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Full Length Research Paper

Microbiological air quality in tie-stall dairy barns and some factors that influence it

Silvana Popescu*, Cristin Borda and Eva Andrea Diugan

Department of Animal Hygiene and Welfare, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania.

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The aim of this study was to assess the microbiological quality of the air in tie-stall dairy cattle barns and to investigate some factors that influence it. We evaluated 52 dairy cattle barns with tie-stalls, during two years, in the winter period. The number of bacteria and fungi was slightly higher in the evening in comparison to the one in the morning and significantly higher in large barns (P<0.01), with bedding (P<0.01) and dirty (P<0.05). Both in the morning and in the evening positive correlations were observed between temperature and airborne bacteria, relative humidity and fungi. The prevalent species of bacteria were *Staphylococcus epidermidis, Staphylococcus xilosus, Aerococcus viridans, Enterococcus faecalis, Enterobacter agglomerans* and *Escherichia coli.* Among fungi, Aspergillus, Penicillium and yeasts predominated. This study's results showed that the number of bacteria and fungi is variable and high in many cases in the indoors air of dairy cattle tie-stall barns, with the predominance of gram positive bacteria. Many of the identified bacterial and fungal species have pathogenic potential, posing risks for the health of animals and humans. Based on the obtained results, we consider the improvement of barn hygiene to be the most practical recommendation for decreasing concentrations of bacteria.

Key words: Airborne bacteria, airborne fungi, indoor air quality, barn hygiene.

INTRODUCTION

The indoor air of dairy cattle barns usually harbors variable numbers of microorganisms, depending on different parameters, such as size of the livestock, breeding and production technologies, floor and bedding types, microclimate factors and, especially, ventilation levels (Hillman et al., 1992; Wathes, 1994; Lange et al., 1997). Among different saprophytic species which are numerically very well represented, there are pathogenic microorganisms as well, such as viruses, different bacteria and fungi. In dairy housing facilities, the sources of bioaerosols include food, manure, litter and the animals themselves. As the level of microbial pollution is higher, there is a proportionally increasing risk of pathogenic microbial contamination for animals and humans. Therefore, the concentration and quality of airborne microflora in the air of dairy barns influences the health of animals, human workers and not lastly the degree of bacterial pollution of the nearby environment (Webster, 1985; Müller and Weiser, 1987; Wathes, 1994). More respiratory symptoms and impaired levels of respiratory function among the dairy farmers were reported by many researchers (Dalphin et al., 1998 a,b; Westeel et al., 2000) compared to the control subjects. Westeel et al. (2000) concluded that the respiratory allergy played a role in the impaired respiratory function among the dairy workers and allergic sensitization to some fungi constituted a risk factor for the development of respiratory airflow obstructions.

Furthermore, the fact that fresh cow milk contains micro-organisms coming from the barns' air is well known, the phenomenon being termed postsecretory contamination (Matković et al., 2007). Considering all of these, monitoring the microbiologic quality of the inside

*Corresponding author. E-mail: popescusilvana@yahoo.com. Tel: +40 264/596384. Fax: +40 0264/593792.

Table 1. The characteristics of the farms.

Parameter	Total number of farms	%
Herd size		
Small (<50 cows)	22	42.30
Large (≥50 cows)	30	57.70
Floor type		
Solid	52	100
Concrete	36	69.23
Soil floor	5	9.62
Soil floor + concrete	11	21.15
Bedding		
With bedding	42	80.77
Without bedding	10	19.23
Milking		
Inside of the barn	52	100
Manual	15	28.85
Mechanic	37	71.15
Manure removal		
Twice per day	16	30.77
Once per day	27	51.92
< Once per day	9	17.31
Ventilation		
Natural	52	100
Through inlets and outlets	34	65.38
Through doors and windows	18	34.62

air in dairy barns has become a necessity. The assessment of the airborne microflora is usually made by determining the number of bacteria and fungi, including potentially pathogenic strains. Limited information is available on the microbiologic quality of the indoors air in dairy barns and the factors that influence it. A more comprehensive study was realised by Lange et al. (1997). Therefore, the aim of the present work was to assess the microbiological quality of the air in dairy cattle barns with tie stalls, through determination of the quantity and diversity of bacteria (mesophilic bacteria, Staphylococci, Streptococci, Gram-negative bacteria) and fungi in the winter period. Another objective of the study was to investigate the influences of some factors (e.g. the moment of the determination, the number of the cows in the barns, the presence or absence of bedding, the cleanness of the barn, microclimate parameters) on the number of bacteria and fungi in the air of dairy cow barns.

MATERIALS AND METHODS

The study was done in 52 dairy cattle barns (30 to 120 cows/barn) with tie stalls, in Transylvania, Romania, during two years (2009 and 2010) in the winter period. All the barns were closed, with solid flooring. The main characteristics of the investigated farms are shown in Table 1. The cows were tethered in the barns only during the cold season (pasturing in the rest of the year) or permanently (without pasturing). Each barn was evaluated three times during winter. The farms were not selected at random but were nominated by veterinary practitioners from seven Transylvanian counties, based on several requirements: tie-stall housing, minimum 30 dairy cows, easy access to the farm during the winter and the farmers' agreement to take part in the study. According to herd sizes the farms were classified into two categories, small (<50 cows) and large (≥50 cows). With regard to bedding the farms were divided in two categories: 'with bedding' and 'without bedding'. Furthermore, the cleanness of the farms was evaluated at each visit based on the body hygiene of the cows, using the system (hygiene scoring system) proposed by Cook (2002) and modified by us. Three body regions were assessed: lower leg, udder and flank and upper leg, by awarding points (from 1 to 4), depending on the degree of manure contamination in the respective areas. A total number of 1986 dairy cows were evaluated (all the cows in barns with < 100 heads and 25% of the herd in those with ≥ 100 dairy cows). The hygiene assessment was made in the morning, in the same days in which the number of bacteria and fungi was determined in the air. The mean score per animal and per farm was calculated, but not the percentage of the scores of 3 and 4, as Cook indicated. Based on the mean hygiene score obtained, the farms were classified in

Table 2. Descriptive statistical indicators for all the parameters determined in the 52 barns in the morning and in the evening.

Parameter	Morning (n=52)				Evening (n=52)			
	Mean	SD	Median	Range	Mean	SD	Median	Range
Mesophilic bacteria (CFU/$m^3 \times 10^4$)	14.10	7.02	15.20	2.50 - 22.60	15.9	7.88	18.70	3.54 - 26.30
Staphylococci (CFU/$m^3 \times 10^4$)	8.43	5.46	7.73	1.10 - 19.4	9.98	6.52	8.25	1.30 - 23.3
Streptococci (CFU/$m^3 \times 10^4$)	2.59	2.47	1.56	0.19 - 9.39	3.65	2.92	2.52	0.50 - 9.41
Gram-negatives (CFU/$m^3 \times 10^3$)	2.55	2.49	1.60	0.00 - 9.15	3.50	3.41	2.00	0.00 - 12.7
Fungi (CFU/$m^3 \times 10^4$)	1.61	1.16	1.54	0.27 - 4.10	2.41	1.95	2.18	0.58 - 7.85
Temperature (°C)	8.97	2.18	9.60	4.10 - 12.00	10.10	2.11	10.20	5.50 - 14.40
Relative humidity (%)	84.05	11.70	87.80	59.20-98.65	80.89	8.39	81.50	61.50-94.00
Air flow velocity (m/s)	0.34	0.04	0.34	0.29 - 0.40	0.34	0.03	0.34	0.29 - 0.40

n = Number of barns; CFU/m^3 = colony-forming units in one cubic metre of air; SD = standard deviation.

two categories: clean (mean scores of 1 to 2) or dirty (mean scores > 2 to 4).

The air sampling was done in the morning (5 to 6 a.m.) and evening (6 to 7 p.m.) in three different locations of the barns (at the extremities and in the centre), approximately 1 m above the floor, representing the height of the animals' breathing zone. The total number of samples/barn was 90 (45 in the morning and 45 in the evening). Air samples were taken using a MAS-100 air sampler (Merck, Germany) based on the principle of the Andersen air sampler. Bacteria and fungi were collected in Petri dishes on different standard culture media: Columbia agar for mesophilic bacteria, Chapmann agar for staphylococci, Endo agar for gram-negative bacteria, blood agar for haemolytic bacteria and Sabouraud agar for fungi. The 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 nutrient Columbia agar and with selective culture media 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. The grown colonies were calculated by a mechanical optic colony counter, the results were corrected using the conversion formula devised by Feller (1950). The average number of bacteria and fungi was calculated as colony-forming units in one cubic meter of air (CFU/m^3). The identification of the species was made using the API system (bio-Mérieux, Marcy-l'Etoile, France). The fungi were identified by native preparations.

Air temperature, relative humidity and air flow velocity in the barns were determined simultaneously, using a Testo 400 (GmbH & Co) device. The mean value of each determined parameter was calculated for every barn, both for the morning and for the evening samples. The obtained data were statistically processed with the SPSS version 17 software. The descriptive statistical indicators were calculated (mean, standard deviation, median, minimum and maximum) for the measured parameters. The correlation coefficient (Spearman r) between mesophilic bacteria and fungi and microclimate parameters (air temperature, relative humidity, air flow velocity) were also calculated. In order to compare values the Kruskal-Wallis Test (Nonparametric ANOVA) was used.

RESULTS

In Table 2, the values of the descriptive statistical indicators (mean, standard deviation, median, minimum, maximum) are shown for the numbers of mesophilic bacteria, staphylococci, streptococci, gram-negative bacteria, fungi and for the microclimate parameters (air temperature, relative humidity and air flow velocity) determined in the 52 dairy cattle barns with tie stalls in the morning and in the evening. Although the mean values are slightly higher in the evening than in the morning for the majority of the parameters (excepting the relative humidity and the air currents' speed), the differences were not significant ($P>0.05$). Figure 1 shows the proportions of staphylococci, streptococci and gram-negative bacteria within the total number of mesophilic bacteria, in the morning and in the evening, in the investigated barns. It is noticeable that depending on the moment of the determination (in the morning, in the evening), staphylococci represented 59.79 to 59.05%, streptococci 18.37 to 21.60% and gram-negative bacteria 1.81 to 2.07%. The concentration of the bacteria and fungi in the small and large barns is shown in Table 3. It can be observed that in the barns with more than 50 animals the number of bacteria (mesophilic bacteria, staphylococci, streptococci, gram-negative bacteria) was significantly higher in comparison with the small barns ($P<0.01$).

In the barns with bedding the number of total mesophilic bacteria, staphylococci, streptococci and fungi was significantly higher ($P<0.01$) than in those without bedding (Table 4). The effect of barn hygiene on the concentration of the microorganisms in the air is shown in Table 5. In the clean barns the number of bacteria and fungi was lower. The differences were significant only for the total mesophilic bacteria, staphylococci and streptococci ($P<0.05$). Both in the morning and in the evening increased temperature was found to be positively correlated with increased mesophilic bacteria and the increased humidity of the air with the fungi (Table 6). The type of bacteria and fungi isolated from the air of dairy cattle barns are presented in Tables 7 and 8. Among gram-positive bacteria the predominant strains were: *Staphylococcus epidermidis, Staphylococcus xilosus, Aerococcus viridans* and *Enterococcus faecalis*. Within the group of gram-negatives we identified more frequently *Enterobacter agglomerans* and *Escherichia coli*. The dominant airborne fungi were the members of

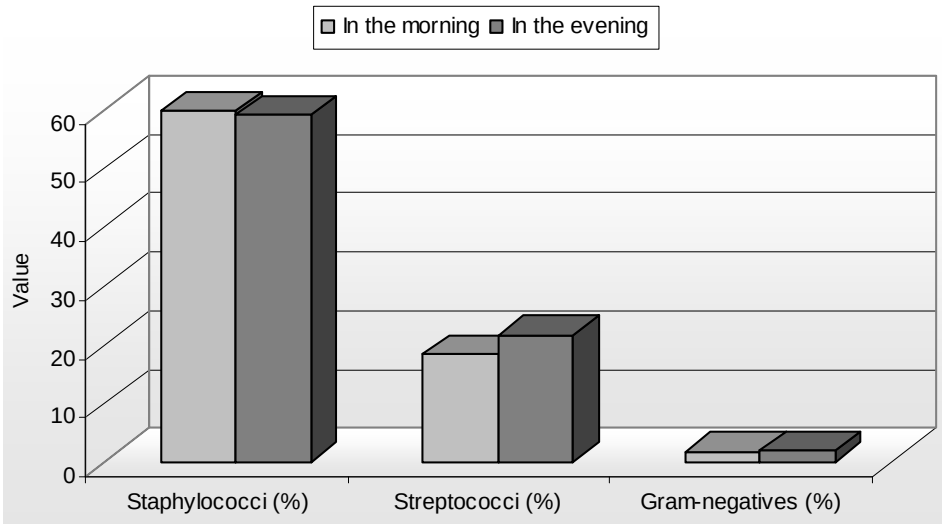

Figure 1. The proportions of the staphylococci, streptococci and gram-negative bacteria within the total number of mesophilic bacteria, in the morning and in the evening, in the 52 investigated dairy barns.

Table 3. Bacterial and fungal parameters classified by the herd size.

Parameter	Small (n = 22)				Large (n = 30)			
	Mean	SD	Median	Range	Mean	SD	Median	Range
Mesophilic bacteria (CFU/m^3 × 10^4)	7.96	4.58	8.05[a]	2.05 - 14.8	18.6	4.67	20.61[b]	7.34 - 22.61
Staphylococci (CFU/m^3 × 10^4)	4.18	2.51	5.00[a]	1.07 - 7.30	8.37	2.73	9.40[b]	2.56 - 11.40
Streptococci (CFU/m^3 × 10^4)	0.93	0.56	1.04[a]	0.20 - 1.80	3.62	1.85	2.77[b]	0.46 - 6.57
Gram-negatives (CFU/m^3 × 10^3)	0.91	1.36	1.60[a]	0.00 - 4.75	3.76	2.78	2.60[b]	0.55 - 9.15
Fungi (CFU/m^3 × 10^4)	1.88	1.32	1.91	0.30 - 4.15	1.41	1.01	0.95	0.27 - 3.07

n = Number of barns; CFU/m^3 = colony-forming units in one cubic metre of air; SD = standard deviation. [a,b] Different superscript within a given row indicates a statistical significant difference (P<0.01).

Table 4. Airborne bacterial and fungal parameters classified by the presence or absence of bedding.

Parameter	Without bedding (n=10)				With bedding (n=42)			
	Mean	SD	Median	Range	Mean	SD	Median	Range
Mesophilic bacteria (CFU/m^3 × 10^4)	6.58	2.87	7.89[a]	2.5 - 8.90	15.91	6.52	19.60[b]	2.91 - 22.61
Staphylococci (CFU/m^3 × 10^4)	3.79	2.62	3.18[a]	1.10 - 7.30	7.27	3.17	8.39[b]	1.07 - 11.40
Streptococci (CFU/m^3 × 10^4)	0.80	0.56	0.78[a]	0.20 - 1.59	2.88	1.97	2.33[b]	0.39 - 6.57
Gram-negatives (CFU/m^3 × 10^3)	1.00	0.89	0.70	0.00 - 2.15	2.92	2.84	1.60	0.00 - 9.15
Fungi (CFU/m^3 × 10^4)	0.32	0.04	0.31[a]	0.28 - 0.39	1.91	1.09	1.99[b]	0.27 - 4.15

n = Number of barns; CFU/m^3 = colony-forming units in one cubic metre of air; SD = standard deviation. [a,b] Different superscript within a given row indicates a statistical significant difference (P<0.01).

the genera *Aspergillus*, *Penicillium, Cladosporium* and yeasts, both in the morning and in the evening.

DISCUSSION

Providing good air quality in farm animal housing is important for the health and welfare of farm animals and staff and for the outdoor environment of farming enterprises (Radon et al., 2002). The microbiological quality of the air in animal houses improves as the number of bacteria and fungi decreases. In our study, the microbiological quality of the air was different from one investigated farm to another, being influenced by several

Table 5. Airborne bacterial and fungal parameters classified by hygiene in the barns.

Parameter	Clean (n=16)				Dirty (n=36)			
	Mean	SD	Median	Range	Mean	SD	Median	Range
Mesophilic bacteria (CFU/m^3 × 10^4)	5.41	2.63	5.43[a]	2.50 - 8.90	17.99	4.35	19.61[b]	8.44 - 22.61
Staphylococci (CFU/m^3 × 10^4)	3.03	2.41	1.93[a]	1.07 - 7.30	8.18	2.33	9.38[b]	3.79 - 11.40
Streptococci (CFU/m^3 ×10^4)	0.73	0.51	0.50[a]	0.20 - 1.56	3.25	1.87	2.34[b]	0.55 - 6.57
Gram-negatives (CFU/m^3 × 10^3)	0.35	0.32	0.20	0.00 - 1.20	3.59	2.63	2.60	0.50 - 9.15
Fungi (CFU/m^3 × 10^4)	1.10	0.89	0.71	0.28 - 2.20	1.83	1.21	1.66	2.70 - 4.15

n = Number of barns; CFU/m^3 = colony-forming units in one cubic metre of air; SD = standard deviation. [a,b] Different superscript within a given row indicates a statistical significant difference (P<0.05).

Table 6. Spearman's correlation coefficients for microclimate parameters with mesophilic bacteria and fungi in dairy barns (n=52) in the morning and in the evening.

Time of day	Parameter	Mesophilic bacteria (CFU/m^3)	Fungi (CFU/m^3)
Morning	Temperature (℃)	0.52*	0.14
	Relative humidity (%)	0.18	0.63*
	Air flow velocity (m/s)	-0.14	0.03
Evening	Temperature (℃)	0.56*	0.22
	Relative humidity (%)	0.23	0.69*
	Air flow velocity (m/s)	-0.26	0.11

*Correlation is significant at the 0.01 level (2-tailed).

factors, such as the number of the animals, the presence of the bedding, the hygiene of the barns, the microclimate parameters and the ventilation level. The number of bacteria (mesophilic bacteria, staphylococci, streptococci, gram-negative bacteria) and fungi varied in the 52 assessed barns, the determined values being similar to those in the scientific literature. Several researches showed that the number of mesophilic bacteria in cattle houses range from 10^3 to 10^6 CFU/m^3 (Dutkiewicz et al., 1994; Karwowska, 2005; Matković et al. 2007; Seedorf et al., 1998; Duchaine et al., 1999), and some authors found even values of 10^8 to 10^9 CFU/m^3 (Eduard, 1997). The great variability of the mesophilic bacterial count in the air of the barns is the reason for which a compulsory hygienic standard for the acceptable number of airborne bacteria is not yet established on an international level. There are different recommendations available for maximum concentrations of major airborne pollutants for both humans and livestock (Donham et al., 2000; Cargill et al., 2002).

However, the recommendation of most of the authors is that the total number of mesophilic bacteria should not exceed 1 × 10^5 CFU/m^3 (Donham et al., 2000; Cargill et al., 2002) in the air of farm animal houses. In our study, in 60% of the assessed barns the total number of mesophilic bacteria exceeded this maximum recommended limit. In these farms several management deficiencies were identified, such as improper hygiene (manure removal once or <once per day) and poor ventilation. It can be observed (Table 3) that the values were slightly higher for the evening determination in comparison with the morning samples, for all the groups of microorganisms. When the values of determinations made in the morning and those made in the evening were compared, no significant differences were found between the two determinations both for the numbers of bacteria (mesophilic bacteria, staphylococci, streptococci, gram-negative bacteria) and for the numbers of fungi (P>0.05). This aspect was observed also in other studies (Wathes, 1994; Seedorf et al., 1998; Duchaine et al., 1999; Matković et al., 2007) and could be explained as a consequence of diurnal animal and barn activities. This result suggests that the air sampling can be performed either in the morning or in the evening, the moment of the day having no significant influence on the number of airborne microorganisms. The conclusion is valid also for the investigated microclimate parameters (temperature, relative humidity, air currents' speed). The general recommendation is that the best moment of the day for sample collection/parameters measurement for the air quality determination would be early in the morning, before the aeration of the barn and before the beginning of the farm activities.

In light of our findings, we believe that this recommendation should be reconsidered. According to our experience in the farms, the evening is a better moment for the microbiologic air quality determination in the barns. The aspect we signalled here is beneficial both

Table 7. Type and frequency distribution (%) of airborne bacteria isolated in dairy barns in the morning and in the evening**.**

Bacteria	Morning	Evening
Staphylococci		
Staphylococcus aureus	11.7	10.3
Staphylococcus epidermidis	25.2	24.6
Staphylococcus lentus	14	15.5
Staphylococcus sciuri	14.7	16.2
Staphylococcus simulans	12.6	13.7
Staphylococcus xilosus	21.8	19.7
Streptococci		
Aerococcus viridians1	20.5	21.6
Aerococcus viridians2	18.2	19.4
Aerococcus viridians3	19.9	17.5
Enterococcus faecalis	24.4	26.3
Streptococcus bovis	7.3	9.2
Streptococcus pneumonie	8.3	5.5
Others Gram-negative bacteria	1.4	0.5
Citrobacter freundii	11.1	8.7
Enterobacter agglomerans	23.7	20.7
Escherichia coli	29.5	33.3
Klebsiella pneumonie	5.5	7.3
Proteus mirabilis	12.4	13.2
Proteus vulgaris	8.8	9.6
Pseudomonas spp.	7.3	5.3
Others	1.7	1.9

Table 8. Type and frequency distribution (%) of airborne fungi isolated in dairy barns in the morning and in the evening.

Fungi	Morning	Evening
Alternaria spp.	1.7	2.4
Aspergillus spp.	33	31.6
Cladosporium spp.	6.8	7.5
Fusarium spp.	3.6	5.2
Mucor spp.	2.6	3.2
Penicillium spp.	24.2	21.2
Rhizopus spp.	5.3	7.2
Scopulariopsis spp.	3.4	2.7
Yeast	19.4	19

for the researchers and for the farm workers. For example, in the evening the researchers can do their work calmly, without disturbing the beginning of the workday in the farms. Furthermore, the farm workers would be more cooperative because their working schedule would not be modified such as in the case of morning determinations, when they must be in the farm at least two hours earlier. Regarding fungi, the results are also consistent with literature data, where the total fungi count in dairy cattle houses ranges from 10^3 to 10^9 CFU/m^3. Hanhela et al. (1995) found concentrations of 10^2 to 10^7 CFU/m^3 for viable fungi in Finnish cow barns and Duchaine et al. (1999) reported about 10^6 CFU/m^3 levels of viable airborne fungi in dairy farms from Quebec, Canada. Adhikari et al. (2004) found a mean concentration level of 1.6×10^2 to 2.2×10^3 CFU/m^3 in cattle shed in India and more recently Matković et al. (2009) reported that fungi numbers in stable housing facilities of dairy cows range from 3.98×10^3 to 5.11×10^4 CFU/m^3. The increasing concentrations of fungi in the air indicate the presence of continuous contamination sources and a raised risk of mycoses and of allergic conditions, through repeated contacts with the fungi. Wet and humid conditions induce decomposition of raw organic materials in cattle barns, which provide suitable condition for growth of fungi and consequently increase the airborne spore load. Within the determined microclimate parameters, only relative humidity exceeds the recommended value in dairy cattle barns in the cold season (NSVFSAR, 2007). Increased humidity seems not to affect the concentrations of total and gram-negative

bacteria (Banhazi et al., 2008), but favours the apparition of fungi (Tang, 2009).

In our study, gram-positive bacteria such as staphylococci and streptococci, predominate in the air of the barns (up to 80%) (Figure 1). This fact is due to their high resistance in the environment (Hartung, 1992). It is asserted that the gram-negative bacteria isolated from the air of the barns represent a minor fraction of the total bacteria, between 0.02 and 5.2% (Zucker et al., 2000 a, b; Matković et al., 2007), as it was ascertained in our study as well (Figure 1). A possible reason for the smaller proportion of airborne gram-negative bacteria in livestock production systems is that they are more vulnerable to environmental risks such as oxidation, radiation, and dehydration, probably because of their thinner cell walls (Pal et al., 2007). As potential endotoxin carriers, gram-negative bacteria represent a very important microorganism group, which may negatively affect animal health. Thus, it is assumed that their numbers in the air should not exceed 10^3 CFU/m^3 (Clark et al., 1983). The low number of gram-negative bacteria isolated in the air samples did not imply that the air was free from their endotoxins. 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 total number of mesophilic bacteria represents a basic assessment criterion of air hygiene quality. The microbial load of the air indicated through the total number of mesophilic bacteria is influenced by several factors, such as the number of housed animals, the breeding technology used, the flooring type, the bedding materials, the microclimate quality, the dust concentration, the ventilation level and so on. As a cause for high air contamination levels, Lange et al. (1997) indicated an improper functioning of ventilation systems, storage moisture of feed rations, kinds of work practice and climatic conditions. In our study, the number of the microorganisms found in the air was higher in barns with more than 50 cows, in those provided with bedding and in dirty barns. Of course, the poor ventilation in most of the investigated barns plays an important role in the high degree of microbial contamination of the air. The increasing concentration of airborne microorganisms concomitantly with the number of animals was observed in several studies (Hinz and Linke, 1998; Predicala et al., 2001). Animals represent an important source of airborne microflora with implications in pathology. They release epiphytic germs from their skin, mucous membranes and upper respiratory tract by exhalation; and potentially pathogenic germs by secretions and excretions eliminated by cough, sternutation, gynaecologic and mammary disorders, various injuries and lesions. Housing systems with bedding causes more air quality problems, although such housing systems are generally thought to be more beneficial for animal welfare (Kim et al., 2008). The type of bedding material also affects the concentration of microorganisms in the air (Banhazi et al., 2008).

In the present study, straw bedding, which generates large amounts of dust and bacteria, was used in approximately 81% of the barns. The effect of barn cleanness on the concentration of airborne bacteria and fungi in our knowledge was not investigated in dairy cow shelters but the studies made on other farm animal species (for example pigs) show that sub-optimal hygiene is one of the main causes of high bacterial concentrations (Banhazi et al., 2008). Although our results confirm this, we still believe that more research is needed in this domain, especially if we take into account that certain authors have views to the contrary. For example, Duchaine et al. (2000) reported that a housing system that appeared cleaner contained more airborne bacteria than one that appeared dirtier. There is little information available, and often contradictory, about the correlation between microclimate factors and airborne microorganisms in farm animal houses. The significant correlation between the air temperature and the concentration of the bacteria in the air was highlighted in a recent study (Dungan et al., 2011) in an open dairy cow barn. In pig houses the relation was signalled by Banhazi et al. (2008). In our study the relative humidity of the air correlated positively with the number of the airborne fungi, an issue also reported by other researchers (Tang, 2009; Reanprayoon and Yoonaiwong, 2011).

Many of the identified species have pathogenic potential, their presence in the air indicating an increased risk of disease for animals and humans. *S. epidermidis* is part of the normal flora of the skin, skin glands and mucous membranes of humans and animals. It is an opportunistic pathogen for humans that can cause urinary tract infections, wound infections, endocarditis, and septicaemia (Siqueira et al., 2002). *S. xylosus* is virtually defined as a nonpathogenic *Staphylococcus*, but a few strains of *S. xylosus* are related to animal and human opportunistic infections (Tompkins et al., 2004). *Staphylococcus simulans* has been a well-documented animal pathogen causing clinical and subclinical mastitis in sheep and cattle (Fthenakis et al., 1994; Jarp, 1991). Members of the *Staphylococcus sciuri* group are widespread in nature, and they can be isolated from a variety of farm animals, pets, and wild animals, as well as from various food products of animal origin (Hauschild and Schwarz, 2003). However, they have been associated with serious infections and, most frequently, wound infections (Shittu et al., 2004). *S. aureus* is a pathogenic bacterium which may cause purulent infections or septicaemia in humans and animals. It is one of the causal agents of mastitis in dairy cows (Roberson et al., 1994). *A. viridans* has been associated with different human infections such as endocarditis, urinary tract infections, arthritis, or meningitis (Gopalachar et al., 2004). *A. viridans* has also been isolated from the milk of cows with subclinical mastitis

(Devriese et al., 1999). Enterococci are part of the normal intestinal flora of humans and animals but are also important pathogens responsible for serious infections. *E. coli* is a common microorganism in the air environment, particularly in animal houses and their surroundings (Zucker et al., 2000a). *E. agglomerans (Pantoea agglomerans*) is member of the Enterobacteriaceae group that inhabits plants, soil, water and such species includes bacteria reported as both commensal and pathogen for animals and humans (Gavini et al., 1989). Zucker et al. (2000a) stated that in animal houses using straw as bedding material *E. agglomerans* was most frequent, whereas in animal houses without litter *E. coli* was mainly found. The main source of *E. agglomerans* is probably the bedding material and the food of the animals and for *E. coli* the faeces of the animals. We observed only that *E. coli* was present more frequently in the dirty barns.

Regarding the fungi, in our study members of the genera *Aspergillus, Penicillium Cladosporium* and yeasts were identified more frequently. These results are similar to the findings reported by many other authors for indoor environments of the same study area (Hartung, 1992; Seedorf et al., 1998; Adhikari et al., 2004; Matković et al., 2007, 2009). The dominant species correlate closely with fungal infections. The most frequent aerosol fungi belong to genus *Aspergillus*, some of which are opportunistic pathogens. Some *Aspergillus* may produce aflatoxins that induce tumors or reduce white blood cell counts. Death due to aflatoxins has been reported in humans, animals and birds (Denning, 1998). The second most frequent species identified belongs to genus *Penicillium* that infects human beings who are affected by leukaemia or lymphoma. Some species may infect brain or lungs, producing ochratoxins (Pitt, 1994). The third most frequent genus was *Cladosporium.* The airborne spores of *Cladosporium* species are significant allergens, and in large amounts they can severely affect asthmatics and people with respiratory diseases (Kurup et al., 2000). Prolonged exposure may weaken the immune system. *Cladosporium* species produce no major mycotoxins of concern, but do produce volatile organic compounds associated with odours. Various yeasts are commonly identified in air samples. Yeasts may be allergenic to certain individuals when present in sufficient concentrations.

The study showed that the number of bacteria and fungi is quite variable and in many cases high in the air of dairy cattle barns with tie stalls. The isolated microbial flora with pathogenic potential was represented mostly by gram-positive species, some of which are cited to have conditioned pathogenic potential. Some recorded fungi were earlier reported as allergenic, toxic and pathogenic for occupational workers as well as cattle population. Even if the number of bacteria and fungi was influenced by all of the investigated factors, we consider barn hygiene to be important in the reduction of the number of airborne bacteria, because it can be improved relatively easily by respecting the elementary hygiene rules in the barns. In order to reduce the microbial load in the air, it is also necessary to ameliorate the ventilation systems in dairy cattle barns in the future.

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