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Assessment of the evolution of the proportion of respiratory and enteric pathogens and diseases in pre-weaned unvaccinated dairy heifers from Québec, Canada

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Abstract

The objective of this study was to describe the proportion of enteric and respiratory pathogens and diseases in unvaccinated pre-weaned dairy heifers, in their first 2 weeks of life (exam 1), and at 4- to 8-weeks old (exam 2). Heifers from 20 dairy herds were examined and sampled twice for respiratory and enteric pathogens and diseases. Respiratory health score and ultrasonographic lung consolidation were assessed, and nasopharyngeal swabs, blood samples, and feces samples were collected. The prevalence for each disease and pathogen was described, and the difference between exams 1 and 2 was assessed. A total of 198 heifers were included at exam 1, and 182 of them were examined again at exam 2. At exam 1, the prevalence of respiratory diseases (positive clinical score or presence of lung consolidation) and diarrhea was 18% and 23%, respectively. At exam 2, the prevalence of respiratory diseases and diarrhea was 62% and 13%, respectively. Heifers were less likely to have respiratory diseases and pathogens at exam 1 than exam 2, and were more likely to have diarrhea at exam 1 than exam 2. These results help in understanding the dynamic of respiratory and enteric pathogens and diseases.

Key words: pneumonia, diarrhea, calves, dairy, housing

Introduction

The health of dairy calves is crucial for ensuring the health and productivity of future dairy cows in a herd.[1,23] Respiratory disease and diarrhea are the most commonly reported conditions in dairy calves, with diarrhea being traditionally reported during the first 3 to 4 weeks of life, and respiratory disease from 3 to 10 weeks of age.[22,28] The presence of respiratory disease has now been, however, repeatedly reported in the first month of life,[36,43] suggesting prevention might be needed at an early stage. For example, the incidence risk of respiratory disease at the individual level was 7.7% in the first 2 weeks of life, 8.0% in weeks 2 to 5, and 9.5% in weeks 5 to 12 in a sample of 19 North American herds.[43] In the same study, the incidence risk of diarrhea at the individual level was 21.2% in the first 2 weeks of life, decreasing to 1.8% in weeks 3 to 5.

Enteric and respiratory pathogens are spread by direct and indirect contact among calves, which could result in increased morbidity in group-housed calves,[28] but the association between group housing (vs individual housing) and increased morbidity has not been clearly demonstrated in previous studies, especially for small groups of calves (≤ 4; reviewed by Costa et al).[11] In Canada, both individual and group housing are used for pre-weaned heifers (64% individually, and 36% group housing),[42] but the national survey did not assess morbidity, and only herd size (number of lactating cows) was associated with mortality in pre-weaned heifers. In a multi-herd (n = 39) study, pre-weaned heifers housed in groups were more likely to have pulmonary consolidation than pre-weaned heifers housed individually,[7] but no respiratory pathogens were evaluated. In another multi-herd (n = 11) study, the difference between the type of housing for heifers was not assessed, but a variability among herd prevalence for respiratory pathogens and diseases was observed (single testing of 2- to 13-week-old heifers).[18]

The transmission patterns and the incidence of diseases, enteric and respiratory, likely differ according to the pathogens involved. Infectious and non-infectious causes of calf neonatal diarrhea have been described. The most studied and identified pathogens are rotavirus, coronavirus, *Salmonella enterica* subsp *enterica*, *Escherichia coli*, and *Cryptosporidium parvum*,[2,3,26,39] and while enteric pathogens have been identified in healthy calves,[12,20] most studies report pathogens in diarrheic calves. Respiratory disease in calves can also be caused by a variety of bacteria and viruses, alone or in combination. The most studied and reported bacteria are *Pasteurella multocida*, *Histophilus somni*, *Mannheimia haemolytica*, and *Mycoplasma bovis*, while the viruses are bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PI3), infectious bovine rhinotracheitis virus (BVH-1), bovine viral diarrhea virus (BVDV), and bovine coronavirus.[18,24] It is, however, unclear which pathogens are associated with diseases at different stages in the pre-weaning period, such as for early respiratory diseases. Describing the diseases and pathogens present in the first weeks of life would contribute to the understanding of the transmission patterns and could hint at how to develop better prevention and management practices to minimize health problems in pre-weaned dairy heifers. The objective of this study was therefore to describe the proportion, at the individual and herd levels, of enteric and respiratory pathogens and diseases at 2 different moments during the pre-weaning period in dairy heifers.

Materials and Methods

This prospective cohort study was approved by the Animal Care Committee of the Université de Montréal (19-Rech-1954), and the STROBE-Vet statement was used to report the findings.[30] The study size was limited due to budgetary restrictions. The budget allowed enrollment of a total of 200 calves (2 visits per calf), which were recruited within 20 herds to allow the assessment of herd prevalence. This sample size was sufficient to identify, with a confidence of 95% and using an intraclass correlation coefficient of 0.1 for 10 heifers per herd, a prevalence of 50% with a precision of 10%, a prevalence of 20% with a precision of 8%, or a prevalence of 10% with a precision of 6%.[14]

Herds from the clientele of the bovine ambulatory clinic of the Faculté de médecine vétérinaire of the Université de Montréal (St-Hyacinthe, QC, Canada) were enrolled between August 2018 and September 2019 (convenience sample). To avoid false positive results for the tested viruses, only herds that did not vaccinate their heifers before weaning were considered for enrollment.[41] In order to favor a good representation of different heifer management systems, 10 herds raising heifers individually until weaning and 10 herds raising heifers in groups from the second week of life until weaning and using a group feeding system were enrolled. Within each farm, the first 10 heifers born after the start of data collection were systematically sampled during a visit following their birth (first 2 weeks of life). The same heifers were sampled again during a second visit at 4- to 8-weeks of age, depending on the enrollment schedule.

At each visit, the heifers' weight was quantified from the measurement obtained with a heart-girth measurement (dairy weigh tape).[a] Heifers were also examined and attributed a respiratory health score following a standardized procedure.[b,28] Briefly, a clinical score between 0 and 3 (0: normal, 1: slightly abnormal, 2: abnormal, 3: severely abnormal) was attributed for body temperature, coughing, nasal and eye discharge, and ear position, and the total score was recorded.[28] An ultrasound of both lungs was performed using a 8.5-MHz probe[c] as previously described,[32] and the presence and size of lung consolidation was recorded. Two nasopharyngeal swabs were then taken using a double guarded mare swab.[d,15] One swab was placed in transport media[e] for conventional bacteriological culture and the other swab was placed in a dry tube for PCR testing. Feces were collected directly from the rectum of heifers. Fecal consistency was scored as 0 = normal consistency, 1 = semiformed or pasty, 2 = loose feces, and 3 = watery feces.[28] For heifers 10 d of age or younger, a blood sample was collected from the jugular vein to evaluate the transfer of passive immunity (TPI). Antimicrobial treatment information for all the enrolled calves was collected from on-farm treatment logs.

Laboratory analyses

Blood samples were transported on ice to the bovine ambulatory clinic of the Université de Montréal where they were centrifuged (1,750 x *g* for 10 min). The TPI was estimated by refractance (% Brix) of the serum using a digital refractometer validated in calves[f] using a threshold of ≥ 8.4% to define adequate TPI.[13]

Fecal samples were transported on ice to a commercial laboratory[g] where a commercial rapid immunochromatographic test kit for detecting bovine enteric pathogens[h] was used to identify bovine coronavirus, rotavirus type A, *E. coli* F5 (K99), and *Cryptosporidium* spp. Briefly, a spoonful of feces (approximately 0.25g) was added to the dilution tube and shaken with the diluent. The 4 strips, 1 for each pathogen, were dipped in the liquid phase of the sample until the liquid reached the top of the strip (10 minutes). The strips were then removed and dried at room temperature for 5 min. If 1 line appeared on the strip, the sample was considered negative for the pathogen; if 2 lines appeared, it was considered positive. If no line appeared, the test was considered invalid and was repeated.

Swabs were submitted for analysis within 24 h to the veterinary diagnostic laboratory of the Faculté de médecine vétérinaire of the Université de Montréal (St-Hyacinthe, QC, Canada). The swabs collected for conventional bacteriological culture were streaked onto 5% sheep blood agar and chocolate agar. Plates were incubated in a 5% CO_2 incubator at 95°F ± 3.6°F (35°C ± 2°C) and examined after 24 and 48 h for evidence of *P. multocida*, *M. haemolytica*, and *H. somni* growth. Initial selection was based on colony morphology,

and bacterial identification was performed using MALDI-TOF MS.[i] The other swab was also assessed within 24 h of sampling. Real time polymerase chain reaction (rtPCR) testing included detection of *M. bovis*, BRSV, BHV-1, BVDV, PI-3, and BCV. All rtPCR testing was performed at the molecular diagnostic laboratory of the Faculté de médecine vétérinaire de l'Université de Montréal, and were considered positive if ct < 35, according to the laboratory recommendations.

Statistical analysis

Statistical analyses were performed using R version 4.0.3 with the RStudio interface version 1.3.1093.[34] Heifers that were enrolled at an age greater than 14 d of life were excluded from data analyses. At exam 2, exclusion criteria were death or having an exam before 28 d or after 56 d of age. Heifers (≤ 10 d) were classified as having a failure of TPI when the Brix result was < 8.4%.[13] Diarrhea was defined as a fecal score ≥ 2 (loose feces).[28] Respiratory health was categorized as active pneumonia (AP; respiratory health score ≥ 5, and lung consolidation ≥ 1 cm), non-active pneumonia (NAP; respiratory health score < 5, and lung consolidation ≥ 1 cm), upper respiratory tract disease (URTD; respiratory health score ≥ 5 and lung consolidation < 1 cm) or no respiratory tract disease (respiratory health score < 5, and lung consolidation < 1 cm).[8,32]

Descriptive analyses for all diseases and pathogens were obtained for exams 1 and 2 separately, at the herd level (prevalence only, by type of housing) and at the individual level. The prevalence of a pathogen or disease was calculated as the number of positive heifers divided by the total of heifers tested, for both exam 1 and exam 2.

The prevalence for each pathogen and disease at exams 1 and 2 was compared at the individual level using a mixed logistic regression model including herd (clustering) and heifer (repeated measures) as random intercepts (lme4 package).[4] Mixed logistic regression models were used to assess the association between the presence of at least 1 respiratory and enteric pathogen with active pneumonia and diarrhea, respectively. As multiple models were assessed, all the final *P*-values were adjusted using the Benjamini-Hochberg approach (stats package)[27] to minimize type I errors.

Results

A total of 200 Holstein heifers from 20 herds (6 to 12 calves per herd) were originally enrolled in the study. All enrolled herds milked between 50 and 315 cows, and 11 of them (55%) were closed herds. Heifers were fed milk replacer in 15 herds, cow milk in 4 herds, and 1 herd fed both cow milk and milk replacer. Peak feeding levels varied between 6 and 14 L per day (median = 8 L). Antimicrobials were added to milk in 2 herds. Heifers were weaned at 7 to 9 weeks of age (median = 8 weeks). Bedding used was sawdust only (n = 4), a combination of sawdust and straw (n = 9), straw only (n = 6), or a combination of straw and peat moss (n = 1). Heifers housed individually were kept inside (n = 9) or in hutches outside (n = 1). Heifers housed in groups were transferred to group housing between 7 and 15 d of age to groups of 2 to 15. The maximum age difference between heifers in a group varied between 7 and 90 d.

All herds were visited for the first time between August and December 2018, and for the last time between September 2018 and September 2019, with a study duration from 2 to 10 mo (median = 5 mo). Two heifers were older than 14 d old at exam 1, resulting in 198 heifers being included for the exam 1 analysis. Twelve heifers died from 7 different herds between their first and second exam, and 6 heifers were sampled outside of the 28 to 56 d old period at their second exam, resulting in 182 heifers being included for the exam 2 analysis. To describe herd prevalence, 1 herd (group housing) was removed as it had only 6 and 2 heifers sampled at exams 1 and 2, respectively.

In addition to the heifers receiving oral antimicrobial treatment in milk (2 herds), 2 heifers (1%) had received antimicrobial treatment orally (n = 1) and parenterally (n = 1) in the week prior to their first exam, and 6 heifers (3%) had received antimicrobial treatment parenterally in the week prior to their second exam.

Exam 1 – birth to 2 weeks old

The median age at exam 1 was 5 d (range = 1 to 12, mean = 5.2, SD = 2.7). The heifers weighed between 95 and 170 lb (43 and 77 kg [median = 55, mean = 54.7, SD = 5.5; 5 missing values]) at enrollment. Of the calves examined in their first 10 d of age, 37% (n = 70/192) had a failure of TPI. The herd prevalence of failure of TPI varied between 18 and 70% (median = 35%; Figure 1). In the first 2 weeks of life (exam 1), the herd prevalence of AP, NAP, and URTD ranged from 0 to 10% (median = 0%), 0 to 33% (median = 10%), and 0 to 33% (median = 0%), respectively, and the herd prevalence of diarrhea ranged from 0 to 50% (median = 29%; Figure 1, details in Appendix).

At the individual level, nasopharyngeal samples were positive for 0 (79%; n = 157/198), 1 (20%; n = 40/198), or 2 (1%; n = 1/198) respiratory bacteria, and 0 (94%; n = 185/197) or 1 (6%; n = 12/197) respiratory viruses. Each respiratory pathogen was absent in at least half of the herds (median herd prevalence = 0%; details in Appendix). At the individual level, the most commonly identified respiratory pathogen was *P. multocida* (Table 1). Heifers with URTD were more likely to be positive for least 1 respiratory pathogen than heifers without respiratory disease (OR = 1.40, 95% CI = 1.05 – 1.87, *P* = 0.02). Indeed, more than half (n = 5/9) of the heifers with URTD were positive for at least 1 respiratory pathogen, while a third of the heifers with AP (n = 1/3) and NAP (n = 9/27) had at least 1 respiratory pathogen, and 21% (n = 34/159) of the heifers without respiratory disease had at least 1 respiratory pathogen. The distribution of pathogens in heifers according to their respiratory health status are listed in Figure 2.

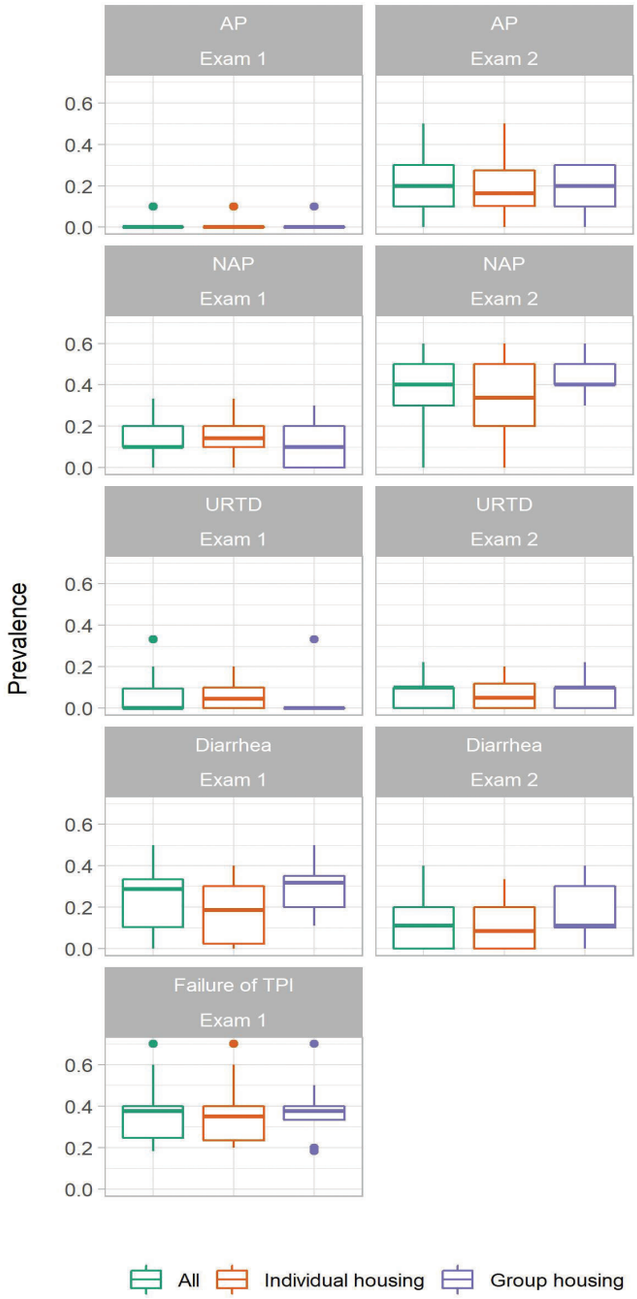

Figure 1. Boxplot of the distribution of herd prevalence of respiratory and enteric diseases, and failure of transfer of passive immunity (TPI) of 1- to 2-week-old (exam 1) and 4- to 8-week-old (exam 2) heifers in 19 Québec dairy herds (All; 10 with individual housing, 9 with group housing before weaning). Active pneumonia (AP) was defined as a respiratory health score ≥ 5* and presence of lung consolidation ≥ 1 cm†, non-active pneumonia (NAP) was defined as a respiratory health score < 5 and presence of lung consolidation ≥ 1 cm, upper respiratory tract disease (URTD) was defined as a respiratory health score ≥ 5 and presence of lung consolidation < 1 cm, and diarrhea was defined as a fecal score ≥ 2 (loose feces).[28]

*Respiratory health score was attributed following a standardized procedure (Wisconsin Clinical Respiratory Scoring Chart).[28]

† Lung consolidation was evaluated by ultrasonography.[32]

Fecal samples were positive for 0 (81%; n = 161/198), 1 (16%; n = 31/198), 2 (2%; n = 4/198), or 3 (1%; n = 2/198) enteric pathogens. Enteric pathogens, except for *Cryptosporidium* spp, were absent in at least half of the herds (median herd prevalence = 0%; details in Appendix), and the most commonly identified enteric pathogen at the individual level was *Cryptosporidium* spp (Table 1). Heifers with diarrhea were more likely to have at least 1 enteric pathogen than heifers without diarrhea (OR = 4.0, 95% CI = 1.7 – 9.5, $P < 0.01$). A total of 36% (n = 16/45) of the heifers with diarrhea had at least 1 enteric pathogen while 13% (n = 18/141) of the heifers without diarrhea did.

Of the calves that died before exam 2, 3 (25%) had a failure of TPI, 4 (33%) were categorized as NAP, 2 (17%) had diarrhea, and 2 (17%) were positive for a pathogen (respiratory BCV, n = 1; and rotavirus, n = 1).

Exam 2 – 4- to 8-weeks old

At exam 2, the heifers were between 28 and 56 d of age (median = 43, mean = 43.2, SD = 5.9), and weighed between 97 and 262 lb (44 and 119 kg [median = 87, mean = 85.6, SD = 12.8; 1 missing value]). At exam 2, the herd prevalence of AP, NAP, URTD, and diarrhea ranged from 0 to 50% (median = 20%), 0 to 60% (median = 40%), 0 to 22% (median = 10%), and 0 to 40% (median = 11%), respectively (Figure 1).

At the individual level, nasopharyngeal samples were positive for 0 (34%; n = 61/182), 1 (57%; n = 104/182), or 2 (9%; n = 17/182) respiratory bacteria, and no (91%; n = 166/182) or 1 (9%; n = 16/182) respiratory viruses. The respiratory pathogen most commonly identified was, again, *P. multocida* (Table 1). While 80% (n = 28/35), 70% (n = 50/71), and 83% (n = 10/12) of the heifers with AP, NAP, and URTD, respectively, were positive for at least 1 respiratory pathogen, 58% (n = 37/64) of the heifers without respiratory disease had at least 1 respiratory pathogen ($P = 0.26$). The distribution of pathogens in heifers according to their respiratory health status is presented in Figure 2.

Fecal samples were positive for 0 (91%; n = 166/182), 1 (8%; n = 14/182), or 2 (1%; n = 2/182) enteric pathogens. Again, more than half of the herds did not have enteric pathogen (median prevalence = 0%; details in Appendix). The most commonly identified enteric pathogen was *E. coli* (Table 1). None of the 24 heifers with diarrhea at exam 2 had enteric pathogens identified, while 10% (n = 16/156) of the heifers without diarrhea did.

Differences between exams 1 and 2

Overall, heifers at exam 1 were less likely to have AP and NAP than at exam 2, and were more likely to have diarrhea at exam 1 than exam 2 (Table 2). Heifers were, however, as likely to have URTD at exam 1 than at exam 2. Indeed, healthy heifers at exam 1 subsequently (exam 2) had NAP (n = 61/149), AP (n = 27/149), or UTRD (n = 8/149), or stayed healthy (n = 53/149). Heifers that had UTRD at exam 1 were subsequently healthy (n = 3/7), remained with UTRD (n = 2/7), or had AP

Table 1. Odds ratio (OR) of respiratory and enteric pathogens in heifers from 20 dairy herds during their first 2 weeks of life (exam 1) and at 4- to 8-weeks of age (exam 2), obtained from mixed logistic regression models (1 per pathogen), including herd and heifer as random intercepts. The pathogens* were identified using conventional culture (*Histophilus somni*, *Mannheimia haemolytica*, and *Pasteurella multocida*) and PCR (*Mycoplasma bovis*, bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), parainfluenza virus (PI3), infectious bovine rhinotracheitis virus (BVH-1), and coronavirus). The 4 enteric pathogens (coronavirus, rotavirus, *Escherichia coli*, and *Cryptosporidium* spp) were identified using a commercial ELISA (Bovine Enterichek®, Biovet Inc., Saint-Hyacinthe, Canada).

	n	OR (95% CI)	*P*-value†
P. multocida			
Exam 1	28/198	Ref.	
Exam 2	95/182	9.3 (5.4 – 16.9)	< 0.01
M. haemolytica			
Exam 1	10/198	Ref.	
Exam 2	19/182	2.4 (1.0 – 5.8)	0.06
M. bovis			
Exam 1	4/198	Ref.	
Exam 2	14/182	2.37 (1.04 – 5.75)	0.04
BRSV			
Exam 1	1/198	Ref.	
Exam 2	2/182	9.7 (0.4 – 250)	0.19
Coronavirus			
Exam 1	9/198	Ref.	
Exam 2	14/182	10.3 (1.3 – 78.0)	0.03
E. coli			
Exam 1	5/198	Ref.	
Exam 2	9/182	2.0 (0.7 – 6.2)	0.24
Rotavirus			
Exam 1	13/198	Ref.	
Exam 2	5/182	0.4 (0.1 – 1.2)	0.11
Coronavirus			
Exam 1	2/198	Ref.	
Exam 2	3/182	1.6 (0.3 – 9.9)	0.59
Cryptosporidium spp			
Exam 1	25/198	Ref.	
Exam 2	1/182	0.03 (0.00 – 0.27)	< 0.01

* No samples were positive for *Histophilus somni* at exam 1 (10 samples positive at exam 2), no samples positive for PI3 and BVH-1 at exam 2 (1 sample positive at exam 1 for both viruses), and no sample positive for BVDV at both exams.

† *P*-values were adjusted for multiple comparisons using the Benjamini-Hochberg approach (stats package).[27]

(n = 1/7) or NAP (n = 1/7). Heifers that had NAP at exam 1 were subsequently healthy (n = 7/21), remained with NAP (n = 6/21), or had AP (n = 7/21) or UTRD (n = 1/21). Heifers that had AP at exam 1 all had NAP at exam 2 (n = 3/3). Heifers were also less likely to have *P. multocida*, BCV, and *M. bovis* at exam 1 than at exam 2 (Table 1). Contrary to what was observed with respiratory pathogens, there was a difference between exams 1 and 2 only for *Cryptosporidium* spp for which heifers were more likely to be positive at exam 1 than exam 2 (Table 1).

Discussion

In the present study, the prevalence of clinical respiratory diseases (categorized in this study as AP and URTD) was less than 5% in the first 2 weeks of life, which is consistent with research suggesting respiratory diseases happen later (3 to 10 weeks).[22,28] However, almost 20% of the heifers had a respiratory condition in their first 2 weeks of life when NAP was also included. As shown previously, pulmonary consolidation as detected by ultrasonography develops quickly after infection and remains stable for several days.[31] This could suggest that the prevalence of respiratory diseases in the first 2 weeks of life is underestimated, and more research would be necessary to understand the dynamic of the introduction of the different pathogens and their impact on health in this period of the heifers' life. Indeed, almost a quarter of the heifers without a respiratory disease when sampled between birth and 2 weeks old was positive for at least 1 respiratory pathogen. As expected, the prevalence of AP and NAP was greater in 4- to 8-week-old heifers than in 0- to 2-week-old ones, but the prevalence of URTD was not. The absence of change in URTD prevalence could have been due to false positives for the respiratory health score, as latent class analyses

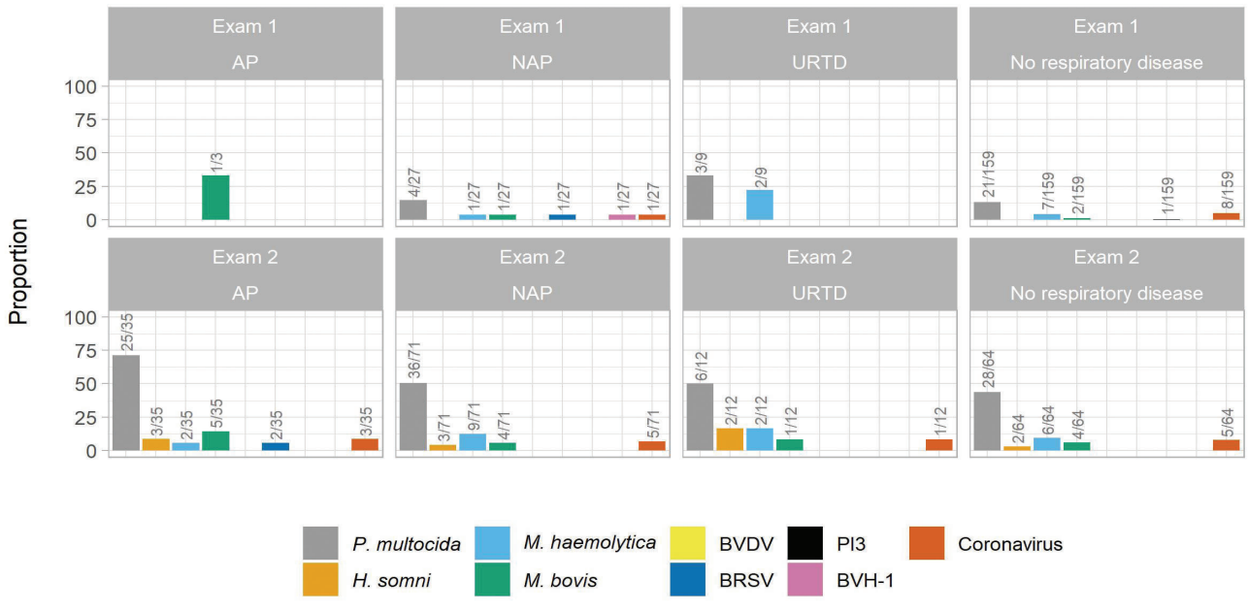

Figure 2. Proportion of heifers from 20 Québec dairy herds with a positive sample for different respiratory agents* according to their pneumonia status from birth to 2 weeks old (exam 1) and 4- to 8-weeks old (exam 2). Active pneumonia (AP) was defined as a respiratory health score ≥ 5† and presence of lung consolidation ≥ 1 cm‡, non-active pneumonia (NAP) was defined as a respiratory health score < 5 and presence of lung consolidation ≥ 1 cm, upper respiratory tract disease (URTD) was defined as a respiratory health score ≥ 5 and presence of lung consolidation < 1 cm, and no respiratory disease was defined as a respiratory health score < 5 and presence of lung consolidation < 1 cm.

* *Pasteurella multocida*, *Histophilus somni*, and *Mannheimia haemolytica* were identified by conventional culture and *Mycoplasma bovis*, bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), parainfluenza virus (PI3), infectious bovine rhinotracheitis virus (BVH-1), and coronavirus were identified by PCR.

† Respiratory health score was attributed following a standardized procedure (Wisconsin Clinical Respiratory Scoring Chart).[28]

‡ Lung consolidation was evaluated by ultrasonography.[32]

Table 2. Odds ratio (OR) of active and non-active pneumonia, and diarrhea in heifers from 20 dairy herds during their first 2 weeks of life (exam 1) and at 4- to 8-weeks of age (exam 2), obtained from mixed logistic regression models (1 per condition), including herd and heifer as random intercepts. Active pneumonia was defined as a respiratory health score ≥ 5* and presence of lung consolidation ≥ 1 cm†, non-active pneumonia was defined as a respiratory health score < 5 and presence of lung consolidation ≥ 1 cm, upper respiratory tract disease was defined as a respiratory health score ≥ 5 and presence of lung consolidation < 1 cm, and diarrhea was defined as a fecal score ≥ 2 (loose feces).[28]

	n	OR (95% CI)	*P*-value‡
Active pneumonia			
Exam 1	3/198	Ref.	
Exam 2	35/182	16.9 (5.8 – 72)	< 0.01
Non-active pneumonia			
Exam 1	27/198	Ref.	
Exam 2	71/182	4.1 (2.5 – 6.8)	< 0.01
Upper respiratory tract disease			
Exam 1	9/198	Ref.	
Exam 2	12/182	4.3 (0.7 – 35.2)	0.22
Diarrhea			
Exam 1	45/186	Ref.	
Exam 2	24/180	0.43 (0.27 – 0.83)	0.02

* Respiratory health score was attributed following a standardized procedure (Wisconsin Clinical Respiratory Scoring Chart).[28]

† Lung consolidation was evaluated by ultrasonography.[32]

‡ *P*-values were adjusted for multiple comparisons using the Benjamini-Hochberg approach (stats package).[27]

suggested a specificity for this test of 74% (95% credible interval = 65 – 83%).[8] In the present study, however, heifers with URTD at the first exam had greater odds than heifers without respiratory disease to have at least 1 respiratory pathogen. This suggests that URTD were not the only clinical scores that were falsely positive, and the absence of change in prevalence between exams 1 and 2 could be due to the size of the sample.

At both exams, the most common pathogen was *P. multocida*, with an estimated prevalence increasing from 10% to 52% between exams 1 and 2. The individual-level prevalence found at exam 2 was similar to previous research in Canada and Belgium,[9,18] but higher than the 17% found in Scottish dairy calves.[25] This supports that our findings in the first 2 weeks of life could be occurring in other populations, but more research is necessary to assess how infection and development of diseases occur at this early stage. It was suggested that bacteria in the upper respiratory tract are part of the respiratory microbiota, and evolve in disease under specific conditions. This could explain, at least partly, the pathogens isolated in healthy heifers in the present study.[37] The present study, however, aimed to only describe the presence of these bacteria, without inferring if they caused the observed diseases.

Our findings showed the quasi-absence of common respiratory viruses in the first 2 weeks of life, except for coronavirus, which had a prevalence of < 5% in this period. In the 4- to 8-week period, the prevalence of respiratory viruses, except for coronavirus, was again negligeable. It is unclear if this is due to the sampling frame, the sampling technique, or a true low prevalence in the studied population. Information about the duration of pathogen shedding and overlapping with clinical signs is limited, but the review by Grissett et al showed shedding of different viruses was 2 to 3 weeks, which is shorter than the time between the exams in the present study.[21] Moreover, the sampling technique (nasopharyngeal swab in the present study) could have affected the results, with identification of BRSV in dairy calves with naturally occurring respiratory disease being optimal when using a bronchoalveolar lavage.[15] It is, however, possible that respiratory viruses are uncommon in the studied population,[18] which could be due, in part, to vaccinations for respiratory pathogens of most adult dairy cows.

A better understanding of the viral and bacterial dynamic in the first weeks of life would require more frequent sampling. More frequent sampling could also allow calculation of incidence rates, which was not possible in the present study as no information was available between exams 1 and 2. A more intensive sampling could also inform how the presence of pathogens in heifers without respiratory disease, as found in the present study, evolve and affect their health. Previous research found viral vaccination had no impact on morbidity and mortality of dairy heifers in a population with low failure of TPI.[44] Earlier studies showed that vaccination of heifers resulted in cell-mediated immunity development and clinical protection against experimental challenge,[16,19] which could result in lower incidence of respiratory diseases in a vaccinated heifer population. This highlights that the findings of the present study were for a population of unvaccinated dairy heifers, and the prevalence of respiratory diseases observed could have been lower in a vaccinated population. Another characteristic of the population of the present study was the prevalence of failure of TPI, which was higher than previously reported.[5,29,38] Failure of TPI has been associated with increased risks of mortality, diarrhea, and respiratory disease,[35] which could have influenced the prevalence found in the present study. It is likely that a population with better TPI would have had lower prevalence of diseases and pathogens than the population in the present study.

Another limitation of the present study that could have influenced these results was the variation in the season of sampling among herds. Indeed, the number of calves enrolled in bigger herds was reached rapidly, mostly at the end of summer and the fall, while smaller herds were sampled over longer periods of time, including winter, spring, and summer. This could have influenced the results observed, as respiratory diseases and viral pathogens have been shown to follow seasonal patterns.[33]

It would also be interesting to explore how management, such as housing or vaccination, affects infection and diseases in the entire pre-weaning period. The herd-level prevalence described in the present pilot study suggests a wide variation, both in individual and group housing herds. This could explain the inconsistencies among studies assessing the association between housing and morbidity in pre-weaned heifers.[11] A recent study in a similar population to the present study found, however, greater odds of lung consolidation in heifers housed in groups than in heifers housed individually.[7] While the current herd sample size was insufficient to assess differences in pathogen and disease incidences between housing types, the descriptive statistics can be used to plan studies with this objective. Moreover, including herds with different management strategies in the present study is likely to better represent the general population of pre-weaned heifers where both individual and group housing are used.

The herd prevalence of diarrhea in the present study also varied among herds, with an individual-level prevalence at exams 1 and 2 aligned with the 19% morbidity observed in 2- to 4-week-old heifers from a previous US study.[40] Our results also support the traditional early timeline for observing diarrhea,[22,28] with heifers up to 2 weeks old being more likely to have diarrhea than 4- to 8-week-old heifers. Enteric pathogens, however, were mostly absent in the collected samples. In the present study, most herds had no heifer positive for the enteric pathogens tested for, but some herds had high prevalence (e.g., up to 50% for rotavirus), which was similar to a recent Argentinian study, but different from what was found in New Zealand.[2,20] At the individual level, the prevalence of the different pathogens was also low, with

Cryptosporidium spp having the highest prevalence, followed by rotavirus. As for diarrhea, these 2 pathogens were more likely to be identified at exam 1 than at exam 2. This pattern for rotavirus and *Cryptosporidium* spp matches observations in a longitudinal study,[12] and a cross-sectional study at different ages,[3,20] respectively. A recent worldwide meta-analysis also found that the rotavirus - *C. parvum* combination was the most common in dairy heifers (pooled prevalence = 7%).[6] As shown in the meta-analysis, most studies focused on the pathogens identified in diarrheic animals.[6] The present study contributes to understanding the prevalence of the different pathogens in healthy heifers. It is unclear, however, if the low prevalence observed in the present study could be partly due to the accuracy of the test used. Indeed, the commercial test used in this study has shown low sensitivity for coronavirus and rotavirus in diarrheic animals, and has not been validated in healthy animals.[10] Another limitation of the commercial rapid ELISA test is that the presence of *Salmonella* spp, 1 of the main enteric pathogens, was not evaluated. Previous research has shown that *Salmonella* was isolated from fecal samples of 4% of dairy heifers (31% of dairy herds).[17] The gap in the enteric pathogens tested in the present study could partly explain why no pathogens were identified in more than half of the heifers with diarrhea (exam 1: 64%, exam 2: 100%). As for respiratory pathogens, the specific sampling schedule could also have contributed to this,[12] but also non-infectious causes (e.g., diet)[11] or untested pathogens (e.g., coccidia).

For all bacterial pathogens, a limitation of the present study was the inclusion of heifers that were treated prior to sampling, or concurrently to sampling (supplemented milk). This could have biased the prevalence of bacterial pathogens observed in the present study, and the results should be interpreted considering that the reported prevalence could be lower than the true prevalence.

Conclusion

The present study found that a small proportion of unvaccinated pre-weaned heifers had respiratory pathogens and developed respiratory conditions in the first 2 weeks of their life, but were more likely to be positive for these pathogens and diseases when 4- to 8-weeks old. On the contrary, heifers were more likely to have diarrhea or to be positive for *Cryptosporidium* spp in their first 2 weeks of life than when 4- to 8-weeks old.

Endnotes

[a] The Coburn Company, Whitewater, Wisconsin, US
[b] Wisconsin Clinical Respiratory Scoring Chart
[c] ExaGo, IMV, Toulouse, France
[d] Continental Plastic, Delavan, Wisconsin, US
[e] BBL Port-A-Cul tubes, Becton, Dickinson and Company, Sparks, Maryland, US
[f] PA203, MISCO, Cleveland, Ohio, US
[g] Biovet Inc., Saint-Hyacinthe, QC, Canada
[h] Bovine Enterichek®, Biovet Inc., Saint-Hyacinthe, QC, Canada
[i] MALDI Biotyper^{smart}, Bruker Daltonics, Bremen, Germany

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Appendix

Table A1. Detailed distribution of herd prevalence of respiratory and enteric diseases and pathogens of 1- to 2-week-old (exam 1) and 4- to 8-week-old (exam 2) heifers in 19 Québec dairy herds (All; 10 with individual housing, 9 with group housing before weaning). Failure of transfer of passive immunity (TPI) was defined as a serum Brix refractance < 8.4%.[13] Active pneumonia was defined as a respiratory health score ≥ 5* and presence of lung consolidation ≥ 1 cm†, non-active pneumonia was defined as a respiratory health score < 5 and presence of lung consolidation ≥ 1 cm, upper respiratory tract disease was defined as a respiratory health score ≥ 5 and presence of lung consolidation < 1 cm, and diarrhea was defined as a fecal score ≥ 2 (loose feces).[28] From nasopharyngeal swabs, *Pasteurella multocida*, *Histophilus somni*, and *Mannheimia haemolytica* were identified by conventional culture and *Mycoplasma bovis*, bovine viral diarrhea virus (BVDV)‡, bovine respiratory syncytial virus (BRSV), parainfluenza virus (PI3), infectious bovine rhinotracheitis virus (BVH-1), and coronavirus were identified by PCR. From feces samples, *Escherichia coli*, rotavirus, coronavirus, and *Cryptosporidium* spp were identified by a commercial ELISA (Bovine Enterichek®, Biovet Inc., Saint-Hyacinthe, Canada).

	Exam 1					Exam 2				
	Min	**Q1**	**Med**	**Q3**	**Max**	**Min**	**Q1**	**Med**	**Q3**	**Max**
Failure of TPI (1 to 10 d)										
All herds	18.2	24.7	37.5	40.0	70.0					
Individual housing	20.0	23.5	35.0	40.0	70.0					
Group housing	18.2	33.3	37.5	40.0	70.0					
Active pneumonia										
All herds	0	0	0	0	10.0	0	10.0	20.0	30.0	50.0
Individual housing	0	0	0	0	10.0	0	10.3	16.3	27.5	50.0
Group housing	0	0	0	0	10.0	0	10.0	20.0	30.0	30.0
Non-active pneumonia										
All herds	0	10.0	10.0	20.0	33.3	0	30.0	40.0	50.0	60.0
Individual housing	0	10.0	14.1	20.0	33.3	0	20.0	33.8	50.0	60.0
Group housing	0	0	10.0	20.0	30.0	30.0	40.0	40.0	50.0	60.0
Upper respiratory tract disease										
All herds	0	0	0	9.5	33.3	0	0	10.0	10.0	22.2
Individual housing	0	0	4.5	10.0	20.0	0	0	5.0	11.9	20.0
Group housing	0	0	0	0	33.3	0	0	10.0	10.0	22.2
Diarrhea										
All herds	0	10.3	28.6	33.3	50.0	0	0	11.1	20.0	40.0
Individual housing	0	2.3	18.6	30.0	40.0	0	0	8.3	20.0	33.3
Group housing	11.1	20.0	31.7	35.0	50.0	0	10.0	11.1	30.0	40.0
Pasteurella multocida										
All herds	0	0	0	15.0	80.0	0	35.0	50.0	80.0	88.9
Individual housing	0	0	10.0	25.0	80.0	0	32.5	50.0	80.0	88.9
Group housing	0	0	0	8.33	70.0	0	40.0	50.0	80.0	80.0
Histophilus somni										
All herds	0	0	0	0	0	0	0	0	5.0	44.4
Individual housing						0	0	0	0	10.0
Group housing						0	0	0	10.0	44.4
Mannheimia haemolytica										
All herds	0	0	0	10.0	30.0	0	0	0	11.8	55.6
Individual housing	0	0	5.0	10.0	11.1	0	0	0	11.9	50.0
Group housing	0	0	0	10.0	30.0	0	0	10.0	11.1	55.6
Mycoplasma bovis										
All herds	0	0	0	0	30.0	0	0	0	5.0	80.0
Individual housing	0	0	0	0	10.0	0	0	0	0	33.3
Group housing	0	0	0	0	30.0	0	0	0	11.1	80.0
BRSV										
All herds	0	0	0	0	10.0	0	0	0	0	0
Individual housing	0	0	0	0	10.0					
Group housing	0	0	0	0	0					
PI3										
All herds	0	0	0	0	9.1	0	0	0	0	0
Individual housing	0	0	0	0	9.1	0	0	0	0	0
Group housing	0	0	0	0	0	0	0	0	0	0

	Exam 1					Exam 2				
	Min	Q1	Med	Q3	Max	Min	Q1	Med	Q3	Max
Coronavirus (nasopharyngeal)										
All herds	0	0	0	5.0	40.0	0	0	0	10.6	33.3
Individual housing	0	0	0	7.5	40.0	0	0	0	7.5	22.2
Group housing	0	0	0	0	10.0	0	0	10.0	20.0	33.3
BVH-1										
All herds	0	0	0	0	10.0	0	0	0	0	0
Individual housing	0	0	0	0	10.0					
Group housing	0	0	0	0	0					
Escherichia coli										
All herds	0	0	0	5.0	11.1	0	0	0	5.0	33.3
Individual housing	0	0	0	0	0	0	0	0	0	33.3
Group housing	0	0	0	10.0	11.1	0	0	0	20.0	20.0
Rotavirus										
All herds	0	0	0	4.5	50.0	0	0	0	0	25.0
Individual housing	0	0	0	6.8	50.0	0	0	0	8.3	25.0
Group housing	0	0	0	0	20.0	0	0	0	0	10.0
Coronavirus (feces)										
All herds	0	0	0	0	10.0	0	0	0	0	12.5
Individual housing	0	0	0	0	10.0	0	0	0	0	12.5
Group housing	0	0	0	0	0	0	0	0	0	11.1
Cryptosporidium spp										
All herds	0	0	10.0	22.5	40.0	0	0	0	0	10.0
Individual housing	0	0	0	16.1	40.0	0	0	0	0	0
Group housing	0	0	11.1	25.0	30.0	0	0	0	0	10.0

***** Respiratory health score was attributed following a standardized procedure (Wisconsin Clinical Respiratory Scoring Chart).[28]

† Lung consolidation was evaluated by ultrasonography.[32]

‡ No samples were positive for bovine viral diarrhea virus (BVDV).

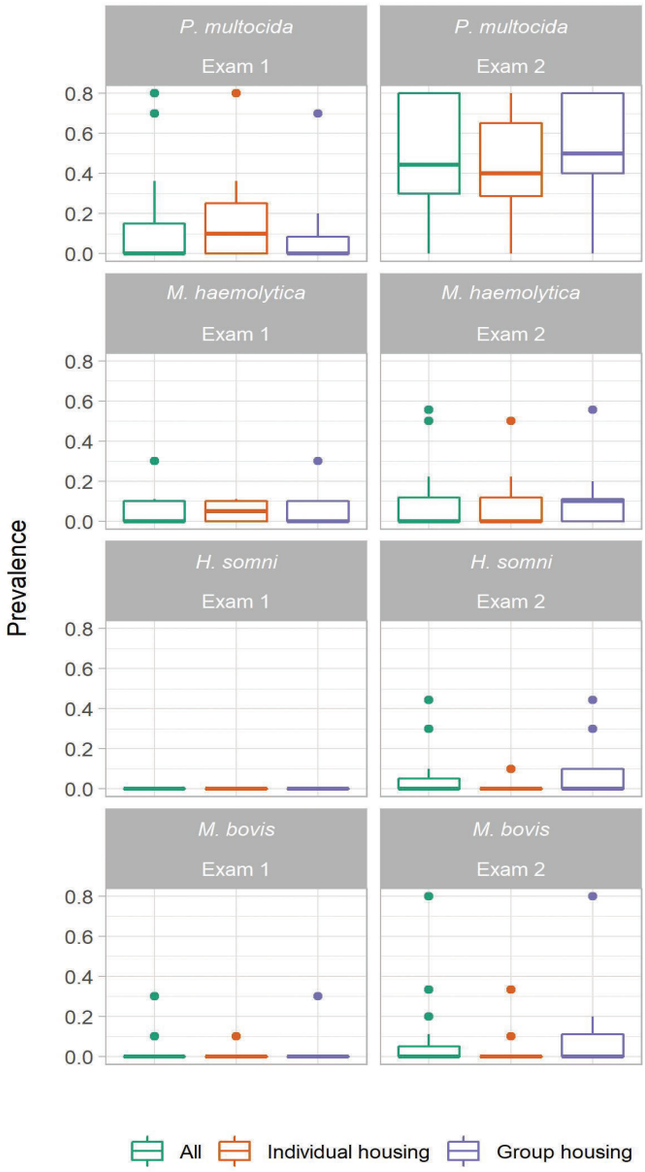

Figure A1. Boxplot of the distribution of herd prevalence of respiratory bacteria identified by conventional culture (*Histophilus somni*, *Mannheimia haemolytica*, and *Pasteurella multocida*) and PCR (*Mycoplasma bovis*) of 1- to 2-week-old (exam 1) and 4- to 8-week-old (exam 2) heifers in 19 Québec dairy herds (All; 10 with individual housing, 9 with group housing before weaning).

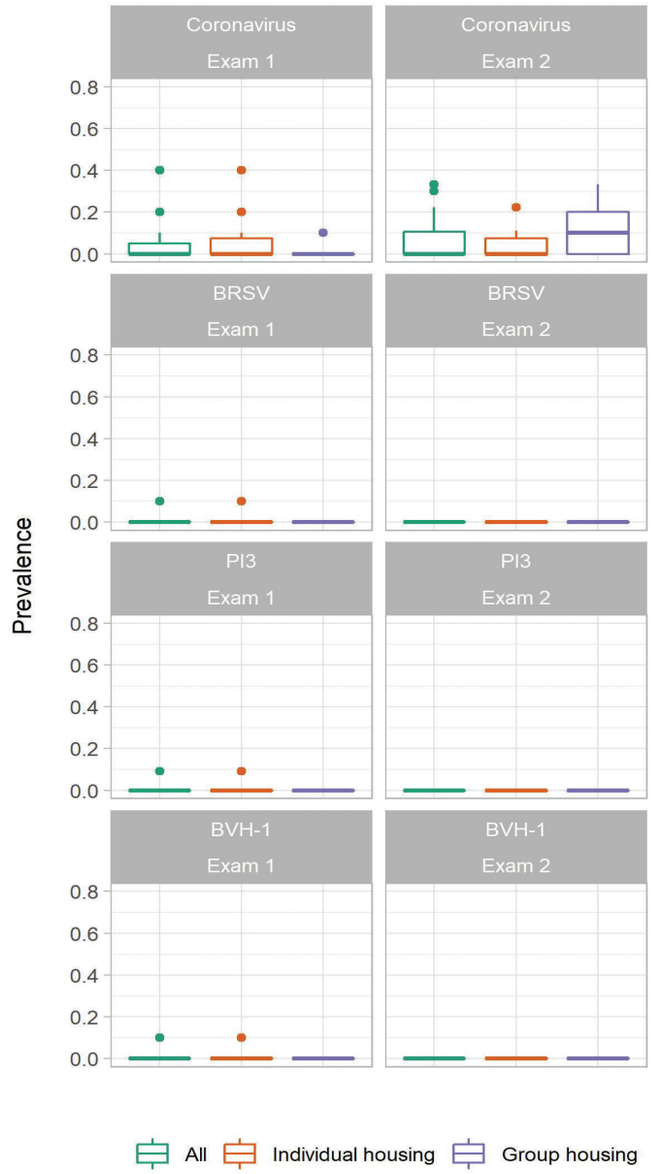

Figure A2. Boxplot of the distribution of herd prevalence of respiratory viruses identified by PCR (bovine respiratory syncytial virus (BRSV), parainfluenza virus (PI3), infectious bovine rhinotracheitis virus (BVH-1), and coronavirus*) of 1- to 2-week-old (exam 1) and 4- to 8-week-old (exam 2) heifers in 19 Québec dairy herds (All; 10 with individual housing, 9 with group housing before weaning).
*No samples were positive for bovine viral diarrhea virus (BVDV).

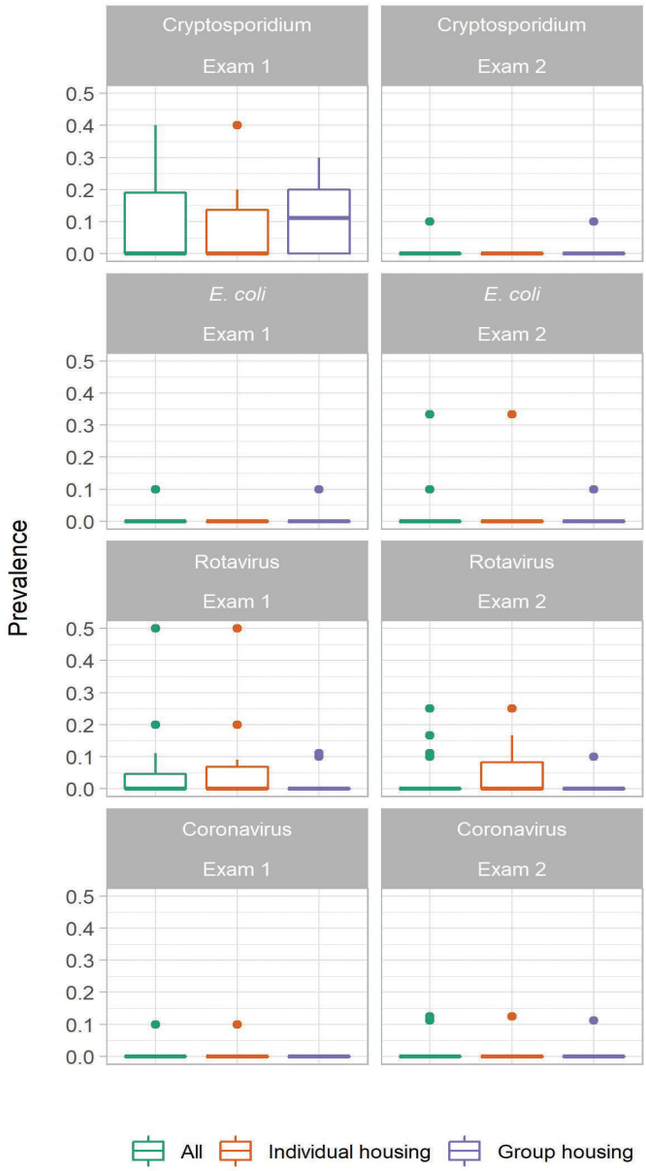

Figure A3. Boxplot of the distribution of herd prevalence of enteric pathogens identified by a commercial ELISA (Bovine Enterichek®, Biovet Inc., Saint-Hyacinthe, Canada; coronavirus, rotavirus, *Escherichia coli*, and *Cryptosporidium* spp) of 1- to 2-week-old (exam 1) and 4- to 8-week-old (exam 2) heifers in 19 Québec dairy herds (All; 10 with individual housing, 9 with group housing before weaning).