Pol. J. Environ. Stud. Vol. 21, No. 4 (2012), 1061-1066

Original Research

Microbiological Contamination of Air Inside and Around Stables during Different Seasons of the Year

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Received: 27 July 2011
Accepted: 8 December 2011

Abstract

The aim of the study was to determine the level of air bacteria and fungi in the horse stable and the emission of microorganisms to the external environment during different seasons of the year. The predominating fungi in the stable and outside were also identified. These results demonstrate that both in the stables and outdoors, the number of microorganisms was the highest in the summer. This tendency was especially noticeable in the case of the concentrations of fungi outside the building, when the average value was significantly higher ($P<0.01$) than in other seasons. No statistically significant differences in the concentrations of airborne microorganisms at different distances from the stable were observed. *Penicillium* sp., *Fusarium* sp., and yeast predominated inside and outside the stable.

Keywords: horses, air sampling, bacteria, fungi, emission

Introduction

The microbiological contamination of air presents an important aspect of zoohygiene and the concentration of bacteria, fungi and their toxins in animal houses, as well as other factors, influence animal welfare. There are no regulations that determine the maximum permissible level of microbial air contamination in livestock buildings. It is very difficult to determine the criteria, which would be commensurate with current animal keeping conditions and systems. Therefore, it seems important to conduct research in this overlooked area.

Much attention is given to microbiological contamination and the possible reduction of contamination in buildings housing poultry and pigs, where the high animal density and favourable microclimatic conditions cause a high concentration of bioaerosol [1-3]. Such studies rarely are carried out in stables. Despite the relatively clean air in horse buildings, the concentration of airborne microorganisms that exceed the range of 103 CFU/m^3 may increase the risk of animal diseases [4]. Bacteria, viruses, moulds, bacterial endotoxins, dusts, etc. are potentially able to induce equine airway inflammation by initiating infection, by inducing allergy, by direct toxicity, or indirectly by overwhelming the pulmonary defence mechanism [5]. Horses, kept in closed stables, inhaling air with organic pollutants, mainly from the litter and hay, suffer from a disease called recurrent airway obstruction (RAO). The prognosis in the treatment of RAO largely depends on the elimination or reduction of environmental allergens [6]. Elfman et al. [7] point out that horses, enthusiasts, and caretakers who spend much time in stables may be affected by the harmful bioaerosol and they could show signs of bronchial obstruction.

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Depending on various technical and management systems in the stables (their construction, size, horse density, ventilation system, microclimatic conditions, and type of bedding) the microbiological contamination can reach different levels [7-9]. Therefore, examinations concerning the concentration of airborne microorganisms in different stables should be performed to determine the optimal concentration of contamination in indoor air, where horses and people reside.

Material and Methods

Our investigation was conducted in a wooden stable with a total area of 394 m^2. There were 10 stalls in the building (16 m^2 each), situated on one or both sides of a central walkway. A utilitarian attic, where the hay was kept during the whole year, was located above the building. In the stable were 7 different horses used for recreation. Microbiological examinations of the air were conducted in each season of the year, from February to November 2010, twice a month. Samples to be analyzed were collected at 7 a.m., before the feeding time in the stable. A series of three duplicated samples were taken for each group of microorganism, at three sites in the building, at approximately 1.5 m above ground level, in front of the stall entrance. The emission level from the stable was determined similarly, with sampling points situated 1.5 m, 5 m, and 15 m from the stable, taking into account the direction of the wind. Air sampling was collected by the collision method using the Air Ideal sampler at a flow rate of 10 l/min. Two groups of microorganisms were determined: total aerobic mesophilic bacteria and total fungi. The airborne bacteria collected on a commercial agar medium (TSA, Envirocheck® Settle plates, Merck) were incubated at 37ºC for 24 hours, and airborne fungi were incubated on commercial Sabouraud's medium (Envirocheck® Settle plates, Merck) at 25ºC for about one week. The number of colonies on Petri dishes was determined with a Colony Star counter. The total concentration of aerobic mesophilic bacteria and fungi were corrected using Feller's formula. Based on morphological criteria [10-11] the predominating fungi were identified after incubation.

Thermal and humidity conditions both inside and outside the stable also were monitored during the entire period of investigation. The temperature (ºC) and relative humidity (%) were recorded every 15 minutes using the thermohigromether LB 520.

The data concerning microbial contamination levels were verified statistically by a one factor analysis of variance. The significance of differences between the mean values of the investigated parameters were determined by Duncan's test, using Statistica 9.0 software.

Results and Discussion

The average temperature and relative humidity inside and outside the stable throughout the study are presented in Figs. 1-2. During the period from February to November the temperature of the air in the stable was only slightly higher (0.7-2.6ºC) than outside. It seems that the wooden construction and incomplete stocking density were influenced by the poor thermal insulation of this building. The relative humidity of air in winter was about 20% higher in the stable than outside, and it even reached 95%, but after that, in the following months the difference was reduced to a few percent. In November the air humidity in the stable was approximately 1% lower than the humidity outdoors (in the stable 89%, outside 90%). The lowest average monthly humidity was observed in April (indoors 81%, outdoors 62%).

Relatively high temperatures and humidity in the summer season encouraged the formation of microorganisms in this environment. Tables 1-4 present average values of airborne bacteria and fungi inside and outside the stable. These results demonstrate that both in the stables and outdoors, the number of microorganisms was the highest in the summer. This tendency was especially noticeable in the case of the concentrations of fungi outside the building (Table 4), when the average value was significantly higher ($P < 0.01$) than in other seasons. In the winter, the outdoor concentration of fungi was by 1.4 $\log_{10}$ CFU/m^3 lower than in summer, and in the spring and autumn by 0.8 $\log_{10}$ CFU/m^3. A statistically significant difference ($P<0.01$) in the number of bacteria in the stable was also observed between the summer and the autumn (Table 1), but it should be mentioned that during the winter the concentration of

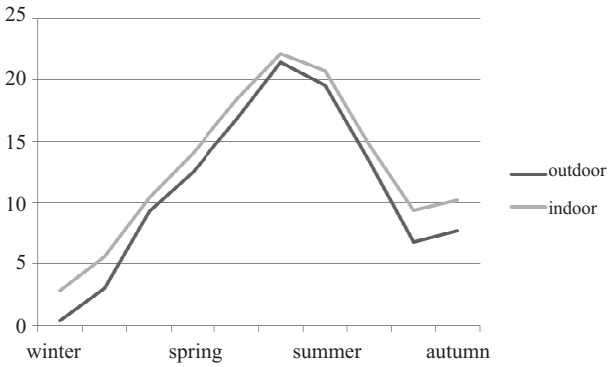

Fig. 1. Average temperatures (ºC) outdoors and in the stable.

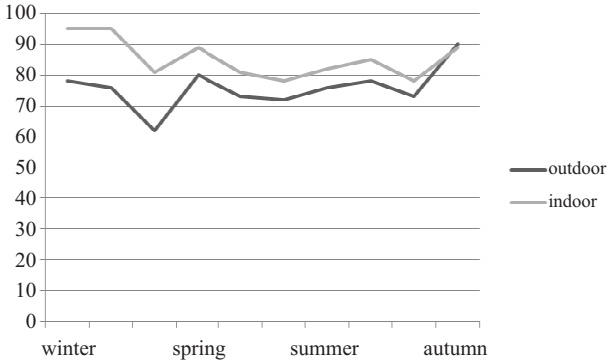

Fig. 2. Average relative humidity (%) outdoors and in the stable.

Table 1. Average values (x±SD) of aerobic mesophilic bacteria in the stable during particular seasons (log_{10} CFU/m^3).

Measuring sites	Seasons				Total sites
	winter	spring	summer	autumn	
I	3.88±0.67	3.63±0.29	4.40±0.59	3.50±0.32	3.89^{C}±0.61
II	4.12±0.92	4.08±0.29	4.76±0.48	4.09±0.44	4.30^{B}±0.63
III	4.90±0.56	4.76±0.57	4.80±0.61	4.66±0.46	4.78^{A}±0.63
Total seasons	4.30^{a}±0.83	4.15±0.61	4.66^{A}±0.57	4.08^{Bb}±0.62	4.32±0.69

Values denoted by different letters are significantly different:
A, B, C – at a level of p≤0.01
a, b – at a level of p≤0.05

Table 2. Average values (x±SD) of fungi in the stable during particular seasons (log_{10} CFU/m^3).

Measuring sites	Seasons				Total sites
	winter	spring	summer	autumn	
I	3.48±0.19	3.80±0.15	3.74±0.29	3.37±0.26	3.61^{Bb}±0.28
II	3.64±0.18	3.78±0.24	3.75±0.23	3.73±0.28	3.73^{Ba}±0.23
III	3.94±0.24	3.89±0.09	3.96±0.29	3.83±0.31	3.91^{A}±0.25
Total seasons	3.69±0.28	3.82±0.17	3.82±0.28	3.65±0.34	3.75±0.28

Values denoted by different letters are significantly different:
A, B, C – at a level of p≤0.01
a, b – at a level of p≤0.05

Table 3. Average values (x±SD) of outdoor aerobic mesophilic bacteria during particular seasons (log_{10} CFU/m^3).

Distance of stable	Seasons				Total distances
	winter	spring	summer	autumn	
1.5 m	2.60±0.51	2.68±0.37	3.14±0.74	2.68±0.64	2.80±0.61
5 m	2.56±0.50	1.91±0.74	2.45±0.31	2.65±0.55	2.40±0.57
15 m	2.07±0.96	2.34±0.31	2.60±0.55	2.33±0.53	2.36±0.62
Total seasons	2.41±0.69	2.31±0.58	2.73±0.62	2.56±0.57	2.52±0.63

bacteria in the stable was slightly lower than in the summer (the difference was only 0.4 log_{10} CFU/m^3), but it also was significantly different (P<0.05) when compared to the autumn. During the spring season, the average value of airborne bacteria was similar to the autumn period, and these values were lower than in the summer and winter, but the differences were not statistically significant. A similar trend was reported by Elfman et al. [7], who conducted the study of microbiological contamination of air in the stable (also constructed from a wooden frame) in the summer and winter seasons. In their investigation, the concentration of bacteria was higher in January and in September (above 4-5 million CFU/m^3) and lower in March (about 2 million CFU/m^3). According to the mentioned authors, the greatest microbiological contamination of air during the winter was due to the poor natural ventilation, because in a temperate climate such as Sweden, stables are often closed during winter time. The high concentration of bioaerosol during the summer is likely connected with the high temperatures and humidity, which support the growth of microorganisms.

The average value of outdoor bacteria was the highest in summer (2.7 log_{10} CFU/m^3) and the lowest in spring (2.3 log_{10} CFU/m^3), and it should be noted that in every investigated season the concentration of bacteria was the highest when closest to the stable (Table 3). In the summer it was over 3 log_{10} CFU/m^3 and in other seasons 2.6-2.7 log_{10} CFU/m^3. In further areas of measurement (5 m and 15 m) the average number of growth colonies was approximately 2.4 log_{10} CFU/m^3. The lowest variability was observed in

Table 4. Average values (x±SD) of outdoor fungi during particular seasons (log_{10} CFU/m³).

Distance of stable	Seasons				Total distances
	winter	spring	summer	autumn	
1.5 m	2.34±0.77	3.09±0.13	3.60±0.59	3.01±0.36	3.06±0.68
5 m	2.12±0.88	2.97±0.25	3.59±0.50	2.97±0.37	2.96±0.75
15 m	2.13±0.64	2.92±0.32	3.71±0.76	2.86±0.51	2.97±0.81
Total seasons	2.20^{C}±0.73	2.99^{B}±0.24	3.64^{A}±0.60	2.95^{B}±0.40	3.00±0.74

Values denoted by different letters are significantly different:
A, B, C – at a level of $p \leq 0.01$

the case of the airborne fungi in the stable (Table 2), and the average values were from 3.8 log_{10} CFU/m³ in the spring and summer to 3.7 log_{10} CFU/m³ during the autumn. Similar results are presented by Nardoni et al. [8], who observed significantly more airborne fungi in the summer, winter and spring when compared to the autumn.

According to Dutkiewicz et al. [4], the risk of the harmful effects of bioaerosol on the animal's respiratory system is increased by large concentrations of airborne microorganisms in the range of 10^3-10^8 (3-8 log_{10}) CFU/m³ of air. In our own investigation the numbers of bacteria ranged from 10^2 to 10^5 (2.8-5.7 log_{10}) CFU/m³ and the mean of airborne bacteria concentrations during the whole investigative period was 10^4 (4.3 log_{10}) CFU/m³. Similar results were reported by Dutkiewicz et al. [4], who conducted analyses of bacterial contamination of air in the stables of reproductive stallions, race horses, mares, and foals. In their study, the concentration of mesophilic bacteria ranged from 2.6×10^4 CFU/m³ to 1.5×10^5 CFU/m³. Higher concentrations of airborne bacteria in the stable were noted by Elfman et al. [7]. In their examination, the levels of colony-forming units exceeded 10^6 CFU/m³ (the range was $2\text{-}5\times10^6$ CFU/m³). The cause of this fact might be connected with the number of horses in that stable, which was more than twice as crowded as the stable in our investigation. However, some results showed that the concentration of airborne microorganisms did not always depend on animal density [4].

In their study comparing two stables, bacterial contamination was higher in the stable for 9 rather than for 19 horses. The type and quality of bedding material as well as the ventilation system may have a great effect on bioaerosol concentration. Houben [9] conducted a study concerning microbiological analysis in 11 stables with different ventilation systems and beddings during winter. One of the buildings used mechanical ventilation and wood shaving bedding and others used natural ventilation and straw bedding. The number of total bacteria was lower in the stables with natural ventilation and straw bedding (2.9×10^3 CFU/m³) than in the stable with mechanical ventilation and shaving bedding (4.8×10^3 CFU/m³), but the mean levels of bacteria in stables with natural ventilation were variable (10^2-10^4 CFU/m³) due to the different construction of the stables. Dutkiewicz et al. [4] reported that Gram-positive cocci, mostly coagulase-negative strains of *Staphylococcus,* were the most numerous mesophilic bacteria in horse stables. Gram-negative flora (mainly *Pseudomonas*, *Acinetobacter calcoaceticus*, and *Erwinia herbicola*) did not exceeded 8% of the total count of bacteria.

In our own investigation, the concentration of fungi ranged from 10^3 CFU/m³ to 10^4 CFU/m³, and the mean level of air fungi during the whole examination was 10^3 (3.8 log_{10}) CFU/m³. A similar trend was reported by Dutkiewicz et al. [4]. They observed a range from 1.7×10^3 CFU/m³ to 2.8×10^4 CFU/m³ of airborne fungi in the stables for different groups of horses. Nardoni et al. [8] analyzed fungal air contamination in two different stables with individual stalls and on the open paddock. The values of fungal concentrations showed differences ranging from 1.8×10^3 CFU/m³ (paddock) to more than 3.0×10^3 CFU/m³ in stables. It should be emphasized that the buildings were much more intensively ventilated than in our own study due to the warmer climate, so the fungal concentrations were lower. In the Swedish study [7] the concentration of airborne fungi was even twice as high as in our investigation, and during all seasons this value exceeded 1×10^6 CFU/m³. Houben [9] reported that in the stable with mechanical ventilation the mean value of the fungi concentration (7.7×10^2 CFU/m³), unlike bacterial concentration, was lower than in stables with natural ventilation (3.4×10^2 CFU/m³).

The analysis of microbiological contamination of air in the stable (Tables 1-2) showed differences between the numbers of bacteria and fungi colonies in particular sites. The statistically highest concentration of bioaerosol was noted at the third point, which was situated furthest from the door. The velocity of air was lower there, so the high humidity and temperature encouraged the growth of microorganisms. In addition, the stalls were situated on both sides from that point, while at the other two points the stalls were only on one side of the walkway. Such a high number of bacterial and fungal colony-forming units may also result from the fact that the hay and straw were stored near that area.

No statistically significant differences in the concentrations of airborne bacteria at specific distances from the stable were observed. However, the average value of aerobic mesophilic bacteria was greater at the nearest point (1.5 m) from the stable (Table 3). The lowest variability was observed in the case of fungi. In the summer the average concentrations of airborne fungi were even the highest at

Table 5. Genera of fungi cultured in the stable and outside during particular seasons.

Genera of fungi	In stable				Outside			
	winter	spring	summer	autumn	winter	spring	summer	autumn
the most common								
yeast	25%	26%	38%	7%	-	21%	66%	25%
Penicillium	50%	34%	44%	43%	22%	7%	3%	12%
Fusarium	14%	21%	8%	41%	50%	59%	18%	49%
Aspergillus	5%	5%	4%	2%	13%	3%	-	2%
Cladosporium	1%	6%	4%	5%	12%	7%	9%	6%
others (less than 2%)								
Botrytis	-	+	+	-	-	+	+	+
Mucor	+	+	+	+	-	+	-	-
Rhizophus	-	+	-	-	-	-	-	-
Rhizomucor	-	-	+	-	-	-	-	-
Trichoderma	-	+	-	+	-	-	-	-
Trichothecium	+	+	+	-	-	-	-	-
Alternaria	-	+	+	+	-	+	+	+
Epicoccum	-	-	+	-	-	+	+	-
Trichophyton	+	-	+	-	-	+	+	-

the furthest distance (15 m) from the stable. It should be noted that there were mainly fungi of the genera *Fusarium*, *Cladosporium*, *Alternaria*, which are typical for the external environment, whereas nearest the building the most common were *Penicillium*, *Aspergillus,* and yeast.

Tables 5 show values of fungi cultured from the stable and from outside in particular seasons. In the stable, *Penicillium* sp. predominated, forming an average from 34% in the spring to 50% in the winter on total count. Yeast was also a relatively common fungi in the stable. In summer it reached an average of 38%, but in autumn the percentage of yeast decreased to 7%. In contrast to yeast, the percentage of *Fusarium* sp. was the highest in fall, forming on average 41%. These fungi were also common in the stable during other seasons, but the proportions were lower. The population of *Aspergillus* sp. was considerably lower, but that genera was isolated in each season. During the experimental period, *Cladosporium* sp. were incubated, but the results demonstrated that this genera is more typical for the external environment. In winter the percentage of outside *Cladosporium* sp. formed 12%, and in the stable the proportion was considerably lower, (probably due to poorer ventilation). Some results showed [7] that *Cladosporium* and the *Alternaria* species could be the most common in stables.

Outdoors, the most frequent fungi were *Fusarium* sp., which are classed as a typical field fungi. Similarly, as in the stable, the lowest percentage of *Fusarium* sp. was identified in summer, where at the same time yeast was most popular. The other common outdoor fungi were *Penicillium*, especially in summer, *Aspergillus* and *Cladosporium*. Occasionally, in the stable as well as outdoors were isolated *Mucoraceae*, *Botrytis*, and *Trichoderma*. In the stable air *Trichothecium roseum* was rarely incubated and dermatophytes (*Trichophyton* and *Epicoccum*) sporadically appeared. Yeast and mold also contribute to mycoses, and genera such a *Fusarium*, *Aspergillus*, and *Penicillium* are important moulds that produce mycotoxins.

In a study conducted by Dutkiewicz et al. [4], *Aspergillus*, *Penicillium*, *Eurotium*, *Monilia,* and *Alternaria* were isolated most frequently. The authors point out that these were allergenic species, which might complement the pathogenic effect of termophilic actinomycetes. They may increase a horses predisposition to the occurrence of RAO. Similarly as in our own study the results of Nardoni et al. [8] showed that *Penicillium* was most frequently encountered by their investigators from samples of stable air (73%). *Aspergillus* and *Mucoraceae* were detected in 61% and 28%. The *Mucoraceae* in the stable were isolated during all seasons, while outside (on the paddock), similarly as in our own study (Table 5), they were not cultured in the spring and autumn periods as in our own study. Other fungi such as *Alternaria*, *Cladosporium*, *Fusarium*, *Beauveria*, and *Drechslera* were recovered occasionally in spring and summer only. *Fusarium* and *Cladosporium* were cultured in summer from air in the open paddock [8], which is consistent with our own results found outside the stable (Table 5).

Conclusion

To summarise the study results, it can be said that the high temperature and humidity in summer stimulated the formation of air bacteria and fungi in stables. In the winter the microbial air contamination in stables can also be high, because the ventilation rate in this period is low. Even in a small stable for 7 horses, the concentration of the microorganisms can exceed 10^3 cfu/m^3, which may promote allergy and inflammation of the respiratory system in people and/or horses. Some of the stable's airborne microflora may contain potential disease agents. This type of stable is not a risk to the external environment, because the emissions of bacteria and fungi are relatively low.

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