Microbial Air Contamination in Indoor and Outdoor Environment of Pig Farms

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Abstract

Ensuring a good air quality in pig farms is important for the health of animals and human workers. The aim of this study was the assessment of the microbiological quality of the air inside the pig houses and outside of these. The study was accomplished in two pig-fattening farms in Cluj County. The microbiological air quality was assessed in the cold and warm season, by determination of the total counts of mesophilic bacteria, staphylococci, streptococci, gram-negative bacteria and fungi. The bacterial and fungal counts varied in the air of the investigated farms. In relation to the season the mean counts of bacteria and fungi were significantly higher ($P < 0.05$) in the cold season. No significant differences ($P > 0.05$) were found between the values of the parameters determined from the indoor air and those obtained outside, from a distance of 5 m from the pig houses. The numbers of the bacteria and fungi in the outdoor air lowered as the distance from the farms increased, the differences being significant at 25 and 50 m ($P < 0.05$). The results of the study show a high bacterial contamination of the indoor and outdoor air of the pig farms.

Keywords: bacteria, fungi, microbial air contamination, pig farms.

1. Introduction

Ensuring good air quality in pig farms is important for the health and welfare of animals and caretakers, as well as for the external environment [1-3]. In pig farms, animals and farm workers are exposed to high concentrations of bacteria and fungi as well as endotoxins and mycotoxins produced by them [4]. Airborne microorganisms may cause various negative effects, especially infectious and allergic diseases [2]. The concentration of microorganisms in the indoor air depends on many factors, such as the type of the building, the number of animals, the ventilation type and the microclimatic conditions [5-7]. Improper hygienic conditions may be causes of considerable microbial air pollution in pig farms [8]. As a result, controlling the microbiological quality of the air in pig houses is extremely important and should be performed regularly. Urgent hygienic measures are required in those barns where the microbiological air quality is poor, having significant deviations from the recommended standards.

The aim of this study was the assessment of the microbiological quality of the air inside the pig houses and outside of these, at different distances. The indoor and outdoor air quality was established based on the number of airborne bacteria (mesophilic, staphylococci, streptococci, Gram-negative) and fungi.

2. Materials and methods

The study was conducted in two modern growing-fattening pig farms in Cluj County (farm A and farm B). Farm A housed 3100 Duroc pigs in three barns and farm B had 1000 Pietrain pigs, housed in two barns. The ventilation was mixed type in Farm A (natural and artificial), and only mechanical in farm B.

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The microbiological indoor and outdoor air quality was assessed in both farms, both in cold season (January-February) and in warm season (May-June). The outdoor samples were taken at 5m, 25m and 50m away from the pig houses.

To analyze the airborne micro-flora, the number of mesophilic bacteria, staphylococci, streptococci, gram negative bacteria and fungi was considered. Eight different determinations were done in each farm, one week apart, both in the cold and in the warm season. The samples were collected using the MAS-100 device (Merck, Germany), from three different places in each farm building, finally calculating the average value.

The volumes of the samples were of 10 litres for the indoor air and 100 litres for the outdoor air, based on previous studies that have shown that these amounts of air are sufficient for the microbiological analysis.

Bacteria and fungi were collected in Petri plates on different standard culture media: Columbia agar for mesophilic bacteria, Chapman agar for staphylococci, Endo agar for Gram negative bacteria, blood agar for haemolytic bacteria and Sabouraud agar for fungi. The bacterial culture plates were incubated at 37^{o}C for 24 hrs, while the fungal culture plates were incubated at 22^{o}C for 5 days.

After incubation, the total number of colony forming units (CFU) for the bacterial and fungal air-flora was recorded and converted to colony forming units per cubic meter (CFU/m^3), using Feller's formula [9].

The results were analyzed using the SPSS 17.0 software. The descriptive statistics included mean, standard deviation, median, minimum and maximum for each parameter. The data obtained were analyzed to determine the significance of the differences using the Mann-Whitney nonparametric test.

3. Results and discussion

Table 1 shows the results obtained in farm A (barns 1-3) in cold and warm season. Staphylococci in the first building (1A) ranged from 11.63 to 13.74%, streptococci from 54.41 to 75.31%, and Gram-negative bacteria from 0.22 to 0.59% of total mesophilic bacteria, depending on the season.

Table 1. Airborne bacterial and fungal parameters in farm A (barns 1-3) in cold and warm season

Season	Parameter	Barn 1A		Barn 2A		Barn 3A	
		Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)
Cold	Mesophilic bacteria (x10^5 CFU/m^3)	3.51 (1.92)	3.09* (0.73-6.72)	6.57 (3.37)	7.32* (8.07-8.10)	9.25 (0.47)	9.31* (8.42-9.89)
	Staphylococci (x10^4 CFU/m^3)	4.82 (0.39)	4.92 (4.31-5.41)	4.43 (0.69)	4.47 (3.40-5.15)	4.56 (0.40)	4.59 (3.94-5.04)
	Streptococci (x10^5 CFU/m^3)	1.91 (0.55)	1.84 (1.20-2.62)	5.30 (1.06)	5.38 (3.27-6.43)	7.63 (0.44)	7.76x10^5 (6.95-8.13)
	Gram-negatives (x10^2 CFU/m^3)	7.62 (1.30)	8.00 (5.00-9.00)	8.12 (0.83)	8.00 (7.00-9.00)	8.12 (0.83)	8.00 (7.00-9.00)
	Fungi (x10^4 CFU/m^3)	7.23 (0.12)	0.735 (0.50-8.70)	0.897 (0.05)	0.885 (0.83-0.99)	0.85 (0.05)	8.40 (7.80-9.40)
Warm	Mesophilic bacteria (x10^4 CFU/m^3)	5.21 (1.04)	1.04* (3.12-6.37)	7.24 (0.66)	7.30* (6.22-8.05)	7.48 (0.50)	7.43* (6.89-8.20)
	Staphylococci (x10^3 CFU/m^3)	6.06 (3.30)	3.30 (1.40-9.40)	3.36 (0.59)	3.30 (2.70-4.30)	5.68 (0.46)	5.60 (5.10-6.50)
	Streptococci (x 10^4 CFU /m^3)	3.93 (0.91)	9.15 (2.00-5.04)	5.96 (0.28)	5.95 (5.61-6.52)	6.65 (0.84)	6.71 (5.53-7.96)
	Gram-negatives (x 10^2 CFU/m^3)	3.12 (1.55)	1.55 (1.00-5.00)	37.5 (1.03)	4.00 (2.00-5.00)	3.87 (1.12)	4.00 (2.00-5.00)
	Fungi (x 10^4 CFU/m^3)	2.00 (0.07)	7.55 (0.10-3.00)	0.32 (0.07)	0.30 (0.20-0.40)	0.21 (0.11)	0.20 (0.10-0.40)

CFU/m^3 = colony-forming units in one cubic metre of air.
SD = standard deviation.
* P < 0.05 the difference is significant between cold and warm season.

In the second barn (2A) the staphylococci counts were from 4.64% to 6.73%, the streprococci from 80.59 to 82.32% and a range of 0.12 to 0.52% Gram-negative bacteria. In the third pig house (3A) the proportion of the bacterial groups ranged as follows: 5.05 to 7.60% staphylococci, 82.56 to 88.95% streptococci and 0.08 to 0.52% Gram-negative bacteria.

The descriptive statistics for the results obtained in farm B (mesophilic bacteria, staphylococci, streptococci, Gram-negative bacteria, and fungi) are listed in Table 2.

Table 2. Airborne bacterial and fungal parameters in farm B (barns 1-2) in cold and warm season

Season	Parameter	Barn 1B			Barn 2B		
		Mean (SD)	Median	Range	Mean (SD)	Median	Range
Cold	Mesophilic bacteria ($x10^6$ CFU/m^3)	1.32 (0.21)	1.24*	1.05-1.72	1.56 (0.17)	1.48*	1.38 -1.82
	Staphylococci ($x10^5$ CFU/m^3)	2.42 (0.33)	2.60	1.89-2.73	2.92 (0.63)	2.90	1.71 -3.89
	Streptococci ($x10^5$ CFU/m^3)	8.80 (0.69)	8.89	7.43-9.41	9.18 (0.64)	9.33	7.93 -9.98
	Gram-negatives ($x10^2$ CFU/m^3)	8.50 (0.92)	8.50	7.00-10.00	7.50 (1.51)	7.50	5.00 -9.00
	Fungi ($x10^2$ CFU/m^3)	5.50 (2.44)	5.50	2.00-9.00	7.12 (1.95)	7.50	4.00 - 9.00
Warm	Mesophilic bacteria ($x10^5$ CFU/m^3)	1.86 (0.43)	1.80*	1.33 -2.63	1.79 (0.28)	1.75*	1.49 -2.22
	Staphylococci ($x10^4$ CFU/m^3)	5.03 (0.35)	5.07	4.45 -5.49	4.21 (0.15)	4.22	4.02 -4.45
	Streptococci ($x10^5$ CFU/m^3)	1.50 (0.19)	1.54	1.23 -1.79	1.60 (0.26)	1.57	1.30 -2.01
	Gram-negatives ($x10^2$ CFU/m^3)	4.50 (1.19)	5.00	2.00 - 6	2.62 (1.30)	2.50	1.00 -4.00
	Fungi ($x10^4$ CFU/m^3)	1.53 (0.48)	1.37	1.03 -2.14	1.18 (0.09)	1.18	1.02 -1.30

CFU/m^3 = colony-forming units in one cubic metre of air. SD = standard deviation.
* $P < 0.05$ the difference is significant between cold and warm season.

In the first barn (1B) staphylococci were 18.3 to 27.08% out of the total mesophilic bacteria, streptococci from 66.63 to 81.28% and Gram-negative bacteria from 0.06 to 0.24%. In the second building (2B), staphylococci ranged from 18.72 to 23.48%, streptococci from 58.85 to 89.61% and Gram-negative bacteria from 0.05 to 0.15%. Other papers reported highly variable data regarding the total number of airborne bacteria in pig fattening units, ranging from 10^4 to 10^6 CFU/m^3 [10-13]. Our results are similar (Table 1, 2), with significantly higher numbers ($P < 0.05$) in farm B. Although there is no standard regulation for the airborne mesophilic bacteria in animal houses, the maximum count recommended by most specialists, also valid in Romania, is 250,000 CFU/m^3. In farm A, the bacterial count exceeded this recommendation by 1.4-1.9 times, while in farm B it was 5.2 to 6.2 times higher. The large number of bacteria could have a negative impact on the health of pigs and caretakers [1, 11].

The number of mesophilic bacteria was significantly higher in the cold season ($P < 0.05$) than in the warm season, in both farms. This was also shown in other studies [14]. The increased ventilation to reduce the high temperatures in the summer months is the main reason for lower levels of microorganisms inside the barns. Yet, the same procedure (increased ventilation) may cause greater diffusion of the particles in the air, therefore leading to higher emissions into the environment. However, other authors reported a higher value for the number of microorganisms during the warm season [15], which causes difficulties in the interpretation of our results in respect to the season.

The airborne flora is of great importance, given its potential pathogenic effects on the health of pigs [16]. The predominant microorganisms revealed

by our study were Gram-positive bacteria, followed by Gram-negative bacteria and fungi (Tables 1, 2). Hartung, 1992 [17] showed that the airborne flora in the animal houses consisted mainly of Gram-positive bacteria, such as staphylococci and streptococci, which may be explained by their higher resistance in the environment. Our results confirmed this finding. In this study, the most frequent bacterial isolates were Gram-positive bacteria, up to 90%. Regarding the hygienic significance, in both farms and both seasons, streptococci prevailed (54.41 to 89.91%). This group of bacteria is an indicator of contamination with nasopharyngeal and oral flora. Some of the streptococci come from other sources (manure, pathological material).

Airborne Gram-negative bacteria in animal houses are a minor proportion of the total number of germs, between 0.02% and 5.2% [18], fact confirmed by the present study. The proportion of Gram-negative bacteria is always lower because of their lower survival rate in the air [18]. Airborne Gram-negative bacteria are an important group of microorganisms, which could adversely affect the animals' health, therefore their number should not exceed 10^3 CFU/m^3 [10].

The concentration of fungi varied in the investigated farms, with averages from $5.5x10^2$ to $7.23x10^4$ CFU/m^3 (Table 1, 2). These results are also consistent with other studies, which indicate that the total numbers of airborne fungi range from 10^3 to 10^5 CFU/m^3 [11, 12]. Fungi are a part of the normal microflora in animal houses, but some species, such as *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp., can cause allergies, asthma and fungal infections [15].

Table 3. The descriptive statistics for the parameters used to assess the outdoor air quality in farm A in cold and warm season

Distance	Parameter	Cold season		Warm season	
		Mean (SD)	Median (Range)	Mean (SD)	Median (Range)
5 m	Mesophilic bacteria (x 10^3 CFU/m^3)	163 (0.29)	161 (1.30-2.22)	19 (0.03)	18 (1.54-2.62)
	Staphylococci (x10^4 CFU/m^3)	4.00 (0.48)	4.05 (3.14-4.77)	1.46 (0.16)	1.43 (1.25-1.71)
	Streptococci (x10^3 CFU/m^3)	121.0 (13.0)	119.0 (107.0-148.0)	4.0 (0.3)	5.0 (3.97-5.04)
	Gram-negatives (x10^2 CFU/m^3)	6.62 (1.59)	7.0 (4.0-9.0)	-	-
	Fungi (x 10^2 CFU/m^3)	56.70 (9.3)	58.0 (40.0-68.0)	4.0 (0.4)	3.9 (3.4-4.8)
25 m	Mesophilic bacteria (x10^3 CFU/m^3)	11.90 (3.7)	18.4* (14.2-26.2)	8.8 (3.9)	4.15 (5.0- 24.4)
	Staphylococci (x10^2 CFU/m^3)	-	-	2.06 (1.33)	1.80 (0.05-4)
	Streptococci (x 10^2 CFU/m^3)	-	-	7.07 (1.04)	6.95 (5.30-8.50)
	Gram-negatives (CFU/m^3)	-	-	-	-
	Fungi (x10 CFU/m^3)	-	-	3.30 x 10 (1.60)	3.50 (1.00-6.00)
50 m	Mesophilic bacteria (x10^3 CFU/m^3)	9.61 (0.86)	9.59* (8.43-10.8)	1.87 (0.32)	1.96 (1.22-2.24)
	Staphylococci (x10^2 CFU/m^3)	-	-	1.15 (0.24)	1.15 (0.8-1.50)
	Streptococci (x10^2 CFU/m^3)	-	-	2.07 (0.55)	2.05 (1.20-2.80)
	Gram-negatives (CFU/m^3)	-	-	-	-
	Fungi (x10 CFU/m^3)	-	-	6.12 (2.70)	6.50 (1.00-9.00)

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

* $P < 0.05$ the difference is significant between inside and outside of the barns.

The airborne flora of enclosed pig houses depends on the production technology and environmental parameters (temperature, relative humidity, air flow rate). The reasons of high levels of air contamination in pig houses, indicated by Lange [6], are: malfunctioning ventilation systems, high humidity of the feed, the growth technology and climatic conditions. Gustaffson, 1997 [5] stated that the operation and location of inlets and exhaust chimneys may have a strong influence on the spread and concentration of indoor air pollutants.

Improper hygiene can also cause considerable microbial pollution. Effective ventilation ensures that the level of air contamination is kept below the critical values reported as threats to animal and human health [19]. The better microbiological quality of the air in the farm A is due to its better ventilation. Although some evidence suggests that reducing the number of animals significantly reduced all species of microorganisms in the air, we did not establish this in the current study. Conversely, a higher number of microorganisms was observed in farm B, where fewer pigs were housed compared to farm A.

The descriptive statistics for the parameters used to assess the outdoor air quality in farms A and B (at 5, 25 and 50m away from the pig houses), in cold and warm season are listed in Tables 3 and 4.

Although smaller, the average values obtained at a distance of 5m from the pig houses are not significantly different from those determined inside. In the same time, the values measured at 25 and 50m were significantly lower ($P < 0.05$) compared to those inside the buildings. This was noticed in both farms in cold and warm season (Tables 3, 4).

Table 4. The descriptive statistics for the parameters used to assess the outdoor air quality in farm B in cold and warm season

Distance	Parameter	Cold season		Warm season	
		Mean (SD)	Median (Range)	Mean (SD)	Median (Range)
5 m	Mesophilic bacteria ($x10^3$ CFU/m^3)	1330.0 (130.0)	1310.0 (1190.0-1350.0)	24.00 (3.00)	25.00 (18.00-26.00)
	Staphylococci ($x10^3$ CFU/m^3)	204.0 (89.0)	223.0 (29.0-289.0)	14.00 (1.00)	17.50 (13.00-27.00)
	Streptococci ($x\ 10^4$ CFU/m^3)	62.20 (16.2)	61.00 (43.3-89.8)	16.00 (0.2)	1.68 (1.2-1.9)
	Gram-negatives ($x10^2$ CFU/m^3)	4.25 (2.12)	4.50 (1.00-7.00)	-	-
	Fungi ($x10^2$ CFU/m^3)	4.87 (2.41)	5.50 (1.00-8.00)	118.00 (46.30)	126.0 (12.3-159)
25 m	Mesophilic bacteria ($x10^3$ CFU/m^3)	475.0 (253.00)	390.0* (184.0-810.0)	19.00 (3.00)	18.4 (14.00-26.00)
	Staphylococci ($x10^2$ CFU/m^3)	444.0 (84.00)	464.00 (323.0-541.0)	5.00 (0.7)	4.8 (4.00-6.00)
	Streptococci ($x\ 10^2$ CFU/m^3)	274.0 (183.0)	184.0 (121.0-643.0)	1.90 (0.2)	0.30 (0.1-0.7)
	Gram-negatives (CFU/m^3)	-	-	-	-
	Fungi (x10 CFU/m^3)	-	-	20.00 (7.60)	17.5 (12.00-35.00)
50 m	Mesophilic bacteria ($x10^3$ CFU/m^3)	3.14 (2.10)	2.62* (1.28-7.43)	1.60 (0.11)	1.58 (1.43-1.77)
	Staphylococci ($x10^2$ CFU/m^3)	-	-	4.02 (1.04)	4.15 (2.10-5.60)
	Streptococci ($x10^2$ CFU/m^3)	-	-	0.40 (0.20)	0.40 (0.10-0.70)
	Gram-negatives (CFU/m^3)	-	-	-	-
	Fungi (x10 CFU/m^3)	-	-	6.00 (2.90)	6.00 (2.00-10.00)

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

* $P < 0.05$ the difference is significant between inside and outside of the barns

The air in both farms was polluted, more intensely in farm B, but outside the farms, it has better quality. Tables 3 and 4 show the reduction of the overall airborne flora with the increasing of distance from the farms. These results are comparable to those obtained in other studies [20]. Sawickaa and Wieland, cited by Karwowska, 2005 [20], showed that the concentration of microorganisms in the vicinity of a pig farm was $2.6x10^3$ CFU/m^3 for bacteria and $3.2x10^2$ CFU/m^3 for fungi.

4. Conclusions

As the study revels, the level of the airborne bacterial contamination is high both inside and outside the pig farms, representing a risk factor for the health of animals and caretakers.

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