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DISTRIBUTION OF AIRBORNE BACTERIA IN SWINE HOUSING FACILITIES AND THEIR IMMEDIATE ENVIRONMENT

VITOMIR BILIĆ[1], BORIS HABRUN[1], IVO BARAČ[2], AND ANDREA HUMSKI[1]

Croatian Veterinary Institute, Zagreb[1], Swine farm, Dubravica[2]; Croatia

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This paper describes a bacteriological analysis of air samples taken from swine housing facilities and the immediate environment. The air volume of the samples was pre-programmed by a standard air sampler (MAS-100, Merck) and was directly impacted onto the bacteriologic agar surface (Petri dishes, standard diameter of 90 mm). The bacterial contamination in forty-eight samples was $2.59x10^5$ CFU/m^3 (ranging from $8.46x10^4$ to $5.30x10^5$ CFU/m^3). Potentially pathogenic bacterial agents predominated in all samples (100%), while primarily pathogenic bacteria were isolated in a minor proportion of samples (33%–66%). Airborne bacterial contamination in samples (N=16) obtained from emptied facilities ranged from $1.8x10^3$ CFU/m^3 (that is, after coarse mechanical washing) to $0.8x10^2$ CFU/m^3 (upon completion of disinfection). Control measurements at different locations and distance from the farm (N=32) pointed to the presence of non-pathogenic airborne bacteria, ranging from $1.55x10^2$ to $3.70x10^2$ CFU/m^3. The results of this preliminary study showed that the emission of potentially pathogenic bacteria from animal housing facilities to the immediate farm environment *via* aerosol was very low.

Key words:
bacteriological analysis, farm environment, pathogenic agents

Respiratory diseases pose the most significant health problem in intensive pig breeding all over the world. The control of respiratory diseases in the conditions of closed and crowded swine housing facilities with mechanical ventilation is highly demanding and includes quality control of the air as an important factor in aerosol dissemination of respiratory infections (1–3). Beside noxious gas emission, humidity, temperature, ven-

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tilation, and dust particles in the air, it is of utmost importance to determine total bacterial contamination (CFU/m^3), especially the presence of potentially and primarily pathogenic bacteria (4–7). It should be emphasized that, in spite of ever more sophisticated monitoring devices, the borderline levels of bacterial air contamination such as those established for noxious gas emission (NH_3 and CO_2) (1, 2, 6) have not yet been determined. The International Animal Hygiene Association has enlarged the programme of basic research for the next millennium with the improvement of housing conditions (sophisticated housing) such as housing air quality and significant reduction and prevention of possible environmental emission. As a part of the programme, this paper brings the results of a study of bacterial contamination of housing air and immediate surroundings in a closed farrowing-finishing farm with a population of 1,500 sows and annual production of some 30,000 fattened hogs. The aim was to test the value of air samplers and highly selective media for isolation of airborne bacteria pathogenic to animal respiratory tract in air samples from intensive breeding facilities and to assess bacterial dissemination in the immediate farm environment.

MATERIAL AND METHODS

A farm of about 13 ha with 22 objects, feed mixing facilities, and two ponds with a manure separator permanently accommodated up to 17,000 animals of all age groups. Animal-age-adjusted microclimate is automatically maintained in all housing facilities. The standards for air temperature and relative humidity in every step of the breeding process are as follows: farrowing 22 °C and 65%; nursery 28–22 °C and 65%; and fattening 18 °C and 70–72%. Automatic ventilation control of the facilities (air flow <1 m/s) is based on the principle of pressure difference, lateral openings, and central air pumping by a ceiling fan.

The nearest settlement of agricultural type is at a distance of 1,000 m from the farm. The high prevalence of respiratory infections recorded in pigs toward the end of 1998 and at the beginning of 1999 pointed to the need of comprehensive analysis of the farming conditions including control of bacterial air contamination. Emissions were simultaneously determined at various locations on the farm and at different distances from the farm facilities.

Mean values for three air samples taken from different facilities holding an average number of animals according to technologic standards were considered as a mean for each facility. The mean value from all technologic steps was taken as the mean value of total bacterial count in the farm air (CFU/m^3). Air samplers can be preprogrammed to so-called delayed sampling, which was used to assess the real condition in the facilities with the animals at rest. Each sample was obtained with a 30-min delay, thus eliminating the effect of abrupt increase in the total particle count in the air due to agitation of the animals caused by the study team when it entered the facilities and installed the measuring devices. The air samples from the immediate farm surroundings were taken at distances of 4.0, 7.5, 12.5, 50.0, 100 m, and 500 m from the farm facilities. The air was sampled with an MAS-100 System air sampler

(Merck) according to the EC standards (8, 9). The device enables that a pre-programmed air volume (1–1,000 L) be directly impacted on the bacteriologic medium surface (standard 90-mm Petri plates). Air particles pass through 360 perforated pores and are impacted onto the medium surface at a mean rate of 11 m/s, corresponding to Andersen sampler degree 5 (9). This rate ensures collection of all >1-μ particles. The air volume can be pre-programmed to 2, 5, 10, 100, and 200 L, depending on the type of the selective medium. Various selective media were used to determine total and individual bacterial count: blood agar base, plate count agar, Chromocult coliform agar, Chromocult *Enterococci* agar, violet red bile agar, xylose lysine deoxycholate agar, and Rambach agar (10). The impacted media were incubated at 37 °C for 24–48 h and then the colony-forming unit (CFU) count was determined. The results were calculated according to the tables provided by the air sample manufacturer. A specific mathematical equation with correction for possible impaction of a greater number of particles/bacteria onto the same site of the medium surface was used in case of a high CFU count (9).

RESULTS AND DISCUSSION

Table 1 shows that the bacterial species of *Streptococcus* D+*, Micrococcus* spp.*, Escherichia (E.) coli, Staphylococcus aureus*, and *Streptococcus suis* were detected in all (100%) air samples obtained from pig-breeding facilities. These results are consistent with those reported from similar studies (2, 3, 6, 11). *Pasteurella multo-*

Table 1 *Rate of isolation (%) and total bacterial count (CFU/m^3) in air samples taken from within farm housing facilities (farm mean, N=48)*

Bacterial species	% of isolation	CFU/m^{3}x10^5
Streptococcus D+*	100	0.840
Micrococcus spp.*	100	0.740
*Escherichia coli**	100	0.260
*Staphylococcus aureus***	100	0.180
*Streptococcus suis***	100	0.130
*Pasteurella multocida***	66.6	0.060
*Actinobacillus suis***	50.0	0.008
*Escherichia coli haemolytica***	41.6	0.045
*Pasteurella haemolytica***	33.3	0.006
*Actinobacillus pleuropneumoniae***	33.3	0.011
*Bordetella bronchiseptica***	33.3	0.009
Total		2.590

* potentially pathogenic bacteria
** primarily pathogenic bacteria

cida and *Actinobacillus suis* were present in 66% and 50% of air samples, respectively. *E. coli haemolytica* was detected in 41%, and *Pasteurella haemolytica, Actinobacillus pleuropneumoniae* and *Bordetella bronchiseptica* in 33% of air samples each.

The mean bacterial count in the air samples from the farm housing facilities was $2.5x10^5$ CFU/m^3. These results are consistent with literature data (1–3, 6, 11). Among the potentially pathogenic bacteria, *Streptococcus* D+, *Micrococcus* spp. and *E. coli* showed highest individual counts ($8.4x10^4$, $7.4x10^4$, and $2.6x10^4$ CFU/m^3, respectively), while the primarily pathogenic bacteria had a prevalence of $6.0x10^2$ to $1.8x10^4$ CFU/m^3.

The mean bacterial contamination of emptied and mechanically washed objects (8 samples) was $1.8x10^3$ CFU/m^3. Upon disinfection and before receiving new animals (8 samples) the mean contamination of the objects with potentially pathogenic agents (*Streptococcus* spp., *Micrococcus* spp., *E. coli* and *Bacillus* spp.) was $0.8x10^2$ CFU/m^3.

Table 2 shows that the potentially pathogenic bacteria of *Streptococcus* spp., *Micrococcus* spp., *E. coli*, and *Bacillus* spp. were isolated from all tested samples

Table 2 *Rate of isolation (%) and total bacterial count (CFU/m^3) in air samples taken from immediate housing environment (farm mean, N=32)*

Bacterial species	% of isolation	CFU/m^3x10^2				
		Location 1	Location 2	Location 3	Location 4	Location 5
Streptococcus D+*	100	0.70	1.65	0.80	1.00	0.60
Micrococcus spp.*	100	0.25	0.55	0.50	0.50	0.70
*Escherichia coli**	100	0.10	1.10	0.90	0.70	0.30
Bacillus spp.*	100	0.15	0.40	0.30	0.45	0.30
*Staph. aureus***	56.2	0.35	0	0.10	0	0.70
Total		1.55	3.70	2.65	2.65	2.60

* potentially pathogenic bacteria

** primarily pathogenic bacteria; location distance from a manure pond: 1=5 m; 2=7.5 m; 3=14 m; 4=50 m; 5=500 m and 50 m from feed mixing facilities

(N=32), while the primarily pathogenic *Staphylococcus aureus* was detected in 56.2% (N=18) of the tested samples.

Total bacterial concentration in the air samples obtained from immediate farm surroundings (samples from locations 1 to 4 in Table 2) ranged from $1.55x10^2$ to $3.70x10^2$ CFU/m^3. It should be noted that highest rates were determined at location 2, that is, at a 7.5-m distance from the fattening facilities. We believe that these results should be attributed to the large, wide-open windows for natural ventilation rather than to the distance. It is also worth mentioning that location 5, although at the greatest distance from the pig housing facilities, had an almost identical bacterial count in the air to other, closer locations. However, it had the highest count of the primarily pathogenic species, *Staphylococcus aureus*. As the feed mixing facility is

located at a distance of some 50 m, the feed preparation technology as well as storage of various agricultural and animal raw materials may have influenced the result obtained at this location. This location certainly calls for more attention in future studies.

In comparison with the rate of bacterial contamination determined in the pig accommodating facilities, the bacterial count found in the air from immediate farm environment was considerably lower and did not exceed the values generally found outdoors (Bilić V. Monitoring of bacterial diseases in swine [invited lecture]. Scientific and Professional Assembly »Health protection of pigs«. 6 May 1999; Zagreb, Croatia, not published). These results are consistent with the latest international reports (12). However, caution is warranted on making any definite conclusions, as the values may greatly depend on particular farm-bred animal species and related mechanical or natural ventilation conditions.

CONCLUSIONS

Studies performed to date have shown that the presence of pathogenic bacteria can be monitored with a standard air sampler and a proper choice of various highly selective media. Despite ever more sophisticated devices for bioaerosol monitoring, limits for air contamination with microorganisms – such as those established for noxious gas concentration (NH_3 and CO_2) – have not yet been determined. In-depth studies using high-quality and ever more selective media as well as sophisticated air samplers will hopefully give a better insight into the issue and result in appropriate standards for the overall breeding process in the future. The isolation of individual, primarily pathogenic agents in the air could be correlated with the occurrence and prevalence of respiratory diseases in pigs. The results of this preliminary study in Croatia also showed that the emission of potentially pathogenic bacteria via aerosol from animal housing facilities to the immediate farm environment is very low.

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Sažetak

PRISUTNOST BAKTERIJA U ZRAKU NASTAMBI ZA SVINJE I NEPOSREDNOM OKOLIŠU

Pored kontrole emisija štetnih plinova, vlage i čestica prašine u nastambama za životinje, važna je i detekcija ukupne bakterijske kontaminacije zraka. Od posebnog su značenja i moguće emisije u okoliš, osobito potencijalno ili primarno patogenih bakterija. U raspravi su prikazani rezultati prvih istraživanja bakterijske kontaminacije zraka u nastambama, kao i u neposrednom okolišu provedenih na jednoj farmi svinja.

Programirani volumen zraka standardnim je skupljačem zraka (MAS-100-Merck) direktno naslojen na površine bakterioloških podloga (Petrijeve ploče promjera 90 mm). U pretraženih 48 uzoraka u nastambama ustanovljena je prosječna bakterijska kontaminacija zraka za farmu od $2,59^5$ CFU/m^3 (od $8,46^4$ do $5,30^5$). U svim su uzorcima dominantno bili prisutni *Streptococcus* spp., *Micrococcus* spp., *Escherichia coli*, *Staphylococcus aureus* i *Streptococcus suis*. U manjem broju uzoraka izdvojeni su potencijalno patogeni uzročnici *Pasteurella multocida* (66%), *Actinobacillus suis* (50%), hemolitični sojevi *E. coli* (41%) te *Pasteurella haemolytica*, *Bordetella bronchiseptica* i *Actinobacillus pleuropneumoniae* (33%). Mjerenjima u praznim objektima, prije useljenja životinja (16 uzoraka), ustanovljena je srednja kontaminacija zraka od $9,0^1$ do $0,4^1$ CFU/m^3 s bakterijskim uzročnicima *Streptococcus* spp., *Staphylococcus* spp. i *E. coli*.

Kontrolna mjerenja izvan objekata (32 uzorka) upozorila su na prosječnu kontaminaciju zraka od $0,26^1$ CFU/m^3 (od $0,15^1$ do $0,37^1$) i uz zastupljenost apatogenih uzročnika *Bacillus subtilis*, *Bacillus cereus*, *E. coli*, *Streptococcus* spp., *Micrococcus* spp. i *Staphylococcus* spp. Rezultati pokazuju da su neznatne emisije potencijalno patogenih bakterijskih uzročnika putem aerosola iz nastambi za svinje u neposredni okoliš.

Unatoč sve sofisticiranijim uređajima za monitoring, ni danas još, u okviru programa animalne higijene, nisu određene granične vrijednosti za bakteriološku kontaminaciju zraka kao što je to slučaj s emisijom štetnih plinova NH_3 i CO_2.

Ključne riječi:
animalna higijena, distribucija bakterija, emisija u okoliš, kontaminacija zraka

Requests for reprints:

Vitomir Bilić, DVM, Ph.D.
Croatian Veterinary Institute, Department of Bacteriology
P.O. Box 883, HR–10001 Zagreb, Croatia