Airborne Microbial Contents in Two Types of Swine Confinement Buildings in Quebec*

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Airborne microorganisms were isolated with a sampler in two types of swine confinement buildings (farrowing units and fattening units). Respirable (particles < 5 μm) and total dust fractions were obtained. Samplings were repeated every 2 weeks for a total of 6 samplings per unit between January and April. The predominant microorganisms isolated were bacteria (up to 1.25 × 10^6 CFU/m^3) with an important fraction in the respirable size range (up to 0.5 × 10^6 CFU/m^3). Only small quantities of gram-negative bacteria, yeasts, and molds were found. Identification of the colonies isolated revealed a great diversity of microorganisms present in the air of the different buildings. *Enterobacter agglomerans, Moraxella, Acinetobacter calcoaceticus,* and *Pseudomonas* were the most frequently identified bacteria. *Scopulariopsis, Aspergillus, Penicillium,* and *Candida* were the most numerous fungi. *Faenia rectivirgula,* the causative agent of farmer's lung, was not a major contaminant. The results show some differences in airborne microbial contamination between farrowing and fattening units; the distinction, however, is not clear-cut and was observed only for the total bacteria. The level of airborne microbial contamination in swine units does not significantly vary as a function of the outside temperature. Some species of bacteria and fungi isolated in this study are known to induce extrinsic allergic alveolitis. Other fungi are known to be potentially pathogenic for man. The air of swine confinement buildings is highly contaminated with bacteria, yeasts, and molds at a level up to 1200 time higher than so-called "normal air."

Many studies have identified a high percentage of respiratory and other occupational health problems of workers in swine confinement buildings.[1-8] The air in these buildings is known to be contaminated by numerous potentially hazardous airborne materials; these include viral, fungal, and bacterial agents carried on particulate matter.[7,9,10] Particles of less than 5 μm in diameter are particularly hazardous since they may penetrate deep into the lungs.[11] Correlation was found between the impairment in pulmonary functions, chest tightness, and dyspnea and the exposure to several contaminants, among them endotoxin, molds, and fermentative bacteria.[12,13] However, potential lung toxicity is correlated not only with the total number of airborne bacteria but also with the bacterial species involved; certain bacteria with leukocyte-mobilizing activity being more damaging.[14] It is important, therefore, to count not only the number of microorganisms present but also to identify them. Precipitating antibodies against *Aspergillus fumigatus* and *Faenia rectivirgula,* two aetiologic agents of farmer's lung disease,[15] were found in swine workers.[7] However, antibody levels against several species of molds were not different in swine workers compared to non-farming controls.[12] No correlation was found between the presence of antibodies and respiratory complaints.[7,8]

Results of studies on airborne dust, microorganisms, and endotoxin levels in swine confinement buildings differ from one country to another.[13,16-23] This is probably related to differences in the types of buildings, the climatic conditions, or the time of the year when the samplings were done. Some studies do not mention these variables.[13,18,19,21,24] Previous Canadian studies on hog confinement farms did not report the level of airborne microbial contamination.[5,6]

Currently available data characterize certain taxonomic groups but often not the genera or species of microorganisms found. Emphasis has been on total airborne bacteria. In an Iowa study,[19] a viable bacterial count of 1.7 × 10^4 colony forming units/m^3 (CFU/m^3) was reported; the predominant type being gram-positive. Thermophilic *Actinomycetes* have also been isolated.[19] The same workers reported 0.4–1.4 × 10^6 CFU/m^3 bacteria in Swedish buildings.[13,19] Again the predominant bacterial type was the gram-positive, the gram-negative count was 8.4 × 10^3 CFU/m^3. In another Swedish study,[18] airborne levels of total and gram-negative bacteria averaged 3 × 10^5 CFU/m^3 and 8 × 10^4 CFU/m^3, respectively. *Enterococcus* accounted for 68%–96% of total bacteria. Other predominant species were *Acinetobacter calcoaceticus, Alcaligenes odorans, Enterobacter agglomerans, Escherichia coli, Klebsiella* spp., and *Pseudomonas* spp.. Total bacterial counts averaged 1.3–3.4 × 10^5 CFU/m^3

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with only a small percentage of fecal coliforms in a swine-growing-finishing unit in Nebraska.[20] Some coagulase-positive *Staphylococcus* were also isolated in that study.

Information on concentrations of airborne molds and yeasts is less well documented. This is surprising considering the fact that some fungi, such as *Penicillium* spp. and *Aspergillus* spp., are known to produce extrinsic allergic alveolitis, a clinical entity found in swine workers.[3,4,25] Previous reports mentioned concentrations of 300 CFU/m^3 and 30 CFU/m^3 for fungi and *Aspergillus* spp., respectively.[18] Other investigations, conducted in the U.S. and Sweden, reported higher level of molds, namely 1.9×10^3 CFU/m^3 and 3.0×10^4 CFU/m^3, with *Penicillium, Alternaria, Aspergillus, Fusarium, Rhizopus, Verticillium, Cladosporium, Scopulariopsis,* and *Hormodendrum* as predominant genera.[19] Concentrations as high as 2.5×10^5 CFU/m^3 and 3.4×10^5 CFU/m^3 in a farrowing and a prefattening unit, respectively, have been observed in Czechoslovakia.[21] However, the relative contribution of each genera to the total mold contamination has not been mentioned (except for *Verticillium* which was higher in the U.S.). No report of airborne yeast concentrations in swine confinement buildings has, to the authors' knowledge, been published.

Although a number of studies on swine confinement buildings have been published, current data lack a complete identification of the microflora found in this environment and provide incomplete information on respirable fraction. This study was done (1) to further identify the bacteria, molds, and yeasts found in swine confinement environment, (2) to quantify the respective bacteria and fungi in respirable and in total dust fractions, (3) to verify the difference in microbial contamination between farrowing and fattening units and the variability over time, and (4) to compare bacterial contamination in the authors' cold climate to data of other countries.

MATERIAL AND METHODS

Environment

The buildings studied consisted of two farrowing units and two fattening units located in a rural county 35 km from Quebec City, Canada. All four buildings had a central manure pit that was emptied when full (about once every two weeks). The two farrowing units (Farms A and C) housed approximately 400 pigs each. These farms also had an additional 60 sows housed separately and brought to the farrowing units for about 1 month at each litter. These two units had electrical heating, and the temperature was maintained fairly constant at about 20° C. The two fattening units (Farms B and D) housed 800 and 400 pigs, respectively, and had no heating. The temperature was adjusted by changing the output of fan ventilators, depending on the outside temperature. Each piggery was visited every 2 weeks for six visits between January 29 and April 30. The sampling schedule is described in Table I. The time of day and the day of the week was different for each visit.

Sampling Strategy and Procedure

Six-stage Andersen samplers (Andersen 2000 Incorporated, Atlanta, Ga.) were used for collecting airborne bacteria and fungi.[11] Total microorganisms reported represent the microorganisms isolated on the six stages of the sampler, whereas the respirable microorganisms (dust diameter < 5 μm) were the ones isolated on Stages 3 to 6. Preliminary experiments showed that if the time of sampling was too long, the number of microorganisms on the agar media was so large that their counting and isolation were difficult when not impossible. The ideal sampling times were 4 min for the total fraction and 20 min for the respirable fraction. All samplings were done at these times, except for January 29 when the total fraction specimens were collected for only 30 sec. Plastic Petri dishes containing 35 mL of the appropriate media were placed in the Andersen samplers for all sampling.[18] The samplers were disinfected with 70% ethanol and dried with acetone to remove moisture between each sampling. Calibration of airflow through the Andersen samplers was done without the Petri dish in place. Previous experiments showed that the presence of the plates in the sampler did not modify the calibration. The average airflow in the samplers was 28.3 L/min and the sampling was done at 1 m above the ground in the central aisle. The total dust sampling was carried out using 37-mm polyvinylchloride membrane filters (0.8-μm pore size) each housed in a plastic cassette (Nuclepore Corporation, Pleasanton, Calif.). The samplings were done using a suction pump (Gilian Instrument Corp., Wayne, N.J.) at a flow rate of 2

TABLE I
Details of Samplings Done at Each Visit for Each Swine Confinement Building and Outdoor Temperatures for Each Sampling Date

Date of Sampling	Outdoor Temperature (°C)	TSA[A] (35° C) T[C]	TSA (35° C) R[D]	MacConkey T	MacConkey R	SDA[B] T	SDA[B] R	Czapek T	Czapek R	TSA (52° C) T	TSA (52° C) R	Dust
1/29/87	-11.4	Y[E]	Y	Y	Y	Y	Y	N[F]	N	N	N	Y
2/10/87	-20.0	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Y
3/9/87	-11.4	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N
3/25/87	7.2	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
4/7/87	3.4	Y	Y	Y[G]	Y[H]	Y	Y	Y	Y[I]	Y	Y	Y
4/30/87	5.7	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

[A]TSA = trypticase soy agar.
[B]SDA = Sabouraud dextrose agar.
[C]T = total fraction.
[D]R = respirable fraction.
[E]Y = sampling.
[F]N = no sampling.
[G]Except for Farms B and D.
[H]Except for Farm B.
[I]Except for Farm D.

L/min during 1 hr. This method was standardized by the Institut de recherche en santé et en sécurité du travail.[26] Sampling took about 2 hr at each farm. Apart from air sampling, temperature and relative humidity were recorded with a psychrometer (Cole-Parmer Instrument Co., Chicago, Ill.).

Counting and Identification of Microorganisms

Total bacterial counts were determined on trypticase soy agar (BBL) plates. MacConkey agar (Difco Laboratories, Detroit, Mich.) was used as a selective media for gram-negative bacteria. Sabouraud dextrose agar with chloramphenicol (Institut Pasteur Production, Marnes la Coquette, France) was used to determine total yeast and mold, and Czapek solution agar (Difco) with 200 mg/L of chloramphenicol was used for the isolation of *Apsergillus* species. Bacterial and fungi plates were incubated at 35°C for 40 to 120 hr. TSA plates incubated at 52°C for 120 hr were used for the determination of *Faenia rectivirgula* (formerly *Micropolyspora faeni*[27]). Bacterial counts were done using a dissecting-type microscope. All colonies growing on MacConkey agar plates and showing different morphological characters were re-inoculated on fresh media for further identification. Total bacteria were counted on TSA; different colonies on this media were identified to species level. Yeasts and molds were counted macroscopically and all different colonies were further identified.

Identification of microorganisms was done using MicroScan (Pos BP panels) (Baxter Healthcare Corporation, West Sacramento, Calif.) for gram-positive bacteria, MicroScan (Neg BP panels) for gram-negative bacteria and API 20C (Analytab Products, Plainview, N.Y.) for yeasts. Most of the molds grown on slide culture and stained with cotton blue were identified microscopically using morphological characters; others were identified using biochemical characteristics.

The concentrations of microorganisms in 1 cubic meter of air were calculated from the colony count and airflow, and expressed as CFU/m^3.

Statistical Analysis

For normally distributed data, analysis of variance followed by Student-Newman-Keuls (SNK) test was performed to detect significant difference between the means. For non-normally distributed data, Friedman's test followed by a multiple comparison analysis for ranked data, similar to the Tukey procedure, was used.[28] However, for reason of uniformity, all microbiological data are expressed as median (range) even if they are normally distributed. Simple linear correlation was used to determine the intensity of association between microorganisms and dust concentrations.

RESULTS

Airborne Microbial Levels

The median airborne concentrations of microorganisms detected in the swine confinement buildings are presented in Table II (total) and Table III (respirable). The airborne concentration of

TABLE II
Median Numbers of Total Microorganisms (CFU/m^3) Found in the Four Swine Confinement Buildings

	Farrowing Units				Fattening Units			
	A		C		B		D	
Total bacteria (10^3)*	151	(123–289)[a]	183	(112–401)[a]	492	(199–1248)[b]	544	(292–996)[b]
Gram-negative	80	(26–4593)	80	(0–238)	140	(71–495)	180	(18–371)
Yeasts	50	(9–115)	50	(9–256)	40	(0–291)	40	(0–124)
Molds	100	(44–353)	10	(0–88)	150	(141–389)	40	(0–557)
Aspergillus sp.§	0	(0–71)[a,b]	0	(0–9)[a]	40	(18–212)[b]	10	(0–796)[a,b]
Faenia rectivirgula	0	(0–2)	0	(0–5)	9	(0–23)	0	(0–9)

*Medians followed by a different letter are significantly different (SNK, $p < 0.025$)

§Medians followed by a different letter are significantly different (nonparametric multiple comparisons, $p < 0.05$)

TABLE III
Median Numbers of Respirable Microorganisms (CFU/m^3) Found in the Four Swine Confinement Buildings

	Farrowing Units				Fattening Units			
	A		C		B		D	
Total bacteria (10^3)*	81.0	(61–111)[a]	86.3	(51–115)[a]	167.8	(138–234)[a,b]	204.9	(140–497)[b]
Gram-negative	11	(0–67)	11	(0–18)	25	(2–122)	22	(2–81)
Yeasts	12	(0–32)	14	(2–23)	5	(0–16)	10	(0–32)
Molds§	40	(19–51)[a]	8	(7–16)[b]	29	(5–62)[a]	15	(9–37)[a,b]
Aspergillus sp.	2	(0–19)	2	(0–9)	16	(0–39)	2	(0–5)
Faenia rectivirgula	0		0		0		0	

*Medians followed by a different letter are significantly different (SNK, $p < 0.025$)

§Medians followed by a different letter are significantly different (SNK, $p < 0.05$)

total bacteria was higher in the two fattening houses than in the farrowing units (SNK, $p < 0.025$). The only other difference was observed for *Aspergillus* concentration, which was higher in Farm B than in Farm C (multiple comparison analysis for ranked data, $p < 0.05$). For the respirable fraction, the concentration of total bacteria in Farm D was higher than those in both farrowing houses (SNK, $p < 0.025$), and molds were more numerous in Farms A and B than in Farm C (SNK, $p < 0.05$). Small, non-statistically significant, variations were found in the level of microorganisms between the dates of sampling (data not shown). The only variation in regard to the outside temperature were for the yeast, total, and respirable fractions, in Farm C.

Respectively, 48% (28%–72%); 15% (0–84%); 52% (6%–100%); and 19% (0–60%) of the total bacteria, gram-negative bacteria, molds, and yeasts of the two farrowing units were in the respirable size range (<5 μm). These percentages were 38% (16%–69%); 12% (1%–100%); 16% (2%–60%); and 7% (0–30%) for the two fattening units. However, these differences between farrowing and fattening units were not significant (Friedman's test). The percentage of *Aspergillus* was not calculated because of their small numbers; colonies were sometimes more frequent in the respirable size range than in the total.

Identification of the colonies isolated revealed a great diversity of microorganisms present in the air of the different buildings. Table IV presents the genera and species of isolated gram-negative bacteria, molds, and yeasts in order of frequency. *E. agglomerans, Moraxella* spp., *A. calcoaceticus,* and *Pseudomonas* spp. were the most frequently identified bacteria on MacConkey agar. Molds belonging to the genera *Scopulariopsis, Aspergillus,* and *Penicillium* were the most numerous. Amongst the yeasts, *Candida* was the genus most often isolated. A diversity of genera and species was also found in the bacteria grown on trypticase soy agar (Table V). *Faenia rectivirgula* was not a major contaminant.

Dust Concentration, Indoor Temperature and Humidity

The airborne dust levels are given in Table VI. Concentration of dust in Piggerie B was higher when compared to the three others (SNK, $p < 0.001$). Data from only one piggerie (A) showed a significant correlation between the quantity of dust and the number of total bacteria ($r^2 = 0.992$, $p = 0.0003$). For the other three housing units (C, B, and D), no correlation was found between these variables ($r^2 = 0.636$, $p = 0.106$; $r^2 = 0.377$, $p = 0.2706$; and $r^2 = 0.006$, $p = 0.9049$, respectively). There was only a small significant difference between dry temperatures inside the swine confinement buildings (Table VI): the two fattening units being colder than Farrowing Unit D, and Farm C being colder than Farm B (SNK, $p < 0.05$). No difference was observed between the dates of sampling in regard to these three variables.

DISCUSSION

Airborne concentrations of bacteria found in this study were comparable to those previously reported in other swine confinement buildings, that is, similar to results reported by Clark et al.[18] and Elliott et al.[20] but higher than that of Donham et al.[18,19] In the latter study, no mention is made on the types of buildings and time of the year. These variables could modify the microflora and explain the differences between the studies. Results of the total bacteria were close to those in the present study; however, the concentration of gram-negative bacteria was greater than that obtained in the present study. This could be explained by the different media used and the country where the study was done (Sweden). The concentrations of fungi (no distinction between molds and yeasts) and *Aspergillus* found by Clark et al.[18] were similar to the present study's findings. Donham et al.[19] found a difference in the concentration of molds between Iowa and Sweden. The results from both of these locations are higher than what the present authors found. *Penicillium, Aspergillus,* and *Scopulariopsis* were the most frequently found genera in both the present and Donham[19] studies; however, the molds *Alternaria, Rhizopus, Cladosporium,* and *Hormodendrum* were not isolated in the present study.

Two very different types of hog raising units are found in the authors' farming community: fattening units and farrowing units. In farrowing units the environment is more controlled (T°) and

TABLE IV
Identification of Colonies Isolated on MacConkey Agar and Sabouraud Dextrose Agar from Air Samples of Swine Confinement Buildings[A]

	Farrowing		Fattening	
	A	C	B	D
Gram-negative bacteria				
Acinetobacter calcoaceticus	4	4	3	1(49%)
Enterobacter agglomerans	2	2	1(59%)	6
Escherichia coli	7	5	6	5
Moraxella sp.	1(34%)	3	4	3
Pasteurella sp.[B]	5	5	6	7
Pseudomonas sp.[C]	3	1(42%)	5	4
Others[D]	6	5	2	2
Molds				
Aspergillus sp.[E]	2	1(42%)	2	3
Circinella sp.	6	5	7	5
Fusarium sp.	8	5	5	8
Geotrichum sp.	5	5	8	2
Mucor sp.	4	2	4	6
Penicillium sp.	3	4	3	4
Scopulariopsis sp.	1(47%)	3	1(31%)	1(32%)
Others[F]	7	5	6	7
Yeasts				
Candida sp.[G]	1(39%)	2	1(61%)	1(61%)
Torulopsis candida	2	3	2	3
Trichosporon beigelii	3	1(45%)	3	4
Others[H]	4	4	4	2

[A] Numbers represent the rank of the number of different isolated microorganisms for each group. The percentage of the most frequently isolated microorganism is given in parentheses.

[B] *P. aerogenes* and *P. haemolytica*

[C] *P. fluorescens, P. picketti, P. putida, P. vesicularis,* and others

[D] *Alcaligenes* sp., *Flavobacterium* IIb, *Proteus vulgaris, Serratia marcescens, Xanthomonas* VE-2, CDC IV-E

[E] *A. flavus, A. fumigatus, A. glaucus, A. terreus,* and others

[F] *Acremonium, Cephalosporium, Chrysosporium, Paecilomyces, Thrichosporon, Tritiarchium,* and *Verticillium*

[G] *C. albicans, C. guilliermondii, C. lambica, C. paratropicalis, C. rugosa,* and others

[H] *Hansenula anomala, Rhodotorula rubra,* and others

TABLE V
Bacteria Isolated on Trypticase Soy Agar at 35° C

Gram Positive	Gram Negative
Aerococcus sp.	*Acinetobacter calcoaceticus*
Bacillus sp.	*Enterobacter agglomerans*
Micrococcus luteus	*Flavobacterium* IIb
varians	*Moraxella* sp.
sp.	*Pseudomonas stutzeri*
Staphylococcus aureus	*testosteroni*
cohnii	
epidermidis	
haemolyticus	
hominis	
saprophyticus	
sciuri	
simulans	
xylosus	
warneri	
Streptococcus acidominimus	
morbillorum	
sp.	
Coryneform type	

TABLE VI
Means ± SEM of Relative Humidity, Dry Temperature, and Dust Concentration of the Four Confinement Buildings

	Farrowing Units		Fattening Units	
	A	C	B	D
Relative humidity (%)	67.7 ± 5.2	70.5 ± 8.6	71.8 ± 6.4	61.3 ± 7.7
Dry temperature (°C)*	19.7 ± 1.3^{a,b}	21.4 ± 0.4^{a}	18.2 ± 0.6^{b,c}	16.4 ± 0.6^{c}
Dust (mg/m^3)*	1.9 ± 0.4^{a}	1.6 ± 0.4^{a}	8.8 ± 1.7^{b}	3.1 ± 0.7^{a}

*means followed by a different letter are significantly different (SNK, $p < 0.05$).

diseases in animals are more prevalent. Little is known on the influence of this variable on microbial contamination of environmental air. In one study the bacterial concentrations were higher in finishing than in farrowing units.[24] In a Czechoslovakian study conducted by Fiser,[21] it was shown that the concentrations of microbial contamination were similar in prefattening and farrowing houses. The present authors' results show some differences in airborne microbial contamination between farrowing and fattening units; the distinction, however, is not clear-cut and is observed only for the total bacteria. Temperature and relative humidity are two important factors associated with dust concentrations[29] and survival of bacteria in dust.[30] However, these variables cannot account for the differences in airborne microbial and dust concentrations seen in the present study since the relative humidity was similar in all farms and only a small temperature difference was observed.

The authors found no statistically significant change in the level of airborne microbial contamination in swine units as a function of the date of sampling. Studies by Curtis et al.[24] demonstrated annual fluctuations of aerial microbial contamination; the CFU/ m^3 was negatively correlated with the outside temperature. They concluded that this phenomenon probably resulted from different ventilation rates during periods of cool and warm weather. Since the temperature in the fattening units that the present authors visited was controlled by changing ventilation with changing outside temperature, such a variation in the present study's results was expected. Perhaps the authors' inability to find such a correlation in their data is due to the short period of the study (January–April). The fact that the samplings were done at a different time of the day at each visit is probably not responsible for this lack of correlation; previous studies have failed to show that one time of the day was preferable for sampling.[24] Fluctuations could be explained by variations in the activity of the farmers with regard to feeding the animals, maintenance, and cleaning of the buildings.

Some species of bacteria and fungi isolated in this study are known to induce extrinsic allergic alveolitis: *Aspergillus* spp., *Bacillus* spp., *Cephalosporium* spp., *Enterobacter agglomerans* (synonym: *Erwinia herbicola*), *Mucor* spp., *Penicillium* spp., and *Trichosporon* spp.[15,31,32] Other fungi are known to be potentially pathogenic for man. These include *Aspergillus* spp., *Scopulariopsis brevicaulis, Mucor pusillus,* and *Candida* spp.[33] Also, *Acinetobacter calcoaceticus* is known as a potential pathogen.[34]

The authors did a more detailed analysis of the various species of microorganisms found in swine confinement buildings than was done in previous reports; however, their data still represent an incomplete picture of microbial contamination of these buildings. The use of other media and different incubation temperatures would certainly result in the identification of additional species.[14] Although there are some fluctuations in the number of microorganisms over time and between different types of piggeries, the air of swine confinement buildings is always highly contaminated with bacteria, yeasts, and molds—up to 1200 times higher than so-called "normal air."[35] Further studies are needed to correlate alterations in respiratory symptoms and functions present in swine workers[1-8,13] and the prevalence of these microorganisms and the presence of precipitins to the potentially pathogenic microorganisms.

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