Article

Temporal patterns of bovine leukemia virus infection in dairy herds in Atlantic Canada

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Abstract

Objective
The primary objective was to determine the youngest age group where bovine leukemia virus (BLV)-infected dairy animals were identified. The secondary objective was to investigate associations between age-specific management practices and BLV infection status of different age groups of dairy calves and heifers.

Procedure
For enrolled herds, BLV status was determined using blood samples from pre-weaned calves, weaned calves, and breeding-age heifers; and bulk tank milk from the adult herd. A questionnaire investigating age-specific management factors was administered for each herd. Ordinal logistic regression was performed to identify management factors associated with the youngest age range in which BLV was identified.

Results
Fifty-three dairy herds from the 4 provinces in Atlantic Canada were enrolled. Bovine leukemia virus was most commonly earliest identified in pre-weaned heifers (18 herds, 32.1%) and the adult herd (18 herds, 32.1%). Ordinal logistic regression revealed that BLV was first identified in older age groups more often than in younger age groups when herds regrouped weaned heifers at least once, when fly control was used for breeding-age heifers, when herds practiced foot trimming on breeding-age heifers, and when bred heifers were brought in.

Conclusion
Producers can use results to identify the youngest age group(s) in which BLV is identified and to tailor management strategies to prevent new infections.

Résumé

Tendances temporelles de l'infection par le virus de la leucémie bovine dans les troupeaux laitiers des provinces atlantiques canadiennes

Objectif
L'objectif principal était de déterminer le groupe d'âge le plus jeune dans lequel les animaux laitiers infectés par le virus de la leucémie bovine (BLV) ont été identifiés. L'objectif secondaire était d'étudier les associations entre les pratiques de gestion spécifiques à l'âge et le statut d'infection par le BLV de différents groupes d'âge de veaux et de génisses laitiers.

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Procédure

Pour les troupeaux inscrits, le statut BLV a été déterminé à l'aide d'échantillons de sang provenant de veaux pré-sevrés, de veaux sevrés et de génisses en âge de se reproduire; et de lait de réservoir en vrac du troupeau adulte. Un questionnaire portant sur les facteurs de gestion spécifiques à l'âge a été administré pour chaque troupeau. Une régression logistique ordinale a été réalisée pour identifier les facteurs de gestion associés à la tranche d'âge la plus jeune dans laquelle le BLV a été identifié.

Résultats

Cinquante-trois troupeaux laitiers des quatre provinces atlantiques canadiennes ont été inscrits. Le virus de la leucémie bovine a été le plus souvent identifié le plus tôt chez les génisses pré-sevrées (18 troupeaux, 32,1 %) et dans le troupeau adulte (18 troupeaux, 32,1 %). La régression logistique ordinale a révélé que le BLV a été identifié pour la première fois plus souvent dans les groupes d'âge plus âgés que dans les groupes d'âge plus jeunes lorsque les troupeaux regroupaient au moins une fois les génisses sevrées, lorsque le contrôle des mouches était utilisé pour les génisses en âge de se reproduire, lorsque les troupeaux pratiquaient le parage des pattes des génisses en âge de se reproduire., et quand les taures saillies étaient intégrées au troupeau.

Conclusion

Les producteurs peuvent utiliser les résultats pour identifier le(s) groupe(s) d'âge le plus jeune dans lequel le BLV est identifié et pour adapter les stratégies de gestion afin de prévenir de nouvelles infections.

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Introduction

Enzootic bovine leukosis is a disease of cattle caused by persistent infection with bovine leukemia virus (BLV), a delta-retrovirus (1). Although all cattle can be infected, it is primarily of concern for dairy herds in Canada, compared to beef herds. The virus does not cause overt clinical signs in most BLV-infected cattle, but ~30% of infected cows will develop persistent lymphocytosis, and up to 5% of infected cows will develop lymphoid tumors in a number of organ systems (2). There has historically been relatively little emphasis placed on control of enzootic bovine leukosis in North America due to its limited clinical expression and presumed minimal economic effect. However, recent investigations of the impact of BLV have identified a negative effect on cow health and susceptibility to disease, as well as overall farm economic impact, resulting in increased interest among dairy producers to control BLV on their farms (3–7).

Bovine leukemia virus is mainly transmitted to naïve cows *via* blood transfer from infected cows (1,8). For adult cattle, this has resulted in control measures focused on minimizing blood transfer between cows; *e.g.,* single use of hypodermic needles and rectal sleeves, cleaning of communal equipment such as hoof trimming and dehorning implements, and segregation of BLV-infected and -negative cows in particularly committed herds (1). However, there is also evidence that cows can become infected with BLV as calves or young heifers, either through blood contamination or possibly through ingestion of colostrum or milk from BLV-infected cows (9–11). Less commonly, BLV can be transmitted *in utero* or at parturition (10–12).

Despite increased awareness of BLV infection and its adverse effects, herd-level prevalence of BLV infection is high in Atlantic Canada, with ~90% of dairy herds having at least one BLV-seropositive cow (13). Communication with local animal owners has suggested that disease-control measures for older heifers and adult cattle have been implemented, but specific practices for calves and young heifers have not been prioritized. The primary objective of this study was to determine the youngest age group in which BLV infection was identified in dairy herds in Atlantic Canada. The secondary objective was to investigate associations between age-specific management practices and BLV infection status of different age groups on dairy farms in Atlantic Canada.

Materials and methods

Sample collection

Inclusion criteria for herd recruitment included participation in the concurrent regional BLV surveillance program and completion of a BLV-specific risk assessment and management program workbook with each herd's regular veterinarian (14). The goal was to recruit 60 herds in total across all 4 Atlantic Canada provinces (New Brunswick, NB; Newfoundland and Labrador, NL; Nova Scotia, NS; Prince Edward Island, PE), in proportions approximating the total number of dairy herds present in each province. This would result in recruiting 20 herds in NB, 2 herds in NL, 21 herds in NS, and 17 herds in PE. Information regarding each herd's bulk tank status and estimated within-herd prevalence for the adult milking herd was obtained from data collected for the ongoing regional BLV surveillance program, for the year in which the samples were collected.

For each herd, 6 blood samples were collected from individual animals in each of 3 age groups: pre-weaned heifer calves (generally younger than 2 mo of age), weaned heifers that were not old enough for breeding (generally between 2 and 14 mo of age), and breeding-age heifers (generally older than 14 mo of age, until calving). Animals were selected randomly from each age group. For smaller farms where the number of animals in a certain group was less than 6, all animals in that age group were sampled. Blood was collected by the primary author from either the jugular vein or coccygeal vein/artery into plain, red-top vacutainer tubes, depending on calf or heifer size. Some herds were sampled by the regular veterinarian, who then shipped the blood samples to the Maritime Quality Milk laboratory at the Atlantic Veterinary College, University of Prince Edward Island;

samples were shipped chilled on ice within 48 h of collection. All blood samples for a single farm were collected on the same day, and all samples were allowed to clot at room temperature before either being shipped chilled on ice and/or refrigerated at 4°C until sample processing. Samples were processed on a weekly basis during the sample collection phase.

Sample processing

Blood samples were brought to room temperature and then centrifuged at 2500 × g for 15 min. Serum was collected from each sample and used for further analyses. For pre-weaned calves, samples were tested for the presence of BLV genetic material using qPCR for the BLV *pol* gene (Bovine leukemia virus pol gene qPCR, PCRmax; Stone, Staffordshire, United Kingdom), following the manufacturer's instructions, after RNA extraction (QIAmp Viral RNA Mini Kit; Qiagen Canada, Montreal, Quebec). The test results were reported as PCR cycle threshold values (Ct), where a Ct of > 38 indicated a negative result, a Ct of > 0 and ≤ 38 indicated a positive result, and a Ct of ≤ 0 indicated a failed reaction or lack of DNA template. Weaned heifer and breeding-age heifer samples were tested for the presence of anti-BLV antibodies using a commercial indirect ELISA kit (SVANOVIR BLV gp51-Ab; Svanova, Uppsala, Sweden), following the manufacturer's instructions. The test results were reported as percent positivity (PP) values, PP = (OD$_{corrected}$ sample/OD$_{corrected}$ positive control) × 100, where OD is optical density. A PP of ≥ 15 indicated a positive result and a PP of < 15 indicated a negative result. However, after using this kit on the first set of weaned heifer samples and obtaining a higher proportion of BLV-seropositive results compared to the pre-weaned and breeding-age heifers, we tested weaned heifers instead using the qPCR assay used for the pre-weaned calves. This was due to likely persistence of BLV from maternal antibodies in weaned heifers younger than 6 mo that were included in this age group. The same commercial ELISA kit was used on bulk tank milk samples to determine if the adult milking herd was BLV-infected. The PP cutoff for bulk tank milk was < 5 to classify a herd as BLV-negative and ≥ 5 to classify a herd as BLV-positive. Pre-weaned calves and weaned heifers were considered BLV-positive if they had a Ct value of > 0 and ≤ 38 on qPCR from a serum sample, breeding-age heifers were considered BLV-positive if they had a PP of ≥ 15 on ELISA from a serum sample, and the milking herd was considered BLV-positive if it had a PP of ≥ 5 on ELISA from a bulk tank milk sample.

Questionnaire administration

A questionnaire with questions about age-specific management practices was administered either at the time of sample collection or *via* telephone after sample collection, depending on producer availability. The questionnaire is included in Appendix 1 (available online from: www.canadianveterinarians. net). Data were entered into EpiInfo v7.2.2.6 software (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) and then exported to either Excel 2013 (Microsoft Corporation, Redmond, Washington, USA) or Stata 16.1 (Statacorp, College Station, Texas, USA) software for analysis.

Statistical analysis

All analysis was performed using Stata 16.1 software. Each age group on a farm was considered BLV-positive if at least 1 calf or heifer in the age group was PCR-positive or ELISA-positive, depending on the test used for each age group. The adult herd was considered BLV-positive if at least 1 bulk tank milk sample was BLV-positive on ELISA testing. The number of calves or heifers per age group testing positive was divided by the total number of calves or heifers sampled for each age group, to determine the BLV prevalence of the animals sampled in each age group for each farm.

The outcome variable used for statistical analysis was the youngest age group in which BLV infection was identified: pre-weaned calves, weaned heifers, breeding-age heifers, or adult cows. The 4 values that the outcome variable could take were 1 = BLV infection identified in pre-weaned calves, 2 = BLV identified in weaned heifers, 3 = BLV identified in breeding-age heifers, and 4 = BLV identified in adult cows as the youngest age group. For outcome variable 1, herds that had BLV-positive pre-weaned calves did not necessarily have BLV infection identified in the weaned heifers or breeding-age heifers; similarly, for outcome variable 2, herds that had BLV-positive weaned heifers did not necessarily have BLV infection identified in the breeding-age heifers. However, all the pre-weaned calves in these herds were BLV-negative. Because the outcome variable was ordinal categorical and had 4 distinct values (corresponding with the 4 age groups stated above), ordinal logistic regression was used, rather than standard logistic regression (15). With the 4 age group categories, ordinal logistic regression uses 3 thresholds that correspond to dichotomizing the ordinal outcome at different groupings of the age group categories. These thresholds in this model correspond to the odds of BLV first being identified in the 3 older age groups (weaned heifers, breeding-age heifers, and adult cows) *versus* in pre-weaned calves, BLV first being identified in breeding-age heifers or adult cows *versus* in pre-weaned calves or weaned heifers, and BLV first being identified in adult cows *versus* in all calf and heifer age groups. The probabilities and odds generated from the model are for the outcome (youngest age group in which BLV infection was identified) being in a higher age group category *versus* being in a lower age group category. Both the "ologit" and the "gologit2" commands were used in Stata to explore whether the predictors met the proportional odds assumption for ordinal logistic regression. Predictors that meet the proportional odds assumption have the same OR for all investigated thresholds, corresponding to the predictor having the same effect on the model across all the investigated thresholds. Predictors that do not meet the proportional odds assumption have different ORs at different thresholds.

The questionnaire contained a total of 75 variables relating to management factors in the 4 different age groups. Univariable analyses were performed for all independent variables, and those with a *P*-value of < 0.2 were retained for further analysis (15). The Wald test was used to assess overall *P*-values for variables with more than 2 categories and to assess whether a variable met the proportional odds assumption. Table S1 (available online from: www.canadianveterinarians.net) contains results of all the univariable analyses; 16 variables were excluded from analysis

Table 1. Summary of herds enrolled in each province compared to the total number of herds active in the Atlantic Canada region in 2016 to 2017.

Province[a]	Herds enrolled	Total herds	% of total enrolled	Goal for her enrollment	% of goal
NB	19[b]	195	9.7	20	95
NL	2	27	7.4	2	100
NS	25[c]	217	11.5	21	119
PE	10	166	6.0	17	59
Total	56	605	9.3	60	93

[a] NB — New Brunswick; NL — Newfoundland and Labrador; NS — Nova Scotia; PE — Prince Edward Island.
[b] Two herds were removed from data analysis: 1 herd had no bovine leukemia virus (BLV)-positive animals identified and 1 herd was removed due to questionnaire nonresponse.
[c] One herd was removed from data analysis due to questionnaire nonresponse.

due to > 90% of herds having the same option selected from the list of possible answers. Due to the large number of potential predictors and the small size of the dataset, forward selection was used to build the final multivariable ordinal logistic regression model. For predictors that did not meet the proportional odds assumption, model-building used the "gologit2" software package to allow for nonproportional odds for that predictor(s) while maintaining proportional odds assumptions for the other predictors. A P-value of < 0.05 was considered significant for variable inclusion in the final model.

Results

A total of 56 herds were recruited across all 4 Atlantic Canadian provinces; detailed information is included in Table 1. The anticipated numbers of herds were recruited from NL and NS, but fewer herds than anticipated were recruited from NB and PE. Additional herds from NS were interested in participating, so those herds were included to increase the total number of participating herds. All but 1 herd were BLV-infected based on bulk tank milk samples. The single BLV-negative herd was excluded from data analysis (all 4 age groups were BLV-negative). Two additional herds were excluded from statistical data analysis due to nonresponse when contacted to complete the questionnaire, leaving a total of 53 herds included in the data analysis. The number of calves and heifers sampled in each age group per farm ranged from 2 to 7; when < 6 calves or heifers were sampled per age group on a farm, the number of sampled animals comprised the entire age group present at the time of sampling.

Table 2 shows the youngest age group where BLV-positive animals were identified. Approximately 1/3 of herds had pre-weaned calves as the youngest BLV-infected age group, 11% of herds had weaned heifers as the youngest BLV-infected age group, 23% of herds had breeding-age heifers as the youngest BLV-infected age group, and 32% of herds had no BLV-positive calves or heifers and but had BLV-positive adults. Not all herds where BLV-positive pre-weaned heifers were identified also had BLV-positive weaned heifers or breeding-age heifers identified; similarly, not all herds with BLV-positive weaned heifers also had BLV-positive breeding-age heifers identified. All 53 herds contained BLV-positive adult cows based on bulk tank milk ELISA results.

For the 18 herds where pre-weaned heifers were the youngest identified BLV-positive age group, the median prevalence

Table 2. Summary of herd infection status for each of the 4 age groups tested, showing the youngest age group in which bovine leukemia virus (BLV)-positive animals were identified.

Age at first infection	Number of herds (%)
None[a]	1 (1.8)
Adults	18 (32.1)
Breeding-age heifers[b]	13 (23.2)
Weaned heifers	6 (10.7)
Pre-weaned heifers	18 (32.1)

[a] This herd was removed from analysis because no BLV-positive animals were identified.
[b] Two herds in this group were removed from analysis due to questionnaire nonresponse.

within the sampled calves was 20.0% (range: 14.3 to 83.3%). Thirteen herds had 1 BLV-positive pre-weaned calf, 3 herds had 2 BLV-positive pre-weaned calves, and 1 herd each had 3 and 5 BLV-positive pre-weaned calves. For the 13 herds where weaned heifers were the youngest identified BLV-positive age group, the median prevalence within the sampled heifers was 16.7% (range: 14.3 to 50.0%). Ten herds had 1 BLV-positive weaned heifer, 2 herds had 2 BLV-positive weaned heifers, and 1 herd had 3 BLV-positive weaned heifers. For the 29 herds where breeding-age heifers were the youngest identified BLV-positive age group, the median prevalence within the sampled heifers was 25.0% (range: 16.7 to 50.0%). Fifteen herds had 1 BLV-positive breeding-age heifer, 9 herds had 2 BLV-positive breeding-age heifers, and 5 herds had 3 BLV-positive breeding-age heifers.

Table S1 (available online from: www.canadianveterinarians.net) displays the univariable analyses for all 59 variables from the questionnaire that were analyzed, and Table 3 displays the variables retained for model-building after univariable analyses. Table 4 displays the variables included in the final multivariable model. After forward selection model-building, the final model incorporated 4 variables: the number of times weaned heifers were regrouped, use of fly control in weaned heifers, the use of foot-trimming implements in breeding-age heifers; and whether the farm purchased bred heifers.

Herds that regrouped heifers after weaning had higher odds of older age groups being the youngest in which BLV was identified, compared to younger age groups. The highest odds were identified in herds where heifers were regrouped twice before they were old enough to enter the breeding-age heifer group (OR: 30.06), followed by herds that regrouped heifers 3 or more times (OR: 6.05), then by herds that regrouped heifers

Table 3. Variables retained for forward selection model-building using the youngest age group where bovine leukemia virus (BLV) was identified as the outcome variable in ordinal logistic regression. For variables that met the proportional-odds assumption, the *P*-value displayed is the *P*-value for each age group comparison.

Variable	Levels	OR	95% CI	*P*-value
Treatment of milk fed to calves	Pasteurized or acidified	N/A		
	Not treated	3.299	0.845, 12.880	0.086
Type of calf housing, pre-weaned calves	Individual pens or hutches	N/A		
	Group pens or hutches	2.051	0.705, 5.965	0.187
Fly control in pre-weaned calves	None	N/A		
	Environmental or topical	3.327	0.935, 11.841	0.063
	Environmental and topical	5.287	0.654, 42.711	0.118
	Overall *P*-value			0.133
Number of times weaned heifers are regrouped	Not regrouped	N/A		
	Regrouped once	2.791	0.502, 15.529	0.241
	Regrouped twice	9.644	1.791, 51.931	0.008
	Regrouped three times	2.146	0.384, 12.001	0.384
	Overall *P*-value			0.033
Fly control in weaned heifers	None	N/A		
	Environmental or topical	3.431	1.207, 9.760	0.021
	Environmental and topical	5.286	0.386, 72.452	0.213
	Overall *P*-value			0.051
Age when heifers enter the breeding group		0.809	0.590, 1.110	0.190
Breeding-age heifer housing related to adults	> 200 m away	N/A		
	< 200 m away	1.420	0.372, 5.416	0.608
	Same building	3.181	0.836, 12.105	0.090
	Overall *P*-value			0.177
Foot-trimming in breeding-age heifers[a]	No	N/A		
	Yes	2.753	0.808, 9.381	0.105
	Overall *P*-value			0.011
Fly control in breeding-age heifers	None	N/A		
	Environmental or topical	4.645	1.1493, 14.454	0.008
	Environmental and topical	4.439	0.660, 29.877	0.125
	Overall *P*-value			0.019
Farm buys bred heifers	No	N/A		
	Yes	3.169	0.810, 12.403	0.098
Farm buys mature cows	No	N/A		
	Yes	2.142	0.680, 6.749	0.193
Any other method of contact with other herds	No	N/A		
	Yes	0.315	0.080, 1.239	0.098

N/A — Not applicable.
[a] For the variable that did not meet the proportional-odds assumption, the estimate and *P*-value displayed are the one for the relevant age group comparison, along with the overall *P*-value.

once (OR: 5.35). Herds using some type of fly control for their breeding-age heifers were more likely to have older age groups as the youngest in which BLV was identified, compared to younger age groups, with the highest odds for herds using either environmental or topical fly control methods (OR: 13.06); however, the OR for herds using both environmental and topical fly control methods was only slightly lower (OR: 12.98). Herds where foot-trimming instruments were used on breeding-age heifers had 2.70× higher odds of adult cows being the youngest group in which BLV was identified, compared to younger age groups. The final predictor associated with older age groups being the youngest in which BLV was identified was the farm purchasing bred heifers; this resulted in odds 12.75× higher that older age groups were the youngest in which BLV was identified, compared to younger age groups.

Discussion

This study showed that, on dairy farms in Atlantic Canada, the most common age groups in which BLV infection is present are pre-weaned heifer calves and the adult milking herd. Also, BLV was commonly identified in breeding-age heifers, with the weaned heifers being the least likely in this study to be the youngest age group in which BLV was identified. The fact that BLV was identified in all age groups of calves, heifers, and cows suggests that there is a range of management practices on dairy farms in this region that may influence BLV transmission. However, on most farms where BLV-positive calves or heifers were identified, the apparent prevalence (*i.e.,* the number of BLV-positive animals divided by the number of sampled animals) was low.

Table 4. Final multivariable ordinal logistic regression model investigating management factors associated with the youngest age group of calves and heifers in which bovine leukemia virus (BLV)-positive animals were identified.

Variable	Levels	OR	95% CI	P-value
Number of times weaned heifers are regrouped	Not regrouped			
	Regrouped once	5.348	0.724, 38.542	0.096
	Regrouped twice	30.058	3.686, 245.136	0.001
	Regrouped three times	6.049	0.785, 46.591	0.084
	Overall P-value			0.013
Fly control in breeding-age heifers	None			
	Environmental or topical	13.057	2.666, 63.937	0.002
	Environmental and topical	12.978	1.544, 109.069	0.018
	Overall P-value			0.003
Foot-trimming in breeding-age heifers[a]				
All older age groups *versus* pre-weaned calves	No			
	Yes	0.338	0.071, 1.607	0.173
Adult cows + breeding-age heifers *versus* younger age groups	No			
	Yes	0.180	0.037, 0.861	0.032
Adult cows *versus* all calf and heifer age groups	No			
	Yes	2.704	0.519, 14.077	0.237
	Overall P-value			0.035
Farm buys bred heifers	No			
	Yes	12.745	1.908, 85.132	0.009

[a] For the variable that did not meet the proportional-odds assumption, all estimates and P-values for the 3 difference comparisons are shown, as well as the overall P-value.

The results of this study revealed 4 management practices that were statistically significantly associated with identifying BLV-positive animals in different age groups of calves and heifers on dairy farms in Atlantic Canada: if weaned heifers were regrouped before entering the breeding-age heifer group, if fly control was used for breeding-age heifers, if foot-trimming implements were used on breeding-age heifers, and if the farm purchased bred heifers.

For farms where weaned heifers were regrouped after weaning — where heifers did not remain solely with the heifers they were grouped with after weaning, but either were intermingled with other heifers or had their original group merged with another small group of heifers — the odds of older age groups being the youngest in which BLV was identified was higher than in herds where weaned heifers stayed in their original group until entering the breeding-age heifer group. This finding is contrary to what was initially expected. Moving animals and introducing them to new groups is stressful and can influence immune function, theoretically making heifers that experience more movement or regroupings more susceptible to infection, including BLV. Additionally, having more animals in a pen or more frequent mixing of animals will result in more direct contact between animals, and has the potential to allow more frequent blood transfer from a BLV-infected heifer to a BLV-negative one. However, the results of this study suggest that farms where heifers are moved more often have higher odds of adult cows being the youngest group in which BLV was identified, compared to any of the calf and heifer age groups. One possible explanation could be that farms regrouping heifers more often are doing so to optimize their feed intake and average daily gain, and so in general are implementing a larger proportion of more intensive heifer management practices. Another possibility is that these farms have implemented infection control practices in pre-weaned calves (*e.g.,* pasteurizing/freezing colostrum,

feeding milk replacer or milk from BLV-negative cows), and so there is no risk to regrouping heifers, as none (or very few) are infected with BLV.

Fly-control practices in breeding-age heifers were also significantly associated with the youngest age group in which BLV was identified. Herds in which environmental or topical fly control was used had 13.06× higher odds of older age groups being the youngest in which BLV was identified, and herds using both types of fly control had 12.98× higher odds compared to herds using no fly control for breeding-age heifers. Univariable analysis also suggested that use of one or both methods of fly control in pre-weaned calves and weaned heifers resulted in higher odds of older age groups being the youngest in which BLV was identified, with the use of both methods of fly control having the highest odds. These findings suggest that the use of fly control may help to prevent spread of BLV *via* biting flies in calf and heifer groups and may result in BLV first becoming prevalent in adult cows. It is also interesting to note the link between fly control practices and the lower odds of BLV being first identified in younger age groups, as multiple studies have investigated the role of flies in BLV transmission in adult cows (16–21).

The practice of foot care in breeding-age heifers resulted in higher odds of adult cows being the youngest group in which BLV was identified, compared to any of the younger age groups. This could be explained by the fact that foot-trimming implements can become contaminated with blood, especially if foot infections such as strawberry foot rot are present. If not properly disinfected between animals, the implements could serve as fomites to transmit BLV. It is unlikely that farms would use a separate set of implements for heifers and adult cows, and so BLV-positive adult cows could be the infection source for naïve breeding-age heifers. Although the use of foot-trimming implements in breeding-age heifers resulted in higher odds of adult cows being the youngest group in which BLV was identified, it

did not result in higher odds of breeding-age heifers being the youngest group. This could be due to timing of foot trimming in breeding-age heifers; a possibility is that producers may be performing foot trimming within a few weeks or months of the heifer's anticipated entry into the adult herd at the time of calving, and so any infections that occur in these heifers may not be apparent until they are in the adult milking herd.

The final management factor that was associated with the youngest age group where BLV was identified was whether the farm purchased bred heifers. Farms purchasing bred heifers had 8.65× higher odds of older age groups being the youngest in which BLV was identified, compared to herds that did not purchase bred heifers. An explanation for this finding is that these farms could be practicing good disease-control measures in young age groups but purchasing bred heifers from farms where BLV infection occurs in the younger age groups, thus introducing BLV into their herds *via* older heifers. In this case, it would appear as though good infection control practices are in place until heifers enter the adult milking herd, when actually the BLV pressure on the farm is very low until BLV-infected heifers are purchased from an outside source and determined to be BLV-seropositive as adult cows.

None of the other management factors investigated for the 3 calf and heifer age groups was statistically significantly associated with the youngest age group in which BLV was identified. This included management factors previously associated with BLV transmission in adult cows; for example, the reuse of hypodermic needles and syringes (1). A possible explanation is the small dataset in this project prevented some important management factors from showing statistical significance, and a larger sample size of farms may allow for identification of further management factors associated with the youngest age group in which BLV is identified.

There were several limitations of this study. We did not manage to recruit the anticipated number of herds, and 3 herds that were sampled were excluded from analysis either due to being BLV-negative or due to an inability to complete the questionnaire. This limited the number of data points available for analysis and may have affected the overall results. In terms of the number of herds recruited compared to the total number of dairy herds present in Atlantic Canada, < 10% of herds were enrolled in this study. This could have resulted in a sample size too small to detect significant results for other management factors included in the survey. Due to the small number of herds and the large number of questions asked on the questionnaire, there was also the risk of overfitting the available data points if too many predictors remained statistically significant in the final model.

The number of individual animals sampled on each farm was also a limitation, for several reasons. Regardless of herd size, the same maximum number of calves or heifers was sampled on each farm. In some small herds, this resulted in 100% of an age group being included in the study; in the largest herds, this resulted in 2% of an age group being included in the study. It is possible that some of the age groups sampled that were classified as BLV-negative may have been BLV-positive if a larger proportion of animals had been sampled. For example, there were several herds where BLV-positive pre-weaned calves were identified but all the sampled weaned heifers and breeding-age heifers were BLV-negative. These herds were still classified as having pre-weaned calves as the youngest group in which BLV was identified, as, presumably, if a larger subset of each age group was sampled, BLV-positive weaned heifers or breeding-age heifers would have been identified. This could have effects on the overall validity of the model in terms of using management factors to predict the odds of an age group being BLV-positive. In addition, the adult herd was only sampled using the results of bulk tank milk samples collected as part of a concurrent surveillance program, and no individual adult blood samples were collected. However, studies have shown that ELISA testing of bulk tank milk is both sensitive and specific for identifying BLV-infected herds, as well as for estimating the within-herd prevalence of BLV infection in lactating animals (13,22–23).

The literature is sparse in terms of prevalence studies of BLV in pre-lactating dairy animals, but 1 study (24) described a prevalence of 11.5% in naturally infected calves < 12 mo old in a dairy herd with very high adult within-herd prevalence, and another study (12) reported 10.8% of calves born to BLV-infected mothers were BLV-positive at birth. Both these studies used nested PCR to test calves, whereas our study used qPCR on calves of the same ages. Although this estimate of prevalence in calves may be too high for dairy herds in Atlantic Canada, it illustrates that, in herds where a very small percentage of an age group was sampled, age group may have been falsely classified as BLV-negative, especially if the prevalence of BLV was low in that herd.

The calves and heifers sampled on each farm were intended to be randomly sampled, to prevent veterinarian or producer bias and the preferential inclusion of "BLV-suspect" animals. However, practicality on farms sometimes necessitated convenience sampling of whichever calves or heifers were available; *e.g.,* some farms had breeding-age heifers at a separate location, or loose in a pasture, or pastured with a bull. This reduced the proportion of herds where animals were sampled randomly and could have introduced sampling bias. Also, as noted above, the sampling fraction was 100% in some age groups due to the small size of the herd, and so sampling fraction was not consistent across all herds.

The different age groups of calves and heifers were also assessed with different tests for BLV. Due to the potential presence of maternal anti-BLV antibodies, the initial plan was to test the pre-weaned calves with RT-qPCR for viral RNA and the older calves and heifers with indirect ELISA for anti-gp51 antibodies. However, as the age range of weaned heifers was 2 to 14 mo, some of the younger heifers could have still retained maternal antibodies in their serum due to colostrum ingestion. After inconsistent results were obtained with the first 2 herds tested, where a higher prevalence was identified in weaned heifers compared to pre-weaned calves or breeding-age heifers, all of the pre-weaned and weaned calves/heifers were tested using qPCR, and only the breeding-age heifers were tested with ELISA. As these tests were investigating different measures of infection; *i.e.,* the qPCR was directly looking for viral genetic material after reverse transcription and the ELISA was looking

for the host response to BLV infection through antibodies, it is difficult to directly compare prevalence in the different age groups. There is currently no evidence that BLV-infected animals can clear the infection, and so the BLV-seropositive heifers presumably would also be BLV PCR-positive if tested with qPCR. However, seroconversion after infection can take up to 57 d (25), and so there may have been false negative results in the breeding-age heifer group. Additional false negatives could result from diagnostic test performance; however, the ELISA test used has a reported sensitivity of 99% and specificity of 99.4%, and the qPCR test has a reported sensitivity to detect < 100 copies of the target DNA, making the presence of false negatives unlikely.

In conclusion, on dairy farms in Atlantic Canada, BLV was identified in all age groups of calves and heifers sampled, with pre-weaned calves and the adult milking herd most often the youngest age groups in which BLV was identified; however, there was no clear pattern of infection seen in all participating herds. In BLV-positive herds, management factors involving all age groups of calves and heifers were associated with the youngest age group in which BLV was identified. These results can be used by producers to identify the youngest age group where BLV is most likely to be identified, and tailor disease-control methods accordingly. CVJ

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