Qualitative structure of airborne bacteria and fungi in dairy barn and nearby environment

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ABSTRACT: The study was conducted in a dairy barn and nearby environment to determine the level of air bacterial and fungi contamination. Measurements were performed in morning, noon and evening sampling periods once a week during two autumn months inside the barn and in a 25 m distant barn environment. A Merck MAS-100 air sampler was employed with different medium and incubation combinations for the capture and identification of bacteria and fungi. The results of the study showed a statistically significant difference ($P < 0.05$) between the bacterial and fungi counts measured inside and outside the barn, whereby total outside bacterial count was by 97.4% to 98.0% lower, and total outside fungi count by 85.2% to 99.4% lower than the respective indoor counts in various sampling periods. In 125 analyzed colonies, gram-positive bacteria, especially those of the genus *Staphylococcus* and *Streptococcus*, were most commonly identified in the air samples from both inside and outside the barn. Gram-negative bacteria were present at a low rate, predominated by enterobacteria and by the genus *Moraxella* and *Pseudomonas*. Nine mould genera were identified in 325 fungi colonies, predominated by the genus *Aspergillus*, *Penicillium*, *Rhizopus* and yeasts, both in the barn and in the nearby environment.

Keywords: airborne bacteria; airborne fungi; dairy barn

Stock breeding is inevitably associated with various forms of air pollution, a potential risk for both the human and the animal health. Stable air is known to contain a high level of microorganisms found in both solid and liquid bioaerosols (Wathes, 1995; Lange et al., 1997). Saprophytes are the most common findings in this setting; however, pathogenic bacteria, moulds and viruses may also be occasionally detected.

Any impairment of the animal environment due to a change in the air and microclimate quality, or in animal keeping and manipulation conditions is stressful for animals and produces factors contributing to the occurrence of multicausal diseases (Müller and Weiser, 1987; Wathes, 1994; Wathes et al., 1998). Therefore, both indoor and outdoor factors, which do not act as causative agents by themselves, may be implicated in the pathogenesis of these diseases.

Air quality inside the barn also influences the health of people working with animals, whereas the spread of bioaerosol outside animal housing facilities may result in local or even more extensive environmental pollution. The grade of environmental contamination depends on the microorganism count, their potential pathogenicity and biological viability, and current topographic and atmospheric characteristics of the environment (Cox, 1989; Wilson et al., 2002).

Considering that freshly milked milk contains a certain number of microorganisms originating from the barn air, so-called postsecretory contamination (Pavičić et al., 2003), the aim of the present study was to help identify the microbiological structure

Table 1. Arithmetic means and standard deviations of total bacterial count inside and outside the dairy barn

Dairy barn air		*n*	Arithmetic mean			Standard deviation		
			morning	noon	evening	morning	noon	evening
Bacteria (CFU/m^3)	inside	8	$1.14^a \times 10^5$	$8.81^a \times 10^4$	$1.26^a \times 10^5$	8.39×10^4	8.01×10^4	9.03×10^4
	outside	8	$1.23^b \times 10^3$	$8.60^b \times 10^2$	$1.72^b \times 10^3$	8.80×10^2	8.45×10^2	2.70×10^3

CFU = colony-forming unit; *n* = number of measurements;
[a,b]arithmetic means marked with different superscript letters showed a statistically significant difference at $P < 0.05$

Table 2. Arithmetic means and standard deviations of total fungi count inside and outside the dairy barn

Dairy barn air		*n*	Arithmetic mean			Standard deviation		
			morning	noon	evening	morning	noon	evening
Fungi (CFU/m^3)	inside	8	$8.35^a \times 10^4$	$5.23^a \times 10^4$	$7.07^a \times 10^4$	9.10×10^4	5.49×10^4	8.17×10^4
	outside	8	$2.36^b \times 10^3$	$2.59^b \times 10^3$	$5.67^b \times 10^3$	1.96×10^3	1.71×10^3	6.51×10^3

CFU = colony-forming unit; *n* = number of measurements;
[a,b]arithmetic means marked with different superscript letters showed a statistically significant difference at $P < 0.05$

of the air that may result in this type of contamination. Therefore, the study was carried out in and around a dairy barn, where air samples were analyzed to determine the bacterial and fungi genera most commonly grown on nutrient agars.

MATERIAL AND METHODS

The study was conducted in a dairy barn, 15.80 × 12.5 × 2.5 m in size, with the usual area distribution to accommodate 20 head of cattle. During the study, there were 12 cows and 3 calves in the barn. The cows were tied along the feedlot supplied with usual fodder (hay, haylage, concentrate) with a daily ration of feeding at 07:00 and 18:00 h. Water was supplied from local waterworks via appropriate automatic watering troughs. Cow dung was manually removed from the barn and bedding was replaced with fresh straw twice a day, at 07:30 and 18:30 h. Measurements were done in the morning (at 08:00 h), at noon (12:00 h) and in the evening (at 18:30 h), once a week, during two autumn months. Measurements were performed in the barn, in the area of animal stay along the feedlot, and outside the barn at a distance of 25 m. Air was sampled with a Merck MAS-100 device (Merck KgaA, Darmstadt, Germany) on a commercially available nutrient agar. Columbia agar was used for bacteria and Sabouraud agar (Biolife, Milan, Italy) for fungi.

Air was sampled in a volume of 10 l because preliminary studies showed it to be optimal for the subsequent plate analysis and type of agar. Plates with the usual bacterial nutrient Columbia agar were then incubated for 24 h in an incubator at a working temperature of 37°C. The material sampled on Sabouraud agar was incubated for 5 days at 22°C.

In 8 measurements at three sampling times a total of 432 plates with Columbia agar, 216 from the barn and 216 outside the barn, were analyzed. The same number of plates with Sabouraud agar was analyzed. The grown colonies (CFU/m^3) were calculated with a mechanical optic colony counter, and the results were corrected by the respective table and mathematical equation (Anonymous, 1998). Upon determination of the mean total bacterial count, the most common colonies were reinoculated on a selective medium (McConkey agar, mannitol salt agar). The identification of bacterial colonies was carried out according to a procedure described by Quinn et al. (1994) and API system (bio-Mérieux, Marcy-l'Etoile, France). The fungi were identified by a native preparation. The values obtained in this way were analyzed by Microsoft Excel and Statistica 6 Softwares using Wilcoxon matched pair test at a level of statistical significance of 5% ($P < 0.05$) (Anonymous, 1994).

RESULTS AND DISCUSSION

After air sampling onto the respective nutrient agar and incubation, mean values of total bacterial and fungi counts were determined (CFU/m^3) (Tables 1 and 2). A statistically significant difference ($P < 0.05$) in total bacterial and fungi counts

Table 3. Determination of airborne bacteria (in %) in morning, noon and evening sampling periods (n = 125)

Bacteria	Morning		Noon		Evening	
	inside	outside	inside	outside	inside	outside
Alcaligenes sp.	0.00	0.00	0.00	0.00	0.50	0.00
Bacillus sp.	13.00	30.00	15.50	27.00	16.00	27.60
Corynebacterium sp.	6.00	7.50	1.30	4.50	5.80	3.60
Other enterobacteria	0.09	1.20	0.70	0.50	0.40	0.20
Micrococcus sp.	1.20	2.10	2.50	4.50	8.60	6.60
Moraxella sp.	0.23	0.00	0.70	0.00	0.00	0.00
Proteus sp.	0.19	0.00	0.30	0.00	0.01	0.00
Pseudomonas sp.	0.19	0.00	0.00	0.00	0.05	0.00
Staphylococcus sp.	39.00	32.50	43.00	31.00	38.00	35.00
Streptococcus sp.	40.10	26.70	36.00	32.50	30.64	27.00

n = bacteria grown on nutrient media determined after incubation

was observed between the barn air and the outer environment in all sampling periods (morning, noon, and evening). The highest difference in total bacterial count was recorded at noon (98.0%) and the lowest one in the evening (97.4%). The highest difference in total fungi count was observed in the morning (99.4%) and the lowest one in the evening (85.2%). Then, 125 most common bacterial colonies were analyzed and 325 air samples with grown fungi were identified by a native preparation.

The results of this study documented that the highest proportion of gram-positive bacteria present in air samples belonged to the genus *Staphylococcus* and *Streptococcus* (79%), followed by the genus *Bacillus* (13%) and *Corynebacterium* (6%). Gram-negative bacteria predominated by the genus *Proteus* and other enterobacteria besides the genus *Alcaligenes, Moraxella* and *Pseudomonas,* accounted for 0.2% of the total sample bacterial count. Outdoor air samples were also predominated by the bacteria of the genus *Staphylococcus* and *Streptococcus*, with a considerable rise in the proportion of bacteria of the genus *Bacillus*. Similar results were recorded in all three sampling periods (Table 3).

The great difference in the proportion of gram-positive and gram-negative bacteria was explained by the resistance of the former bacteria to environmental stressors, whereas the latter bacteria lack it due to their cellular structure, especially their phospholipid membrane losing its thermodynamic stability with temperature and relative humidity variation (Cox, 1995). The rising proportion of the genus *Bacillus* bacteria in the air outside the barn indicated the spore-forming bacteria to be most resistant in the atmosphere, which is a very harsh environment for the survival of microorganisms due to various unfavourable conditions, primarily desiccation (Shaffer and Lighthart, 1994). The increased concentration of bacteria of the genus *Bacillus* in outdoor air samples could be related to the presence of bacteria on the ground surface and their ascending to the atmosphere, depending on weather conditions. Therefore, it seems reasonable to ask what proportion and what genera of bacteria identified in the air sampled around the barn actually migrated from the barn, and what had already been present in the atmosphere or deposited on the ground surface.

The low number of gram-negative bacteria isolated in the air samples did not imply that the air was free from their endotoxins because the activity of endotoxins is not terminated with the degradation of the bacteria (Zucker et al., 2000b), thus posing a health risk for both animals and farm workers.

The qualitative structure of fungi in the barn air sampled in the morning (Table 4) showed the highest proportion of moulds of the genus *Aspergillus* (31%), *Penicillium* (25%), *Rhizopus* (13%), and yeasts (22%). A comparable proportion of these mould genera was also identified in the air sampled outside the barn, along with an increased proportion of moulds of the genus *Fusarium*. In addition to these, air samples collected at noon and in the evening inside and outside the barn showed a higher proportion of the genus *Fusarium* (8%), *Cladosporium* (5%) and *Mucor* (5%).

The results of the present study assessing the qualitative air structure in a dairy barn are consist-

Table 4. Determination of airborne fungi (in %) in morning, noon and evening sampling periods (n = 325)

Fungi	Morning		Noon		Evening	
	inside	outside	inside	outside	inside	outside
Alternaria sp.	1.50	0.00	0.50	0.50	0.00	0.00
Aspergillus sp.	31.00	32.00	30.00	25.00	33.00	31.00
Cladosporium sp.	2.60	1.50	5.00	2.50	4.50	3.50
Fusarium sp.	1.30	5.30	8.30	7.40	7.50	8.00
Yeast	22.00	25.00	22.00	33.00	22.00	24.00
Mucor sp.	2.30	1.60	1.30	1.50	4.35	5.50
Penicillium sp.	25.00	26.00	25.00	26.00	21.00	23.00
Rhizopus sp.	13.00	8.00	7.83	4.10	7.50	5.00
Scopulariopsis sp.	1.30	0.60	0.07	0.00	0.15	0.00

n = fungi colonies determined after incubation

ent with literature data. Hartung (1992) reported that the qualitative air microorganism structure in animal housing facilities was predominated by gram-positive bacteria such as staphylococci and streptococci (90%), along with *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Cladosporium* sp. *and Alternaria* sp. as the most common mould genera. Wilson et al. (2002) determined bacterial flora outside a cattle barn, down the wind, between two feeding times. The most common isolates belonged to the genus *Bacillus* sp., *Corynebacterium* sp. and *Micrococcus* sp. Gram-negative bacteria isolated from the air represent only a minor proportion (0.02–5.2%), (Zucker and Müller, 2000; Zucker et al., 2000a).

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