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The assessment of gram-negative bacteria in the air of two swine nursery buildings

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ABSTRACT

Farm environments represent a source of gram-negative bacteria and endotoxins that generate the significant health risk of developing respiratory diseases in farm workers and animals. The aim of this study was to conduct a quantitative analysis of gram-negative airborne bacteria and their correlation with the total number of mesophilic bacteria and environmental parameters (temperature, humidity, air velocity, ammonia and carbon dioxide). Air sampling was conducted in two swine nursery buildings. The average values of the total number of mesophilic bacteria were 76×10^3 and 99×10^3 CFU/m^3, while the range of gram-negative bacteria were 1410^3 and 22×10^3 CFU/m^3 in both pig houses. The average concentration of gram-negative bacteria was approximately 18-22% in comparison with the total number of mesophilic bacteria. The number of total mesophilic and gram-negative bacteria was significantly lower in House 1 than in House 2 ($P = 0.05$). In both houses, there was no significant correlation between bacteria count and environmental parameters ($P>0.05$), except for a small negative correlation between gram-negative bacteria count and air velocity ($r = -0.48$, $P = 0.05$). The most prevalent species in both pig houses was *Escherichia coli*. The number of gram-negative bacteria in both swine houses exceeded recommended threshold limit values.

Key words: mesophilic bacteria, gram-negative bacteria, air, swine nursery building

Introduction

Farm environments represent a source of gram-negative bacteria and endotoxins that generate the significant health risk of developing respiratory diseases in farm workers

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and animals. Airborne endotoxins are very often present in swine housing as components of the outer cell membranes of the gram-negative bacteria: *Escherichia coli, Neisseria* sp.*, Haemophilus* sp.*, Pseudomonas* sp. (CLARK et al., 1983; ZUCKER and MÜLLER, 2002). In general, endotoxins consist of a lipid A region associated with an oligo- or polysaccharide chain (ALEXANDER and RIETSCHEL, 2001). Their concentration in the air can be assessed using the kinetic, chromogenic *Limulus* amebocyte lysate (LAL) assay, very often applied in evaluating air quality in occupational settings within the United States (THORNE, 2000). The inhalation of endotoxins can lead to acute or chronic lung inflammatory responses, not only in pigs but also in farm workers (DONHAM et al., 1989). Some studies have reported gram-negative bacterial exposures in swine barns between 7×10^3 and 65×10^5 CFU/m^3 (ATTWOOD et al., 1987; SEEDORF et al., 1998), while others (CHANG et al., 2001) reported significantly lower concentrations of 0.42 to 4.52×10^2 CFU/m^3. To date, an average of 1 to 2% of the total bacteria count in the air of swine barns has been determined to be gram-negative bacteria (SEEDORF et al., 1998). All the above mentioned reasons have led to different studies of gram-negative bacteria in swine confinements and the recommended threshold limit value (TLV) of 1000 CFU/m^3 air (CLARK et al*.,* 1983).

The concentration of airborne microorganisms in a controlled, enclosed swine confinement building depends on production technology and different environmental parameters; temperature, relative humidity, air velocity, and the presence of commonly detected gases (ammonium, carbon dioxide, and hydrogen sulphide). The use of different ventilation systems (natural, artificial or a combination of both) ensures that the air contamination is kept below critical values reported as posing a threat to animal and human health. Previous studies have shown that the facility's air temperature is negatively correlated with the number of microorganisms present at a high ventilation rate (HARTUNG, 1995). However, a Korean survey found a positive correlation of the temperature with bacteria, ammonia and hydrogen sulphide, while the correlation of humidity with the same air pollutants was negative (KIM et al., 2005). The experiment performed in an aerobiocontamination system showed that humidity above 85% causes inactivation of gram-negative bacteria within thirty minutes, demonstrating the proportional surveillance of these bacteria with a decrease of relative humidity in the air (ROBINE et al., 2000). Although it is stated that specified air exchange rates in swine housing keep air contamination under control, an independent Korean study observed that the number of microrganisms in the air was not influenced by the ventilation rate (KIM et al., 2007).

The aim of this study was to conduct a quantitative analysis of gram-negative airborne bacteria in the air of two swine nursery housings and compare this with the total mesophilic bacteria present in the facilities. Furthermore, the correlation of both

airborne bacteria with the environmental parameters (temperature, humidity, air velocity, ammonia and carbon dioxide) was investigated.

Materials and methods

This research was performed in two swine nursery buildings. The first building (Farm A) was 72.5m long, 9m wide and 4m high and had two rooms separated by a corridor. Bacteriological sampling was performed in the room (House 1; 34×8.20×3.27m) with 60 aluminum pens (2.20×1.35×0.70m), a fully slatted floor, and 600 animals. The second building (Farm B) was 40.5m long, 14.5m wide and 6m high. Measurements were done in the room of the building (House 2), which was 13×14×2.50m. Animals were assigned to two types of pens: large (3.28×3.04×0.80m) and small (size 2.98×2.74×0.80 m). Both types of pens had synthetic partitions. All the pens had fully slatted floors with rubber mats (2.98m×0.58m×0.40m) placed in the centre. House 2 had 16 pens and 560 animals. In House 1, ventilation was achieved by natural (windows) and mechanical (fans) system. In House 2 the air exchange was completely regulated by a computerised ventilation system.

The experimental stage was conducted in the cooler part of year (winter and spring) with 8 visits altogether. An SAS 100™ air sampler (PBI International) was loaded with Petri dishes containing nutrient agar (Biolife, Italy) to obtain the total number of mesophilic bacteria. For the enumeration of gram-negative bacteria, chromogenic and fluorogenic C-EC agar (Biolife, Italy) was used. Bacteriological air samples were collected approximately 20 cm above the floor (animal breathing zone) at three different places in each house. The duration of sampling was 10 seconds and the total aspirated volume of air 10 L. Plates were incubated at 37 ºC within 24 to 48 hours. On the C-EC agar only blue-green colonies of gram-negative bacteria were counted. The final numbers of colonies counted were corrected and calculated according to the table supplied by manufacturer. Temperature, relative humidity, and air velocity were recorded by Testo instruments (Testo, Germany), and carbon dioxide and ammonia by Dräger Multiwarn II (Dräger, Germany). Isolated bacterial strains were identified using Gram staining and the API 20 E system (20 100 bioMérieux, France). Data are presented as the number of colony forming units per m^3 of air (CFU/m^3).

Statistical analyses. Statistical analyses were performed using SAS software (version 8.1, Cary, SAS Institute Inc., NC, USA). Data in the graph and tables are presented as means and standard errors of means. In order to normalise data distributions, the total mesophilic bacteria, gram-negative bacteria and ammonia were logarithmically transformed. Square root transformation was used for the air velocity and carbon dioxide data. The effect of building and the season were analysed using repeated measurements analysis of variance (PROC MIXED). The relationship between the total number of mesophilic and gram-

negative bacteria *versus* microclimate parameters was analyzed by Pearson's correlation test. In all analyses P values equal and below 0.05 ($P \leq 0.05$) were considered statistically significant.

Results

The average values of total mesophilic and gram-negative bacteria in the air of House 1 (76×10^3 and 14×10^3 CFU/m^3) were significantly lower ($P = 0.05$) than in House 2 (99×10^3 and 22×10^3 CFU/m^3) (Table 1 and Fig. 1). Concentrations of gram-negative bacteria in both pig houses were four to five times lower than the concentration of total mesophilic bacteria (approximately 18-22% of the total number of mesophilic bacteria). There were no statistically significant differences in the numbers of total mesophilic bacteria and gram-negative bacteria between visits within the testing period. Moreover, seasonal influence was not determined (data not shown). Mean values and standard errors of means of the environmental parameters (temperature, humidity, carbon dioxide and ammonia) are presented in Table 2. From the environmental parameters, relative humidity (87.72% vs. 56.98%) and the concentration of carbon dioxide (6100 ppm vs. 2200 ppm) were significantly higher in House 1 than in House 2 ($P<0.01$). There was no significant correlation between total mesophilic and gram-negative bacteria and temperature, relative humidity, carbon dioxide, and ammonia. A small, but statistically significant correlation between gram-negative bacteria count and air velocity ($r = -0.48$, $P = 0.05$) was determined (Table 3).

Table 1. Mean values and standard errors of means of total mesophilic and gram negative bacteria in the air samples of two pig houses (n = 24)

Culturable bacteria (CFU/m^3)	Number of air samples (n)	House 1 mean ± SEM	House 2 mean ± SEM
Total number of mesophilic bacteria	24	$76 \times 10^3 \pm 5 \times 10^3$	$99 \times 10^3 \pm 10 \times 10^3$
Gram-negative bacteria	24	$14 \times 10^3 \pm 2 \times 10^3$	$22 \times 10^3 \pm 3 \times 10^3$

Table 2. Mean values and standard errors of means of the environmental parameters in the air of two pig houses (number of air samples, n = 24)

	Nº of air samples	Temperature (ºC)	Relative humidity (%)	Air velocity (m/s)	Carbon dioxide (ppm)	Ammonia (ppm)
House 1	24	21.18 (± 1.16)	87.72* (± 2.85)	0.07 (± 0.01)	6100$^{\#}$ (± 0.09)	3.38 (± 0.45)
House 2	24	21.20 (± 0.32)	56.98* (± 3.04)	0.10 (± 0.03)	2200$^{\#}$ (± 0.02)	2.67 (± 1.70)

*,$^{\#}$ $P<0.01$ statistically significat difference between House 1 and House 2

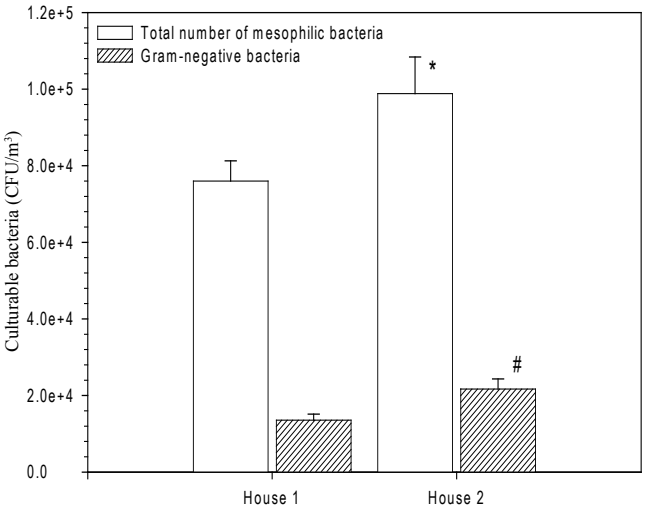

Fig. 1. Mean values and standard errors of means of total mesophilic and gram-negative bacteria *P = 0.05, total number of mesophilic bacteria in the House 1 compared with House 2; #P = 0.05, gram-negative bacteria in the House 1 compared with House 2

Table 3. Relationship between total mesophilic and gram-negative bacteria with microclimate parameters

	Temperature	Relative humidity	Air velocity	Carbon dioxide	Ammonia
Total number of mesophilic bacteria	0.04 (0.85)	-0.30 (0.25)	-0.08 (0.74)	-0.39 (0.12)	0.04 (0.87)
Gram-negative bacteria	-0.34 (0.18)	-0.33 (0.20)	-0.48 (0.05)*	-0.32 (0.22)	-0.39 (0.12)

*P value is statistically significant

Discussion

The main objective of this study was the assessment and quantification of gram-negative bacteria in the air of two swine nursery houses. Since gram-negative bacteria are usually isolated in very small quantities, selective C-EC agar for the detection of total gram-negative bacteria was used. Coliforms belong to the group of gram-negative bacteria that ferment lactose and include the following genera: *Escherichia, Enterobacter*

and *Klebsiella* (NAGLIĆ et al., 2005). The range of gram negative-bacteria in this study was from 10×10^3 to 67×10^3 CFU/m^3, which is in accordance with values reported in previous studies (CLARK et al., 1983; ATTWOOD et al., 1987; CORMIER et al., 1990; SEEDORF et al., 1998). Significantly lower levels of gram-negative bacteria, compared to the level of the total number of mesophilic bacteria, were identified in the air of both swine houses (14×10^3 vs. 76×10^3 CFU/m^3 and 22×10^3 vs. 99×10^3 CFU/m^3). This may be due to the loss of gram-negative bacteria culturability after transmission into the air. Earlier studies demonstrated that gram-negative bacteria remained viable after the process of aerosolisation but failed to grow on the culture media (HEIDELBERG et al., 1997). In addition, the survival rate of gram-negative bacteria in the airborne state is much lower than those of gram-positive bacteria (MÜLLER and GRÖNING, 1981). The impaction method used by the SAS sampler can be very stressful for microorganisms, and can damage bacterial structure or their metabolism, decreasing their potential for recovery on the collection media (STEWART et al., 1995). In the present study, the average concentration of gram-negative bacteria was approximately 18-22% of the total number of mesophilic bacteria. This level is similar to those observed in earlier studies by CLARK et al. (1983) and ATTWOOD et al. (1987). In contrast, ZUCKER and MÜLLER (2002) reported only 0.02 to 5.2% of gram-negative bacteria in the air of swine houses. The higher percentage of gram-negative bacteria in this study could be explained by differences in the type of selective media and in the technical performance of the SAS sampler used in this study (CHANG et al., 2001). During laboratory analysis, we found that a certain percentage of gram-positive bacteria grew on selective C-EC agar. ZUCKER and MÜLLER (2002) demonstrated that different *Staphylococcus* strains grew very well on Endo agar, which is supposed to inhibit the growth of gram-positive bacteria.

Air velocity in the swine buildings represents the main dilution and elimination factor of microorganisms in the air (HILLIGER, 1984). In order to maintain optimal air circulation in the animal houses, recommended values for the winter period are set at 0.1 to 0.2 m/s (BLENDL, 1979). A negative correlation of gram-negative bacteria and air velocity in this study indicates that their concentration decreased in parallel with an increase of air velocity in the building, which is consistent with the findings of a previous study by METHLING et al. (1981). Despite the fact that House 2 had completely computerised technology and a higher predisposition for superior hygiene conditions, it contained a significantly higher content of total mesophilic and gram-negative bacteria than House 1. An analysis of the gases present revealed that mean values of ammonia concentrations in both houses were in the acceptable range (3.38 and 2.67 ppm, respectively) when assessed against an exposure limit of 25 ppm recommended by GROOT KOERKAMP et al. (1998) while mean values of carbon dioxide exceeded suggested exposure limits of 1540 ppm (DONHAM, 1995) (6100 ppm and 2200 ppm). Carbon dioxide levels in the air of a swine facility are a very important indicator of its hygiene conditions. It is released into

the air as the product of different metabolic processes, primarily by animal respiration and faeces degradation (VERSTEGEN et al., 1994). Since both buildings shared a similar animal density, the significantly higher concentration of carbon dioxide in the air of House 1 indicates regulatory problems with the ventilation system (SEEDORF et al., 1998a) that need to be explored in the future. Additionally, the poor health status of animals recorded in the House 1 during the study (diarrhoea outbreak, fever) could have contributed to the increased frequency of the animals' breathing and, consequently, a surplus of carbon dioxide in the air.

The most commonly identified gram-negative bacteria in swine barns were: *Enterobacteriaceae, Pseudomonadaceae* and *Neisseriaceae* (ZUCKER and MÜLLER, 2002). In this study, the prevalent species in the air of both swine houses was *E. coli*. It is likely that *E. coli* was transferred into an airborne state during cleaning and manure pit management procedures. In addition, post-weaning diarrhoea caused by *E. coli* contributes to its higher concentration in the air. Previous studies have shown that *E. coli* can be detected in the intestines of weaned pigs suffering from post-weaning diarrhoea and pigs with no clinical manifestation of the disease (WATHES, 1989).

In conclusion, the number of gram-negative bacteria in both swine houses exceeded suggested threshold limit values. As emphasised in previous studies, gram-negative bacteria, with their endotoxic properties, represent a significant occupational health hazard, and their presence in the air should be addressed. More extensive measurements of gram-negative bacteria and endotoxin concentrations in the air of swine buildings will be investigated in future studies.

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SAŽETAK

Stočne farme kao glavni izvor gram-negativnih bakterija i endotoksina predstavljaju velik rizik za pojavu bolesti dišnoga sustava ljudi i životinja. Cilj istraživanja bio je odrediti prisutnost gram-negativnih bakterija u zraku u odnosu na ukupan broj mezofilnih bakterija i njihovu povezanost s mikroklimatskim čimbenicima (temperatura, vlaga, brzina strujanja zraka, amonijak i ugljični dioksid). Bakteriološko uzorkovanje zraka provodilo se u dva odgajališta svinja. Prosječne vrijednosti ukupnoga broja mezofilnih bakterija bile su 76×10^3 i 99×10^3 CFU/m^3, te gram-negativnih bakterija 14×10^3 i 22×10^3 CFU/m^3. Prosječne vrijednosti gram-negativnih bakterija iznosile su od 18 do 22% u odnosu na ukupni broj mezofilnih bakterija. Koncentracija mezofilnih i gram-negativnih bakterija bila je statistički značajno niža u odgajalištu 1 u odnosu na odgajalište 2 ($P = 0,05$). U oba odgajališta nije ustanovljena statistički značajna povezanost između broja mezofilnih i gram-negativnih bakterija te mikroklimatskih pokazatelja ($P>0,05$), osim male ali statistički značajne korelacije između gram-negativnih bakterija i brzine strujanja zraka ($r = -0,48$, $P = 0,05$). Najčešće izdvojena vrsta u oba odgajališta prasadi bila je *E. coli*. U oba odgajališta prasadi navedene koncentracije gram-negativnih bakterija prelazile su preporučene dozvoljene vrijednosti u zraku.

Ključne riječi: mezofilne bakterije, gram-negativne bakterije, zrak, odgajalište, prasad