Development and implementation of a risk assessment and management program for enzootic bovine leukosis in Atlantic Canada

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ABSTRACT

Over the past 30 yr, the prevalence of bovine leukemia virus (BLV) infection has increased in North America, including Atlantic Canada, at both the herd and individual cow levels. This has occurred despite increased awareness of the disease and its deleterious effects and despite implementation of management practices aimed at reducing disease transmission. Our objectives were to identify risk factors associated with the within-herd prevalence of BLV-infected cows by using a risk assessment and management program workbook, as well as to determine the current level of BLV prevalence in the Atlantic Canada region. We hypothesized that previously established risk factors, including management practices associated with calf rearing and fly control, would affect within-herd BLV prevalence. Bulk tank milk (BTM) samples were collected in January and April of 2016 and again during the same months in 2017 and 2018 from all dairy farms shipping milk in the region. Samples were tested with ELISA for levels of anti-BLV antibodies to estimate within-herd prevalence. Regional BLV prevalence at the herd level was 88.39% of dairy herds infected in 2016 and 89.30% in 2018. All dairy farms shipping milk and who had BTM samples collected in 2017 ($n = 605$) were eligible to participate in the risk assessment and management program questionnaire (RAMP), which was developed and distributed to all bovine veterinarians in Atlantic Canada. One hundred and six RAMP were returned, with representation from all 4 provinces. The RAMP results were combined with the mean BTM ELISA results, and univariable logistic regression followed by multivariable logistic regression was performed to investigate the association between RAMP risk factors and the estimated within-herd BLV prevalence. Factors in the multivariable model significantly associated with the odds of a herd being classified as $>25\%$ estimated within-herd prevalence included history of diagnosis of

clinical BLV and calves receiving colostrum from cows with unknown BLV status. Differences in within-herd prevalence were not associated with hypodermic needle and injection practices, rectal sleeve practices, or using bulls for natural breeding, based on these 106 dairy farms.

Key words: bovine leukemia virus, risk assessment, bulk tank milk

INTRODUCTION

Enzootic bovine leukosis (EBL) is a disease of cattle, mainly of dairy cows, caused by persistent infection with bovine leukemia virus (BLV). Because the proportion of infected cows displaying overt clinical signs is low (usually $<5\%$), EBL has historically been considered a disease of low economic importance in the dairy industry (Bartlett et al., 2014). However, recent research shows that BLV-infected cows are more likely to be culled earlier (Erskine et al., 2012a), reduce the average milk production of the herd (Erskine et al., 2012a; Norby et al., 2016), and are potentially prone to reduced immune response to vaccination (Erskine et al., 2011). Financial losses also occur when BLV-infected cows with occult tumors are sent to slaughter, resulting in condemnation of the carcass (Government of Canada, 2018a). Relatively recently, many European countries have eradicated BLV (EFSA AHAW Panel, 2015; OIE World Animal Health Information System, 2018) and have established import restrictions for semen and embryos from countries that have BLV-infected cattle.

Because lymphocytes are the main target of BLV infection, blood transfer from infected to naïve animals is considered the major form of disease transmission (Evermann et al., 1986). Iatrogenic transfer of blood from infected to naïve cows via the use of blood-contaminated equipment such as gouge-type dehorners, or via reuse of hypodermic needles and rectal sleeves, is currently thought to be a major mode of BLV transmission in modern dairy herds (Bartlett et al., 2014). Biting flies have been suggested as an additional transmission route, but this has not been confirmed under natural

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field conditions (Kohara et al., 2018; Panei et al., 2019). Ruiz et al. (2018) recently reviewed mechanisms of BLV infection in neonatal calves and determined that, although infection may occur in utero, during birth, or through the ingestion of colostrum from a BLV-infected cow, the current evidence is equivocal regarding the relative importance of these transmission routes compared with infection in adolescent or adult cows.

As interest in reducing the prevalence of BLV has increased in North America, many dairy producers have implemented infection control practices aimed at minimizing transmission from infected to naïve cows. These include discarding hypodermic needles after a single use, disinfecting hoof trimming equipment between animals, transitioning to the use of cautery-type dehorning equipment, and changing rectal sleeves after each animal during reproductive activities. However, despite anecdotal reports from producers reporting that they have implemented and are adhering to these control measures, BLV prevalence has continued to increase at the herd level in the Atlantic Canada region (New Brunswick, **NB**; Newfoundland and Labrador, **NL**; Nova Scotia, **NS**; and Prince Edward Island, **PE**; Richardson and MacAulay, 1992; Nekouei et al., 2015a,b).

The primary objective of this study was to design and implement a risk assessment and management program (**RAMP**) in the Atlantic Canada region, with the secondary objective to identify management practices associated with increased within-herd BLV prevalence in the region. The hypothesis was that, in addition to previously established risk factors, management practices associated with calf rearing and fly control would also be associated with within-herd BLV prevalence. An additional objective was to determine the current level of BLV infection in dairy herds in Atlantic Canada.

MATERIALS AND METHODS

Regional Surveillance

All dairy herds in Atlantic Canada shipping milk during the period between January 2016 and December 2018 had 2 bulk tank milk (**BTM**) samples collected annually, in January and April, as part of the ongoing regional surveillance program. These samples were collected by the governing bodies of dairy producers from the 4 Atlantic Canadian provinces for regulatory purposes and were released to the Atlantic Veterinary College after standard components and quality analyses were completed for each BTM sample in the local laboratories in each province. Information collected for each sampled farm consisted of the province in which

the farm was located as well as the bulk tank barcode, to be used as a unique herd identifier.

For each sample collection, 30-mL BTM samples were collected by milk truck drivers during routine milk pickups from the dairy farms. Drivers followed standard procedures used for the collection of samples for regulatory and payment purposes. The BTM samples were kept at 4°C, and the samples were preserved with one Brotab milk preservative tablet (Advanced Instruments Inc., Norwood, MA) after components and quality analyses. The BTM samples were transferred to the Maritime Quality Milk laboratory at the Atlantic Veterinary College, University of Prince Edward Island, for BLV antibody testing. All samples were tested within 7 d from their sampling dates, using a commercial indirect ELISA kit (SVANOVIR BLV gp51-Ab, Svanova, Uppsala, Sweden), as described elsewhere (Nekouei et al., 2015a). The test results were reported as percent positivity values (**PP**) = $(OD_{\text{corrected sample}} / OD_{\text{corrected positive control}}) \times 100$, where OD is optical density.

RAMP Development

A RAMP workbook was developed to be used on-farm by producers. This was designed to be administered by each farm's herd veterinarian, and the format and layout were chosen to mimic the appearance of the Atlantic Johne's Disease Initiative Risk Assessment Workbook currently in use in the region (AJDI, 2015). A copy of the RAMP has been included in the online Supplemental Material (<https://doi.org/10.3168/jds.2019-17434>).

The RAMP was constructed with 8 sections, each focused on 1 category of management practices or age group of cattle. Each section was also assigned a risk category, based on perceived risk of the factors associated with that section being involved in BLV transmission. These risk categories were determined by examination of the available literature on BLV transmission, by adaptation of similar risk assessments published in several US states (Hovingh, 2016; New York State Cattle Health Assurance Program, 2016), and by expert opinion. During RAMP development, general biosecurity (visitor access to cows, cow contact with other herds, cow cleanliness) and calving and calving area (BLV status of dam, calf access to dam, use and cleaning of calving pens) were considered low-risk categories; general BLV (history of clinical BLV, purchase of untested cattle, fly control methods), preweaned heifer calves (colostrum and milk sources, location of calf housing in relation to adult cows), weaned heifer management (location of housing in relation to adult cows, use of shared equipment), and adult cows (BLV status

and its influence on housing, milking order, and culling decisions) were considered medium-risk categories; and shared equipment and other fomites (cleaning of dehorning, ear tagging, and foot trimming equipment; use of syringes and needles; sharing of feed equipment) and reproduction (practices associated with pregnancy checking and ultrasound use, estrus synchronization, use of bulls for live breeding and their BLV status) were considered high-risk categories.

The management plan included in the RAMP was also modeled after the current AJDI Risk Assessment Workbook (AJDI, 2015), in which the veterinarian and producer prioritized up to 3 separate areas where the farm could improve in terms of minimizing BLV transmission. After developing a final draft of the RAMP booklet, copies were sent to 4 dairy veterinarians (2 each in Nova Scotia and Prince Edward Island) in the region for opinions on the format, layout, and content. After incorporation of any suggestions and edits, a final copy was printed and distributed to all bovine practitioners in the Atlantic Canada region.

RAMP Implementation

Veterinarians were informed of the research project through communication on the regional bovine practitioner email list, as well as through written communication sent to each practice with the hard-copy RAMP booklets. Follow-up communication during the first year of the project occurred at the regional bovine practitioners' annual continuing education meeting as well as via the email list. Communication with dairy producers in the region occurred separately in each province, via their respective dairy board newsletters.

Veterinarians completed RAMP booklets with their clients and faxed or emailed completed forms to the Atlantic Veterinary College. As incentives for participa-

tion, producers who participated received annual information regarding their herd's estimated within-herd BLV prevalence and had access to subsidized individual adult cow milk or serological testing for BLV. Veterinarians were encouraged to submit invoices for reimbursement of their time for completing the RAMP, so that any increased professional time required for RAMP completion was not paid by the producer.

Reminders were sent via the bovine practitioners' email list at regular quarterly intervals, and a reminder was also published in each province's dairy board newsletter.

Data Analysis

All statistical analyses were performed in Stata 14.2 (StataCorp, College Station, TX).

BLV Prevalence Calculations. The ELISA test values for each herd were converted into a binary outcome, where $PP \geq 5$ was considered a positive herd-level test result and $PP < 5$ as a negative test, as per the manufacturer's instructions. For each year, a herd was considered negative if both samples collected during the same year had a PP value < 5 , and positive if at least 1 sample from each year had $PP \geq 5$. The apparent prevalence of BLV in each province and in the Atlantic region was calculated for each year (Dohoo et al., 2009). McNemar's test was used to test for a significant change in the prevalence from 2016 to 2018, both for the individual provinces and for the whole region.

Herds were also annually classified into 4 infection tiers. The 2 BTM samples for each year were averaged, and the mean PP was used to estimate within-herd prevalence. Table 1 details the infection tiers, which were the primary method of communicating herd status to producers during this study.

RAMP Analysis. Data collected from each herd that completed a RAMP included demographic information (farm and producer name, province of origin, regular herd veterinarian, bulk tank barcode), type of housing for adult cows, size of milking herd, and use of frozen colostrum, as well as answers for each of the 31 questions included in the 8 sections of the RAMP. Other than size of milking herd and the scores for the 8 sections of the RAMP, all variables were categorical.

Data were analyzed using univariable and multivariable logistic regression. Infection tiers 1 and 2 were combined to form the "low-prevalence herds" category, and infection tiers 3 and 4 were combined to form the "high-prevalence herds" category. The outcome variable was the dichotomized prevalence category (low vs. high) of the 2017 BTM samples. Initial unconditional analyses were performed for each individual predictor,

Table 1. Infection tiers used to classify dairy herds based on estimated within-herd bovine leukemia virus prevalence¹

Infection tier	PP used to classify herds	Range of estimated within-herd prevalence (%)
1	<15	<5
2	15 to <50	5–25
3	50 to <80	15–60
4	≥ 80	>50

¹Mean percent positivity (PP) of Jan. and Apr. 2017 bulk tank milk samples was used for each herd shipping milk in 2017 to determine the overall range of estimated within-herd prevalence during that calendar year (Nekouei et al., 2015a). Percent positivity was calculated as $PP = (OD_{\text{corrected sample}} / OD_{\text{corrected positive control}}) \times 100$, where OD is the optical density result from the ELISA.

as well as of the aggregate scores for each section and of the overall total RAMP score. Any variable for which the unconditional analysis *P*-value was <0.30 was retained for further consideration in developing the final multivariable model.

Due to the large number of potential predictors, forward selection was performed while building the multivariable logistic regression model, starting with the predictors with the smallest unconditional *P*-values. Herd size and barn type were retained in the final model as potential confounders.

RESULTS

Regional Surveillance

In 2016, BTM samples were received from 620 dairy herds across the region (NB, 199; NL, 27; NS, 222; PE, 172). This number decreased to 605 herds in 2017 (NB, 195; NL, 27; NS, 217; PE, 166) and to 589 herds shipping milk in 2018 (NB, 191; NL, 25; NS, 211; PE, 162). Table 2 shows the prevalence of BLV-positive herds based on ELISA testing of BTM as well as the change in prevalence over time. The number of active dairy herds shipping milk declined in all 4 provinces as well as across the whole region during the study period; this is consistent with overall changes seen in the number of dairy farms across Canada during the same period (Government of Canada, 2018b). No significant change was detected in herd-level BLV prevalence in Atlantic Canada during the 3-yr study period.

RAMP Implementation and Risk Factor Analysis

Of the 605 herds shipping milk that had 2 BTM samples collected in 2017, 106 completed RAMP (17.52%). Completed RAMP were received from 10 BLV-negative herds (9.43%) and 96 BLV-positive herds (90.57%), similar to the proportion of BLV-negative and positive herds based on regional surveillance. When broken down by province, 24 RAMP were received from NB (2

negative and 22 positive herds; 12.31% of the herds active in 2017), 3 were received from NL (3 positive herds; 11.11% of the herds active in 2017), 50 were received from NS (6 negative and 44 positive herds; 23.04% of the herds active in 2017), and 29 were received from PE (2 negative and 27 positive herds; 17.47% of the herds active in 2017). Twelve herds (11.32%) had bulk tank ELISA values that categorized them as infection tier 1; 22 (20.75%) were categorized as infection tier 2; 64 (60.38%) were categorized as infection tier 3; and 8 (7.55%) were categorized as infection tier 4. This resulted in 34 herds in the “low-prevalence” group and 72 in the “high-prevalence” group. Figure 1 shows the distribution of bulk tank ELISA values for herds participating in this study.

Question B3 was removed from data analysis (whether or not the herd had performed BLV testing within the past 12 mo), as initial examination of the results indicated that the question could have been misunderstood by veterinarians and producers. After univariable logistic regression of the 42 variables, 8 were retained for multivariable model building, listed in Table 3. Forward stepwise inclusion resulted in the final logistic regression model shown in Table 4.

Management practices associated with higher odds of a herd being classified as high-prevalence included history of clinical BLV in the herd and feeding colostrum from cows with unknown BLV status. Table 5 summarizes the use of different types of colostrum in herds classified as low-prevalence and high-prevalence. A significant difference was detected in the colostrum source fed in low- and high-prevalence herds ($P = 0.02$), with 29.41% of low-prevalence herds feeding colostrum from a commercial source, pasteurized colostrum from their farm, or colostrum from a known BLV-negative cow. Only 11.11% of high-prevalence herds fed colostrum from any of these sources, with the remaining 70.59% of low-prevalence and 88.89% of high-prevalence herds feeding colostrum from a single cow of unknown BLV status or pooled colostrum from cows of unknown BLV status.

Table 2. Estimated herd-level bovine leukemia virus prevalence and change in prevalence from 2016 to 2018, determined via bulk tank milk sample testing, for each province as well as the total for the region of Atlantic Canada

Province ¹	Prevalence (%) [95% CI]			Prevalence change (%), 2016–2018	<i>P</i> -value, 2016–2018
	2016	2017	2018		
NB	85.93 [80.31, 90.44]	87.18 [81.66, 91.53]	87.96 [82.48, 92.21]	2.03	0.22
NL	96.30 [81.03, 99.91]	92.59 [75.71, 99.09]	92.00 [73.97, 99.02]	−4.30	1.00
NS	86.49 [81.27, 90.69]	86.98 [81.73, 91.17]	88.15 [83.01, 92.18]	1.66	0.63
PE	92.44 [87.42, 95.91]	92.17 [86.98, 95.76]	91.97 [86.67, 95.66]	−0.47	1.00
Total	88.39 [85.60, 90.80]	88.72 [85.92, 91.14]	89.30 [86.52, 91.68]	0.91	0.45

¹NB = New Brunswick; NL = Newfoundland and Labrador; NS = Nova Scotia; PE = Prince Edward Island.

Figure 1. Distribution of bulk tank milk ELISA percent positivity (PP) results of anti-bovine leukemia virus antibodies for the 106 dairy farms participating in the study. Vertical dashed lines have been added at PP values of 15, 50, and 80 to indicate cutoff values for infection tier 1 (PP <15), infection tier 2 (PP 15 to <50), infection tier 3 (PP 50 to <80), and infection tier 4 (PP ≥80).

DISCUSSION

Regional Surveillance

Based on annual BTM surveillance, the proportion of BLV-positive herds is currently very high in Atlantic

Canada, with an overall herd-level prevalence of 89.30% of herds in 2018. This number is consistent with BLV herd-level prevalence in other regions of Canada and the United States (Scott et al., 2006; Nekouei et al., 2015b; LaDronka et al., 2018). Although this proportion has not changed appreciably since the last regional

Table 3. Variables retained for logistic regression model building after univariable analysis; outcome variable: classification of herd as low or high prevalence of bovine leukemia virus (BLV)¹

Variable	Response	Odds ratio	95% CI	P-value
History of clinical BLV	No (n = 42)	Referent		
	Yes (n = 64)	3.92	1.66, 9.26	<0.01
Cattle purchased	No (n = 48)	Referent		
	Yes (n = 58)	1.58	0.69, 3.58	0.28
Visitors can access	No (n = 37)	Referent		
	Yes (n = 69)	1.03	0.99, 1.07	0.17
Colostrum source ²	BLV-negative (n = 18)	Referent		
	BLV-unknown (n = 88)	3.33	1.18, 9.44	0.02
Fly control	Consistent environmental and topical (n = 29)	Referent		
	Consistent environmental or topical (n = 37)	0.38	0.13, 1.16	0.09
	Occasional environmental or topical (n = 29)	0.69	0.20, 2.30	0.54
	None (n = 11)	0.31	0.07, 1.39	0.13
General BLV score ³		1.01	1.00, 1.02	0.11
Shared equipment score ³		1.00	0.99, 1.00	0.30
Prewaned Heifers score ³		1.01	1.00, 1.02	0.19

¹Low-prevalence herds were classified as infection tier 1 or 2. High-prevalence herds were classified as infection tier 3 or 4.

²BLV-negative colostrum source = pasteurized colostrum, colostrum from a cow confirmed BLV-negative, or commercial colostrum replacer.

³Individual questions contained in these categories are outlined in the RAMP Development, Materials and Methods section.

Table 4. Variables retained in the final logistic regression model investigating risk factors associated with the odds of a herd being classified as having a high prevalence of bovine leukemia virus (BLV; classified as infection tier 3 or 4)

Variable	Response	Odds ratio	95% CI	P-value
Herd size		1.00	0.99, 1.00	0.37
Barn type	Freestall (n = 71)	Referent		
	Tiestall (n = 35)	2.87	0.94, 8.79	0.07
History of clinical BLV	No (n = 42)	Referent		
	Yes (n = 64)	4.68	1.83, 11.94	<0.01*
Colostrum source ¹	BLV-negative (n = 18)	Referent		
	BLV-unknown (n = 88)	4.03	1.27, 12.79	0.02*

¹BLV-negative colostrum source = pasteurized colostrum, colostrum from a cow confirmed BLV-negative, or commercial colostrum replacer.

* $P < 0.05$.

surveillance study performed in our region (Nekouei et al., 2015a), it is markedly higher than the proportion of BLV-positive herds in our region when the first herd-level surveillance was performed in the 1990s (Richardson and MacAulay, 1992). This is in spite of regional producers reporting implementation of measures to prevent virus transmission between cows.

Over the study period of this project, no significant change occurred in the proportion of BLV-positive herds in any of the provinces or in the overall region. Although interest in BLV and reducing transmission appeared to increase among local dairy producers during the study period, the short 2-yr study period may have prevented us from observing any changes in both overall herd BLV status and within-herd BLV prevalence.

RAMP Analysis

Despite promotion of the BLV RAMP project among regional veterinarians and provincial dairy boards, only 17.52% of regional herds completed and returned the RAMP. This could be due to prioritization of other management issues and diseases ahead of EBL by producers: the recently completed National Dairy Study in Canada showed that, in needs assessment discussions, producers ranked EBL fourth in terms of priority diseases after lameness, *Staphylococcus aureus* mastitis, and calf diarrhea (National Dairy Study, 2015). Another

potential reason could be the time required by both producers and veterinarians to complete the RAMP. The RAMP was intended to be completed in less than 30 min, but as its design was based on the AJDI RAMP for Johne's disease, producers or veterinarians unfamiliar with the AJDI RAMP may have required longer to complete the BLV RAMP. Although the small proportion of herds that completed the RAMP may have resulted in selection bias, herds that completed the RAMP did not have statistically different within-herd prevalences of BLV infection compared with herds that did not complete the RAMP (data not shown).

Despite the modest number of herds completing the RAMP, a small number of herd attributes and management practices were significantly associated with the odds of a herd being classified as high-prevalence. It was unsurprising that in a herd with a history of clinical BLV, the odds of the herd being classified as high-prevalence (>25% estimated within-herd prevalence) were 4.68 times higher than those of herds without confirmed clinical BLV. It is likely that this is more a consequence of a herd having a large proportion of BLV-positive cows rather than a factor influencing their within-herd prevalence of BLV infection.

In this study, herds that fed colostrum from cows of unknown BLV status were 4.03 times as likely to be high-prevalence herds compared with those whose calves received commercial colostrum, pasteurized colostrum, or untreated colostrum from confirmed

Table 5. Colostrum sources fed to calves in farms classified as low or high prevalence based on estimated within-herd prevalence of bovine leukemia virus (BLV) in the adult lactating herd

Herd status ¹	Pasteurized, commercial, or BLV-negative colostrum	Unknown BLV status or pooled colostrum	Total
Low prevalence	10	24	34
High prevalence	8	64	72
Total	18	88	106

¹Low-prevalence herds were classified as infection tier 1 or 2. High-prevalence herds were classified as infection tier 3 or 4.

BLV-negative cows. Because no individual calf testing was performed in this study, it was not possible to determine whether the colostrum source that calves received directly influenced individual calves' BLV status, or whether the colostrum source acted more as a general indicator of management practices for calves that could predispose them to BLV infection. However, as several other calf management practices were not associated with herd prevalence (e.g., dehorner and ear tagger use, needle and syringe practices), it is possible that colostrum source plays an important role in BLV transmission despite conflicting conclusions from previous studies (Kanno et al., 2014; Gutiérrez et al., 2015; Konishi et al., 2018).

Despite the association noted between feeding colostrum from cows of unknown BLV status and a higher within-herd prevalence, the majority of low-prevalence herds also fed colostrum from either a single cow or a pool of cows of unknown BLV status. This may be due to these herds having prior knowledge of their BLV status and considering the risk of transmission via colostrum to be marginal or nonexistent. The feeding of commercial, pasteurized, or BLV-negative colostrum in a small number of high-prevalence herds may also be due to prior knowledge in these herds of their BLV status and implementation of control measures to minimize BLV transmission; alternatively, the producers may have other motivations related to calf management for their decision to use these types of colostrum that are unrelated to BLV herd status. Further research into the motivations of producers in Atlantic Canada in regard to calf feeding practices would help to clarify the producers' justifications for their colostrum source, especially concerning the use of colostrum from BLV-positive cows.

Barn type, although initially retained to control for different types of housing, also displayed a relatively low *P*-value, with tiestall barns having higher odds of being classified as high-prevalence herds. A previous study reported earlier seroconversion of BLV-negative cows when housed adjacent to BLV-positive cows in tiestall barns (Kobayashi et al., 2015). Tiestall barns in Atlantic Canada have a higher proportion of older cows (3+ lactations; 45% of lactating cows) than freestall barns do (38% of lactating cows; E. E. John, unpublished data); because the prevalence of BLV infection tends to increase with cow age (Erskine et al., 2012b), this would support a larger proportion of BLV-positive cows being present in tiestall barns compared with freestall barns.

The results of this study did not fully support the hypothesis that calf rearing and fly control practices would be associated with within-herd BLV prevalence, except for the type of colostrum fed to calves. Several

possible reasons may explain why the data did not support the hypothesis. One of the most likely is that the number of farms that participated in the survey was low and the study did not have sufficient power to determine the effects of these factors on the adult herd prevalence. It is also possible that, in Atlantic Canada, calf rearing practices do not have a direct association with prevalence of BLV within the adult milking herd. In terms of fly control, it is possible that biting flies are not a significant source of infection compared with other transmission routes, and thus the presence or absence of fly control is not an important factor in relation to BLV prevalence.

Several limitations existed in this study. Our response rate to the RAMP was low compared with the total number of dairy herds in the region, so we likely were unable to achieve adequate power to see the effects of some management practices on BLV within-herd prevalence. A power calculation was not performed before beginning this study, but it is very possible that we would have obtained different results with a higher response rate. Also, because participation was voluntary it is possible that selection bias affected our results, and we received responses from producers who perceive BLV as more important than the average producer in our region. However, 90.57% of the RAMP received were from BLV-positive herds and 9.43% from BLV-negative herds, which is a similar proportion to the overall numbers of BLV-positive and negative herds in our region.

A second limitation was that the BLV infection tier of a herd was determined by testing of BTM samples rather than by individual testing of the youngstock and adult cows on a farm. This introduced imprecision into the estimate of within-herd prevalence for each herd, and so some herds may have been misclassified in terms of infection tier. The ranges of within-herd prevalence were also wide, especially for the higher infection tiers. This also meant that we received no direct information regarding the BLV status of any nonlactating animals. Thus, we were not able to determine any associations between herd management factors investigated in the RAMP and any estimates of BLV prevalence in calves, heifers, dry cows, or bulls. Additionally, because we performed the RAMP at a single time point for each dairy herd, after producers were given their initial BTM results, we were unable to determine whether the management factors the producers reported were directly associated with the current BLV within-herd prevalence or whether the producers had recently altered some management practices in response to information about their BLV infection tier.

Based on the results of this study, a revised version of the RAMP is being developed to reduce the amount

of time needed for producers and veterinarians to complete it. Future directions also include direct investigation of BLV prevalence in different populations of cattle on each farm (calves, young heifers, and breeding-age heifers) to determine whether any age-specific management factors are associated with the timing of new BLV infections in dairy herds in our region.

CONCLUSIONS

This study confirmed the high prevalence of BLV-positive dairy herds in Atlantic Canada. Herd-level factors associated with herds being classified as high-prevalence included a history of clinical BLV cases as well as the feeding of untreated colostrum from cows of unknown BLV status; additionally, we found a trend toward significance of tiestall herds being more likely to be classified as high-prevalence. Potential reasons for poor response rate include the time commitment for both producers and veterinarians to complete the RAMP; future versions of the RAMP may need to be modified to allow producers to complete it in a shorter amount of time.

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REFERENCES

- AJDI (Atlantic Johne's Disease Initiative). 2015. Documents and Forms. Accessed Feb. 12, 2019. <https://atlanticjohnes.ca/documents.php>.
- Bartlett, P. C., L. M. Sordillo, T. M. Byrem, B. Norby, D. L. Grooms, C. L. Swenson, J. Zalucha, and R. J. Erskine. 2014. Options for the control of bovine leukemia virus in dairy cattle. *J. Am. Vet. Med. Assoc.* 244:914–922. <https://doi.org/10.2460/javma.244.8.914>.
- Dohoo, I. R., S. W. Martin, and H. Stryhn. 2009. *Veterinary Epidemiologic Research*. 2nd ed. VER, Inc., Charlottetown, PEI, Canada.
- EFSA AHAW Panel (European Food Safety Authority Panel on Animal Health and Welfare). 2015. *Enzootic bovine leukosis*. EFSA J. 13:4188. <https://doi.org/10.2903/j.efsa.2015.4188>.
- Erskine, R. J., P. C. Bartlett, T. M. Byrem, C. L. Render, C. Febvay, and J. T. Houseman. 2012a. Association between bovine leukemia virus, production, and population age in Michigan dairy herds. *J. Dairy Sci.* 95:727–734. <https://doi.org/10.3168/jds.2011-4760>.
- Erskine, R. J., P. C. Bartlett, T. M. Byrem, C. L. Renders, C. Febvay, and J. T. Houseman. 2012b. Using a herd profile to determine age-specific prevalence of bovine leukemia virus in Michigan dairy herds. *Vet. Med. Int.* 2012:350374. <https://doi.org/10.1155/2012/350374>.
- Erskine, R. J., P. C. Bartlett, K. M. Sabo, and L. M. Sordillo. 2011. Bovine leukemia virus infection in dairy cattle: Effect on serological response to immunization against J5 *Escherichia coli* bacterin. *Vet. Med. Int.* 2011:915747. <https://doi.org/10.4061/2011/915747>.
- Evermann, J. F., R. F. DiGiacomo, J. F. Ferrer, and S. M. Parish. 1986. Transmission of bovine leukosis virus by blood inoculation. *Am. J. Vet. Res.* 47:1885–1887.
- Government of Canada: Agriculture and Agri-Food Canada. 2018a. Red Meat Condemnation Report by Species for Federally Inspected Plants. Accessed Feb. 12, 2019. <http://aimis-simia.agr.gc.ca/rp/index-eng.cfm?action=pR&pdctc=&r=134>.
- Government of Canada: Agriculture and Agri-Food Canada. 2018b. Number of Farms with Shipments of Milk by Province. Accessed Feb. 12, 2019. <https://aimis-simia-cdic-ccil.agr.gc.ca/rp/index-eng.cfm?action=gR&r=220&signature=F4BE2DFA27E9E65EFDEAF794D1EEE9C0&pdctc=&pTpl=1#wb-cont>.
- Gutiérrez, G., M. Lomonaco, I. Alvarez, F. Fernandez, and K. Trono. 2015. Characterization of colostrum from dams of BLV endemic dairy herds. *Vet. Microbiol.* 177:366–369. <https://doi.org/10.1016/j.vetmic.2015.03.001>.
- Hovingh, E. 2016. Risk assessment: Bovine leukemia virus (BLV). Accessed Feb. 12, 2019. <https://extension.psu.edu/risk-assessment-bovine-leukemia-virus-blv>.
- Kanno, T., R. Ishihara, S. Hatama, Y. Oue, H. Edamatsu, Y. Konno, S. Tachibana, and K. Murakami. 2014. Effect of freezing treatment on colostrum to prevent the transmission of bovine leukemia virus. *J. Vet. Med. Sci.* 76:255–257. <https://doi.org/10.1292/jvms.13-0253>.
- Kobayashi, S., T. Tsutsui, T. Yamamoto, Y. Hayama, N. Muroga, M. Konishi, K. Kameyama, and K. Murakami. 2015. The role of neighboring infected cattle in bovine leukemia virus transmission risk. *J. Vet. Med. Sci.* 77:861–863. <https://doi.org/10.1292/jvms.15-0007>.
- Kohara, J., M. Takeuchi, Y. Hirano, Y. Sakurai, and T. Takahashi. 2018. Vector control efficacy of fly nets on preventing bovine leukemia virus transmission. *J. Vet. Med. Sci.* 80:1524–1527. <https://doi.org/10.1292/jvms.18-0199>.
- Konishi, M., H. Ishizaki, K. Kameyama, K. Murakami, and T. Yamamoto. 2018. The effectiveness of colostral antibodies for preventing bovine leukemia virus (BLV) infection in vitro. *BMC Vet. Res.* 14:419. <https://doi.org/10.1186/s12917-018-1724-5>.
- LaDronka, R. M., S. Ainsworth, M. J. Wilkins, B. Norby, T. M. Byrem, and P. C. Bartlett. 2018. Prevalence of bovine leukemia virus antibodies in US dairy cattle. *Vet. Med. Int.* 2018:5831278. <https://doi.org/10.1155/2018/5831278>.
- National Dairy Study. 2015. Canadian dairy producers' priority management and disease issues. Accessed Feb. 14, 2019. <http://www.nationaldairystudy.ca/new-page-1>.
- Nekouei, O., H. Stryhn, J. VanLeeuwen, D. Kelton, P. Hanna, and G. Keefe. 2015a. Predicting within-herd prevalence of infection with bovine leukemia virus using bulk-tank milk antibody levels. *Prev. Vet. Med.* 122:53–60. <https://doi.org/10.1016/j.prevetmed.2015.10.009>.
- Nekouei, O., J. VanLeeuwen, J. Sanchez, D. Kelton, A. Tiwari, and G. Keefe. 2015b. Herd-level risk factors for infection with bovine leu-

- kemia virus in Canadian dairy herds. *Prev. Vet. Med.* 119:105–113. <https://doi.org/10.1016/j.prevetmed.2015.02.025>.
- New York State Cattle Health Assurance Program. 2016. Bovine leukosis virus module. Accessed Feb. 12, 2019. <https://ahdc.vet.cornell.edu/programs/NYSCHAP/modules/bovineleukosis/index.cfm>.
- Norby, B., P. C. Bartlett, T. M. Byrem, and R. J. Erskine. 2016. Effect of infection with bovine leukemia virus on milk production in Michigan dairy cows. *J. Dairy Sci.* 99:2043–2052. <https://doi.org/10.3168/jds.2015-10089>.
- OIE World Animal Health Information System. 2018. List of countries by disease situation. Accessed Feb. 12, 2019. http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statuslist.
- Panei, C. J., A. E. Larsen, N. A. Fuentealba, G. E. Metz, M. G. Echeverría, C. M. Galosi, and A. R. Valera. 2019. Study of horn flies as vectors of bovine leukemia virus. *Open Vet. J.* 9:33–37. <https://doi.org/10.4314/ovj.v9i1.6>.
- Richardson, G. F., and E. B. MacAulay. 1992. The prevalence of bovine leukemia virus infection in dairy cows on Prince Edward Island. *Can. Vet. J.* 33:188–189.
- Ruiz, V., N. G. Porta, M. Lomonaco, K. Trono, and I. Alvarez. 2018. Bovine leukemia virus infection in neonatal calves: Risk factors and control measures. *Front. Vet. Sci.* 5:267. <https://doi.org/10.3389/fvets.2018.00267>.
- Scott, H. M., O. Sorensen, J. T. Y. Wu, E. Y. W. Chow, K. Manninen, and J. A. VanLeeuwen. 2006. Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis*, *Neospora caninum*, bovine leukemia virus, and bovine viral diarrhea virus infection among dairy cattle and herds in Alberta and agroecological risk factors associated with seropositivity. *Can. Vet. J.* 47:981–991.

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