacteria, and total dust. Although, only the difference in concentration of yeasts was significant ($p < 0.05$).

Lime is used to absorb moisture and disinfect barn floors. The application of lime is typically the final step of cleaning the barns, which was not completed on a daily basis. Additional activities of barn cleaning were the removal of manure and old bedding, and the distribution of fresh straw. Not surprisingly, the application of lime was highly correlated ($r = 0.7$) with the use of fresh straw. Hence, the relationships of lime use to bioaerosol concentrations were consistent with the results evaluating bedding. (Data for lime not shown).

Multiple Regression Analyses of Environmental Factors. Multiple regression analyses were used to describe which environmental factors exhibited the strongest correlation with the concentration of bioaerosols. Results of separate analyses for yeasts, molds, mesophilic bacteria, and thermophilic bacteria are given in Table 8. Each model ranks the independent variables from most to least important in terms of their correlation with the concentration of a bioaerosol. The higher the F to remove a variable from the model, the more important that variable was to the concentration of a bioaerosol. The most significant correlates for the concentration of yeasts in the air of dairy barns were the number of cows in the barn, the temperature during sampling, and the use of a supply ventilation system. The feeding of dry grain and hay were the strongest correlates of mold concentration. For regression analysis of mesophilic bacteria, the feeding of moist grain was the strongest correlate. The feeding of silage was the best correlate of the concentration of thermophilic bacteria. Overall, the percent of variance in concentration of bioaerosols explained by the independent variables ranged from 38% to 51%.

Table 8. Regression analyses of the culturable concentrations of bioaerosols in dairy barns.

Variables	Partial R^2	F (p-value) to remove variable from model
Yeasts model $R^2 = 0.41$ ($p < 0.001$)		
Number of cows in barn	0.14	8.9 ($p < 0.01$)
Temperature during sampling	0.13	8.4 ($p < 0.01$)
The use of supply ventilation	0.12	7.7 ($p < 0.01$)
Animal density	0.10	6.4 ($p < 0.05$)
Distribution of bedding during sampling	0.07	4.7 ($p < 0.05$)
Mold model $R^2 = 0.38$ ($p < 0.001$)		
Feeding of dry grain	0.24	14.8 ($p < 0.01$)
Feeding of hay	0.14	8.5 ($p < 0.01$)
No use of bedding	0.08	5.2 ($p < 0.05$)
Mesophilic bacteria model $R^2 = 0.51$ ($p < 0.001$)		
Feeding of moist grain	0.23	14.8 ($p < 0.001$)
The use of tunnel ventilation	0.17	10.6 ($p < 0.01$)
Number of cows in barn	0.16	9.9 ($p < 0.01$)
Thermophilic bacteria model $R^2 = 0.42$ ($p < 0.001$)		
Feeding of silage	0.28	20.3 ($p < 0.001$)
Use of mixing ventilation	0.11	7.8 ($p < 0.01$)
Feeding of hay	0.05	3.9 ($p < 0.05$)
Feeding of moist grain	0.05	3.8 ($p < 0.05$)

Correlation Among Environmental Analytes. Correlation among the environmental analytes would suggest shared sources and would support the feasibility of using one analyte as a surrogate for others. Pearson's correlation coefficients were calculated between each of the time-weighted average concentrations of yeasts, molds, mesophilic bacteria, thermophilic bacteria, total dust, and CO_2, see Table 9. Generally, correlation was poor between the environmental analytes. There was moderate correlation only between the concentration of CO_2 and total dust ($r = 0.51$); and that of yeasts and mesophilic bacteria ($r = 0.46$).

DISCUSSION

The etiology of occupational respiratory disease in agricultural producers has not been completely elucidated. There are two types of bioaerosol exposure related to

Table 9. Correlation among environmental analytes in a dairy barn.

	Total dust	Yeasts	Molds	Mesophilic bacteria	Thermophilic bacteria
Carbon dioxide	0.52**	—	—	—	—
Total dust		—	0.32*	0.34*	—
Yeasts			—	0.46**	—
Molds				—	0.34*
Mesophilic bacteria					—

**$p < 0.05$ of a significant correlation; *$p < 0.10$ of a significant correlation; — no correlation.

disease in dairy farmers that are generally recognized: 1) exposure to any species of microorganism at an airborne concentration of $10^{10}/m^3$, which results in an acute non-infectious disease termed organic dust toxic syndrome [12] and 2) exposure to a specific microorganism such as *Saccharopolyspora rectivirgula* at concentrations sufficient to induce hypersensitivity pneumonitis [20]. For the most part, dairy farmers are not faced with these levels of exposure on a daily basis. However, dairy farmers are exposed to concentrations of bioaerosols within the dairy barn greater than ambient for a duration of five to ten hours a day for up to 365 days a year. The average exposure in the dairy barns was greater than 10^5 total cfu of fungi and bacteria per m^3 of air (Tab. 2). This concentration is on the same order of magnitude of exposure as in other agricultural buildings, such as turkey and swine barns [18, 24]. In comparison, indoor ambient air in non-complaint homes has approximately 10^3 cfu/m^3 [4]. The health outcomes from daily exposure to bioaerosol concentrations greater than ambient have not been documented as extensively as they have for organic dust toxic syndrome and hypersensitivity pneumonitis. However, several studies have shown the daily exposure concentration to be related to disease. In a cross-sectional epidemiologic study of dairy farmers, farmers with selected respiratory symptoms experienced higher average daily exposure to bioaerosols than farmers free of respiratory symptoms [10]. Similarly, in an investigation of respiratory disease in turkey farmers, respiratory symptoms were greatest in the winter months when exposure to bioaerosols was at its highest concentration [19].

In this study, the variation in bioaerosol concentrations ranged over two to three orders of magnitude (Tab. 2). This wide range indicates all barns did not have equal daily concentrations of bioaerosols, even after considering the high environmental variability in the collection of bioaerosols (e.g., work pace of farmers, distance of area sampling sites from multiple distribution sites, and subtle management practices) [21] and analytical variability in the culture method [26].

This study was designed to test the null hypothesis that the amount of ambient rainfall received by crops had no association with bioaerosol concentrations when those crops were used in the barn. The null hypothesis, evaluated with a sample size of 19 *wet farms* and 22 *dry farms*, was not rejected (Tab. 3). There are several interpretations of accepting the null hypothesis. One interpretation is that feed and bedding are, in general, not a main source of bioaerosols. Surprisingly, in milking parlors, which did not have feed and bedding materials distributed inside them, the bioaerosol concentrations were not significantly different from those of the stanchion barns (Tab. 2). An alternative interpretation is that similar storage conditions of the crops before their use may have equalized microbial colonization. Five to seven months had passed between fall harvest and environmental sampling in late spring. A third interpretation is that the time-weighted average concentration of cfu/m^3 is a poor measurement of short term bioaerosol exposures associated with feed and bedding distribution. Generally, distributions of the feed and bedding did not last more than 15 minutes of approximately 3 hours of environmental sampling. Therefore, long term sampling may have obscured important short term peaks in concentration.

In addition to ambient rainfall, the effects on bioaerosol concentrations by other factors were evaluated. Feed and bedding materials have been shown to be sources of bioaerosol exposure on dairy farms [8]. In this study, the type of fodder was the strongest correlate of all farm management practices with concentration of molds, mesophilic bacteria, and thermophilic bacteria (Tab. 8). The use of bulk feeds and nutritional feeds that had a relatively high moisture content were associated with a lower concentration of bioaerosols than similar feeds stored at a lower moisture content (Tables 5 and 6). This work is consistent with other investigations that have shown fodder with the most moisture to be associated with the lowest airborne concentration of dust and bacteria [1, 18]. A likely explanation is that aerosols of moist fodder compared to dry fodder are hydrated and therefore have a higher sedimentation rate.

As one would expect, an increase in the rate of ventilation within agricultural buildings has been shown to be negatively correlated with airborne concentration of dust, endotoxin, and Gram-negative bacteria [18]. Many new barns or those being remodeled are installing either mixing or tunnel ventilation. In the present study, the concentration of CO_2 was used to indicate the influx of make-up air. Even with a limited sample size to compare

the two types of ventilation, barns with tunnel ventilation had a significantly lower concentration of CO_2 than barns with mixing ventilation (Tab. 4).

The quantitation of culturable bioaerosols is tedious and subject to error [26]. Thus, it would be desirable to identify a surrogate marker for bioaerosol exposure that is readily measured and that correlates well with the concentration of bioaerosols. Carbon dioxide is both a product of cellular respiration and a natural constituent of ambient air. The concentration of CO_2, which is readily obtainable from direct reading instruments, is a common surrogate measurement for the quality of indoor air in office buildings [17]. Carbon dioxide is used, not because of any health risks of CO_2, but with the assumption that its concentration correlates with known or unknown causal agents that are present. In a swine barn, the concentration of CO_2 has been shown to have moderate correlation (all $r > 0.5$) with airborne concentrations of endotoxin, bacteria, and total microbes [5]. In the present study, the concentration of CO_2 did not correlate with the concentration of any culturable bioaerosols. Hence, the use of CO_2 as a surrogate measurement of culturable bioaerosols in dairy barns was not supported.

Since daily exposure to bioaerosols in dairy barns may be a risk factor for respiratory disease, a reduction in the concentrations of bioaerosols may benefit the health of farmers. The results of this study suggest that several environmental factors are related to bioaerosol concentrations. An option to reduce bioaerosol concentrations within dairy barns may be to use feed rations stored at high moisture (silage, moist grain), and save the feed rations of dry storage (hay, dry grain) for the outdoor feedlot. In addition, this study suggests tunnel ventilation is superior to mixing fans for reduction of bioaerosol exposure. The use of these mentioned dairy management practices should have minimal effects on milk production.

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