

Calf Respiratory Disease and Pen Microenvironments in Naturally Ventilated Calf Barns in Winter

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ABSTRACT

Relationships between air quality, a variety of environmental risk factors, and calf respiratory health were studied in 13 naturally ventilated calf barns during winter. A minimum of 12 preweaned calves were randomly selected and scored for the presence of respiratory disease in each barn. An air sampling device was used to determine airborne bacteria colony-forming units per cubic meter (cfu/m³) of air in calf pens and central alleys within the barns. Airborne bacteria samples were collected on sheep blood agar (BAP) and eosin methylene blue (EMB) agar plates. Temperature and relative humidity were recorded in each calf pen, the barn alley, and outside the barn. Samples of bedding were collected in each pen and DM was measured. Pen bedding type and a calf nesting score (degree to which the calves could nestle into the bedding) was assigned to each barn. Calf numbers, barn and pen dimensions, ridge, eave, and curtain openings, and exterior wind speed and direction were determined and used to estimate building ventilation rates. Factors that were significantly associated with a reduced prevalence of respiratory disease were reduced pen bacterial counts (log₁₀ cfu/m³) on BAP, presence of a solid barrier between each calf pen, and increased ability to nest. Individual calf pen bacterial counts were significantly different from barn alley bacterial counts on both BAP and EMB. Significant factors associated with reduced calf pen bacterial counts on BAP were increasing pen area, increasing number of open planes of the calf pen, decreasing pen temperature, and wood-particle bedding. Significant factors associated with reduced alley bacterial counts on BAP were increased ventilation changes per hour, increased barn volume per kilogram of calf, reduced pen bacterial counts, and barn type.

Key words: calf barn, respiratory disease, airborne bacteria, natural ventilation

INTRODUCTION

Although hutches are frequently recommended as the preferred housing for preweaned dairy calves (Brand et al., 1996; McFarland, 1996), dairy owners continue to build calf barns because of the discomfort and inconvenience of cold weather, snow, and rain for calf caregivers. In recent years, naturally ventilated barns with individual pens to house calves from birth through weaning (McFarland, 1996; Holmes, 2000) have been constructed on many dairy farms. Although the barns share the common features of a ridge opening and adjustable curtain sidewalls, they vary widely in terms of construction materials, pen size and enclosures, bedding, and management of the sidewall openings. Greenhouse or conventional frame buildings enclose pens made from wire mesh, plywood, or plastic-coated panels. Some are fitted with solid covers, called a hover, over the rear half of the pen. Bedding varies in terms of quantity and material, ranging from shallow sawdust to deep, long straw.

Based upon field investigations of herds referred to the University of Wisconsin's School of Veterinary Medicine, enzootic pneumonia of calves is common in these barns, particularly during the winter months. Enzootic pneumonia of calves is traditionally associated with poorly ventilated housing conditions (Radostits et al., 2000; Callan and Garry, 2002), with a general acceptance that there is a need to improve air quality in outbreak situations. The design features of these barns meet the general recommendations for natural ventilation of livestock buildings in winter by providing eave or sidewall openings that allow prevailing winds to force fresh air into the building, and ridge openings that allow warmed air to rise by thermal buoyancy and exit the building (Albright, 1990). However, the ventilation requirements of naturally ventilated calf barns are frequently compromised by the need to avoid cold stress for the calves. Some operators close the sidewall openings in cold weather and prevent ventilation of the building by prevailing winds. Inside the barns, many calf pens are enclosed by solid panels on 3 or 4 sides, some also with covers, to minimize drafty conditions in cold weather (Holmes, 2000), and these enclosures may restrict ventilation of the

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pens. In addition, calves produce relatively little heat compared with adult cows, which limits the potential to ventilate the pen by thermal buoyancy. These factors could result in poorly ventilated microenvironments within the pens that house the individual calves.

Traditional tools used in ventilation troubleshooting include manometers to measure pressure differentials in mechanically ventilated spaces, anemometers to measure speed of air movement through inlets, and smoke generating devices to help visualize air movement. However, these devices did not appear to be appropriate to evaluate the naturally ventilated calf pen microenvironment. Noxious gas concentration detectors are sometimes used to evaluate ventilation adequacy (Feddes et al., 1984; Gerber et al., 1991), but preliminary investigations in these types of barns by the authors showed normal concentrations of carbon dioxide, low concentrations of ammonia, and minimal variability between barns. Airborne bacterial counts have been suggested as a method to evaluate air hygiene in animal houses (Blom et al., 1984; Wathes et al., 1984). After preliminary field work with an air sampling device, we conducted a field survey of commercial dairy calf barns to quantify air quality within these barns using airborne bacterial counts, explored differences between alley and pen environments, and examined the relationship between airborne bacterial concentrations, various environmental factors, and calf respiratory health.

MATERIALS AND METHODS

Barn Selection

The study population was a convenience sample of barns referred by practicing veterinarians and extension dairy specialists who were contacted late in 2003 and early 2004 for assistance in locating suitable buildings. Barn selection criteria included erection within the previous 6 yr, frame or greenhouse construction, natural ventilation with curtain sidewall inlets and an open ridge, no mechanical ventilation, individual calf pens, and a minimum of 12 preweaned calves. The request for barns specified that selection be based upon building design criteria and herds should not be selected based upon the presence or absence of disease problems. Seventeen farmers were contacted regarding the project and all were willing to participate. However, 2 farms did not have the minimum 12 nursing calves at the time, 1 barn had hutchers inside a naturally ventilated building, and 1 farm had just changed calf barn personnel and operation policies regarding the ventilation system and was excluded from the study. A single visit was made to each of 13

calf barns between January 15 and March 15, 2004. During the initial telephone contact with the owner to schedule the visit, an inquiry was made whether the calf health at the time was representative of the season (i.e., not a current outbreak). The inquiry was repeated with the owner, manager, or caregiver on the date of the visit. Health status of the calves at the time of the visit in all of the barns was considered to be typical by the farm personnel.

Environmental Assessment of Barns

Dimensions of the barn, ridge, sidewall and curtain openings, alleys, and calf pens were measured. Building and pen construction materials such as steel and greenhouse roofs, insulation, woven-wire or solid-sided pen panels and covers, and so on, were recorded. In the woven-wire or mesh panels, the wire was typically spaced on 15- to 20-cm grids. Substantial variation between barns in the width of alley and size of storage areas could affect airborne bacterial concentration, so the proportion of pen area within the barn was calculated by dividing total pen area by total barn interior area.

Calf pens were assigned a box factor score related to the number of solid planes around the calf. Box factor 1 was assigned to pens with 1 to 3 solid planes around the calf; that is, a solid floor with open wire-mesh on all sides, or floor with solid rear panel and 3 mesh sides, or floor, 2 solid sides, and mesh ends. Box factor 2 was assigned to pens with 4 solid planes around the calf; that is, floor, 3 solid sides, mesh front, and open top. Box factor 3 was assigned to pens with 5 solid planes around the calf; that is, floor, 4 solid sides, and open top, or floor, 3 solid sides, solid cover, and mesh front panel.

The type and approximate quantity of bedding was recorded. Bedding samples from the pens of subject calves were collected using a bulb planter inserted to a depth of approximately 10 cm in the center of the pen. The samples were weighed, oven dried at 60°C for 48 h, and reweighed for DM estimation. Each barn was also assigned a nesting score based upon an estimate of the ability of the calf to nestle into the bedding. Nesting score 1 was assigned when most of the calves appeared to lie on top of the bedding with legs exposed. Score 2 was assigned when calves would nestle slightly into the bedding, but part of the legs were visible above the bedding. Score 3 was used when the calf appeared to nestle deeply into the bedding material and legs were not visible. Because all of the calves were not observed while lying down, a nesting score was assigned to each barn based upon the most frequently observed score.

A Dickson TR320 Pro Series temperature and humidity data logger (Dickson, Addison, IL) was placed exterior to the barn under shade and another was placed in the building central alley floor for the duration of the visit, during which temperature and relative humidity data were recorded. Temperature data for a 2-h period during each visit was averaged and used to estimate ventilation due to thermal buoyancy. Prevailing wind speed was measured using an anemometer (model 840003, Sper Scientific, Scottsdale, AZ). Wind direction relative to the building was noted and used to estimate ventilation due to wind. The barn ventilation rate (Q_{total}) was calculated using estimates of thermal buoyancy-induced ($Q_{thermal}$) and wind-induced (Q_{wind}) ventilation rates summed through quadrature, where $Q_{total} = \sqrt{Q_{thermal}^2 + Q_{wind}^2}$ as described by Albright (1990).

Environmental Assessment of Calf Pens

Depending on the number of preweaned calves in the barn, between 12 and 21 pens were selected at evenly distributed locations around the barn. The respiratory health of each calf in each of these pens was assessed as described in the next section and air from each of these pens was sampled to determine the concentration of airborne bacteria. Airborne bacterial samples were collected using an impaction-type air sampler (airIDEAL, bioMérieux, Inc., Hazelwood, MO). Five liters of air was sampled onto a sheep blood agar plate (BAP) for total bacterial counts and 50 L of air was sampled onto an eosin methylene blue agar plate (EMB) for gram-negative bacterial counts from calf pens and from the central alleys. The pen samples were collected by moving the calf quietly to the front of the pen and setting the air sampler on a tripod located in the rear of the pen. The air sampler was positioned approximately 0.6 m above the bedded surface, 0.75 m from the rear side of the pen, and at least 1 m from the calf with the air sampler intake plate directed away from the calf. Alley samples were collected in the center of the alley using the same tripod setting at 5 to 6 evenly spaced sites along the entire length of the barn. One barn had 4 rows of stalls with 2 alleys so 11 alley sites were sampled in that barn.

The inoculated plates were incubated at $35 \pm 2^\circ\text{C}$ for 36 h before bacterial colonies were counted. The bacterial counts (cfu per cubic meter of air) were estimated from counting the clusters of colonies on the agar and using the conversion table in the user's manual (airIDEAL, 2001). The maximum count measurable by the air sampler was 326,418 cfu/m³.

Temperature and relative humidity were measured in each selected pen at the time of air microbiological

sampling using a handheld Dickson TH300 temperature and humidity indicator (Dickson).

Ammonia concentrations were measured using Gastec Precision Gas Detector System tubes (Sensidyne, Clearwater, FL). Samples were collected approximately 0.25 m above the bedding near the center of each selected pen. The average pen ammonia (mg/kg) of all pens sampled was assigned to the barn.

Calf Respiratory Disease Assessment

The total numbers of calves in each barn were counted. In each selected pen, the calf was identified, birth date was recorded, and age in days was calculated. Calf weight was estimated using heart girth measurements as described by Heinrichs et al. (1992). Stocking density (m³/kg) was calculated by dividing the barn volume by the product of total number of calves and their average estimated weight. A respiratory disease score was assigned based on rectal temperature, the character of nasal discharge, eye or ear appearance, and presence of a cough (McGuirk, 2005). As shown in Table 1, the respiratory disease score is the sum of points from the 4 categories of clinical signs, with increasing values representing progressive severity. The scoring system resulted in a minimum score of 0 and a maximum score of 12. Calves with score 6 or higher had at least 2 clinical signs of respiratory disease, and thus were considered sick. Prevalence of calf respiratory disease was calculated as the percentage of weekly age cohorts of calves with respiratory disease score 6 or more. The calf assessment process was approved by the University of Wisconsin Research Animal Resources Center Animal Care and Use Committee.

Statistical Analysis

Factors associated with the prevalence of calf respiratory disease, calf pen bacterial counts (log₁₀ cfu/m³), and alley bacterial counts (log₁₀ cfu/m³) were examined using the MIXED procedure of SAS (SAS Institute, 2001) in 3 separate models. These fixed effects included factors related to ventilation, which included air changes per hour, difference in absolute water, percentage increase in absolute water, and temperature difference between the inside and outside of the barn, and pen and alley relative humidity and air temperature; factors related to stocking density, which included measured area of an average pen in each barn, total pen area divided by barn area for each farm, and interior barn volume per kilogram of calf; factors related to pen design, which included the use of a solid divider between pens or an open mesh di-

Table 1. Scoring system for calf respiratory disease

Clinical sign	Points allocated for signs below			
	0	1	2	3
Rectal temperature, °C (°F)	37.8–38.2 (100–100.9)	38.3–38.8 (101–101.9)	38.9–39.3 (102–102.9)	≥39.4 (>103.0)
Cough	None	Induce single	Induce repeated or occasional spontaneous cough	Repeated spontaneous coughing
Nasal discharge	Normal serous	Small amount of unilateral, cloudy	Bilateral, cloudy or excessive mucus	Copious bilateral, mucopurulent nasal discharge
Eye or ear	Normal	Mild ocular discharge	Bilateral purulent ocular discharge or unilateral ear drop	Head tilt or both ears dropped

vider, pen box factor (1 to 3), mean pen bedding DM and nesting score (1 to 3) for each barn, and bedding type (straw, sawdust, other); mean calf age for each barn and barn type (conventional or green-house). The relationship between each of these factors and each of the outcomes of the study was first examined using univariate analysis with biologically relevant fixed effects that were significant at $P < 0.5$ being offered to the multivariate analyses.

Factors Associated with the Prevalence of Calf Respiratory Disease

The fixed effects used in the MIXED model for calf respiratory disease prevalence included barn nesting score, barn type, type of pen divider, mean pen bacterial counts (\log_{10} cfu/m³) on both BAP and EMB, mean calf age, and mean bedding DM. Farm served as the experimental unit and \log_{10} transformations were used for concentrations of cfu/m³ to ensure an approximate normal distribution and to avoid heteroscedasticity in residual plots. Because of the limited degrees of freedom, residual plots were also examined critically to ensure that the model was not over-fitting the data. The stepwise manual backward elimination method was used to build the model, retaining factors significant at $P < 0.05$. Plausible 2-way interactions between significant effects were added in and a manual stepwise backward elimination of nonsignificant effects ($P > 0.05$) was performed to create the final model.

Factors Associated with Airborne Bacterial Counts in Calf Pens

For the model describing pen bacterial counts, the fixed effects included barn variables of box factor (1 to 3), bedding type (straw, sawdust, or other), barn ventilation rate, mean calf age, mean pen area; and

individual pen observations of temperature, and relative humidity, and bedding DM. Farm was included as a random effect to control for clustering of the data, and the containment method was used to estimate degrees of freedom. \log_{10} transformations were used for concentrations of cfu/m³ to ensure an approximate normal distribution and to avoid heteroscedasticity in residual plots. The final model, with fixed effects significant at $P < 0.05$ was built as previously described.

Factors Associated with Airborne Bacterial Counts in the Alleys

The fixed effects used to build the alley bacterial count model included; barn type, barn ventilation rate, barn volume per kilogram of calf, mean pen bacterial counts (\log_{10} cfu/m³) on BAP, mean alley temperature and relative humidity, and area occupied by pens within the barn. Farm was included as a random effect to control for clustering of the dependent variable data, and the containment method was used to estimate degrees of freedom. \log_{10} transformations were used for concentrations of cfu/m³ to ensure an approximate normal distribution and to avoid heteroscedasticity in residual plots. The final model, with fixed effects significant at $P < 0.05$, was built as previously described.

In both the pen and alley bacterial count models, one barn emerged as a consistent outlier. The curtain and ridge openings on this barn at the time of sampling were completely closed, impeding natural ventilation (one of the inclusion criteria for the study). Mean pen and alley cfu/m³ in this barn were tested against those of the other 12 barns in PROC GLM (SAS Institute, 2001) using Bonferroni-adjusted pairwise comparisons. The outlier barn had significantly higher bacterial concentrations in both the pen and the alley compared with the other barns and was removed from the models. Because the model for calf respiratory disease

Table 2. Descriptive statistics of barn level data from 13 naturally ventilated calf barns

Parameter	Mean	SD	Minimum	Maximum
Calves, no.	59.2	39.7	13	161
Average calf age, d	36.7	12.4	24	66
Avg. calf weight, kg	60.6	11.4	43.1	86.2
Average no. calves scored for respiratory disease	17.5	2.1	12	21
Prevalence of calves with respiratory disease, %	14.3	11.2	0	37
Mean respiratory score	3.4	0.9	1.6	4.6
Barn area per calf, m ²	8.3	2.8	4.2	15.6
Barn volume per calf, m ³	38.4	16.9	17.5	85.6
Pen area, m ²	3.0	0.5	2.3	4.1
Outside temperature, °C	2.3	5.3	-8.9	10.6
Inside temperature, °C	3.9	5.3	-6.7	12.2
Temperature difference, inside minus outside, °C	1.6	5.8	-4.2	11.8
Wind speed, m/min	161	80	58	300
Ventilation rate, m ³ /h per calf	556	647	0	2,106
Ventilation rate, changes per h	18.1	27.8	0.0	94.3
Outside relative humidity, %	53.9	9.7	38.8	67.4
Inside relative humidity, %	53.7	13.6	23.5	70.9
Bedding DM, %	47.6	14.5	27.3	68.2
Ammonia in pen air, mg/kg	2.2	1.4	0	4
Average pen airborne bacterial count on blood agar, cfu/m ³	112,280	100,288	29,644	>326,418
Average alley airborne bacterial count on blood agar, cfu/m ³	44,482	89,636	5,274	>326,418
Average pen airborne bacterial count on eosin methylene blue (EMB) agar, cfu/m ³	632	464	119	1,446
Average alley airborne bacterial count on EMB agar, cfu/m ³	325	324	58	1,021

did not depend on factors related to ventilation, the herd was retained in the analysis of factors related to prevalence of respiratory disease.

Difference Between Alley and Pen Airborne Bacterial Counts

To determine whether there was a significant association between pen bacterial counts (\log_{10} cfu/m³) and alley bacterial counts (\log_{10} cfu/m³) across farm, a rank test had to be used because pen counts were truncated by the sampling procedure, limited to a maximal concentration of 326,418 cfu/m³; PROC FREQ (SAS Institute, 2001) was therefore used to create rank scores and generate Cochran-Mantel-Haenszel statistics, with significance tested using Friedman's χ^2 at $P < 0.05$.

RESULTS AND DISCUSSION

Barns, Stocking Density, Ventilation Rates, and Ammonia

The 13 barns (9 conventional, 4 greenhouse) presented considerable differences in almost all variables as shown in Table 2. The interior volume per calf varied greatly among barns, ranging from 17 to 86 m³/calf, far in excess of the minimal recommended guidelines of 6 m³/animal for young calves (Mitchell, 1976; Callan and Garry, 2002).

Pens and bedding also showed substantial differences between barns. Nine barns had solid dividers between each pen, 3 had mesh dividers, and 1 barn had half solid dividers and half mesh. Four barns were assigned to each one of the box factor categories, whereas in one barn, half of the pens were assigned to the box factor 1 category and half to the box factor 2 category. Straw was used as bedding in 7 barns, wood products (either shavings or sawdust) in 4 barns, and other materials (shredded cornstalks and blended straw with shavings) in 2 barns. A nesting score of 1 was assigned to 5 barns, nesting score 2 to 6 barns, and nesting score 3 to 2 barns.

There are differing recommendations for minimal ventilation rates of calf barns. All barns except one exceeded recommended ventilation guidelines of 25 m³/h calf for mechanically ventilated barns (Midwest Plan Service, 1990). Other ventilation recommendations are based upon theoretical air changes of the interior volume per hour and 5 of the 13 barns did not meet the 4 air changes per hour recommended by Bates and Anderson (1979). However, this failure to meet recommended volume changes per hour may be related to the large interior volume per calf provided in these barns.

Wind provided the major ventilation force in 8 of 13 barns, whereas thermal buoyancy was the major ventilation force in 4 barns, and 1 barn was essentially unventilated. Exterior wind speed averaged 163 m/

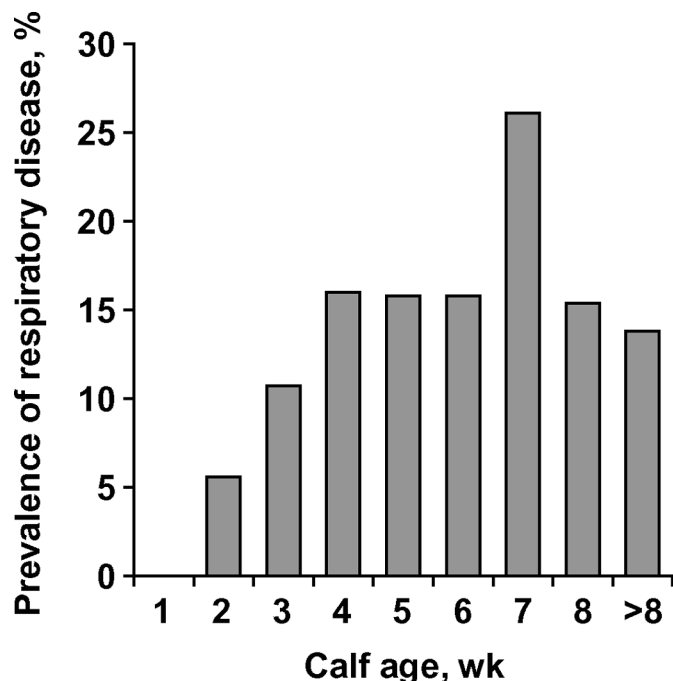


Figure 1. Prevalence of calf respiratory disease by week of age.

min (range 58 to 300), and the average difference between inside and outside temperature of the barns was less than 2°C (range -4.2 to +11.8). In 3 of the 8 barns in which wind was the predominant ventilating force, the interior temperature was equivalent to, or colder than, the exterior temperature.

Ammonia concentrations in pen air (Table 2) were consistent with the 3 to 7 mg/kg reported by Seedorf and Hartung (1999) from calf barns in Germany. The levels were well below the recommended safe thresholds of 15 to 20 mg/kg (Urbain et al., 1994), and also lower than the 5 mg/kg level at which ammonia begins to show a synergism with *Pasteurella multocida* in inducing rhinitis in swine (Hamilton et al., 1996).

Prevalence of Respiratory Disease

In the current study, the mean prevalence of respiratory disease in 225 calves in the 13 calf barns was 14% (range 0 to 37%). Most surveys of calf respiratory disease report incidence, but Virtala and Mechor (1996) reported a weekly incidence rate and a mean duration of pneumonia of 4 wk, which yields prevalence rates similar to our findings.

None of the scored calves in their first week of life showed signs of respiratory disease. Prevalence of respiratory disease increased from the second week of age and peaked during the seventh week of life (Figure 1). This pattern is similar to other surveys of calf respi-

ratory disease that reported peak incidence at 5 wk of age (Virtala and Mechor, 1996) and 6 wk of age (Waltner-Toews et al., 1986).

Factors Associated with the Prevalence of Calf Respiratory Disease

The prevalence of calf respiratory disease in 13 barns increased with increasing mean \log_{10} cfu/m³ on BAP in the pens ($P < 0.003$, 1 df), and decreased with the presence of solid dividers between pens ($P < 0.002$, 1 df) and increasing nesting score ($P < 0.004$, 2 df; Table 3). Mean calf age, bedding DM, mean pen bacterial counts (\log_{10} cfu/m³) on EMB, and barn type were not significant in the model. Figure 2 shows the modeled relationship between counts on BAP and prevalence of respiratory disease with various combinations of nesting scores and the presence or absence of solid barriers between pens.

Although the association between pen bacterial counts (\log_{10} cfu/m³) and prevalence of respiratory disease was significant, this study does not prove a causal relationship. Webster (1984) offered hypotheses for a relationship between bacterial counts and calf respiratory disease and both Pritchard et al. (1981) and Hillman et al. (1992) associated filtered air (lower bacterial and dust concentrations) with reduced incidence of respiratory disease. However, Blom et al. (1984) reported no differences in calf respiratory disease between 3 barns with significant differences in airborne bacteria and fungi counts. Wathes et al. (1984) point out that the majority of airborne bacteria are non-pathogenic, but that even dead airborne bacteria can provide a burden to respiratory tract defenses. Air that is highly contaminated with noninfectious microorganisms in work environments of people is recognized as a risk factor for respiratory disease and, although specific exposure limits have not been established, air with bacterial concentrations an order of magnitude greater than found outdoors or in "uncontaminated" areas (usually less than 10⁴ cfu/m³) is considered to be "contaminated" (Eduard and Heederik, 1998).

The practice of placing solid dividers between calf pens is a traditional recommendation for reducing risk of respiratory disease (Callan and Garry, 2002) and the practice is supported by this study. Solid panels would likely reduce the exchange of airborne pathogens between pens, as well as prevent direct nose-to-nose contact. In a Dutch study, the incidence of respiratory disorders of calves housed individually in solid-sided pens was 38.5%, compared with 60.0% for group-penned calves (Hanekamp et al., 1994). The current study confirms the value of solid dividers between individually housed calves in reducing respiratory dis-

Table 3. Final mixed model describing prevalence of calves with respiratory disease in 13 naturally ventilated calf barns

Variable	Level	Prevalence of calves with respiratory disease score of >5 (%) ¹			
		Estimate	SE of estimate	P-value for referent	P-value for effect
Intercept	—	-1.4490	0.3528	—	0.0026
Mean pen log cfu/m ³ on blood agar	—	0.2738	0.06682	—	0.0027
Pen divider	Mesh	0.2419	0.05424	0.0016	0.0016
	Solid	Referent	—	—	—
Nesting score	1 (legs visible when lying)	0.3014	0.07159	0.0021	0.0031
	2 (legs partially visible when lying)	0.2215	0.04936	0.0015	—
	3 (legs not visible when lying)	Referent	—	—	—

¹Mean = 14.3; SD = 11.2; range = 0 to 37.

ease. Although solid barriers between calves were beneficial, additional solid barriers on the ends of the pens or as a solid roof increased pen bacterial counts (cfu/m³), which is a risk factor for respiratory disease (discussed later).

Nesting score reflected the ability of the calf to nestle into the bedding when lying down. Although the bedding material did not dictate the nesting score, nesting score 3 was assigned only to pens with deep, long straw bedding and nesting score 1 was assigned only to sawdust or sawdust on sand. If the bedding material is too dense, wet, or of inadequate depth, nesting behavior

cannot occur. Inglis and Robertson (1953) showed that deep straw was a more effective insulator for animals than an insulated floor without bedding. Nesting in deep, dry bedding helps to reduce heat loss through conduction and helps the animal to avoid drafts (Webster, 1984). In effect, the calf can create its own micro-environment, trapping a boundary layer of warm air around itself, which reduces the lower critical temperature of the calf (Webster, 1984).

Ambient temperature inside the barns averaged 3.9°C (range -6.7 to 12.2°C) during our visits. These values represented late morning and very early afternoon conditions and would usually be near the daily high within the buildings on the day of the visit. The thermoneutral zone is between 10 and 26°C for a newborn calf and between 0 and 23°C for a 1-mo-old calf (Wathes et al., 1983). Clearly, the young calves were exposed to temperatures below their thermoneutral zone during many days and nights throughout the trial period.

The association of increased nesting score with reduced prevalence of respiratory disease may reflect reduced nutritional needs and immune function. Caloric requirements are increased in cold environments (National Research Council, 2001). The ability to nest reduces the lower critical temperature of the calf, eliminating a portion of the additional caloric needs for cold environments. Pollock et al. (1993) have shown reduced immune response related to inadequate nutrition. We attempted to determine nutritional status of the feeding programs in these herds, but were unsuccessful in gathering high quality data in all herds.

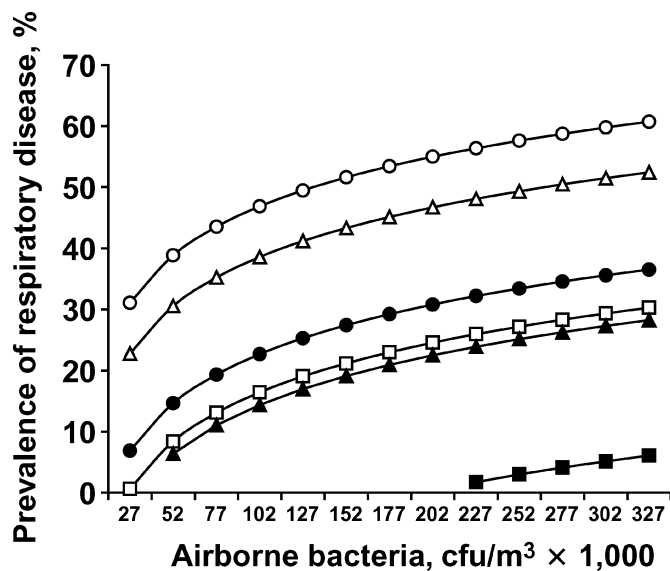


Figure 2. Model of the association between airborne bacterial concentration and prevalence of calf respiratory disease with different combinations of nesting scores and the presence or absence of a solid barrier between each pen. Nesting scores: 1 = legs visible above bedding when lying down; 2 = legs partially visible; 3 = legs not visible. Nesting score 3 and presence of a solid barrier (■); nesting score 3 and absence of a solid barrier (□); nesting score 2 and presence of a solid barrier (▲); nesting score 2 and absence of a solid barrier (△), nesting score 1 and presence of a solid barrier (●); and nesting score 1 and absence of a solid barrier (○).

Difference Between Alley and Pen Airborne Bacterial Counts

Airborne bacterial concentration within each barn depended on the location from which the samples were taken. Descriptive statistics on pen and alley airborne bacterial samples, as well as pen temperature and

Table 4. Descriptive statistics of data from individual calf pens and individual alley airborne bacterial samples from 13 barns

Parameter	No.	Mean	SD	Minimum	Maximum
Bedding DM, %	225	47.4	20.3	17.3	91.8
Pen temperature, °C	222	4.7	5.4	-5.5	20.8
Pen relative humidity, %	222	63.1	12.9	25.3	86.0
Pen airborne bacterial count on blood agar, cfu/m ³	224	110,631	124,416	5,022	>326,418
Pen airborne bacterial count on eosin methylene blue (EMB) agar, cfu/m ³	223	613	611	0	3,151
Alley airborne bacterial count on blood agar, cfu/m ³	69	39,731	82,654	1,212	>326,418
Alley airborne bacterial count on EMB agar, cfu/m ³	63	299	344	0	1,732

humidity, are presented in Table 4. The bacterial counts (cfu/m³) in the pens were significantly higher on both BAP ($P < 0.001$) and EMB ($P < 0.001$) than in the alley. The mean alley count on BAP ranged from 5,274 to greater than 326,418 cfu/m³ (the maximum count) and pen counts ranged from 29,644 to greater than 326,418 cfu/m³. On EMB, the mean alley count ranged from 58 to 1,021 cfu/m³ and pen counts ranged from 119 to 1,446 cfu/m³. The mean count by location from each barn is shown in Figure 3.

The bacterial counts from the alley are comparable to the 18,000 cfu/m³ mean reported by Pritchard et al. (1981) who made collections from unfiltered air in the central aisles of mechanically ventilated calf barns. Blom et al. (1984) reported yearly mean total bacterial

counts of 67,600 to 101,600 cfu/m³ from aisles of 3 different types of calf barn, and noted lower counts in the winter months. To the authors' knowledge, the current study is the first to differentiate concentrations between locations within barns.

There was a significant association between alley bacterial counts and pen bacterial counts (log₁₀ cfu/m³; $P < 0.009$). This is perhaps not surprising as the main sources of airborne microorganisms in barns are the animals themselves and the bedding materials (Goodrich et al., 1974; Wathes et al., 1984). However, the fact that air quality in the alley and the air in the calf pens were markedly different suggests poor mixing of air within the barn and indicates that the pens can be microenvironments within the barns.

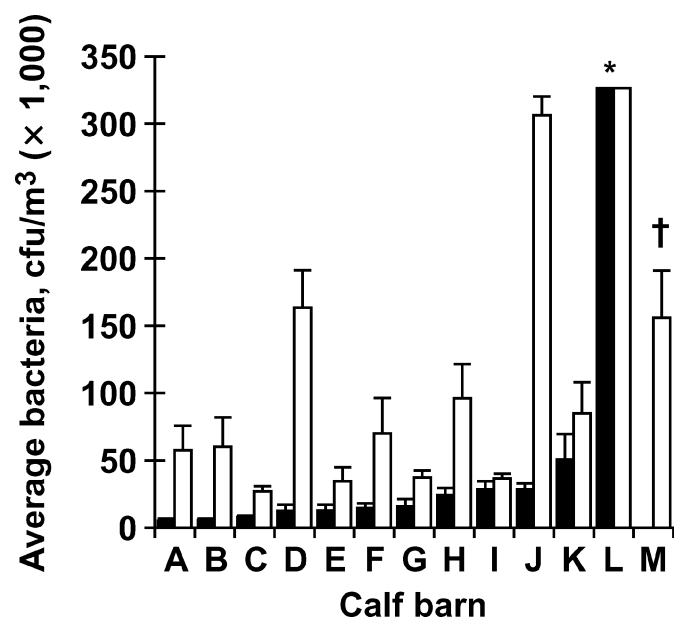


Figure 3. Average bacterial colony-forming units per cubic meter of air on blood agar plates from alley (■) and pens (□) of 13 calf barns, ranked by increasing alley bacterial count (cfu/m³). *All samples from barn L exceeded our ability to count them and were reported as the maximum countable value of 326,400 cfu/m³. †Alley samples were not collected in barn M.

Factors Associated With Airborne Bacterial Counts in Calf Pens

Based on 206 observations of pen bacterial counts (log₁₀ cfu/m³) on BAP in 12 barns, counts decreased in pens with larger area ($P < 0.023$, 1 df), increased with increasing pen temperature ($P < 0.03$, 1 df), were significantly associated with bedding type ($P < 0.001$, 2 df), with higher concentrations (cfu) observed with straw bedding compared with other materials, and increased with increasing number of solid planes surrounding the calf pen ($P < 0.006$, 2 df), called box factor in this study. The final model is shown in Table 5, and Figure 4 shows the modeled effect of these factors on the bacterial counts (cfu/m³) in different sized pens. Ventilation rate, calf age, bedding DM, and pen relative humidity were not significant in this model. Because bacterial counts (log₁₀ cfu/m³) on EMB in pens were not a significant factor related to prevalence of respiratory disease, we did not model factors related to bacterial counts (log₁₀ cfu/m³) on EMB in pens or alleys.

Mean area (SE) of individual pens in all barns was 3 m² (0.05), with a range from 2.3 to 4.1 m². The finding that pen bacterial counts (cfu/m³) decreased as area increased was expected because the calf, not the bed-

Table 5. Final mixed model describing factors associated with airborne bacterial counts in calf pens in 12 barns

Variable	Level	Log cfu/m ³ on blood agar of air samples collected in calf pens ¹			
		Estimate	SE of estimate	P-value for referent	P-value for effect
Intercept	—	4.7507	0.4302	—	<0.0001
Bedding type	Straw	0.8169	0.1615	<0.0001	<0.0001
	Other	0.2787	0.1868	0.1474	—
Box factor	Wood (sawdust or shavings)	Referent	—	—	—
	1 (1 to 3 solid planes)	-0.4513	0.1616	0.0058	0.0057
	2 (4 solid planes)	-0.04115	0.1475	0.7806	—
Pen area, m ²	3 (5 solid planes)	Referent	—	—	—
	—	-0.02641	0.01151	—	0.0228
Pen temperature, °C	—	0.01298	0.005911	—	0.0293

¹Mean = 4.75; SD = 0.50; range = 3.70 to 5.51.

ding, is the primary source of airborne bacteria in the pen (Goodrich et al., 1974; Wathes et al., 1984).

Increasing air temperature within the pen was associated with increased bacterial counts (log₁₀ cfu/m³) on BAP. In laboratory conditions, increasing air temperature decreased bacterial survival time (Donaldson, 1978; Wathes et al., 1984), which would yield reduced counts (cfu/m³). However, several field studies show increased counts in barns in warmer conditions (Jones and Webster, 1981; Blom et al., 1984). Although bacterial survival time may decrease in warmer conditions, increased temperature in the pen may increase production of bacteria and yield higher concentrations.

The finding that straw bedding was associated with higher bacterial counts (log₁₀ cfu/m³) in pens compared with either sawdust or wood shavings is consistent with controlled studies. Kotimaa et al. (1991) forced air through various types of feedstuffs and bedding materials including straw, sawdust, and wood shavings and found that straw released by far the highest counts of the materials tested. This finding may appear to contradict the earlier model where nesting score was associated with reduced prevalence of respiratory disease, but the highest nesting scores were assigned to deep straw bedding. The study suggests that the benefits of nesting in deep straw outweigh the respiratory disease risk associated with increased cfu concentrations that are attributable to straw.

Figure 4 shows the effect of increased numbers of solid sides around the calf on air hygiene within the pen. Increasing the number of solid planes around the calf creates a microenvironment within the pen, preventing ventilation of the pen and reducing diffusion of the airborne microbes out of the pen. This hypothesis is supported by the finding that the calculated ventilation rate of the building was a significant factor in explaining bacterial counts (log₁₀ cfu/m³) in the alley (discussed in the next section), but building ventilation rate was not a significant factor in explaining bacterial counts in the pens.

The recommendation to enclose the pens with solid sides and hovers has been made to reduce drafts and chilling in cold weather (Holmes, 2000). Drafts, described as air speeds of 0.3 (Wathes et al., 1983) and 0.5 m/s (Lundborg et al., 2005) within the pens, may lead to increased heat loss from the calf and chilling in cold weather. Recently, Lundborg et al. (2005) reported that calves in herds with drafts exceeding 0.5 m/s in calf pens had increased risk of moderate to severe increased lung sounds on auscultation com-

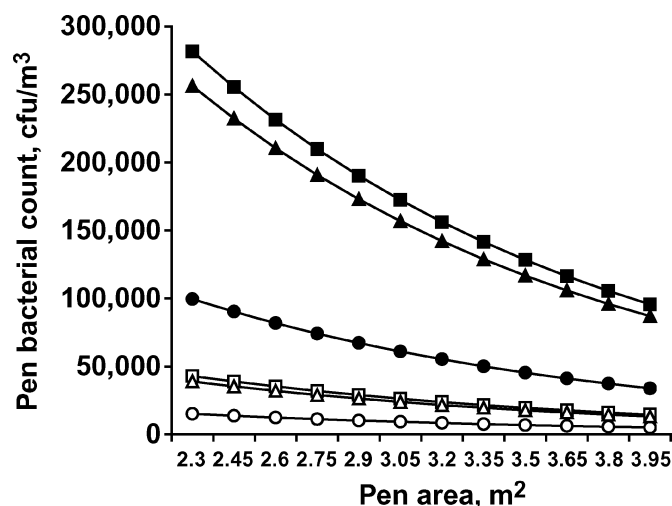


Figure 4. Model showing relationship between pen area and airborne bacteria counts with different combinations of box factor scores and bedding material. Box factor scores: 1 = 1 to 3 solid planes around the calf; 2 = 4 solid planes; 3 = 5 solid planes. Box factor 1 and sawdust (○); box factor 1 and straw (●); box factor 2 and sawdust (△); box factor 2 and straw (▲); box factor 3 and sawdust (□); and box factor 3 and straw (■).

Table 6. Final mixed model describing factors associated with airborne bacterial counts in alleys in 12 barns

Variable	Level	Log cfu/m ³ on blood agar of samples collected in alleys ¹			
		Estimate	SE of estimate	P-value for referent	P-value for effect
Intercept	—	3.1495	0.5822	—	0.0016
Ventilation rate, changes/h	—	-0.00747	0.001412	—	<0.0001
Barn volume, m ³ /kg of calf	—	-0.01027	0.002577	—	0.0002
Pen log cfu/m ³ on blood agar	—	0.3277	0.1206	—	0.0089
Barn type	Conventional	-0.3520	0.08465	0.0001	0.0001
	Greenhouse	Referent	—	—	—

¹Mean = 4.21; SD = 0.53; range = 3.08 to 5.51.

pared with calves in pens without drafts. The findings of our study suggest that it is preferable to manage drafts and cold stress by providing sufficient bedding to allow nesting rather than through enclosure of pens. A pen with mesh ends and solid sides (i.e., box factor 1) can be converted to box factor 2 by replacing the mesh rear panel of the pen with a solid panel. This change may reduce drafts, but is associated with an increase in airborne bacterial concentration (Figure 4).

Factors Associated With Airborne Bacterial Counts in the Alleys

Based on 63 observations of alley bacterial counts (log₁₀ cfu/m³) on BAP in 11 barns, counts decreased with increasing barn volume per kilogram of calf (*P* < 0.001, 1 df), increased with reduced barn ventilation rates (*P* < 0.001, 1 df), were higher in barns with higher pen bacterial counts (*P* < 0.009, 1 df), and were higher in greenhouse-type barns than conventional barns (*P* < 0.001, 1 df). Proportion of pen area within barn, and mean alley relative humidity and temperature were not significant in the final model, which is shown in Table 6.

The antagonistic effect of barn ventilation rate and stocking density on bacterial counts (log₁₀ cfu/m³) was expected. A graphic representation of the final model is shown in Figure 5 and is similar to the model produced by Wathes et al. (1983). These models show that a doubling of ventilation rate will not compensate for a doubling of stocking density, and Wathes et al. (1983) suggest that a 10-fold increase in ventilation rate would be a more realistic rate needed to compensate for a doubling of stocking density.

The finding that pen bacterial count (log₁₀ cfu/m³) was a significant factor in the model for alley bacterial count (log₁₀ cfu/m³) was expected, because the calf and pen are the primary source of airborne organisms in the barn. However, the finding that counts are higher in greenhouse barns was unexpected. Further studies are needed to explain this result.

Although relative humidity is known to be a factor in determining survivability of bacteria in open air (Donaldson, 1978; Jones and Webster, 1981; Wathes et al., 1983), humidity did not remain in the final models explaining either alley or pen airborne bacterial counts. Mean (SE) relative humidity for the barn alley air in this study was 56.3% (1.37), with a range from 35 to 71%. These values are considered to be moderate and did not extend into the ranges of >90% or <30%, where bacterial survival is changed substantially (Sainsbury and Sainsbury, 1979).

CONCLUSIONS

Calf pens in naturally ventilated calf barns frequently become microenvironments of poorer air hy-

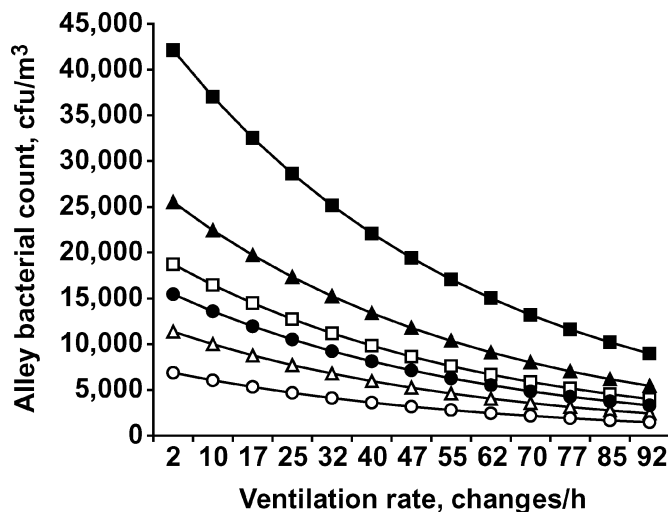


Figure 5. Model showing relationship between ventilation rate and airborne sheep blood agar bacterial counts with various stocking densities in greenhouse and conventional barns. Conventional barn providing 70 m³/50-kg calf (○); conventional barn providing 40 m³/50-kg calf (△); conventional barn providing 10 m³/50-kg calf (□); greenhouse barn providing 70 m³/50-kg calf (●); greenhouse barn providing 40 m³/50-kg calf (▲); and greenhouse barn providing 10 m³/50-kg calf (■).

giene within the barn. Increased ventilation rates effectively improve air hygiene in the alleys, but solid fronts, rear panels, and hovers result in the accumulation of airborne bacteria within the pens. The accumulation of high bacterial counts in the pens was associated with increasing prevalence of calves with respiratory disease. Solid fronts and hovers are sometimes recommended to prevent drafts and chilling, but it appears that supplying deep straw bedding in which the calf can "nest" is a preferable strategy. Although straw bedding was associated with higher pen counts than wood-based bedding, the thermal control benefits of nesting appear to outweigh the increased airborne bacteria associated with straw. Although enclosing the pen with solid fronts or covers should be avoided, a single solid barrier between calves is associated with decreased prevalence of respiratory disease. The study suggests that the ideal pen provides 3 m² or more area, has solid panels on 2 sides to separate each calf from the next, mesh panels in front and rear, and deep loose bedding during months when temperatures fall below the thermoneutral zone of the calf.

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