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# COMPARISON OF BIOAEROSOL SAMPLING METHODS FOR SWINE BARNS [1]

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## Summary

Two bioaerosol sampling methods (Andersen sampler and filtration sampler) were compared. The two samplers were used to assess the bioaerosol loads in two swine finishing barns. They were similar in terms of the species of microorganisms sampled. The persistent strains of microorganisms were various species of the following genera: *Staphylococcus, Pseudomonas, Bacillus, Listeria, Enterococcus, Nocardia, Lactobacillus,* and *Penicillium*. However, the use of Andersen sampler resulted in significantly higher bioaerosol concentrations than the filtration sampler. Thus, it appears that filtration sampling can be used for a qualitative survey of bioaerosols in swine barns while the Andersen sampler is suitable for both quantitative and qualitative assessments.

(Key Words: Airborne Microorganisms, Bioaerosol Sampling, Swine Housing.)

## Introduction

Bioaerosols include airborne particles that are living, as well as large molecules and volatile compounds that were released from a living organism. Previous studies have documented considerably higher bioaerosol concentrations in animal houses than in industrial, residential or ambient settings. Inhalation of bioaerosols can be detrimental to health through infection, allergy or toxicosis. Thus, there is a need to assess potential health risks by measuring work-place exposure to bioaerosols.

Various bioaerosol assessment methods have been reviewed and the characteristics of the different bioaerosol sampling devices have also been evaluated. However, standard methods for bioaerosol assessment have not been established and no bioaerosol sampler has been fully characterized in terms of its physical and biological sampling efficiencies. In the absence of standard methods, existing methods should be validated.

Impaction and filtration are used widely for assessing the airborne microbial loads inside livestock buildings. The six-stage Andersen viable cascade impactor (herein referred to as Andersen sampler) is the most commonly used bioaerosol sampler; it has served as a reference sampler in evaluating other sampling devices. Filtration sampling, on the other hand, is simple and relatively inexpensive compared to other sampling methods. In addition, filters can be assayed by a variety of culture and non-culture methods.

The main objective of the study was to compare bioaerosol sampling by filtration and impaction (i.e., Andersen sampler). The information will be useful to producers and

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researchers in determining appropriate sampling methods for livestock buildings.

## Procedures

Bioaerosol concentrations were measured in two swine finishing barns weekly from November 1999 to June 2000. The barns, one naturally ventilated and one mechanically ventilated, were located on the same commercial swine farm in northeast Kansas. They were similar in terms of outdoor environmental conditions, breed of pigs, type of feeds and supplements, feeding system, veterinary support, and husbandry practices. In addition, both barns had slatted floors, automatic self-feeders, and drinkers. Ground feed from bins outside the barns was distributed through overhead augers to the feeders. Both barns had gas heaters that provided supplemental heat during extremely cold weather, as well as water misting systems for cooling during hot weather.

The naturally ventilated barn (450 ft long, 40 ft wide) had five rooms that were separated from each other by solid partitions from floor to roof. The waste management system consisted of collecting the manure in shallow underfloor pits and flushing the pits twice a week. Environmental conditions inside the barn were regulated by automatically raising or lowering the curtains on either side of the barn, manually adjusting the ridge slot opening, and/or operating the misting system or supplemental heater mentioned above.

The mechanically ventilated barn (175 ft long, 32 ft wide) had two rooms separated by a solid wall and a curtain over a central alley. Ventilation air entered through slot inlets along the top of the side walls and was exhausted by two 3-ft diameter wall fans. The fans were operated intermittently by an electronic thermal controller system. Manure was collected in static pits about 4 ft deep; a submerged stand pipe maintained the manure slurry depth at 3 ft by draining the overflow into the pipe to a nearby lagoon. In each room, two fans with a diameter of 2 ft provided pit ventilation.

The mean stocking densities were 7.3 and 7.0 $ft^2$/head in the naturally and mechanically ventilated barns, respectively. The pigs were brought into the barns when they weighed about 50 to 75 lb each and remained in the barns for about 15 to 17 weeks, until they reached a marketing weight of about 240 to 275 lb.

The air temperatures inside the barns ranged from 58 to 90°F with a slightly lower mean for the naturally ventilated barn at 71°F (SD = 10°F) compared to the mechanically ventillated barn at 76°F (SD = 7/F). The relative humidity (RH) inside the barns ranged from 26 to 61% with a mean of 40% (SD = 11%). The outside air temperatures for the duration of the study (obtained from the nearest weather station) ranged from 28 to 80°F with a mean of 48/F (SD = 15°F), and the outside RHs ranged from 37 to 90%, with a mean of 66% (SD = 18%).

Two bioaerosol sampling methods were used: filtration and impaction. Filtration involved collection of airborne particulates on sterilized cellulose nitrate membrane filters and incubation on plates with R2A agar as culture medium. Air was sampled at a flow rate of 2.0 L/min for 3 minutes. An open-faced filter holder loaded with a 47-mm membrane filter and a 37-mm membrane filter with a respirable dust cyclone preseparator were used for sampling total and respirable bioaerosols, respectively. The cyclone had a 50% cut-point of 4.0 μm aerodynamic diameter. Duplicate samples were obtained inside each barn along the alley; one sample was taken about 5 m upwind outside each barn to determine the background concentration.

Sampling by impaction was done with the Andersen sampler. The sampler was a cascade impactor with 400 holes per stage and was able to separate the particles into the following size ranges: >7, 4.7-7, 3.3-4.7, 2.1-3.3, 1.1-2.1, and 0.65-1.1 μm. It was operated with a Petri dish with R2A agar under each stage. Duplicate samples were collected inside each barn along the alley at an airflow rate of 28.3 L/min for 1 min.

All culture plates were incubated at room temperature (77°F ± 9°F) for 72 h. After incubation, the colony forming units (CFUs) were counted with a colony counter. The colonies on each plate also were categorized based on appearance (i.e., color, surface form, size and surface texture). The commonly encountered strains were isolated and pure cultures of each were preserved on R2A agar stock slants. The stock strains were Gram stained and the species determined whenever possible by inoculating them on different types of selective and differential media (MacConkey, Phenethyl Alcohol Agar, Kligler's Iron Agar, Lysine Iron Agar, Sheep blood agar), which were examined according to the medium protocol.

Bioaerosol concentrations were obtained by dividing the number of CFUs by the volume of air sampled (6 L for the filter sampler and 28.3 L for the Andersen sampler). For the Andersen sampler, the total bioaerosol concentration was the sum of CFU concentrations in all six stages while the respirable fraction was taken as the sum of CFU concentrations in stages 3 to 6 (<4.7 μm aerodynamic diameter). Paired t-tests on the CFU concentrations to compare the two barns and the two sampling methods were conducted using PC-SAS.

## Results and Discussion

In the 2000 Swine Day Report of Progress (p 114) we reported total CFU and respirable CFU values for both the mechanically and naturally ventilated barns. Since that publication, we have continued to monitor these criteria. However, the values remain similar to those collected last year. The total CFU concentration in the NV barn obtained by the filter sampler ranged from $1.2 \times 10^4$ to $2.4 \times 10^5$ CFU/m$^3$ with a mean of $5.8 \times 10^4$ CFU/m$^3$. Corresponding values in the mechanically ventilated barn ranged from $1.3 \times 10^4$ to $1.4 \times 10^5$ CFU/m$^3$ with a mean of $6.5 \times 10^4$ CFU/m$^3$. The respirable CFU concentration in the NV barn ranged from $5.0 \times 10^2$ to $4.5 \times 10^4$ CFU/m$^3$ with a mean of $1.0 \times 10^4$ CFU/m$^3$ and the corresponding values in the mechanically ventilated barn ranged from $1.6 \times 10^3$ to $6.4 \times 10^4$ with a mean of $1.1 \times 10^4$ CFU/m$^3$. The respirable fraction was about 20% (SD = 19.9%) of the total CFU concentration in the naturally ventilated barn and about 18% (SD = 11.9%) in the mechanically ventilated barn. The two barns did not show any significant difference ($P>0.05$) in total and respirable CFU concentrations obtained by filtration.

Similar trends were observed in the total and respirable CFU concentrations in the naturally ventilated and mechanically ventilated barns measured by the Andersen sampler, although the actual concentrations were higher compared to those obtained by filtration. Thus, for both the filter and Andersen samplers the corresponding data from the two barns were combined in subsequent analyses.

The total CFU concentrations inside the barns measured by filtration were about 3.6 times (range = 0.5 to 11.0) the outside concentrations while the inside respirable values were about 2.9 times (range = 0.1 to 9.5) the outside concentrations. This was expected because the main sources of bioaerosols were inside the barns. The measured concentrations were within the range of published values from similar studies in swine buildings.

Comparison of the two samplers showed significant differences ($P<0.05$) in the total and respirable CFU concentrations (Table 1). Filtration had significantly ($P<0.05$) lower (by about 23%) total CFU concentration compared to the Andersen sampler. This could be attributed to the possible desiccation of the microorganisms on the membrane filter during sampling.

The filter sampler with the cyclone preseparator also had significantly ($P<0.05$) lower respirable CFU concentration compared to the Andersen sampler (Table 1). The large disparity in the respirable fraction between the two sampling methods (about 68% in terms of respirable CFU concentration) could be explained by several factors such as the possible desiccation associated with filtration, slightly lower cut-off diameter of the cyclone preseparator (4.0 μm) compared to that of the Andersen sampler

(4.7 μm), and possible wall losses in the cyclone.

Identification of the persistent strains of microorganisms showed that *Staphylococcus* accounted for over 70% of the total CFUs for both sampling methods. The other predominant types of organisms were *Pseudomonas, Bacillus, Listeria, Enterococcus, Nocardia, Lactobacillus*, and *Penicillium*.

Most of the above organisms were observed in all six stages of the Andersen sampler, although in varying proportions. This could indicate that cells or spores of specific microorganisms may have been aerosolized in different sizes or may be attached to particles of various sizes, thus they were deposited in all of the stages of the sampler. Of the various genera identified, *Staphylococcus* and *Lactobacillus* occurred mainly in the top three stages (>3.3 mm), while *Enterococcus*, *Bacillus*, and *Nocardia* appeared almost in uniform percentages throughout all stages, indicating wide variability in particle size. The concentrations of *Penicillium, Pseudomonas* and *Listeria* were higher in stages 3 (size range = 3.3 to 4.7 mm) and 4 (2.1 to 3.3 mm).

Comparison of the filter and Andersen samplers for each of the eight types of organisms showed that they differed significantly ($P<0.05$) in two (*Staphylococcus* and *Pseudomonas*) out of the eight strains in terms of total concentrations and four strains (*Staphylococcus, Bacillus, Listeria, Enterococcus*) in terms of respirable concentration.

From these observations, it appears that filtration sampling combined with the appropriate culture medium and sampling protocol can be used to assess qualitatively the bioaerosols in swine buildings. For both quantitative and qualitative assessments, the Andersen sampler can be used.

**Table 1. Total and Respirable CFU Concentrations Inside the Two Swine Barns Using Filtration and Impaction Sampling Methods, CFU/m$^3$ (n = 22)**

| | Filtration | | | Impaction | | |
|---|---|---|---|---|---|---|
| | Total | Respirable | Respirable Percentage[a] | Total | Respirable | Respirable Percentage |
| Mean | $6.6 \times 10^4$ [b] | $9.0 \times 10^3$ [b] | 15.6% [b] | $8.6 \times 10^4$ | $2.8 \times 10^4$ | 31.6% |
| SD | $3.8 \times 10^4$ | $4.1 \times 10^3$ | 8.0% | $5.1 \times 10^4$ | $2.2 \times 10^4$ | 9.0% |

[a]Respirable percentage = (respirable concentration/total concentration) × 100.
[b]Indicates significant difference ($P<0.05$) compared to corresponding mean for impaction.