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Respiratory Pathogens in Québec Dairy Calves and Their Relationship with Clinical Status, Lung Consolidation, and Average Daily Gain

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Background: Bovine respiratory disease (BRD) is 1 of the 2 most important causes of morbidity and mortality in dairy calves. Surprisingly, field data are scant concerning the prevalence of respiratory pathogens involved in BRD in preweaned dairy calves, especially in small herds.

Objectives: To identify the main respiratory pathogens isolated from calves in Québec dairy herds with a high incidence of BRD, and to determine if there is an association between the presence of these pathogens and clinical signs of pneumonia, lung consolidation, or average daily gain.

Animals: Cross-sectional study using a convenience sample of 95 preweaned dairy calves from 11 dairy herds.

Methods: At enrollment, calves were weighed, clinically examined, swabbed (nasal and nasopharyngeal), and lung ultrasonography was performed. One month later, all calves were reweighed.

Results: Twenty-two calves had clinical BRD and 49 had ultrasonographic evidence of lung consolidation. *Pasteurella multocida*, *Mannheimia haemolytica*, and *Histophilus somni* were isolated in 54, 17, and 12 calves, respectively. *Mycoplasma bovis* was identified by PCR testing or culture in 19 calves, and 78 calves were found to be positive for *Mycoplasma* spp. Bovine coronavirus was detected in 38 calves and bovine respiratory syncytial virus in 1. Only the presence of *M. bovis* was associated with higher odds of clinical signs, lung consolidation, and lower average daily gain.

Conclusions and Clinical Importance: Results suggested that nasopharyngeal carriage of *M. bovis* was detrimental to health and growth of dairy calves in small herds with a high incidence of BRD.

Key words: Bovine respiratory disease; Mycoplasma; Pneumonia; Ultrasonography; Virus.

Bovine respiratory disease (BRD) is 1 of the 2 most important causes of morbidity and mortality in dairy calves and affects both preweaned and weaned calves.[1] BRD is a syndrome of diverse etiology that is caused by 1 or more of a wide range of organisms, including bacteria and viruses. Among bacteria, *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni*, *Mycoplasma bovis,* and *Trueperella pyogenes* are more commonly reported.[2] Viruses involved in the development of BRD are mainly bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PI-3), bovine herpesvirus type 1 (BHV-1), bovine viral diarrhea virus (BVDV), and bovine coronavirus (BCV).[2] Most of these pathogens are considered omnipresent in cattle populations and the exact role of some of them,

Abbreviations:

ADG	average daily gain
BHV-1	bovine herpesvirus type 1
BRD	bovine respiratory disease
BRSV	bovine respiratory syncytial virus
BVC	bovine coronavirus
BVDV	bovine viral diarrhea virus
CRSC	calf respiratory scoring criteria
DEPTH	depth of consolidation
NASA	nasal swab
NASO	deep nasopharyngeal swab
PI-3	parainfluenza virus type 3
ROC	receiver operating characteristic (ROC)

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The study was conducted using 11 herds that were regular clients of the bovine ambulatory clinic of the Faculté de médecine vétérinaire of the Université de Montréal.

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such as *Mycoplasma* spp. or BCV, in the development of BRD is still unclear.[2–4] The relative importance of these organisms in BRD pathogenesis also may be different among farms, depending on which pathogens are most prevalent and how they interact together. Over the past 10–15 years, there has been emergence or re-emergence of respiratory pathogens such as BCV[5] and *Mycoplasma bovis,*[6] which were shown to be involved in the development of BRD in dairy calves. Currently, data on the prevalence and relative importance of these different BRD pathogens in preweaned dairy calves are scant, and available data are usually from large dairy farms. Therefore, it is unclear if the prevalence of BRD pathogens is the same in large herds as in small herds such as those located in Eastern Canada.

Prevalence information of the main BRD pathogens could help to better understand how to control and prevent BRD in Eastern Canada. Therefore, the first objective of this study was to identify the main respiratory

pathogens found in preweaned dairy calves from herds with a high prevalence of BRD. The second objective of this study was to determine if there was an association between the presence of these pathogens and various clinical outcomes such as the presence of clinical signs of BRD, lung consolidation, and average daily gain (ADG). Our first hypothesis was that BRD pathogens found in small dairy herds with a high prevalence of calf respiratory disease problems would be similar to those found in larger dairy herds or beef cattle operations. Secondly, we hypothesized that the presence of some of these pathogens would be associated with cases of clinical BRD, cases of lung consolidation, or have a detrimental impact on ADG.

Materials and Methods

The study protocol was approved by the animal care committee of the Université de Montréal.

Herd and Calf Selection

A cross-sectional study was conducted on 95 dairy calves from 11 herds that were regular clients of the bovine ambulatory clinic of the Faculté de médecine vétérinaire of the Université de Montréal (Saint-Hyacinthe, QC, Canada). Herd selection was based on convenience of proximity (within 25 km radius of the ambulatory clinic), history of BRD diagnosed on the farm, and accessible accurate, detailed health records for the calves. Herds were considered to have enzootic BRD problems if they had at least 2 cases of BRD in calves confirmed by veterinary diagnosis over the last 6-month period, and if the veterinarian overseeing the herd agreed that there was an active problem of BRD in calves.

Within each selected herd, the 10 oldest preweaned female calves were selected for enrollment. If herds had <10 preweaned calves, all available preweaned calves were enrolled. Sample size estimation for the study was based on finding a significant difference of 0.3 kg/d of ADG between 2 groups of animals (pathogen present: 0.5 kg/d; pathogen absent: 0.8 kg/d), considering alpha and beta errors of 0.05 and 0.20, respectively, and after accounting for 1 covariable and herd clustering effect. A similar sample size calculation was performed for a difference of 20% in abnormal clinical status (pathogen present: 60%; pathogen absent: 40%) or lung consolidation (pathogen present: 60%; pathogen absent: 40%). In all of these calculations, a sample size of 45 animals per group (pathogen present or absent) was targeted for an estimated total count of 90 calves to be enrolled.

Procedures

All participating herds were visited twice by 2 veterinarians and a technician between November 2012 and January 2013. During the first farm visit, enrolled calves were identified, clinically examined, weighed, and sampled for BRD pathogen detection. Weight was estimated by girth circumference using a tape measure specific for this purpose.[a] Information on any previous treatments that had been administered to the calf also was collected, with particular attention paid to previous treatments for BRD. Participating producers were asked to collect on a specific sheet all disease and treatment events that occurred after the first farm visit. Participating herds were visited for a second time 1 month after the first visit by the same veterinarians and technician. During the second visit, data collection sheets were saved and enrolled calves were weighed.

Clinical Examination

During the first visit, each calf was identified, clinically evaluated, and scored by the same veterinarian using the Calf Respiratory Scoring Criteria (CRSC) from the University of Wisconsin.[7] Briefly, this 15-point score is based on 5 different criteria including rectal temperature, cough, nasal discharge, eye, and ear scores. Each criterion is scored on a 0–3 scale, with 0 associated with the lowest risk of being sick and 3 with the highest risk of BRD.

The lung area of every calf was scanned by ultrasound from the 8th to the 4th intercostal space, as previously described.[8] The ultrasonography was performed by the same person (GF) using an 8.5 MHz linear probe[b] directly applied on the thorax. Isopropyl alcohol (70%) was applied on the area of interest as contact media for acceptable image quality without clipping body hair.[c,8] The abnormality noted during lung ultrasonography was the presence of consolidated lung. Depth of consolidation (DEPTH) was considered relevant when it was ≥1 cm.[c]

Laboratory Analyses

During the first farm visit, 1 midnasal swab (NASA) was taken from the left nostril of each calf and then placed in transport media[d] for viral and *M. bovis* detection by bacteriologic culture and PCR testing. A deep nasopharyngeal swab (NASO)[e] was taken as previously reported[9] from the right nostril for routine bacterial and *Mycoplasma* cultures and then placed in transport media.[f] All laboratory analyses were performed in veterinary accredited diagnostic laboratories (see Data S1).

All conventional and mycoplasma bacteriologic cultures were performed at the Laboratoire d'épidémiosurveillance animale du Québec of the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec. Deep nasopharyngeal swabs were refrigerated and conventional culture was done within 4–6 hours of sampling. *Mycoplasma* culture was performed on NASO and NASA within 4–6 hours after sampling.

Midnasal swabs were refrigerated and submitted for PCR testing within 24 hours of sampling. Polymerase chain reaction testing included detection of *M. bovis*, BRSV, BHV-1, BVDV, PI-3, and BCV. All PCR testing was performed at the molecular diagnostic laboratory of the Faculté de médecine vétérinaire de l'Université de Montréal.

Statistical Analyses

The experimental unit of this study was the calf. A value of ADG was determined for each calf by calculating the weight gain between first and second farm visits and dividing this result by the number of days between the 2 visits.

Identification of the optimal clinical score (CRSC) threshold to predict lung consolidation was performed. For this purpose, the sensitivity and specificity for each different clinical score from 3 to 8 was calculated using the FREQ procedures of SAS.[g] A receiver operating characteristic (ROC) curve also was obtained and the Youden index calculated with the ROC module of MedCalc[h] to determine the threshold (diagnostic criterion) that maximized the total sum of sensitivity and specificity to predict lung consolidation.

Descriptive statistics were performed using the MEANS and FREQ procedures of SAS for continuous variables (ADG and age at first visit) and binary variables (clinical status, lung consolidation, treatment before first visit, and bacteriologic results), respectively. Univariable analyses were performed using the FREQ procedure of SAS (Pearson chi-square test), considering the dependent variables to be "clinical status" or "lung consolidation." Univariable associations with a $P \leq .20$ were retained for further

modeling. Final multivariable logistic regression models were built (GLIMMIX procedure in SAS) considering the dependent variables to be "clinical status" or "lung consolidation," adjusting for herd as a random effect, and forcing age at first visit into all models. Model building was performed using a backward elimination strategy until P values of all remaining variables were ≤.05.

A univariable linear mixed model (MIXED procedure in SAS) considering ADG as dependent variable and adjusting for herd as a random effect was used to screen for potential predictors. Univariable associations with P values ≤.20 were retained for further modeling. A final multivariable mixed linear model was built for ADG, considering herd as a random effect and forcing the variable age at first visit into the model. Model building was performed using a backward elimination strategy as previously described.

Results

Study Population

A total of 95 preweaned female Holstein calves from 11 different herds were enrolled in the study. The size of participating herds ranged from 36 to 264 cows in lactation (median, 98 cows). The weaning time was between 6 and 8 weeks of age in all herds, except 1 in which it was 12–16 weeks.

Sensitivities and specificities of clinical scores between 3 and 8 to predict lung consolidation are presented in Table 1. Based on ROC curve calculation (Fig 1) and the Youden index, a score of ≥7 on the CRSC was determined to be the optimal threshold for predicting calves with clinical signs of BRD (ie, sick calves). Therefore, 22 calves of 95 (23%) were considered to have clinical pneumonia in this study. Descriptive statistics for age at the first visit, clinical status, transthoracic ultrasonography, number of dead calves between visits, ADG, and prevalence of treatment before the first visit are presented in Table 2. Overall, 49 calves (52%) had ultrasonographic evidence of consolidation (DEPTH ≥1 cm). Among these 49 calves, 32 (65%) had CRSC scores <7. On the other hand, among the 22 calves with

Table 1. Prevalence and proportion of 95 preweaned female dairy calves enrolled in a study on bovine respiratory disease with lung consolidation according to different clinical score thresholds using the Calf Respiratory Scoring Criteria.

| Clinical Score | Prevalence[a] | Proportion of Calves with Lung Consolidation | | Se | Sp | P Value |
		Under TP	Above TP			
≥3	79	44.75	53.16	0.86	0.20	.49
≥4	65	43.33	55.38	0.73	0.37	.27
≥5	47	43.75	59.57	0.57	0.58	.12
≥6	28	44.78	67.86	0.39	0.80	.04
≥7	22	43.84	77.27	0.35	0.89	.006
≥8	10	45.88	100	0.20	1	.0012

Se, sensibility; Sp, specificity; TP, threshold point; which is the cutoff point for the clinical score above which a calf should be considered as clinically affected.

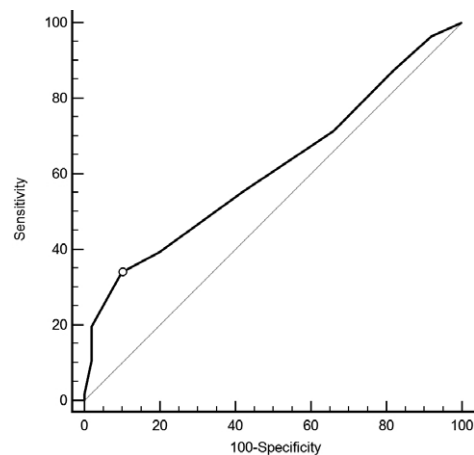[a]Number of calves with a clinical score ≥ to the threshold point.

Fig 1. Receiver operating characteristic (ROC) curve of the clinical score for the diagnosis of lung consolidation (1 or more cm) determined by bilateral thoracic ultrasonography. The dot indicates the Youden index (cutoff point of the clinical score superior to 6). The dotted line indicates the line for a completely random guess which means agreement by chance alone.

scores ≥7, only 5 (23%) had no signs of lung consolidation on ultrasonography.

Results of Laboratory Analyses

Pasteurella multocida was isolated in 54 calves (57%) from 10 herds. *Mannheimia haemolytica* was isolated in 17 calves (18%) from 5 herds. *Histophilus somni* was isolated in 12 calves (13%) from 3 different herds. More than 1 of these 3 bacteria were identified in 5 herds and in 15 calves. *Pasteurella multocida* was cultured in association with *M. haemolytica* and *H. somni* in 7 and 8 calves, respectively. Seventy-eight calves (82%) were positive for *Mycoplasma* species other than *M. bovis*. *Mycoplasma spp.* were cultured only from the NASA in 6 calves, only from NASO in 2 calves, and from both swabs in 70 calves. *Mycoplasma spp.* was cultured at least once in all herds. Nineteen calves (20%) were positive by culture, PCR, or both, of the NASA, NASO, or both swabs for *M. bovis* (Table 3). *Mycoplasma bovis* was identified only by PCR testing in 4 calves, by culture from the NASA and NASO in 4 calves, by PCR testing and culture of the NASO in 1 calf, and by PCR testing and culture of the NASA and NASO in 10 calves. *Mycoplasma bovis* was identified with *M. haemolytica* in 4 calves, *P. multocida* in 5 calves, and *P. multocida* as well as *H. somni* in 3 calves.

Bovine coronavirus and BRSV were the only viruses detected in the study. BRSV was detected in 1 calf in the only herd in which no vaccination program for BRD was carried out. BCV was detected in 38 calves (40%) from 7 different herds.

Association Between the Presence of Respiratory Pathogens and Clinical Outcomes

The presence of *P. multocida* was negatively associated with treatment before first visit ($P = .01$). Conversely,

Table 2. Descriptive statistics for age at the first visit, treatment before the first visit, number of dead calves between visits, ADG, clinical status, and transthoracic ultrasonography of 95 preweaned female dairy calves enrolled in a study on bovine respiratory disease.

Herd	Number of Calves[a]	Median Age at First Visit	Treatment Before First Visit[b]	Dead Calves Between 2 Visits	Median ADG (Range)	Clinically Affected Calves[c]	Consolidated Calves[d]
1	4 (4)	19	2	0	NA	0	2
2	10 (13)	44.5	2	0	0.85 (0.46–1.26)	2	5
3	8 (8)	30	2	1	0.38 (0.19–1.00)	3	3
4	10 (12)	28	7	0	0.68 (0.43–1.16)	0	7
5	9 (9)	35	0	0	0.73 (0.00–0.91)	2	7
6	4 (4)	34	0	0	0.70 (0.48–0.88)	3	3
7	10 (30)	36	3	0	0.63 (0.50–0.91)	2	3
8	10 (13)	38	6	0	0.67 (0.09–1.09)	3	5
9	10 (13)	22	7	4	0.34 (0.00–0.65)	4	6
10	10 (15)	48.5	1	0	0.87 (0.58–1.23)	1	3
11	10 (16)	86	7	0	1.10 (0.52–1.79)	2	5
Total	95	34	37	5	0.70 (0.00–1.79)	22	49

ADG, average daily gain; NA, not available.

[a]Number of calves sampled; the number in parentheses indicates the total number of preweaned calves present in the herd.

[b]Number of sampled calves that had been treated with antimicrobial drugs before the first visit.

[c]Clinically affected calves: number of calves with a clinical score ≥7.

[d]Consolidated calves: number of calves with depth of pulmonary consolidation ≥1 cm on transthoracic ultrasonography.

Table 3. Nasopharyngeal and nasal swab bacteriologic culture and PCR testing results for the 19 preweaned female dairy calves enrolled in a study on bovine respiratory disease with at least 1 test positive for *Mycoplasma bovis*.

Herd	Culture NASO	Culture NASA	PCR	*Mycoplasma bovis*[a]
4	1	0	3	3
7	2	2	2	2
8	3	3	1	3
9	9	9	8	10
10	0	0	1	1
Total	15	14	15	19

NASO, deep nasopharyngeal swab; NASA, Midnasal swab.

[a]Number of calves positive by culture or PCR testing, or both in the nasopharyngeal or nasal swabs, or both for *M. bovis*.

isolation of *M. bovis* in NASO (*P* = .02) and NASA (*P* = .03) cultures was positively associated with treatment before the first visit. No significant associations (*P* > .2) were found between antimicrobial treatment before the first visit and *M. haemolytica*, *H. somni*, *Mycoplasma* spp. in NASO and NASA cultures, as well as detection of *M. bovis* by PCR.

Univariable analyses showed an association between the presence of *M. bovis* in NASO (*P* = .02) and NASA cultures (*P* = .01) and calf clinical status. No other significant associations were found in the univariable association. The final multivariable model showed that, after adjusting for herd clustering and age at the first visit, *M. bovis* in NASA culture was associated with the clinical status of the animal at the first examination (negative NASA culture: 18% clinically affected; 95% CI, 14–22; positive NASA culture: 48% clinically affected; 95% CI, 34–62; *P* = .03).

The final multivariable model to determine the effect of laboratory results on lung consolidation showed that after adjusting for herd clustering and age at the first visit, bacteriologic culture results for *M. bovis* from NASO sampling were associated with lung consolidation (negative NASO culture: 47% consolidated; 95% CI, 40–54; positive NASO culture: 78% consolidated; 95% CI, 67–89; *P* = .05).

Two different models were built to determine the effect of laboratory results on ADG; 1 model was built for *M. bovis* results from NASA sampling and another model was built for *M. bovis* from NASO sampling. After adjusting for herd clustering and age at the first visit, positive NASA results for *M. bovis* were associated with a lower subsequent ADG (negative NASA culture: 0.74 kg/d; 95% CI, 0.70–0.78; positive NASA culture: 0.43 kg/d; 95% CI, 0.35–0.51; *P* < .01). A similar association was found for positive NASO results for *M. bovis* (negative NASO culture: 0.74 kg/d; 95% CI, 0.70–0.78; positive NASO culture: 0.47 kg/d; 95% CI, 0.39–0.55; *P* < .01).

Discussion

The current study focuses on the prevalence of microbial pathogens in cases of BRD in preweaned dairy calves in relatively small herds using an objective measure (lung consolidation) for the definition of cases of BRD in living animals. Among all the BRD pathogens detected in this study, only *M. bovis* isolated from the nasopharynx or the nasal cavity had a negative impact on ADG, and was associated with higher odds of having lung consolidation. *Pasteurella multocida*, *Mycoplasma* spp., and BCV were the main bacteria and virus identified in the study. However, none of them were associated with clinical score, lung consolidation on transthoracic ultrasonography, or ADG. In addition,

results of this study emphasize the importance of the adaptation of respiratory clinical score charts used in the particular population studied.

Mycoplasma bovis was the third most frequently found bacteria in the current study, but it was the only 1 associated with clinical score, lung consolidation, and poor subsequent ADG. *Mycoplasma bovis* already has been shown to be associated with chronic debilitating diseases that respond poorly to treatment.[6,10] In a recent study, *M. bovis* identified by PCR in lung tissues was associated with pneumonic lesions in young dairy calves of <1 month of age.[11] *Mycoplasma bovis* also was reported to be associated with decreased ADG in stocker calves.[12] To our knowledge, ours is 1 of the first reports to show the association between *M. bovis* culture results and decreased ADG in dairy calves. Interestingly, results of the present study showed that antimicrobial treatment before the first examination of participating calves was associated with higher odds of finding *M. bovis* at sampling. A possible explanation for this finding is that because *M. bovis* is well known to have multiple antimicrobial resistance,[10] antimicrobial treatments could have decreased the presence of other bacterial inhabitants of the nasal cavity. Such a situation potentially could promote the growth of *M. bovis*.

Definition of a clinical case is a critical point when studying BRD in cattle.[13] In the present study, the clinical status of calves was determined using previously published clinical score criteria.[7] These criteria have been developed as a useful practical tool for calf ranchers when screening dairy calves for BRD in the context of large dairy herds. The weight given to each parameter studied in the global score may be different according to different management practices such as those used in smaller herds. Consequently, it seemed important to determine a threshold that would be suitable for the particular population studied. In the present study, the optimal clinical score threshold for predicting lung consolidation (an objective measure of lung lesions) was determined based on the highest sum of sensitivity and specificity. In other words, this optimal threshold would provide the lowest possible number of classification errors (diseased or not). Determination of disease definitions in the absence of a gold standard by using an objective outcome for comparison has been commonly used for conditions such as hyperketonemia and postpartum endometritis in dairy cows.[14,15] Such an approach represents an improvement to the current standard procedure for conditions that have substantial detrimental impacts on health, culling, or reproductive performance, and that are difficult to diagnose clinically (mostly subclinical diseases). One could argue that this could have biased the clinical status definition used in the study toward more severe and chronic cases compared with the definition commonly used on farms. In the case of BRD, the use of an objective outcome is crucial considering that, in an experimental model, lung consolidation was seen by transthoracic ultrasonography as soon as 2 hours after infection with *M. haemolytica*.[1] Therefore, it is very likely that lung consolidation could appear much earlier than clinical signs of disease.

This may be an explanation for the fact that 65% of calves with lung lesions had clinical scores <7. On the other hand, 1 other explanation could be that calves have chronic lesions not associated with clinical signs. The use of clinical disease status then should be targeted at predicting the lung consolidation status of animals with the greatest accuracy.

As in previous reports in dairy calves,[16–18] *P. multocida* and *Mycoplasma* spp. were the most common bacteria isolated. *Pasteurella multocida* is well accepted as a respiratory pathogen.[2,3,19] It was not associated with the clinical status of calves, lung consolidation, or ADG in this study, which is in opposition to previous studies in dairy calves or cattle.[17,18,20–22] The presence of *P. multocida* was negatively associated with previous antimicrobial treatment, which could suggest that previous treatment decreased its prevalence. Clinical status and lung consolidation also were only evaluated once. Consequently, it was not possible to determine which stage of the diseases (ie, very acute, clinically active, or chronic) affected the calves. In other words, it was possible that calves were sick before or after the evaluation. Therefore, the role and impact of *P. multocida* in BRD could have been underestimated in the present study.

The role of *Mycoplasma* spp. (other than *M. bovis*) in the development of BRD is not clear. Numerous mycoplasma species have been isolated from the nasal flora of healthy calves,[23] but some of them, such *M. dispar*,[18] *M.* canis,[24] and *M. californicum*,[25] have been associated with BDR in calves. They are mainly associated with subclinical infections and are suspected to play a role in the induction of BRD by negatively affecting the immune system and upper respiratory defense mechanism.[2] In the present study, no association was found between the isolation of *Mycoplasma* spp. and clinical status, lung consolidation, or ADG. Unfortunately, these *Mycoplasma* spp. were not specifically identified in the present study. *Mycoplasma* spp. identified by PCR in lung tissue have been reported to be associated with pneumonic lesions in young dairy calves of <1 month of age.[11] In 1 recent study on young dairy calves, culture of *Mycoplasma* spp. in midnasal and deep pharyngeal swabs was associated with clinical cases of BRD.[17] However, no distinction between *M. bovis* and other *Mycoplasma* species was made, which could be an important confounding factor for the association between *Mycoplasma* spp. and BRD. These discrepancies point out the importance of the identification of *Mycoplasma* spp. in cases of BRD and the necessity for additional studies for the determination of the pathogenic role of *Mycoplasma* species other than *M. bovis* in young dairy calves experiencing BRD.

Among all viruses investigated in the present study, BCV was by far the most commonly detected, but no association was found between the presence of BCV and clinical status, lung consolidation, or ADG. This finding is consistent with the results of another study conducted in dairy calves from large herds in California.[17] The exact role of this virus in the development of BRD is still unknown and its pathogenic role in BRD is still unclear.[4] In young dairy calves, BCV has been

associated with mild clinical signs of pneumonia, cough, rhinitis, fever, and anorexia, usually in association with enteritis.[5] In 1 Norwegian study, dairy calves in herds with BCV seropositive calves were at a higher risk of developing pneumonia compared with calves in herds with only seronegative calves.[26] However, frequent and intermittent nasal shedding has been reported in dairy calves with or without clinical signs of respiratory disease.[27,28]

In opposition to a study in the United States in which BRSV was isolated in 14.1% of dairy calves,[17] it was only detected once in this study (1%). This is similar to the finding of a Scottish study in which 0.7% of dairy calves were positive.[16] The sample size, herd, and calf recruitment procedure in the present study potentially could explain the low prevalence of BRSV in the study population. Bovine viral diarrhea and IBR and PI-3 viruses were not found in the present study. This finding is in agreement with another study conducted in California in which there were no animals positive for BVD or BHV-1.[17] In another study conducted on 295 dairy calves, PI-3 was detected in 17.9% of animals.[16] All herds included in the study except 1 (in which BRSV was detected) had vaccination protocols for the prevention of infection by these viruses, and these protocols may have controlled and precluded dissemination of these viruses within the herds. Nonetheless, viral excretion in the nasal cavity is generally of a short duration (usually <1 week, depending on the virus), which makes viral isolation within a herd or an animal difficult if repetitive sampling is not performed. Consequently, it is difficult to conclude based on the absence of these viruses that they are not important pathogens in BRD in dairy calves in Québec.

A total of 95 calves were included in the present study. This sample size was based on a calculation of the sample size needed to provide 80% power to detect a difference of 0.3 g/d (pathogen present: 0.5 kg/d; pathogen absent: 0.8 kg/d) in ADG between 2 groups, and a difference of 20% in clinical status (pathogen present: 60%; pathogen absent: 40%) or lung consolidation (pathogen present: 60%; pathogen absent: 40%). Unfortunately, the prevalence of some pathogens was lower than expected, leading to the number of infected animals being <45 per group. This was especially important for all viruses, *M. haemolytica*, *H. somni*, and *Mycoplasma*. Therefore, although no association between these pathogens and ADG, clinical status, or lung consolidation was reported in this study, statistical power could have been insufficient in some cases (if the difference between 2 groups was the same as expected in sample size calculation) to support this lack of association with confidence. Also, results of the current study were obtained from herds recruited based on a high incidence BRD problems. Another limitation was the fact that it was a convenience sample of calves. However, in 10 herds, the sample represented all or most of the calves in the herd. In addition, the median age of the selected calves was similar to the median age of the median age of first treatment for BRD in a recent study.[29] It is unclear if sampling herds with few

or no respiratory problems or younger or older pre-weaned calves would have provided similar prevalence results for these pathogens. This situation deserves further assessment.

Footnotes

Acknowledgments

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Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Data S1. Complementory information on the bacteriological cultures and PCR testings perfomed during the study.