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Assessment of dairy calves' microbiological environment using 3M™ Petrifilm™ bacteriology plates

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Abstract

Bovine respiratory disease complex is a major disease in dairy calves. Environmental factors such as microbiological air quality have been associated with greater risk of bovine respiratory disease in calves. Routine air sampling for microbiological testing has not been traditionally performed by practitioners, mainly because of the cost associated with air sampling machines. Here we present an alternative, cost-effective air quality assessment technique using 3M™ Petrifilm™ bacteriology plates. This method can determine total aerobic bacteria, coliform, yeast, and mold counts. We found that a 10-min contact time was optimal for assessing calf environments. In the calf pens, median (ranges) bacterial counts were 340 (90 to 840) cfu/plate for aerobic plate counts, 4 (1 to 11) cfu/plate for coliform counts, 4 (1 to 11) cfu/plate for yeast counts, and 42 (1 to 120) cfu/plate for mold counts. Based on our findings, we propose that this approach can be used to monitor air quality in dairy calf barns.

Key words: BRD, environment, 3M™ Petrifilm™, bacteriology

Résumé

Le complexe respiratoire bovin est une maladie majeure des génisses laitières. Des facteurs environnementaux tels que la qualité microbiologique de l'air ont été associés au complexe respiratoire bovin. L'évaluation de la qualité microbiologique de l'air n'est pas utilisée de routine par les praticiens du fait des coûts importants des systèmes d'échantillonnage d'air. Nous rapportons dans cet article l'utilisation de systèmes de cultures Petrifilm™ pour évaluer la qualité de l'air en termes de comptages de bactéries aérobiques totales, coliformes, moisissures et levures. En utilisant un temps de contact de 10 minutes, l'utilisation des Petrifilm™ est un outil supplémentaire pour investiguer la qualité des bâtiments où sont logés les veaux. Les comptages médians (variations) au niveau des veaux étaient de 340 cfu/plaque (90–840 cfu/plaque) pour le comptage aérobique total, 4 cfu/plaque (1 to 11 cfu/plaque) pour les comptages de coliforme, 4 (1 to 11 cfu/plaque) pour les levures et 42 cfu/plaque (1 to 120 cfu/plaque) pour les moisissures. Cette méthode d'évaluation de l'air peut potentiellement aider à la gestion de la qualité de l'environnement dans les pouponnières de veaux laitiers.

Introduction

The quality of pre-weaned calves' environment is considered a risk factor for the bovine respiratory disease (BRD) complex.[9-11] As such, the air quality in calf barns and increased aerobic bacteria count, but not coliform, in the air have been associated with greater risk of BRD in dairy calves.[6,9,11] Presence of fungal spores found in barns has been associated with increased risk of respiratory disease in horses,[1] but not in calves.[4] Quantification of airborne bacteria, yeasts, and molds in dairy farms is challenging, with no validated practical or inexpensive tools available. Air samplers traditionally have been used as they allow sampling a pre-specified air volume.[9] However, the high cost (>$6,000) of these devises is prohibitive for sporadic use. Recently, 3M™ Petrifilm™[a] on-farm milk culture systems have been validated for implementation of specific udder health surveillance programs.[5] These easy-to-use bacteriology plates allow quantification of various microorganisms including aerobic plate counts (APC), coliform counts (CC), and yeasts and molds (YM) plates. These different plates have also been validated for environmental monitoring procedures.[a] The manufacturer recommends hydration of the dry culture media with sterile solution, and up to 15 min contact with the air to be sampled is recommended for air sampling as an environment monitoring procedure. However, the applicability of such an approach has not been validated on farms. The first objective of this pilot study was to validate the APC, CC, and YM Petrifilm™ plates for the farm environment using different contact times, and to identify factors causing variation of these counts. Since it was anticipated that YM counting could be difficult because of variable morphology or size of YM,[2] a second objective of the study was to assess interobserver variation for YM plates.

Materials and Methods

A 2-step cross-sectional study was performed in July and August of 2015 on 14 commercial dairy farms served

by the bovine ambulatory clinic of the Faculté de médecine Vétérinaire of the Université de Montréal (St-Hyacinthe, Qc, Canada). Farm selection was based on convenience, based principally on willingness to participate. The unit of interest of this study was the farm.

Phase 1. Identification of the appropriate duration of sampling

Because no previous information was available on the use of Petrifilm™ plates on farms, we first studied to determine the optimal contact time of the plates with air. This was performed on 2 farms that group-fed calves using air contact times of 5, 10, 15, and 20 min.

Phase 2. Determination of microbiological air quality in dairy farms

During this phase 12 more study farms were recruited (total of 14), and sampling durations were adjusted based on Phase 1 data. The study was conducted during summer months because it has been previously shown that higher microorganism counts occur during the summer.[4] It was also anticipated that group-raising calves would lead to higher air contamination because of the movement of multiple calves within the pen. A maximum of 1,000 cfu/plate were counted, and plates with > 1,000 cfu were assigned a count of 1,001. On each farm, 2 sites were sampled, the alley of the calf barn and the environment where the calves were raised, i.e., hutch, individual, or group pen. No participating farms utilized outside hutches. Calf pen sampling was always done in occupied pens.

To avoid any movement of the calf, which could contaminate the Petrifilm™ plates, calves were moved away from the individual pen/hutch during the sampling period. One operator remained in pens of group-raised calves to limit the risk of contamination due to calf movements. The alley sampling site was adjacent to the calf pen that was sampled, and the alley sampling site was used by the farmer to feed the calves with milk, milk replacer, and/or solid feed. Samples were taken at 8 to 12 inches (20 to 30 cm) from the ground to more closely mimic the location where a calf breathes when its head is dipped (Figure 1). The system used for the collection was a roof plastic base[b] where the plates (APC, CC, and YM) were disposed of using metal binders, avoiding any contact with growth media. Contact time was controlled with a chronometer.

Air quality was assessed at the same location using an anemometer[c] assessing wind speed, relative hygrometry, and temperature during a 2-min sampling. The recordings were stored in a laptop computer and minimal, maximal, and mean values were recorded. Ammonia levels were recorded with a handheld ammonia analyzer[d] with a detection range of 0 to 50 ppm.

For Phase 1 of the study (2 farms), 5, 10, 15, and 20-min durations were tested for APC and CC to be able to select an appropriate exposure time and to avoid plate overgrowth,

Figure 1. Calf barn air sampling assessment using Petrifilm™ system. The plates (previously hydrated with 1 mL of sterile water) are exposed on a plastic surface at approximately 8 inches (20 cm) from the ground. The picture represents a sample taken at the alley level.

especially for APC, which was anticipated to have higher counts.[9] For Phase 2 of the study (14 farms), only collection durations of 5 and 10 min were used. The Petrifilm™ plates were incubated in a standard incubator[e] and read according to the manufacturer's recommendations. Briefly, the APC and CC were read 48 h and 24 h after incubation, respectively, at 95°F (35°C). YM plates were read 3 to 5 days after incubation at room temperature, 68 to 77°F (20 to 25°C); all plates were read by the same person. To assess inter-observer agreement, a second operator counted yeasts and mold since this had not been previously reported, and because variation in yeast and mold morphology make them more difficult to identify.

Specific farm covariates anticipated to have an association with bacteria and YM counts were also noted. Bedding composition, nesting score (assessed in 5 calves using the previously published 1 to 3 scoring system[9]), and group vs individual housing were recorded.

The statistical analyses were performed using commercial software.[f,g] Descriptive statistics were used. Inter-operator agreement for YM count was determined by calculating the intra-class correlation coefficient (ICC) for

absolute agreement, the coefficient of variation (CV) between 2 measurements, and Bland-Altman agreement plots.[3] Spearman correlation (ρ_s) was used to determine the association between ventilation parameters (mean hygrometry, mean temperature, and maximal wind speed noted during air sampling) and APC, CC, and YM counts. Non-parametric analyses were performed for determining the association between APC, CC, and YM counts and other study covariates. Wilcoxon rank sum test for paired samples was used to determine the association between counts and exposition time, counts, and sampling site (alley or calf). The Kruskal-Wallis test was used to determine the association between counts obtained in the calf pens and calf-raising characteristics (group vs individual calf raising, type of bedding). The level of statistical significance was set at $P < 0.05$.

Results and Discussion

Phase 1. Identification of the appropriate duration of sampling

The APC and CC data for 4 different contact times on 2 farms are shown in Table 1. The counts increased with increasing contact time, with values close to the maximal plate count capacity for APC after 15 and 20 min at the calf and alley sites (close to 1000 cfu/plate) for the first farm. For these reasons, the 5- and 10-min contact times were selected for the second phase of the study. Wide variation of bacterial counts between the 2 farms was observed.

Phase 2. Determination of microbiological air quality on dairy farms

There were 60 to 280 (median 80) milking cows on the 14 study farms. Nine of 14 farms (64%) raised calves in groups during the preweaning period. Bedding types included various combinations of straw (n = 8), wood shaving (n = 7), and peat moss (n = 2). There was no association between APC, CC, or YM counts and bedding type ($P > 0.1$). There was little variability in the mean nesting score (median: 1 (min, 1; max, 1.5)).

Air quality characteristics are shown in Table 2. Descriptive results of APC, CC, and YM counts at different contact times, and sampling locations are shown in Table 3. Increased contact time with air was associated with increased contamination of microorganisms on the plates. Ammonia, temperature, and relative hygrometry were comparable with what has been recorded during the summer on Ontario dairy farms.[8] These parameters were not associated with bacterial, yeast, or fungi counts at the calf or alley sites. However, this study was not designed to detect a specific correlation between these parameters and, for this reason, it is important to validate this preliminary finding in a larger study, taking into account seasonal changes in air quality.

Difference in counts between the calf and alley sampling sites were significant for coliform counts, but not for APC and YM counts. This finding is in partial agreement with a previous study which found a difference on both aerobic and gram-negative bacterial count when sampling 5 and 50 L of air[9] between alley and calf sampling sites. However, there are several differences with the present study. In the current study various calf management systems were used (vs individual calf pens in a Wisconsin study) during the summer months (vs a winter study in the previous report[9]). Also, ventilation characteristics vary greatly between eastern Canada and many areas of the United States. In the current

Table 1. Variation of total aerobic and coliform counts in calf housing environment according to contact time using on-farm (Petrifilm™) culture system on 2 farms with group-fed calves.

Farm	Test	Site	5 min	10 min	15 min	20 min
1	TAC*	alley	600	740	920	1001
2		alley	200	220	320	340
1		calf pen	700	800	940	1001
2		calf pen	240	260	380	360
1	CC*	alley	6	10	6	7
2		alley	0	1	3	2
1		calf pen	12	11	20	30
2		calf pen	5	5	6	12

*TAC = total aerobic count; CC = coliform counts.

Table 2. Air quality characteristics in dairy calf barns at the calf and alley site during summer.

Parameters	Alley site Median (range)	Calf site Median (range)
Ammonia (ppm)	0 (0-3)	0 (0-5)
Hygrometry (%)	57.7 (52.4-63.5)	58.5 (49.8-66.8)
Temperature	68.7°F (65.8-78.6°F)	67.8°F (64.8-80.1°F)
Maximal wind speed velocity ($m.s^{-1}$)	0.31 (0.12-1.17)	0.28 (0.17-0.97)

Table 3. Comparison of aerobic, coliform, yeast and mold counts (median; ranges) with Petrifilm™ plates using 5 and 10 minutes contact time in 14 dairy farms at the calf and alley sites.

Test time	Alley					Calf					
	Median	Mean	Min	Max	*P* values (time*)	Median	Mean	Min	Max	*P* values (time*)	*P* values (site†)
APC 5 min	255	312	125	660	-	300	294	50	700	-	0.76
APC 10 min	410	492	220	1001	0.02	340	416	90	840	<0.01	0.30
CC 5 min	1	2	0	6	-	3	3	0	12	-	<0.01
CC 10 min	2	3	0	10	0.01	4	5	1	11	0.02	0.048
Y 5 min	6	15	2	86	-	6	9	0	29	-	0.46
Y 10 min	14	23	4	101	<0.01	4	5	1	11	0.05	0.15
M 5 min	50	52	6	150	-	19	35	1	105	-	0.52
M 10 min	75	67	10	150	<0.01	42	52	1	120	0.049	0.36

APC = aerobic plate count; CC = coliform counts; YM = yeast and mold counts.
*Corresponds to the association between contact time and plate count within a particular farm at the calf-pen or alley site.
†Corresponds to the association between plate count and sampling site (alley vs calf pen) within a particular farm for a specific contact time.

farm study, ventilation profiles changed significantly between summer and winter. For this reason, findings in the present study cannot be extrapolated to the winter season. Moreover, techniques used to detect coliform *per se* could have an impact on the quantitative assessment of coliform counts. The CC plate has been shown to underestimate coliform counts when comparing counts obtained from water samples vs the reference membrane filtration lactose Tergitol-7, which is considered as a gold standard.[7]

The agreement plots for yeast and mold data are summarized in Figure 2. The ICC and CV were 0.99 (95% confidence interval (CI): 0.99 to 1.00) and 32.7% for yeasts, and 0.97 (95% CI: 0.96 to 0.98) and 29.5% for mold counts, respectively. The YM plate allows detection of a wide range of yeasts and mold, and sometimes the delimitation of the mold contour can be difficult.[2] For these reasons, inter-observer agreement was assessed, which was considered (despite some inter-observer variations (CV around 30%)) to be compatible for future use by operators with limited background in mycology.

Findings in this study suggest that APC, CC, and YM Petrifilm™ plates can be used to assess microbiological air quality on dairy farms. Unfortunately, this preliminary study did not account for the specific calf health and performance outcomes that could potentially be associated with air quality, such as disease prevalence or incidence, as well as growth characteristic. This could be considered as the next step in demonstrating the potential application of this air quality assessment method.

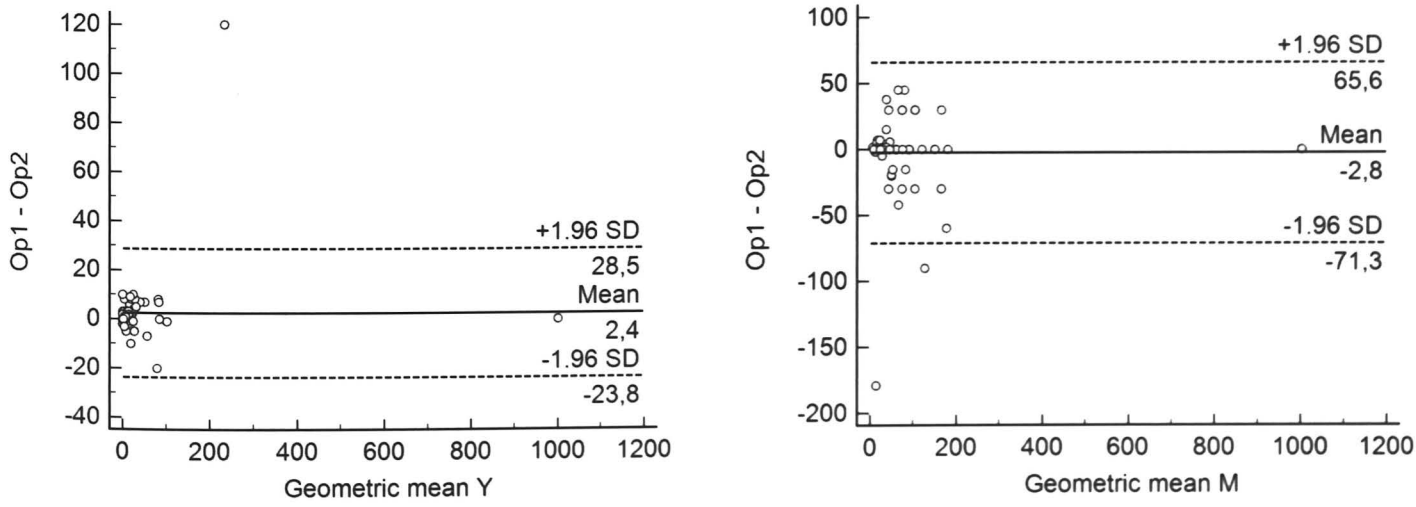

Figure 2. Interobserver agreement plots between 2 operators (Op1 and Op2) for Yeast (Y) and Mold (M) counts using Petrifilm™ plates. The intra-class correlation was 0.993 (95% CI 0.983 to 0.995) for yeast counts (on 88 plates) and 0.973 (0.959 to 0.982) for mold counts (on 87 plates).

Conclusions

In this study 3M™ Petrifilm™ plates were used to assess microbiological air quality on dairy farms. Based on the findings, we propose a 10-min contact time is suitable for detecting aerobic and coliform bacteria. Coliform counts were higher when sampled in pens or hutches compared to sampling in a nearby alley. Mold and yeast were also quantified using Petrifilm™ plates, with a good intra-class correlation coefficient between different operators with limited microbiological background.

Endnotes

[a]3M™ Canada, London, Ontario, Canada
[b]Oatey, Cleveland, OH, USA
[c]Velocicalc 9565, TSI Inc, Shoreview, MN, USA
[d]GasAlertNH3, Honeywell analytics, Lincolnshire, IL, USA
[e]Boeker digital incubator, Boeker Scientific, Feasterville, PA, USA
[f]SAS, v.9.4, Cary, NC, USA
[g]MedCalc Statistical Software version 13.1.0; MedCalc Software bvba, Ostend, Belgium

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