

## Exposure of Workers to Airborne Microorganisms in Open-Air Swine Houses

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**This study quantified the levels of airborne microorganisms in six swine farms with more than 10,000 pigs in subtropical Taiwan. We evaluated breeding, growing, and finishing stalls, which were primarily open-air buildings, as well as partially enclosed farrowing and nursery piggeries. Airborne culturable bacteria, gram-negative bacteria, and fungi were placed on appropriate media by using an all-glass impinger or single-stage Andersen microbial sampler. Results showed that mean concentrations of culturable bacteria and gram-negative bacteria were  $3.3 \times 10^5$  and 143.7 CFU/m<sup>3</sup>, respectively. The concentration of airborne culturable fungi was about  $10^3$  CFU/m<sup>3</sup>, with *Cladosporium* the predominant genus. The highest airborne levels of culturable bacteria and gram-negative bacteria were identified in the finishing units. The air of the nursery stalls was the least contaminated with culturable and gram-negative bacteria. Irregular and infrequent cleaning, high pig density, no separation of wastes from pen floors, and accumulation of water as a result of the processes for cleaning and reducing pig temperature possibly compromise the benefits of the open characteristic of the finishing units with respect to airborne bacterial concentration.**

Modern agricultural methods have changed the way pigs are raised. To increase production with minimum labor, swine have been fed in confinement buildings, which are mainly enclosed structures densely stocked with swine (12, 13, 29). A mechanical ventilation system and a system for handling animal wastes are usually set up to maintain the health status of pigs indoors. Pigs are managed in different types of confinement sectors depending on their growth stage and the operation process. The stages of swine growth can be categorized as lactating sow and offspring, pre-nursery (10 to 30 lb), nursery (30 to 75 lb), growing (75 to 150 lb), and finishing (150 lb to market weight) (29). Accordingly, five types of buildings are generally involved in swine production: breeding (for pre-pregnant and pregnant swine), farrowing (for delivered swine and newborn pigs), nursery (for weaned piglet less than 75 lb), growing (for swine approximately under 150 lb), and finishing (for swine before slaughtered). Some farms may also have a separate house for male pigs, which are brought to the breeding buildings periodically for copulation.

Microorganisms and their components or products, resulting from pig dander, fecal matter, and feed materials, are easily accumulated and aerosolized in such densely populated and enclosed buildings (14). Due to exposure, swine workers may experience upper respiratory irritation, chronic bronchitis, organic dust toxic syndrome, or other respiratory symptoms (15). Exposure assessments show that airborne bacteria in pig confinement buildings in the United States, Canada, The Netherlands, Sweden, and Poland, reach levels of  $10^5$  to  $10^6$  CFU/m<sup>3</sup> (3, 8, 9, 14–16, 21, 27) and up to  $10^7$  CFU/m<sup>3</sup> in the United Kingdom (10), whereas the number of viable fungi is lower,

approximately 10 to  $10^4$  CFU/m<sup>3</sup> (8–10, 14). Gram-positive bacteria are the predominant bacteria present (3, 8, 10, 14, 15, 21, 27), and microbial genera have been identified in many swine confinement units (8–10, 14, 16). Some organisms are recognized as potential agents inducing extrinsic allergic alveolitis and causing pathogenic infection (9).

In Taiwan, a subtropical country, swine production farm industries are mainly located in the southern area, where the relatively high ambient temperature is above 30°C in summer. Because of the weather, swine houses are built and operated in an open-air style, as high temperature affects the adult swine's appetite and fertility, which decreases swine production. An example of open-air swine buildings is shown in Fig. 1. Plastic curtains are occasionally used to cover both sides of the building when the ambient temperature is relatively low in winter. On the other hand, most of farrowing and nursery houses are partially enclosed with many open windows. Some other farrowing and nursery houses are constructed in an open-air style with plastic curtains on sides that are usually rolled up for better ventilation. Sometimes windows are closed or the curtains are rolled down to protect newborn and growing piglets, which are sensitive to low ambient temperature. A mechanical ventilation system is usually built into these types of buildings to help maintain air exchange.

Given the differences in building structure, the magnitude of exposure to airborne microorganisms in such piggeries may be different from those in mainly or completely enclosed buildings. However, to our knowledge, no information regarding the level of airborne microorganisms in such structures has been previously published. Further, exposure in many studies has been measured mostly in swine fattening, nursery, or farrowing buildings (2, 3, 9, 10, 16, 21); little exposure assessment has been conducted simultaneously for all kinds of swine buildings by specific building type, including the breeding unit. Therefore, the major aim of this study was to determine the levels of

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FIG. 1. Photograph of a finishing piggery as an example of open-air swine houses commonly found in subtropical Taiwan.

airborne microorganisms in five major kinds of swine houses. The results were then compared to published data for enclosed swine buildings. The farm characteristics, feeding and cleaning practices, and environmental factors that might affect both the composition and the concentration of airborne microorganisms in piggeries were also investigated.

#### MATERIALS AND METHODS

**Sampling strategy and procedure.** Ten swine farms with five types of swine houses were randomly selected. Walk-through and preliminary surveys of these farms were conducted as a pilot study in September and repeated in December 1994. Airborne microorganisms were sampled from one randomly selected piggery of six of visited swine farms, which included four farrowing, one growing, and one finishing unit. The information was then used to determine the optimal and feasible sampling method and protocol for airborne microorganisms for the follow-up investigation.

In April to May 1995, a 2-day comprehensive sampling was performed on each of five types of swine houses of six swine farms, for a total of 30 swine houses. Samples of airborne culturable bacteria, gram-negative bacteria, and culturable fungi were simultaneously collected 1.5 m above the floor of the central walkway in each of the examined piggeries. Temperature, relative humidity, and wind velocity were recorded at the same floor height in the central walkways as well as two open ends of swine houses, using direct-reading instruments (Testo GmbH, Lenzkirch, Germany). Information on the characteristics of the stalls where environmental measurements were taken was obtained through visual inspection and interviews with swine workers regarding the structure of each pig house; pig density; feeding method, material, and frequency; and cleaning practice and frequency.

**Bioaerosol sampling and analysis.** Three sampling methods that have been used in swine farms for collecting airborne microbes were used in the preliminary survey: filtration (10, 14–16, 32), liquid impingement (3, 10, 16, 32), and impaction on agar plates (8, 9, 14, 15, 20, 21, 32). Samples were simultaneously collected with a single-stage Andersen microbial sampler (AMS; (Graseby, Smyrna, Ga.) operated at 28.3 liter/min; an all-glass impinger (AGI) with 30-mm jet-to-bottom spacing (AGI-30; Ace Glass Incorporated, Vineland, N.J.) operated at 12.5 liter/min; and Nuclepore filter (NF; polycarbonate membrane filter, 0.4- $\mu$ m pore size, 37-mm diameter; Costar, Boston, Mass.) in a three-stage polystyrene cassette (37-mm diameter; SKC, Eighty Four, Pa.) operated at 2 liter/min. Airflow was calibrated before and after field sampling. By using two identical sampling devices, duplicate samples were simultaneously collected at the same sampling time in each swine house.

The AMS was preautoclaved in the laboratory and disinfected by 70% ethanol-immersed cotton balls between each sampling. By using six AMSs simultaneously operated for 0.5 to 10 min, airborne microbes were directly collected onto 20 ml of Trypticase soy agar (TSA), MacConkey's medium (MAC), and malt extract agar with streptomycin sulfate (MEAS) (Difco Laboratories, Detroit, Mich.) in 100-mm-diameter petri dishes. Duplicate samples of each of three media were

simultaneously collected at the same sampling time. Two field blank samples were taken in the same way at each measurement site, except that the samplers were not operated. All of the AMS samples were sent back to the laboratory at room temperature. Concentrations of culturable bacteria, gram-negative bacteria, and culturable fungi were respectively determined after incubation.

The collection medium for AGI-30 was 20 ml of 1% peptone-distilled water with 0.01% Tween 80 and 0.005% antifoam A (Sigma Chemical Co., St. Louis, Mo.) (32). The AGI-30 and collection medium were autoclaved before use. After a 30-min sampling in the field, the neck of each impinger was flushed with the impinger solution, and the liquid was transferred to sterile glass bottles. Samples were then transported under refrigeration to the laboratory for treatment on the same day. The final volume of each sample was measured and corrected for evaporation. After shaking, samples were diluted in 10-fold series with sterile 0.1% peptone-distilled water. An aliquot of 0.1 to 0.5 ml of each sample was plated onto duplicate TSA, MAC, and MEAS plates. One field blank sample for each measured stall was also collected and treated in the same way.

NF and cellulose backing pads were sterilized by UV illumination at 254 nm for 2 h. Three-piece polystyrene cassettes were sterilized with 12% ethylene oxide. The filters supported by pads were then housed into closed-face sterile cassettes, sealed, and brought to swine farms. The sampling times ranged from 20 to 30 min. After sampling, filters were sealed inside the cassettes and within 1 h sent to the laboratory along with one blank field sample at ambient temperature. Samples were then eluted from the filters by injecting 1 ml of sterile 0.1% peptone-distilled water with 0.01% Tween 80 into the support pad and 5 ml of the same medium onto the filter surface. The cassettes were recapped and shaken for 15 min; the solution was drawn off, measured, and then plated in serial 10-fold dilutions onto the culture media (TSA, MAC, and MEAS) with 0.1 to 0.5 ml of solution.

Regardless of sampling method, all TSA, MAC, and MEAS plates were incubated, at 30°C for 2 to 5 days, 30°C for up to 10 days, and 25°C for 5 to 7 days, respectively. Colonies formed on plates were then enumerated. The positive-hole method was applied to AMS samples for corrections of microbial coincidence (1). Fungi were identified morphologically under a microscope by a trained technician. Culture plates were first examined under a dissecting microscope (model SZ-PT; Olympus, Tokyo, Japan). The spores of each colony were picked and placed under a high-resolution microscope (model BH-2; Olympus) for identification of genus according to published references (4, 23).

**Environmental factors.** The temperature, wind velocity, and relative humidity in the stalls were recorded using direct reading instruments at 7:30 to 8:30 a.m., 10:30 to 11:30 a.m., 1:30 to 2:30 p.m., and 4:00 to 5:00 p.m. on each sampling day. Three readings were recorded and averaged for each measurement.

**Statistical analysis.** SAS software was used to test the normality of original and transformed data. For normally distributed data, analysis of variance and Scheffe test were performed to detect significant difference among means of contaminants at various measurement sites. Nonparametric analysis was carried out for nonnormally distributed data.

#### RESULTS

##### Comparison of bioaerosol sampling and analysis methods.

The AGI-30 sampling and analysis method obtained the highest concentration of culturable bacteria: 12 times that obtained using NF and 17.8 times that obtained using AMS (Table 1). In terms of forming countable bacterial colonies, 91% of AGI-30 samples were within the recommended plate count limit of 30 to 300 CFU on 100-mm-diameter plates (19), while only 58% NF samples were within this range. In AMS samples, only 37 and 17% culture plates were countable after sampling for 0.5 and 2 min, respectively. Colonies grown on most culture plates of AMS samples were found to be too numerous to count. The extremely high density of colonies on AMS collection plates resulted in difficulty in enumeration and significant underestimation of concentration of culturable bacteria.

For gram-negative bacteria and culturable fungi, the concentration determined by AGI-30 and NF sampling methods was higher than that determined by AMS. However, at the recommended maximum sampling time of 30 min (32), less than 30% of undiluted samples and none of diluted samples collected by AGI-30 or NF were countable (30 to 300 CFU/

TABLE 1. Comparison of three bioaerosol sampling methods for measuring culturable bacteria, gram-negative bacteria, and fungi in swine houses<sup>a</sup>

Material measured	Sampling method	Concn (CFU/m <sup>3</sup> )			n	% of samples within recommended plate count limit <sup>b</sup> (sampling time or dilution)
		Mean	SD	Range		
Culturable bacteria	AMS	26,304	23,756	4,200–TNTC <sup>c</sup>	12	37 (0.5 min); 17 (2 min)
	AGI-30	467,204	953,104	40,592–3,275,564	11	91 (30 min)
	NF	38,903	26,780	7,631–95,473	12	58 (20–30 min)
Culturable gram-negative bacteria	AMS	1,144	967	285–4,249	28	80 (10 min); 83 (5 min); 100 (1–2 min)
	AGI-30	8,107	8,151	1,041–29,576	11	28 (undiluted)
	NF	2,406	1,864	404–6173	9	17 (undiluted)
Culturable fungi	AMS	1,787	1,079	248–4,300	35	66 (5 min); 100 (1–2 min)
	AGI-30	3,389	1,722	1,041–6,495	11	17 (undiluted)
	NF	3,833	3,245	807–10,138	12	8 (undiluted)

<sup>a</sup> Airborne microbes were sampled from four farrowing, one growing, and one finishing piggery in a preliminary survey.

<sup>b</sup> Recommended plate count limits for standard ca. 100-mm-diameter culture plates: 30 to 300 CFU for total bacteria and gram-negative bacteria (19); 25 to 250 CFU/plate for fungi (26).

<sup>c</sup> TNTC, too numerous to count.

plate for gram-negative bacteria [19] and 25 to 250 CFU/plate for fungi [26]). Colony counts less than the lower plate count limits were frequently observed in the plates of AGI-30 and NF samples. However, the number of colonies on AMS collection plates was all within the enumeration region when sampling was for gram-negative bacteria and fungi for 1 to 2 min. Inclusion of data derived from the plates with substantially low or high counts likely introduces biases and affects the accuracy of data interpretation. Based on the results in Table 1, sampling by AMS was optimal for collection of gram-negative bacteria and fungi, while AGI-30 was considered appropriate for culturable bacteria in swine farms. Both were used in the following 2-consecutive-day sampling, in 1995.

**Airborne microorganisms.** By using optimal sampling methods in a way that would properly minimize colony masking (6, 7), bioaerosol concentrations in five types of swine houses were

determined (Table 2). The results showed that mean concentrations of culturable bacteria among five types of swine houses ranged from  $1.0 \times 10^5$  to  $7.6 \times 10^5$  CFU/m<sup>3</sup>, with an overall average of  $3.3 \times 10^5$  CFU/m<sup>3</sup>. The highest concentration,  $3.5 \times 10^6$  CFU/m<sup>3</sup>, was found in a finishing unit. The airborne concentration of culturable bacteria was significantly higher in the finishing houses ( $7.6 \times 10^5$  CFU/m<sup>3</sup>) than in the farrowing, nursery, or growing piggeries ( $P = 0.009, 0.001, \text{ or } 0.002$ , respectively, by the Scheffe test). The airborne concentration of culturable bacteria in breeding units was the second highest. Relatively low levels of culturable bacteria were determined in the air of the farrowing, nursing, and growing piggeries.

Mean airborne concentrations of gram-negative bacteria were relatively low in all swine units, ranging from 42 to 452 CFU/m<sup>3</sup>. The highest concentration was again found in the finishing houses, which showed a statistical significance by the

TABLE 2. Concentrations of airborne microorganisms measured in five types of swine houses of six swine farms in 2-day consecutive sampling

Material measured	Sampling house type	Concn (CFU/m <sup>3</sup> )			n
		Mean	SD	Range	
Culturable bacteria <sup>a</sup>	Breeding	496,827	423,831	59,533–1,316,410	24
	Farrowing	182,973	191,663	7,121–742,476	23
	Nursery	102,975	115,925	4,941–440,318	24
	Growing	127,213	146,552	214,813–717,421	24
	Finishing	756,244 <sup>d</sup>	1,044,082	16,223–3,496,828	24
	Overall	334,509	571,663	4,941–3,496,828	119
Culturable gram-negative bacteria <sup>b</sup>	Breeding	50	93	4–674	61
	Farrowing	42	37	4–166	66
	Nursery	44	38	4–178	79
	Growing	75	82	4–450	74
	Finishing	452 <sup>e</sup>	690	7–3,545	82
	Overall	143	372	4–3,544	362
Culturable fungi <sup>c</sup>	Breeding	3,576	5,822	430–34,023	33
	Farrowing	3,014	1,931	603–7,111	36
	Nursery	2,297	1,341	283–6,214	36
	Growing	2,152	1,217	554–7,095	35
	Finishing	2,486	2,042	695–10,589	36
	Overall	2,693	2,948	283–34,023	176

<sup>a</sup> Collected by AGI-30 samplers and then cultured on TSA medium at 30°C for 2 to 5 days.

<sup>b</sup> Collected on MAC medium by AMS and incubated at 30°C for up to 10 days.

<sup>c</sup> Collected on MEAS medium by AMS and incubated at 25°C for 5 to 7 days.

<sup>d</sup>  $P < 0.01$  by Scheffe test compared to results for the farrowing, nursery, and growing stalls, respectively.

<sup>e</sup>  $P < 0.001$  by Scheffe test compared to results for the other four types of stalls.

TABLE 3. Identification of fungus genus isolated on malt extract agar from AMS samples collected from selected swine houses

Genus	Occurrence of genus (%)						
	September 1994 <sup>a</sup>	December 1994 <sup>b</sup>	April–May 1995 <sup>c</sup>				
			Breeding	Farrowing	Nursery	Growing	Finishing
<i>Cladosporium</i>	34.23	39.09	92.77	93.35	93.78	95.29	96.02
<i>Cephalosporium</i>	23.26	4.17	— <sup>d</sup>	—	—	—	—
<i>Aspergillus</i>	—	7.81	—	—	—	—	—
<i>A. niger</i>	—	—	0.03	—	0.10	0.11	0.01
<i>A. flavus</i>	—	—	—	0.01	0.05	0.13	0.04
<i>Alternaria</i>	—	18.33	0.31	0.20	1.09	0.69	0.82
<i>Penicillium</i>	4.44	1.81	2.06	0.97	0.62	1.04	0.50
<i>Fusarium</i>	4.16	1.31	1.57	0.40	0.51	0.69	0.72
<i>Curvularia</i>	—	—	—	0.10	—	—	0.03
<i>Sclerotium</i>	1.39	—	—	—	—	—	—
<i>Geotrichum</i>	0.33	—	—	—	—	—	—
<i>Drechslera</i>	0.22	—	—	—	—	—	—
<i>Ulocladium</i>	—	3.33	—	—	—	—	—
<i>Diplococcus</i>	—	0.22	—	—	—	—	—
<i>Oidium</i>	—	3.33	—	—	—	—	—
<i>Aureobasidium</i>	—	2.00	—	—	—	0.04	—
<i>Stemphyllium</i>	—	0.22	—	—	—	—	—
<i>Trichoderma</i>	—	2.33	? <sup>e</sup>	?	?	?	?
<i>Monilia</i>	—	2.84	?	?	?	?	?
<i>Paecilomyces</i>	—	—	—	?	0.11	0.69	0.33
<i>Zygomycetes</i>	—	—	?	?	?	?	?
<i>Botrytis</i>	—	—	0.12	—	—	—	—
Yeast	28.44	12.51	0.38	2.72	1.55	0.14	0.28
<i>Candida</i>	—	—	—	—	—	0.14	—
<i>Actinomycetes</i>	1.91	—	—	—	—	—	—
Others	1.62	0.69	2.77	2.25	2.18	1.04	1.26

<sup>a</sup> Samples were collected from one breeding, two farrowing, and one nursery houses.

<sup>b</sup> Samples were collected from four farrowing and two finishing houses.

<sup>c</sup> Samples were collected on 2 consecutive days from six of each type of swine house (i.e., breeding, farrowing, nursery, growing, and finishing houses).

<sup>d</sup> —, fungus of that genus not identified.

<sup>e</sup> ?, fungus identified but difficult to quantitate.

Scheffe test compared with the airborne levels of gram-negative bacteria determined in the other four types of stalls (all with *P* values of <0.001). Gram-negative bacterial concentrations in the air of the breeding, farrowing, and nursery stalls were only 1/10 of the mean level in the finishing piggery.

The airborne concentration of culturable fungi measured in swine buildings averaged between  $2.2 \times 10^3$  and  $3.6 \times 10^3$  CFU/m<sup>3</sup>. Analysis of variance showed no significant difference in airborne fungi concentration among piggeries (*P* = 0.25). However, a great diversity of fungal genera was found in swine houses (Table 3). Among 21 identified genera of fungi, *Cladosporium* was predominant in all three sampling periods. In the April–May 1995 samples, *Cladosporium* represented more than 90% identified fungi in all five types of swine stalls. Other frequently identified fungi included *Cephalosporium* (4.2 to 23.3%), *Aspergillus* (7.8%), *Alternaria* (0.2 to 18.3%), *Penicillium* (0.5 to 4.4%), *Fusarium* (0.4 to 4.2%), and yeast (0.1 to 28.4%) isolates.

**Characteristics of piggeries.** The floors of pens were mostly concrete in the breeding, growing, and finishing houses and were neither elevated nor slatted in most growing and finishing stalls (Table 4). In contrast, slatted and elevated floors made by metal frames were mainly observed in the farrowing and nursery houses. Open style with no curtains or with curtains rolled up on both sides of the building was observed in all of the breeding, growing, and finishing houses; 50, 20, and 17%, respectively, were ventilated mechanically in addition to natu-

rally. In 84% of farrowing and 67% of nursery houses, the structures were partially enclosed with open windows or with curtains partially rolled down. Mechanical ventilation was used in two-thirds of farrowing and nursery buildings. At least half of all types of swine houses except for the finishing units had natural ventilation in the form of long vents in the roof.

Swine in the breeding and farrowing units were manually fed 1.7 to 2 times per day. An automatic feeding system, with which feed was always available to the pigs via an automated delivery system to troughs, was operated mostly in the nursery, growing, and finishing houses. The feed material was mainly dry powder. Cleaning by water spraying was performed in all types of piggeries except the farrowing units, where dry sweeping was used. The breeding, farrowing, and nursery houses were cleaned routinely on a daily basis. However, the intervals of house cleaning averaged  $25 \pm 53.1$  and  $30.8 \pm 59.5$  days, respectively, for the growing and finishing piggeries.

As shown in Table 5, the finishing stall was the largest in surface area (average of 935.7 m<sup>2</sup>); the surface areas of other stalls averaged between 529.8 m<sup>2</sup> and 585.7 m<sup>2</sup>. Swine density was estimated to be 0.32, 0.85, and 0.78 hog/m<sup>2</sup> in the breeding, growing, and finishing houses and to be 1.23 and 1.63 piglets/m<sup>2</sup> in the farrowing and nursing stalls, respectively.

**Environmental factors.** The averages of wind velocity, temperature, and relative humidity among swine houses are presented in Table 5.

TABLE 4. Characteristics and practices in swine houses

Characteristic or practice	Value					
	Breeding	Farrowing	Nursery	Growing	Finishing	Overall
Stall structure (% occurrence) <sup>a</sup>						
Wall						
Concrete	33	50	83	67	67	60
Brick	50	50	17	33	33	37
Concrete and brick	17	0	0	0	0	3
Ceiling						
Wood	17	50	17	17	17	23
Concrete	67	33	50	50	67	54
Wood and concrete	16	17	33	33	16	23
Pen						
Concrete	0	0	0	17	33	10
Metal	83	100	100	83	67	87
Concrete and steel	17	0	0	0	0	3
Pen floor						
Concrete	100	17	20	67	80	56
Metal	0	83	80	20	0	37
Elevated	0	83	67	17	17	37
Slatted	50	100	83	17	17	53
Ventilation (% occurrence)						
Stall						
Open <sup>b</sup>	100	16	34	100	100	70
Partially enclosed <sup>c</sup>	0	84	67	0	0	30
Long vent at roof	50	83	80	75	33	63
Use of mechanical ventilation	50	67	67	20	17	45
Feeding practice (% occurrence)						
Method						
Manual	83	100	34	0	17	47
Automated	17	0	66	100	83	53
Material						
Dry powder	83	83	67	83	83	80
Dry pellet	17	17	33	17	17	20
Feeding frequency (times/day)	1.7 ± 0.5	2 ± 0	1 ± 0	2 ± 0	2 ± 0	1.7 ± 0.5
Cleaning practice (% occurrence)						
Manual, watering	83	33	67	50	50	57
Manual, sweeping	0	67	0	0	0	16
Automated	0	0	0	17	33	10
Cleaning frequency (times/day)	1 ± 0	1.2 ± 0.4	1 ± 0.1	0.9 ± 0.2	1.2 ± 0.5	1.1 ± 0.3
Cleaning interval (days since last cleaning date)	1 ± 0	1 ± 0	1.3 ± 0.7	25 ± 53.1	30.8 ± 59.5	11.9 ± 35

<sup>a</sup> Total not equal to 100%; some observations not classifiable.

<sup>b</sup> Open structure with no curtains or curtains up.

<sup>c</sup> Including the enclosed building with open windows and the open structure with curtains partially down.

## DISCUSSION

Due to differences in survival capability and culturability among microorganisms, culture methods generally underestimate the actual concentration of airborne microorganisms present in an environment. However, culture is still one of the most popular methods used in bioaerosol field studies because it allows the investigators to determine microbial composition and concentration simultaneously. Among the sampling methods that utilize culture assay, impingement into liquid media tends to give higher colony counts for environments where microorganisms are carried as aggregates, mainly because microbial clusters are broken up during sampling (25). Species of airborne bacteria collected in the present investigation have been identified in a follow-up study, in which *Micrococcus* and *Staphylococcus* were identified as predominant. Both genera are known to form aggregates in nature and were probably collected in AGI-30 liquid media in such form. However, bac-

terial clusters such as *Micrococcus* and *Staphylococcus* were likely broken up in AGI-30 liquid media, as the cells were vigorously scrubbed in liquid for a long (30-min) sampling period. The present study and a previous investigation of swine barns by Thorne et al. (32) demonstrated this possibility. Both studies found that the AGI sampling method gave the highest value of airborne bacteria concentration.

Clusters of bacteria are common in nature, but the same may not be true for dry fungal spores. Besides, the hydrophobicity of fungal spores facilitates their escape from the liquid media of AGI samplers even after they have been captured (26), which in turn decreases colony counts obtained from AGI-30 sampling. This may be one of the reasons why both the present study and that of Thorne et al. (32) found a lower level of fungi obtained by the AGI method than by the NF method.

We also found that the AMS sampling method yielded the highest proportion of data within the limit of plate counts when

TABLE 5. Pig density and environmental factors in swine houses

Factor	Mean $\pm$ SD					
	Breeding	Farrowing	Nursery	Growing	Finishing	Overall
Surface area of stalls (m <sup>2</sup> )	534.3 $\pm$ 190.4	529.8 $\pm$ 165.9	546.5 $\pm$ 193.4	585.7 $\pm$ 542.5	935.7 $\pm$ 419.2	681.0 $\pm$ 362.4
No. of hogs/stall	175.7 $\pm$ 94.1	70.0 $\pm$ 25.9	— <sup>a</sup>	708.7 $\pm$ 413.2	786.7 $\pm$ 479.9	348.2 $\pm$ 429.8
No. of piglets/stall	—	663.3 $\pm$ 245.6	933.8 $\pm$ 557.9	—	—	319.4 $\pm$ 479.5
Hog density (no./m <sup>2</sup> )	0.32 $\pm$ 0.16	0.10 $\pm$ 0.00	—	0.85 $\pm$ 0.24	0.78 $\pm$ 0.25	0.41 $\pm$ 0.39
Piglet density (no./m <sup>2</sup> )	—	1.23 $\pm$ 0.14	1.63 $\pm$ 0.73	—	—	0.57 $\pm$ 0.79
Wind velocity (m/s)	0.80 $\pm$ 0.44	0.61 $\pm$ 0.48	0.60 $\pm$ 0.38	0.74 $\pm$ 0.34	0.74 $\pm$ 0.39	0.7 $\pm$ 0.4
Temp (°C)	30.37 $\pm$ 2.10	30.67 $\pm$ 2.13	30.57 $\pm$ 2.30	30.62 $\pm$ 2.45	30.48 $\pm$ 2.23	30.54 $\pm$ 2.24
Relative humidity (%)	61.80 $\pm$ 8.80	61.43 $\pm$ 8.38	62.58 $\pm$ 8.67	61.85 $\pm$ 8.37	62.45 $\pm$ 8.33	62.02 $\pm$ 8.51

<sup>a</sup> —, not applicable.

sampling for gram-negative bacteria and fungi, but was easily overloaded for collecting culturable bacteria even with very short (30-s) sampling times. Due to microbial overloading and subsequent colony masking, we, like Thorne et al. (32), do not recommend the AMS as a validated sampling method for airborne culturable bacteria in swine houses but consider it optimal for gram-negative bacteria. Our results agree with the conclusion of Thorne et al. that the AGI is the best sampling method for culturable bacteria in swine buildings. However, we did not find an acceptable portion of samples with colony counts falling within plate count limits when sampling for fungi by AGI-30 as did Thorne et al. Thus, we used the AMS method for sampling of culturable fungi.

Although the finishing units were not enclosed, we found that such buildings contained the highest airborne levels of culturable bacteria and gram-negative bacteria. This result agrees with those of other studies conducted in enclosed swine buildings (9, 11). Compared to other types of buildings in the present study, the finishing units were found to be more densely stocked, not only in pig number but also with respect to the body size of growing pigs. Substantial amounts of swine wastes remained on the non-slatted floors (in 83% of cases) where pigs lived. In addition, an automatic water mist spray system to reduce pig body temperature was observed in operation around noon in some breeding, growing, and finishing stalls; for those without an automatic system, workers manually sprayed water directly on pigs via hoses as well as on the floors for cleaning purposes. Wastewater that is not quickly removed from non-slatted floors provides high moisture for microbes to quickly multiply on fecal matter and feed materials abundant in finishing stalls. Furthermore, the interval of house cleaning for finishing piggeries was the longest, averaging 31 days with a great variation of 59 days. Irregular and infrequent cleaning of the densely stocked finishing units offered a great opportunity for bacterial growth, accumulation, and aerosolization. A significant positive correlation has been shown between airborne levels of culturable bacteria and the interval of stall cleaning (3).

In contrast, 83% of the floors in the nursery buildings examined in this study were slatted. Cleaning by water spraying was performed routinely at an average interval of 1.3 days. Produced wastewater was quickly drained from the buildings through floor grates. Consequently, although 67% of the nursery stalls were enclosed, the air in this type of building was the least contaminated with culturable bacteria and gram-negative bacteria.

Wind velocity inside the houses was less than 0.8 m/s on average and did not vary significantly among piggeries (Table 5). In a similar stable atmosphere, a better dilution of airborne contaminants by wind force in open-air buildings (e.g., finishing stalls) might not be as significant as expected. Based on the above findings, poor cleaning practice, high swine density, and no separation of wastes from pen floors tend to compromise the benefit of open-air structures in relation to level of airborne bacteria. Our results also demonstrate the importance of routine sanitation for reducing microbial contamination.

Temperature and relative humidity have been reported as two important factors related to the survival of bacteria in dust (24) and the number of airborne microorganisms (28). However, these variables cannot account for the differences in airborne bacterial concentrations shown in the present study because they were relatively homogeneous among all studied piggeries.

The levels of airborne culturable bacteria in this study were comparable to those documented in many published reports (3, 8, 9, 14–16, 20, 21, 27). However, we found lower airborne concentrations of gram-negative bacteria compared to the level of 10<sup>3</sup> to 10<sup>4</sup> CFU/m<sup>3</sup> previously found in other swine confinement buildings (3, 8, 15, 16, 20, 21). The airborne concentration of average gram-negative bacteria in all piggeries was only 0.04% of that of culturable bacteria, compared to 10, 6.5, and 29% reported by Attwood et al. (3), Heedrick et al. (21), and Clark et al. (8), respectively. The different performance characteristics of the samplers used in assessing culturable bacteria and gram-negative bacteria may partly account for this great deviation. As shown in Table 1, sampling with AGI-30 acquired 17.8 times the levels of culturable bacteria simultaneously collected with AMS. On the other hand, increases in the levels of airborne gram-positive bacteria may provide an explanation. Because 90% of the bacteria isolated from the feces of adult swine are reported as gram positive (31), it is reasonable to attribute the high concentrations of airborne culturable bacteria in stalls to the increase in the number of dominant gram-positive microflora rather than gram-negative bacteria.

Fungal concentrations found in this study were generally also lower than the previously reported levels of 10<sup>3</sup> to 10<sup>5</sup> CFU/m<sup>3</sup> (10, 14, 15, 20, 32) but similar to the 10 to 10<sup>3</sup> CFU/m<sup>3</sup> reported by Cormier et al. (9) and Clark et al. (8). *Cladosporium* was always the predominant fungus in swine houses surveyed, as it was in studies conducted in swine buildings in the United Kingdom (10) and Sweden (14). However,

researchers in Quebec did not isolate this genus from their samples (9). *Aspergillus* and *Penicillium*, the genera found most frequently by both Cormier et al. (9) and Donham et al. (14), were also identified in the present study along with other molds reported elsewhere (9, 14), including *Alternaria*, *Fusarium*, *Verticillium*, *Geotrichum*, and yeast. Nevertheless, we did not detect *Scopulariopsis* (9, 14), *Homodendrum* (14), *Circinella* (9), *Mucor* (9), or *Rhizopus* (14), which are described by Cormier et al. (9) and Donham et al. (14). *Cephalosporium*, which represented 34% of fungi identified in the September 1994 sampling of our study, was neither isolated later (April to May 1995 sampling) nor, to our knowledge, mentioned in any published papers. The reasons for the differences in fungal genera found in various studies are not clear but are probably related to differences in collection media, incubation conditions, climatic condition, types of buildings investigated, or time of year when the samplings were conducted.

Among those identified in the present study, the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Curvularia*, *Stemphyllium*, and *Trichoderma* have been regarded as common allergens (5). *Aspergillus*, *Alternaria*, *Penicillium*, and *Aureobasidium* are associated with extrinsic allergic alveolitis (18, 30). *Penicillium*, *Aspergillus*, *Curvularia*, *Geotrichum*, *Drechslera*, *Stemphyllium*, and *Candida* may cause allergic bronchopulmonary mycoses (17), and *Aspergillus* and *Candida* are known to be potentially pathogenic for humans (22). In addition to the presence of allergic or infectious species, the airborne culturable bacterial concentration in our investigated finishing houses exceeded the level of  $6.3 \times 10^5$  CFU/m<sup>3</sup> reported in a previous study in which significant decrements in baseline of pulmonary function testing were present (15). The high level of airborne bacteria and presence of dangerous fungal genera implied the probability of health hazards for workers in some of the investigated piggeries.

In conclusion, airborne culturable bacterial levels in the open-air swine houses were comparable to those found in enclosed buildings and were probably due to irregular or infrequent cleaning and water accumulation in such densely stocked and non-slatted piggeries. Poor maintenance of piggery sanitation compromises the benefit of open-air piggeries at low wind velocity. As a result, the presence of high concentrations of airborne culturable bacteria and potentially allergic or infectious fungi might pose health risks for workers even in open-air swine stalls.

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