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# Herd-level prevalence of bovine leukemia virus, *Salmonella* Dublin and *Neospora caninum* in Alberta, Canada, dairy herds using ELISA on bulk tank milk samples

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## ABSTRACT

Endemic infectious diseases remain a major challenge for dairy producers worldwide. For effective disease control programs, up-to-date prevalence estimates are of utmost importance. The objective of this study was to estimate the herd-level prevalence of bovine leukemia virus (BLV), *Salmonella* Dublin, and *Neospora caninum* in dairy herds in Alberta, Canada using a serial cross-sectional study design. Bulk tank milk samples from all Alberta dairy farms were collected 4 times, in December 2021 (n = 489), April 2022 (n = 487), July 2022 (n = 487), and October 2022 (n = 480), and tested for antibodies against BLV, *S.* Dublin, and *N. caninum* using ELISAs. Herd-level apparent prevalence was calculated as positive samples divided by total tested samples at each time point. A mixed effect modified Poisson regression model was employed to assess the association of prevalence with region, herd size, herd type, and type of milking system. Apparent prevalence of BLV was 89.4, 88.7, 86.9 and 86.9% in December, April, July, and October, respectively, whereas for *S.* Dublin apparent prevalence was 11.2, 6.6, 8.6, and 8.5%, and for *N. caninum* apparent prevalence was 18.2, 7.4, 7.8, and 15.0%. For BLV, *S.* Dublin and *N. caninum*, a total of 91.7, 15.6, and 28.1% of herds, respectively, were positive at least once, whereas 82.5, 3.6, and 3.0% of herds were ELISA-positive at all 4 times. Compared with the north region, central Alberta had a high prevalence (prevalence ratio (PR) = 1.13) of BLV-antibody positive herds, whereas south Alberta had a high prevalence (PR = 2.56) of herds positive for *S.* Dublin antibodies. Furthermore, central (PR = 0.52) and south regions (PR = 0.46) had low prevalence of *N. caninum*-positive herds compared with the north.

Hutterite colony herds were more frequently BLV-positive (PR = 1.13) but less frequently *N. caninum*-positive (PR = 0.47). Large herds (>7,200 L/day milk delivered ~ > 250 cows) were 1.1 times more often BLV-positive, whereas small herds (≤3,600 L/day milk delivered ~ ≤ 125 cows) were 3.2 times more often *N. caninum*-positive. For *S.* Dublin, Hutterite-colony herds were less frequently (PR = 0.07) positive than non-colony herds only in medium and large stratum but not in small stratum. Moreover, larger herds were more frequently (PR = 2.20) *S.* Dublin-positive than smaller herds only in non-colony stratum but not in colony stratum. Moreover, *N. caninum* prevalence was 1.6 times higher on farms with conventional milking systems compared with farms with an automated milking system. These results provide up-to-date information of the prevalence of these infections that will inform investigations of within-herd prevalence of these infections and help in devising evidence-based disease control strategies.

Keywords: bovine leukosis, neosporosis, *Salmonella* Dublin, dairy farms, surveillance, prevalence

## INTRODUCTION

Infectious diseases have a considerable adverse impact on the productivity and profitability of the dairy industry, directly through treatment and veterinary costs and loss of milk production, and indirectly through reduced cow longevity, reproductive losses, and regulatory implications for export of animals/animal products (Hernandez et al., 2001; Chi et al., 2002; Otta et al., 2003; Haddad et al., 2005; Tiwari et al., 2007; Aghamohammadi et al., 2018; Kuczewski et al., 2019, 2021). Besides economic consequences, animal welfare concerns and health im-

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plications such as transmission of pathogens to humans can also cause consumer concerns that may reduce consumption of cattle products (Bharti et al., 2003; Barkema et al., 2015; Harvey et al., 2017; Mangat et al., 2019). Although outbreaks of emerging diseases often receive most attention, endemic diseases dominate the global burden of infectious diseases of animals (Wierup, 2012). Several important endemic infectious production-limiting diseases, such as contagious mastitis, bovine leukosis, salmonellosis, neosporosis, bovine viral diarrhea, and Johne's disease (JD), remain major challenges for the dairy industry in Canada and worldwide.

Enzootic bovine leukosis, caused by bovine leukemia virus (BLV), is an infection of high economic importance that is associated with decreased milk production, reduced longevity, and impairment of the immune system (Erskine et al., 2012; Bartlett et al., 2014). It was estimated that 83 to 87% of Alberta dairy herds are positive for BLV (Nekouei et al., 2015b; Kuczewski et al., 2019). A recent study indicated that BLV decreased the partial net revenue of $635 per infected cow per year (Kuczewski et al., 2019). However, actual revenue losses are likely higher as BLV modulates the immune response and reduces longevity in the herd (Bartlett et al., 2013).

*Salmonella enterica* ssp. *enterica* serovar Dublin (*S.* Dublin), a zoonotic pathogen that is often multidrug-resistant (Davis et al., 2007; Otto et al., 2018), is adapted to cattle and is associated with abortion, decreased milk production, and increased calf morbidity and mortality on dairy farms (Nielsen et al., 2007a, 2012; McSweeney and McNamara, 2021). A Danish study estimated gross margin losses of €49 – 326 per cow in the first year following *S.* Dublin infection and €8 – 188 per cow per year for next 9 years, depending on level of farm management (Nielsen et al., 2013). An Irish study estimated that *Salmonella* infection on farms reduced annual profits by €112 per cow (O'Doherty et al., 2015). There were 29 *S.* Dublin isolates recovered from 1990 to 1999 in Alberta cattle (Guerin et al., 2005). Furthermore, *S.* Dublin is a reportable disease in Alberta (Alberta Agriculture, 2022) and Alberta Agriculture reported 6 and 2 cases in 2021 and 2022, respectively, in the province (Alberta Agriculture, 2022). However, herd-level prevalence of *S.* Dublin in Alberta, Canada has never been estimated.

Bovine neosporosis, caused by the coccidian parasite *Neospora caninum*, is the most important contributor to abortion and perinatal mortality in dairy cattle in many countries (Hernandez et al., 2002; Brickell et al., 2010; Reichel et al., 2013) including Canada where 41% of abortions with a definitive diagnosis were associated with *N. caninum* during an active surveillance (Haddad et al., 2005; Wilson et al., 2016). The annual costs of neosporosis have been estimated at CA$2,305 for a 50-cow Canadian dairy herd (Chi et al., 2002), which translates to CA$3,740 in 2023 when corrected for inflation (Canada Inflation Calculator) .

Apart from animal health and economic perspectives, some infectious diseases can also have public health implications. For example, *S.* Dublin is transmissible to humans and can cause multidrug-resistant bloodstream infections with severe health complications (Harvey et al., 2017; Mangat et al., 2019).

The importance of controlling endemic infectious diseases is often underrated by producers due to the challenge of linking their presence with demonstrable production or economic losses. Often, in infected herds, typical clinical signs of these diseases are either absent, mild, or vague, and as a result, their presence on dairy farms is commonly accepted (Carslake et al., 2011; Statham, 2011; Ritter et al., 2017).

Effective surveillance and disease control are important for maintaining the health and welfare of dairy cattle, plus the profitability of dairy farming. For effective disease control, up-to-date estimates of disease occurrence are of utmost importance. The prevalence of BLV, *S.* Dublin and *N. caninum* were reported in various regions of the world, but up-to-date data on the prevalence in Canada, especially in western Canada, are limited. Most prevalence estimates for BLV, *S.* Dublin and *N. caninum* in North America are older and may no longer be valid as prevalence can change due to control programs and changes in dairy industry dynamics (Barkema et al., 2015). The objective of this study was, therefore, to generate an up-to-date estimation of the herd-level prevalence and identify spatial trends of and factors associated with BLV, *S.* Dublin and *N. caninum* infection in dairy herds in Alberta, Canada using a serial cross-sectional study design. It is expected that this will inform investigations of within-herd prevalence of these infections and facilitate development of evidence-based disease control and eradication programs not only in Alberta but also in other regions of North America and beyond.

## MATERIALS AND METHODS

The study was approved by the Animal Care Committee (AC21–0070) of the University of Calgary (Calgary, AB, Canada).

### *Study Population*

All active dairy farms that were delivering milk at any of the 4 sampling times in Alberta, Canada, were included in the study. These farms were divided into north, central, and south regions by the provincial milk authority, i.e., Alberta Milk (Alberta Milk, 2022).

### Sample Collection

A 40 mL bulk tank milk (BTM) sample was collected by the milk collectors as an extra sample during the routine milk collection from active dairy producers in Alberta at 4 time points: in December 2021 (n = 489), April 2022 (n = 487), July 2022 (n = 487), and October 2022 (n = 480). Samples were well-mixed and carefully obtained from each tank collected, following standard operating procedures devised by Alberta Milk for routine BTM sample collection. Samples were shipped at 4°C to the laboratory at the Faculty of Veterinary Medicine of the University of Calgary (Calgary, AB, Canada) and stored at 4°C. In the next 2 or 3 d, 10 mL was removed from each sample and centrifuged at 1400 g for 10 min at room temperature and the cream layer was removed. The skim milk samples were aliquoted into 1 mL portions in sterile 2.0 mL centrifuge tubes. Another aliquot before removal of fat layer was also prepared. Original remaining samples and all aliquots were stored at –20°C until testing. The samples remained frozen for a median (Q1 – Q3) of 142 (116 – 145), 17 (4 – 26), 5 (5 – 15), and 18 (17 – 35) days for time point 1, 2, 3, and 4 samples, respectively.

### Bovine Leukemia Virus ELISA

Frozen skim milk aliquots were thawed and tested for antibodies against BLV using commercially available blocking ELISA (Bovicheck BLV; Biovet, Saint-Hyacinthe QC, Canada), following the manufacturer's protocol. Optical density (OD) was measured and percentage inhibition (PI%) was calculated as follows (Equation 1):

$$\mathrm{PI\%} = \left[1 - \left(\frac{\mathrm{OD}_{\mathrm{sample}}}{\mathrm{mean}\mathrm{OD}_{\mathrm{negative}}}\right)\right] \times 100. \quad (1)$$

A PI% ≥ 30 was considered positive, whereas a PI% > 20 and <30 was considered suspected, and a PI% < 20 was considered negative.

### Salmonella Dublin ELISA

Frozen samples, without fat removal, were thawed and tested for antibodies against *S.* Dublin using indirect ELISA (PrioCHECK *S.* Dublin Ab Strip Kit, # 7610640; Thermo Fischer Scientific, Waltham, MA). Plates coated with purified polysaccharide (LPS) isolated from *S.* Dublin were used to detect antibodies against *Salmonella* LPS O-antigens 1, 9 and 12. All tests were conducted according to the manufacturer's instructions. The OD was measured at 450 nm within 15 min. Corrected $OD_{450}$ was calculated by subtracting negative control OD from the sample OD. Percentage positivity (PP%) was calculated as follows (Equation 2):

$$PP\% = \left(\frac{Corrected\,OD\,samples}{Corrected\,OD\,Positive\,Controls} \times 100\right) - 10. \quad (2)$$

Manufacturers recommended cut off-values were used, i.e., PP% ≥ 35 was considered positive whereas PP% < 35 was considered negative.

### Neospora caninum ELISA

Frozen skim milk aliquots were thawed and tested for antibodies against *N. caninum* using commercially available indirect ELISA (IDEXX Neospora X2; IDEXX Laboratories, Westbrook, ME), with small modifications: The dilution was 1:2 for BTM instead of 1:100 recommended for serum using manufacturer's supplied diluent, as described (Bartels et al., 2005; Wapenaar et al., 2007). The OD was measured, and sample-to-positive ratio (S/P) was calculated as follows (Equation 3):

$$S/P = \frac{Sample\,Mean - Negative\,Control\,Mean}{Positive\,Control\,Mean - Negative\,Control\,Mean}. \quad (3)$$

An $S/P \geq 0.50$ was considered to be positive whereas an $S/P < 0.50$ was considered negative for antibodies against *N. caninum* (Nasir et al., 2012).

### Data Management

Data on annual milk volume delivered, geographical region (north, central, south), type of milking system (conventional milking system (CMS) vs. automatic milking system (AMS)) and herd type (Hutterite colony vs. non-colony herds) of each farm was obtained from Alberta Milk. Average daily milk delivered by each farm was calculated by dividing total annual milk delivered by the number of days milk delivered by each farm, which was then categorized into 3 herd size categories: small (≤3,600 L/day milk), medium (3,600 – 7,200 L/day milk), and large (>7,200 L/day milk). To aid interpretation, approximate adult cow herd size was estimated using Alberta Milk's statistics on provincial milk production, number of farms and average herd size (Alberta Milk, 2022) using the following equation (Equation 4):

$$Herd\,size\,of\,farm\,A = \frac{Total\,annual\,milk\,delivered\,by\,farm\,A}{\dfrac{Total\,amount\,milk\,delivered\,in\,Alberta}{(No.\,of\,farms \times Average\,herd\,size)}}. \quad (4)$$

## Statistical Analyses

Data were initially put into spreadsheets using Microsoft Excel for Mac (Microsoft Corporation, Redmond, WA) and then imported to Stata. Statistical analyses were performed using Stata/SE 17.0 for Mac (StataCorp. 2021, College Station, TX). A $P$-value <0.05 was considered statistically significant. Descriptive statistics were calculated for herd characteristics and outcomes. Categorical variables were presented as frequency and proportions while continuous variables were presented as mean and medians along with first and third quartiles. Apparent herd-level prevalence at each time point was calculated by dividing the number of positive herds by the total number of herds tested at the respective time point. Overall apparent herd-level prevalence was calculated as proportion of herds that were positive at least once out of 4 time points. A sensitivity analysis was performed by excluding herds with <4 samples. Additionally, proportion of herds that were positive at all 4 time points was also estimated. Logit-transformation was applied to compute the 95% confidence interval (95% CI) of the proportion estimates. To check any expected differences across the 3 Alberta regions (north, central, south), we compared continuous and binary herd characteristics and outcomes across regions using univariable linear and logistic regression. Histograms were created to illustrate distribution of the PI%, PP% and S/P ratio for the results of the BLV, *S.* Dublin, and *N. caninum* ELISAs, respectively, across time points. Boxplots were created for visualizing interquartile spread of PI%, PP% and S/P ratio across time points and outcomes (positive or negative).

A mixed effect generalized linear model with log link and Poisson family was developed with herd included as random effect to determine association of geographical regions, herd type, herd size, and type of milking system with the prevalence of herds ELISA-positive for each pathogen separately. Robust variance covariance estimator (vce) was used to compute standard errors of the effect estimates using Huber and White sandwich estimator of variance (Huber, 1967; White, 1980, 1982). This approach, called modified Poisson regression is an alternate to more frequently used logistic regression and was chosen considering the prevalence estimation design of the study and the common outcome to avoid inflated odds ratios and associated misinterpretation (Barros and Hirakata, 2003; Coutinho et al., 2008; Fonseca Martinez et al., 2017; Gnardellis et al., 2022).

Predictors were assessed for potential effect modification using 2-way interactions in a manual backward elimination manner. Any statistically significant interactions remained in the model (Dohoo et al., 2010a; b). No effect modification was present except a significant interaction of herd size and herd type for *S.* Dublin model. Consequently, stratum-specific estimates of herd size and herd type were computed for the *S.* Dublin model. Due to small number of observations in one of the strata, i.e., large colony herds, medium and large herds were aggregated into one category for *S. Dublin* model. Akaike's information criterion (AIC) was used to select the best fitting models.

A sensitivity analysis was performed by restricting each pathogen model to herds that were sampled at all 4 times. Equation 5, 6, and 7 represent the final models that we report for BLV, *S.* Dublin, and *N. caninum*, respectively:

$$\log(p_{(BLV)ij}) = \beta_0 + \beta_1 \cdot (\text{Region})_{ij} + \beta_2 \cdot (\text{Herd Size})_{ij} + \beta_3 \cdot (\text{Herd Type})_{ij} + \beta_4 \cdot (\text{Milking Sysem})_{ij} + Zu_i \quad (5)$$

$$\log(p_{(SD)ij}) = \beta_0 + \beta_1 \cdot (\text{Region})_{ij} + \beta_2 \cdot (\text{Herd Size})_{ij} + \beta_3 \cdot (\text{Herd Type})_{ij} + \beta_4 \cdot (\text{Milking Sysem})_{ij} + + \beta_5 \cdot (\text{Herd Size} \times \text{Herd Type})_{ij} + Zu_i \quad (6)$$

$$\log(p_{(NC)ij}) = \beta_0 + \beta_1 \cdot (\text{Region})_{ij} + \beta_2 \cdot (\text{Herd Size})_{ij} + \beta_3 \cdot (\text{Herd Type})_{ij} + \beta_4 \cdot (\text{Milking Sysem})_{ij} + Zu_i \quad (5)$$

where $p_{(OUTCOME)ij}$ is the probability of observing a positive BTM test for the given outcome in jth sample of the ith herd conditional on predictors; $\beta_0$ is the intercept, $\beta_1$ is the regression coefficient representing the difference in logs of the expected probability of outcome between given and the reference categories of the region when other variables are held constant; $\beta_2$ is the difference in logs of the expected probability of outcome between given and the reference categories of herd size when other variables are held constant; $\beta_3$ is the difference in logs of the expected probability of outcome for Hutterite colony herds compared with non-colony herds when other variables are held constant; $\beta_4$ is the difference in log of the expected probability of outcome for AMS herds compared with CMS herds when other variables are held constant; $\beta_5$ is the change in the difference in logs of expected probability between colony and non-colony herds with change in herd size; $Zu_i$ is herd-specific random intercept to account for with-in herd variability.

Final model coefficients were exponentiated to obtain prevalence ratios (PR), which are analogous to the risk ratio, and were presented along with 95% CI and corresponding *P*-values (Fonseca Martinez et al., 2017).

Spatial visualization of the geographical spread of herds was performed using QGIS Version 3.28 Firenze for macOS (QGIS Geographic Information System, QGIS Association) utilizing Statistics Canada boundary files and Alberta census boundaries (Statistics Canada, 2016; Government of Alberta, 2022). The data were transformed and projected into EPSG 3400 (NAD_1983_10TM_AEP_Forest). Heat maps were used to visualize geographical spread of the dairy farms reflecting density across Alberta census sub-divisions.

## RESULTS

Of 495 unique farms in this study, 473 (95.6%) farms were sampled at all 4 time points whereas 13 (2.6%), 3 (0.6%) and 6 (1.2%) farms were sampled only 3, 2, and 1 times, respectively. These herds were spread across 3 regions with varying farm density (Figure 1). There were 139 (28%), 196 (40%), and 160 (32%) herds in the north, central, and south regions, respectively (Table 1). Of the study farms, 49.7, 34.7, and 15.6% of the herds were categorized as small (≤3,600 L/day milk delivered ~ ≤ 125 cows), medium (3,600 – 7,200 L/day milk delivered), and large herds (>8600 L/day milk delivered), respectively, with a mean (median) milk volume delivered of 2,385 (2,530), 4,828 (4,618), and 11,296 (10,180) L/day, respectively (Table 1). Furthermore, 29% of herds used an automated milking system and 27% herds belonged to Hutterite colonies (Table 2). Hutterite colony herds were more common in South Alberta compared with the rest of the province (Table 1).

### *Bovine leukemia virus*

Apparent prevalence of BLV antibody-positive herds ranged from 86.9 to 89.4% at various time points whereas overall herd-level prevalence was 91.7%, with 82.5% of herds consistently positive at all 4 time points (Table 3). The distribution of PI% ranged from –53.7 to 93.6 across time points (Supplementary Figure S1; https://data.mendeley.com/datasets/ypz48nbpmn/1; Shaukat, 2024), whereas mean PI% of the positive samples was 74.5 (median = 77.1, range = 30.4 to 92.6), 69.3 (median = 73.4, range = 30.6 to 90.1), 71.3 (median = 74.6, range = 30.4 to 93.6), and 68.0 (median = 71.2, range = 31.1 to 89.8) at first, second, third, and fourth tests, respectively (Supplementary Figure S2; https://data.mendeley.com/datasets/ypz48nbpmn/1; Shaukat, 2024).

The prevalence of herds positive for BLV antibodies was high (PR = 1.13; 95% CI = 1.04 – 1.23) in Central region compared with north region (Table 4). The proportion of BLV-positive herds was higher in larger (PR = 1.11; 95% CI = 1.03 – 1.20) and non-significantly higher in medium size categories (PR = 1.06; 95% CI = 0.99 – 1.13; $P = 0.11$) compared with small size herds. Hutterite colony herds were more frequently positive (PR = 1.13; 95% CI = 1.07 – 1.20) than non-colony herds. The proportion of BLV-positive farms was not different ($P = 0.78$) between farms milking with a CMS or AMS.

### *Salmonella Dublin*

Apparent prevalence of herds positive for *S.* Dublin antibodies at various time points ranged from 6.6 to 11.2% whereas overall herd-level prevalence was 15.6% (Table 3). The BTM of 3.6% herds tested positive for *S.* Dublin antibodies at all 4 time points, whereas another 2.7% herds were positive at 3 of 4 time points. The PP% ranged from –11.3 to 157.5 across time points (Supplementary Figure S3; https://data.mendeley.com/datasets/ypz48nbpmn/1; Shaukat, 2024) whereas mean PP% of the positive samples was 62.0 (median = 53.2, range = 35.2 to 157.5), 57.0 (median = 49.8, range = 35.9 to 113.2), 59.4 (median = 56.0, range = 36.7 to 136.2), and 57.7 (median = 46.6, range = 35.6 to 108.7) for first, second, third, and fourth test, respectively (Supplementary Figure S4; https://data.mendeley.com/datasets/ypz48nbpmn/1; Shaukat, 2024).

South Alberta had a higher prevalence (PR = 2.56; 95% CI = 1.23 – 5.32) of *S.* Dublin antibody-positive herds compared with north region (Table 5). Larger herd size was associated with an increased likelihood of being positive (PR = 2.20; 95% CI = 1.14 – 4.26) for *S.* Dublin antibodies in non-colony herds stratum, however, herd size did not make any difference ($P = 0.07$) on *S.* Dublin positivity among colony-herds stratum. Hutterite colony herds were significantly less frequently (PR = 0.07; 95% CI = 0.02 – 0.27) positive for *S.* Dublin antibodies compared with non-colony herds, however, this pattern was observed in medium and large herd size only whereas prevalence did not differ ($P = 0.35$) between colony and non-colony herds for small size herds. *S.* Dublin prevalence did not differ ($P = 0.20$) between farms milking with a CMS and AMS (Table 5).

***Neospora caninum.*** Apparent prevalence of *N. caninum* antibody-positive herds ranged from 7.4 to 18.2% across the 4 time points whereas overall herd-level prevalence was 28.1%, with 3% herds positive for *N. caninum* antibodies at all 4 test (Table 3). The S/P ratio ranged from –1.92 to 3.37 across time points Supplementary Figure S5; https://data.mendeley.com/datasets/ypz48nbpmn/1; Shaukat, 2024) whereas mean S/P ratio of the positive samples was 0.81 (median = 0.68, range = 0.50 to 1.79), 0.72 (median = 0.62, range = 0.51 to

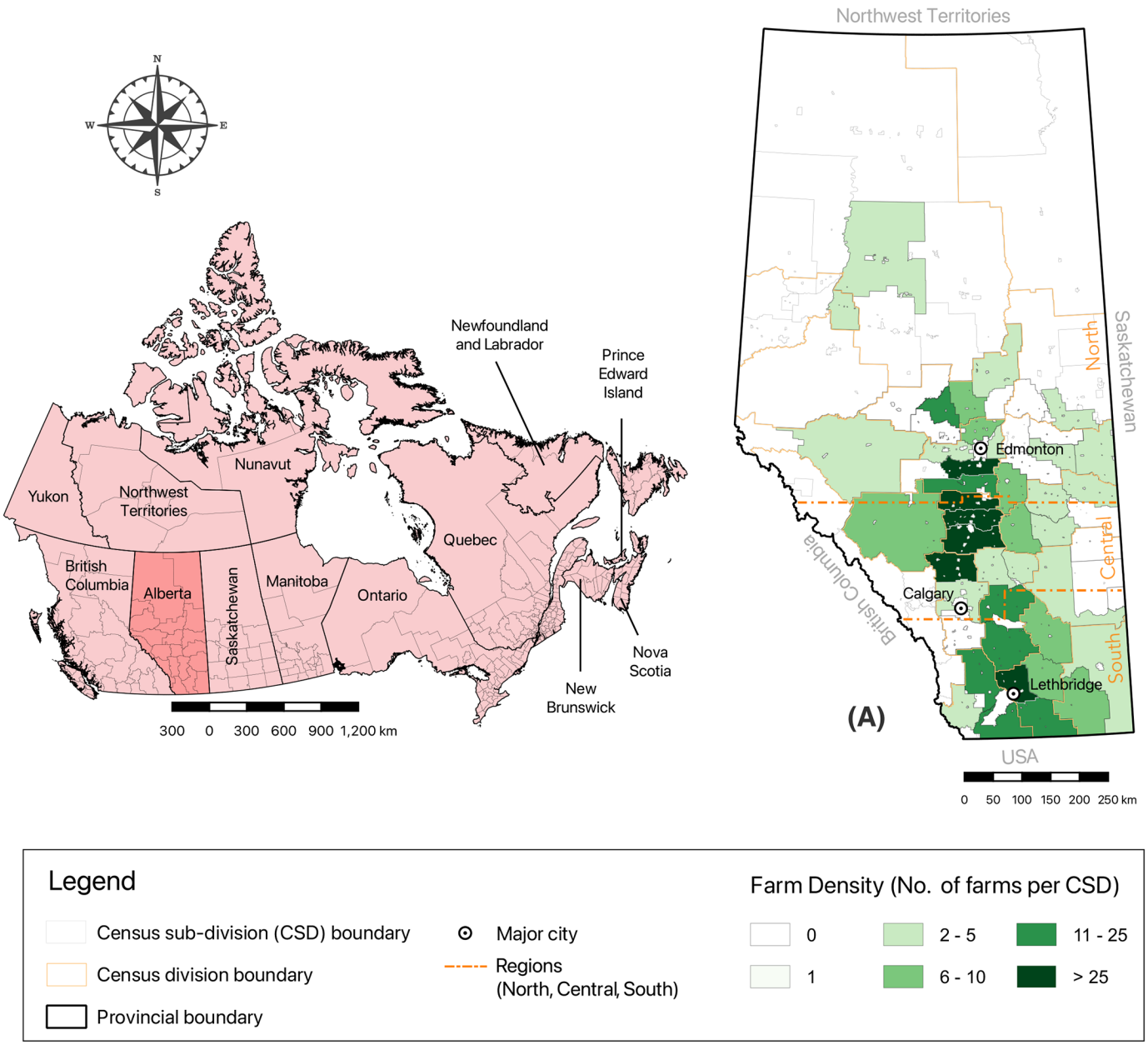


**Figure 1.** Spatial distribution of the dairy farms included in study across Alberta, Canada. Farm density across Alberta represented as number of dairy farms per census sub-division. Dairy farms are categorized into north, central, and south regions.

2.02), 0.99 (median = 0.73, range = 0.51 to 3.37), and 0.80 (median = 0.71, range = 0.50 to 2.15) for first, second, third, and fourth time points, respectively (Supplementary Figure S6; https://data.mendeley.com/datasets/ypz48nbpmn/1; Shaukat, 2024).

The prevalence of *N. caninum* antibody-positive herds was significantly low in central (PR = 0.52; 95% CI = 0.35 – 0.77) and south (PR = 0.46; 95% CI = 0.29 – 0.73) regions compared with north Alberta, with northern herds being 2.2 times more often positive compared with herds in southern Alberta (Table 4). Medium and large herds were less frequently (PR = 0.66; 95% CI = 0.46 – 0.96, and PR = 0.32; 95% CI = 0.16 – 0.62, respectively) positive for *N. caninum* antibodies compared with small herds. The herd-prevalence was significantly low among Hutterite colonies (PR = 0.47; 95% CI = 0.29 – 0.76) compared with non-colony herds. Herds with CMS were 1.6 times more frequently positive than herds with AMS.

**Table 1.** Characteristics of the Alberta dairy herds included in estimation of herd-level prevalence of bovine leukemia virus, *Salmonella* Dublin, and *Neospora caninum* using ELISAs on bulk tank milk samples at four time points

| | North (n = 139) | Central (n = 196) | South (n = 160) | Alberta (n = 495) |
|---|---|---|---|---|
| Alberta milk production (%) | 25 | 45 | 30 | 100 |
| Herd Size [n(%)] | | | | |
| Small (≤3,600 L/day milk delivered; ~ ≤ 125 cows) | 80 (58)[a] | 86 (44)[b] | 80 (50)[ab] | 246 (50) |
| Medium (3,600 – 7,200 L/day milk delivered; ~126 – 250 cows) | 43 (31)[a] | 65 (33)[a] | 64 (40)[a] | 172 (35) |
| Large (>7,200 L/day milk delivered; ~ > 250 cows) | 16 (11)[a] | 45 (23)[b] | 16 (10)[a] | 77 (15) |
| Average milk volume delivered (L/day) [Median (Q1 – Q3)] | | | | |
| Small (≤3,600 L/day milk delivered) | 2,151 (1,433 – 2,772)[a] | 2,356 (1,624 – 3,098)[a] | 2,888 (2,244 – 3,301)[b] | 2,530 (1,710 – 3,110) |
| Medium (3,600 – 7,200 L/day milk delivered) | 4,549 (4,151 – 5,814)[a] | 4,703 (4,182 – 5,328)[a] | 4, 569 (4,055 – 5,428)[a] | 4,618 (4,120 – 5,443) |
| Large (>7,200 L/day milk delivered) | 10,248 (9,201 – 14,994)[a] | 10,206 (8,424 – 14,052)[a] | 9,049 (7,672 – 12,414)[a] | 10,180 (8,424 – 13,253) |
| Farm type [n (%)] | | | | |
| Hutterite colony | 16 (12)[a] | 37 (19)[a] | 81 (51)[b] | 134 (27) |
| Non-colony | 123 (89)[a] | 159 (81)[a] | 79 (49)[b] | 361 (73) |
| Milking system type [n (%)] | | | | |
| Automated milking system | 40 (29)[a] | 59 (30)[a] | 42 (26)[a] | 141 (29) |
| Conventional milking system | 99 (71)[a] | 137 (70)[a] | 118 (74)[a] | 354 (72) |

[a–c]Within a row, proportion or medians without a common superscript differ ($P < 0.05$).

[1]Herd size estimated using annual milk delivered by each farm and Alberta Milk's official statistics on average herd size and total Alberta milk production to aid interpretation.

## DISCUSSION

This study, using ELISA on BTM, provided estimates on herd-level prevalence of 3 important infectious diseases (BLV, *S.* Dublin and *N. caninum*) in Alberta dairy herds. As expected, most dairy herds in Alberta were BLV-positive, but unexpectedly, a substantial number of herds were also positive for *S.* Dublin. The prevalence of herds positive for *N. caninum* was also relatively high. The study also identified important spatial patterns and association of these infections with herd characteristics.

Analysis of BTM samples is a valuable tool for monitoring health status of dairy herds. BTM testing is a convenient, cost-effective and easy to implement strategy, often used in disease surveillance programs (Andersen et al., 2003; Ågren et al., 2018). This non-invasive method can provide valuable information regarding the herd-level prevalence of infectious agents in dairy cattle, enabling early detection plus implementation and evaluation of disease control measures and programs (Nobrega et al., 2023a; b). In addition, BTM samples can also provide a representative sample of dairy herds, allowing for a more comprehensive assessment of disease prevalence compared with individual-animal testing. BTM testing has been successfully used to monitor progress of several disease control programs, including brucellosis control in various countries; paratuberculosis, *Strep. agalactiae*, and *S.* Dublin control programs in Denmark; and a *Mycoplasma bovis* eradication program in New Zealand (Nobrega et al., 2023a).

However, BTM testing could have lower sensitivity compared with individual animal testing due to dilution, especially when within-herd prevalence is low (Nekouei et al., 2015a; Soltau et al., 2017; Nobrega et al., 2023a; b). Another limitation is that BTM does not accurately reflect status of nonlactating animals, i.e., youngstock, bulls and dry cows (Veling et al., 2002; Nielsen, 2013; Petersen et al., 2016). Therefore, the prevalence of infections like *S.* Dublin that primarily affect young stock may be underestimated using BTM testing approach. For example, 25% herds in Ontario had at least 1 positive animal out of 20 animals sampled; that was substantially higher compared with only 4% farms positive in BTM in the same study (Perry et al., 2023). A serial cross-sectional design using repeated BTM testing, as employed in this study, could capture the status of dry cattle and pregnant heifers in subsequent time point testing.

Prevalence studies (cross-sectional or longitudinal) are common in veterinary epidemiology and have value for answering important research questions related to distribution of diseases in a given population (Dohoo et al., 2010c). Often, the outcome is binary (presence or absence of a disease) and prevalence in 1 group is compared with that in another group. Prevalence ratio, which is a ratio

**Table 2.** Alberta herds positive at least once for antibodies against bovine leukemia virus, *Salmonella* Dublin, and *Neospora caninum* across different levels of herd characteristics

| | | No. (%) of antibody positive herds | | |
|---|---|---|---|---|
| | No. (%) herds | BLV | *S.* Dublin | *N. caninum* |
| Region | | | | |
| North Alberta | 139 (28) | 117 (84.2) | 20 (14.4) | 56 (40.3) |
| Central Alberta | 196 (40) | 188 (95.9) | 22 (11.2) | 49 (25.0) |
| South Alberta | 160 (32) | 149 (93.1) | 35 (21.9) | 34 (21.3) |
| Herd size | | | | |
| Small (≤3,600 L/day milk delivered; ~125 cows[1]) | 246 (50) | 219 (89.0) | 32 (13.0) | 83 (33.7) |
| Medium (3,600 – 7200 L/day milk delivered; ~126 – 250 cows[1]) | 172 (35) | 160 (93.0) | 27 (15.7) | 43 (25.0) |
| Large (>7,200 L/day milk delivered; ~ > 250 cows[1]) | 77 (15) | 75 (97.4) | 18 (23.4) | 13 (16.9) |
| Herd type | | | | |
| Non-colony | 361 (73) | 322 (89.2) | 64 (17.7) | 113 (31.3) |
| Hutterite colony | 134 (27) | 132 (98.5) | 13 (9.7) | 26 (19.4) |
| Milking system | | | | |
| Conventional milking system | 354 (72) | 324 (91.5) | 60 (16.9) | 110 (31.1) |
| Automated milking system | 141 (29) | 130 (92.2) | 17 (12.1) | 29 (20.6) |

[1]Approximate adult cows number estimated to ease interpretation of size categories using annual milk delivered by each farm and Alberta Milk's official statistics on average herd size and total Alberta milk production.

between prevalence in 1 group and prevalence in another group, is a suitable measure of association in this type of study design due to its ease of interpretation (Zocchetti et al., 1997; Grimes and Schulz, 2008; Gnardellis et al., 2022). The PR is analogous to the relative risk or risk ratio having the same mathematical formula, but it has a different epidemiological context as it cannot be used in cohort studies or clinical trials (Holcomb et al., 2001; Gnardellis et al., 2022). In prevalence studies, especially when the disease is not rare (>10%), use of PRs should be preferred over commonly used odds ratios obtained from logistic regression to avoid inflated odds ratios and the associated risk of misinterpretation (Holcomb et al., 2001; Behrens et al., 2004; Ospina et al., 2012).

Inferential statistics are useful for sample data whereby estimated sample statistics can be inferred using confidence intervals and *P*-values to the underlying population to predict population parameters. However, when whole population data are available, i.e., census data, use of inferential statistics may not be needed as the true population parameters can directly be computed from the population data, and a finite population correction factor is used to adjust variance estimator for descriptive statistics but not for analytical purposes (Cochran, 1977). Conversely, it can be rightly argued that census data itself acts as a sample, reflecting the population's status at a specific time and representing just one of many possible population states over time (Dohoo et al., 2010d). Thus, it serves as a simple random sample of the broader 'general population'. Consequently, the use of inferential statistics is reasonable to draw inference to the general population especially when goal is analytical (Cochran, 1977; Korn and Graubard, 1999). In this study, data were collected from all active dairy producers in Alberta at 4 different time points representing 4 simple random samples of the general population consisting of all farms at all different times and states. Notably, the population is changing over time as some producers moved out of business and new producers came in within the study period and beyond. Furthermore, this study is based on BTM samples which is a pooled sample from lactating animals in the herd on the specific day of sampling. As composition of lactating herd is changing at a dairy farm with some animals moving out of lactation at drying off and new animals entering the pool after calving, besides usual inflow and outflow of animals from a herd, this leaves an inherent stochasticity in the outcome measurement. Therefore, the design and goals of this study warrants the use of analytical methods enabling to draw inference to the general population.

Although BLV control or eradication programs in Europe (European Commission, 2014) and New Zealand (Voges, 2011, 2012) have been successful, mainly through testing and culling strategies, limited progress has been made in North America. The current findings were similar to other recent studies reporting a high prevalence of BLV in Alberta and in other Canadian provinces (Nekouei et al., 2015a; John et al., 2020). Regardless of using BTM or animal-level samples, Canadian studies have demonstrated that the herd-level prevalence in all provinces except Quebec, consistently ranged from 86 to 97% in the last 15 years (Scott et al., 2006; VanLeeuwen et al., 2006; Nekouei et al., 2015a; b; John et al., 2020; Nobrega et al., 2024). A similar herd-level BLV prevalence of 84 and 94% was reported for the USA using BTM samples and animal-level samples, respectively (APHIS-USDA, 2008; Ladronka et al., 2018). The high prevalence of BLV in North America was attributed to

**Table 3.** Apparent prevalence (% and 95% confidence interval) of herds positive for antibodies in bulk tank milk against bovine leukemia virus, *Salmonella* Dublin, and *Neospora caninum* in Alberta, Canada at four time points

| | December 2021 (n = 489) | April 2022 (n = 487) | July 2022 (n = 487) | October 2022 (n = 480) | Overall Prevalence[l] (n = 495) | Positive four times (n = 474) |
|---|---|---|---|---|---|---|
| Bovine Leukemia virus | | | | | | |
| North Region | 79.6 (71.9 – 85.6)$^{a,i}$ | 80.1 (72.5 – 86.1)$^{a,i}$ | 78.7 (70.9 – 84.8)$^{a,i}$ | 79.1 (71.3 – 85.2)$^{a,i}$ | 84.2 (77.1 – 89.4)$^{i}$ | 73.5 (65.2 – 80.4)$^{i}$ |
| Central Region | 94.3 (90.0 – 96.8)$^{a,ii}$ | 91.7 (86.9 – 94.9)$^{a,ii}$ | 90.6 (85.5 – 94.0)$^{a,ii}$ | 91.1 (86.0 – 94.4)$^{a,ii}$ | 95.9 (92.0 – 98.0)$^{ii}$ | 86.1 (80.3 – 90.4)$^{ii}$ |
| South Region | 91.8 (86.3 – 95.2)$^{a,ii}$ | 92.4 (87.1 – 95.7)$^{a,ii}$ | 89.4 (83.5 – 93.3)$^{a,ii}$ | 88.5 (82.4 – 92.6)$^{a,ii}$ | 93.1 (88.0 – 96.2)$^{ii}$ | 85.8 (79.3 – 90.5)$^{ii}$ |
| Alberta | 89.4 (86.3 – 91.8)$^{a}$ | 88.7 (85.6 – 91.2)$^{a}$ | 86.9 (83.5 – 89.6)$^{a}$ | 86.9 (83.5 – 89.6)$^{a}$ | 91.7 (88.9 – 93.8) | 82.5 (78.8 – 85.7) |
| *Salmonella* Dublin | | | | | | |
| North Region | 10.9 (6.7 – 17.4)$^{a,i,iii}$ | 8.8 (5.1– 15.0)$^{a,i}$ | 8.1 (4.5 – 14.1)$^{a,i}$ | 6.0 (3.0 – 11.6)$^{a,i}$ | 14.4 (9.4 – 21.3)$^{i,iii}$ | 5.3 (2.5 – 10.8)$^{i}$ |
| Central Region | 7.7 (4.7 – 12.5)$^{a,i}$ | 5.2 (2.8 – 9.4)$^{a,ii}$ | 6.3 (3.6 – 10.8)$^{a,i}$ | 5.3 (2.8 – 9.5)$^{a,i}$ | 11.2 (7.5 – 16.5)$^{i}$ | 2.1 (0.8 – 5.6)$^{i}$ |
| South Region | 15.8 (10.9 – 22.4)$^{a,ii}$ | 6.3 (3.4 – 11.4)$^{b,ii}$ | 11.9 (7.7 – 17.9)$^{ab,i}$ | 14.7 (10.0 – 21.3)$^{a,ii}$ | 21.9 (16.1 – 29.0)$^{ii}$ | 3.9 (1.7 – 8.1)$^{i}$ |
| Alberta | 11.2 (8.7 – 14.4)$^{a}$ | 6.6 (4.7 – 9.2)$^{b}$ | 8.6 (6.4 – 11.5)$^{ab}$ | 8.5 (6.3 – 11.4)$^{ab}$ | 15.6 (12.6 – 19.0) | 3.6 (2.2 – 5.7) |
| *Neospora caninum* | | | | | | |
| North Region | 30.7 (23.5 – 38.9)$^{a,i}$ | 15.4 (10.3 – 22.6)$^{b,i}$ | 14.0 (9.1 – 20.9)$^{b,i}$ | 25.4 (18.7 – 33.5)$^{a,i}$ | 40.3 (32.4 – 48.7)$^{i}$ | 8.3 (4.6 – 14.5)$^{i}$ |
| Central Region | 13.9 (9.7 – 19.6)$^{a,ii}$ | 4.7 (2.4 – 8.7)$^{b,ii}$ | 6.3 (3.6 – 10.8)$^{b,ii}$ | 12.1 (8.2 – 17.6)$^{a,ii}$ | 25.0 (19.4 – 31.6)$^{ii}$ | 1.1 (0.3 – 4.2)$^{ii}$ |
| South Region | 12.7 (8.3 – 18.9)$^{a,ii}$ | 3.8 (1.7 – 8.2)$^{b,ii}$ | 4.3 (2.1 – 8.9)$^{bc,ii}$ | 9.6 (5.9 – 15.4)$^{ac,ii}$ | 21.3 (15.6 – 28.3)$^{ii}$ | 0.6 (0.1 – 4.5)$^{ii}$ |
| Alberta | 18.2 (15.0 – 21.9)$^{a}$ | 7.4 (5.4 – 10.1)$^{b}$ | 7.8 (5.7 – 10.6)$^{b}$ | 15.0 (12.1 – 18.5)$^{a}$ | 28.1 (24.3 – 32.2) | 3.0 (1.8 – 4.9) |

[a–c]Within a row, proportions without a common superscript differ ($P < 0.05$).

[i–iii]Within a column, proportions for same infection without a common superscript differ ($P < 0.05$).

[l]Overall prevalence refers to the proportion of farms that tested positive at least once in 4 time point tests.

a lack of effective control over the last decades, despite several voluntary BLV control programs (Brunner et al., 1997; Canadian Food Inspection Agency, 2021). One of the main reasons is that the culling strategy is not feasible in North America due to high within-herd prevalence ~40% (Nekouei et al., 2015b; Ladronka et al., 2018; Kuczewski et al., 2019). Alternative approaches including segregation of infected animals, culling based on high proviral load, and adopting best management practices may be a more practical option in the North American context (Kuczewski et al., 2021; Shrestha et al., 2024a; b). Moreover, genetic selection for BLV-resistant cattle may accelerate BLV control as bovine leukocyte antigen (BoLA) class II haplotypes are known to modulate high proviral load development (Juliarena et al., 2008; Kuczewski et al., 2021).

In our study, prevalence of BLV-positive herds was apparently high in south region of Alberta, however, the effect was statistically non-significant. This was because the association between region and BLV positivity was confounded by herd type as Hutterite colony herds are more concentrated in south region and these herds were also more frequently BLV positive than non-colony herds. "Hutterites are a German speaking religious brotherhood…" (Evans 2019) and an important pillar of agriculture in Western Canada including dairy production, producing 21% of Alberta's milk. The dairy farms at Hutterite colonies are similar to other dairy farms with one distinct difference, i.e., most Hutterite colonies use natural breeding, either fully or partially (Colazo and Whittaker, 2016; Joseph, 2022) which is an important risk factor for BLV transmission (Erskine et al., 2012; Mekata et al., 2015; Kuczewski et al., 2021). However, little is known regarding purchase behavior and general herd management on Hutterite colony herds, which makes inferences challenging.

Larger herds were more likely to be BLV-positive compared with smaller herds, which can be interpreted as either an increased risk for large farms to attract BLV, or a challenge of large herds to eradicate BLV. Effects of herd size on BLV positivity has been demonstrated in Denmark, the USA and Turkey (Gottschau et al., 1990; APHIS-USDA, 2008; Şevik et al., 2015; Sun et al., 2015) whereas others did not report any difference with herd size (Sargeant et al., 1997; Murakami et al., 2011; Nekouei et al., 2015b). Large farms may frequently buy replacement animals or expand their herds, and generally these animals are not tested for BLV before introduction into the herd.

Unexpectedly, herd-level prevalence of *S.* Dublin antibodies in Alberta dairy herds was relatively high (15.5%). *S.* Dublin is an emerging pathogen of concern for the Canadian dairy industry and several provinces have started surveillance for it. The proportion of farms

**Table 4.** Prevalence ratios (PR) and 95% Confidence Intervals (CI) for herd characteristics associated with a positive bulk tank milk sample for antibodies against Bovine Leukemia Virus (BLV) and *Neospora caninum* in all sampled herds (n = 495) using mixed effect modified Poisson regression models

| | | | BLV | | *N. caninum* | |
|---|---|---|---|---|---|---|
| Variable | Herds No. (%) | Samples No. (%) | Prevalence Ratio (95% CI) | *P*-value | Prevalence Ratio (95% CI) | *P*-value |
| Region | | | | | | |
| North | 139 (28) | 543 (28) | Reference | | | |
| Central | 196 (40) | 768 (39) | 1.13 (1.04 – 1.23) | 0.004 | 0.52 (0.35 – 0.77) | 0.001 |
| South | 160 (32) | 632 (33) | 1.08 (0.98 – 1.19) | 0.102 | 0.46 (0.29 – 0.73) | 0.001 |
| Herd Size | | | | | | |
| Small (≤3,600 L/day) | 246 (50) | 956 (49) | Reference | | | |
| Medium (3,600 – 7,200 L/day) | 172 (35) | 679 (35) | 1.06 (0.99 – 1.13) | 0.112 | 0.66 (0.46 – 0.96) | 0.030 |
| Large (>7200 L/day) | 77 (15) | 308 (16) | 1.11 (1.03 – 1.20) | 0.004 | 0.32 (0.16 – 0.62) | 0.001 |
| Herd Type | | | | | | |
| Non-colony farms | 361 (73) | 1411 (73) | Reference | | | |
| Hutterite colony farms | 134 (27) | 532 (27) | 1.13 (1.07 – 1.20) | <0.001 | 0.47 (0.29 – 0.76) | 0.002 |
| Milking System | | | | | | |
| Conventional | 354 (72) | 1388 (71) | Reference | | | |
| Automated milking system | 141 (29) | 555 (29) | 0.99 (0.93 – 1.06) | 0.782 | 0.62 (0.41 – 0.93) | 0.022 |

positive for *S.* Dublin antibodies reported in this study is consistent with *S.* Dublin apparent prevalence using BTM samples reported in Ontario (4%; Perry et al., 2023, and 7.5%; Nobrega et al., 2024), British Columbia (28%; Personnel communication; BC Animal Health Centre, 2023), and Quebec (15.5 and 9.6% in farms with and without a history of *S.* Dublin, respectively; Um et al., 2022). However, differences in sampling strategies may have affected the low prevalence in Ontario and Quebec as 2 studies (Um et al., 2022; Perry et al., 2023) used a convenience sample out of total farms and collected only 2 BTM samples per farm 4–6 mo apart, while the third study used single sample from all Ontario herds (Nobrega et al., 2024) whereas the current study, similar to British Columbia, used a serial cross-sectional design with 4 rounds of BTM samples 3–4 mo apart from all farms in the province, which likely increased sensitivity. The *S.* Dublin prevalence ranged from 6.6 to 11.2% across time points in this study; hence it seemed likely to miss some farms if only 1 or 2 samples were collected instead of 4 BTM samples, as 9 farms sampled only once or twice tested negative. Notably, we used

**Table 5.** Prevalence ratios (PR) and 95% Confidence Intervals (CI) for farm characteristics associated with a positive bulk tank milk sample for antibodies against *Salmonella* Dublin in all sampled herds (n = 495) using mixed effect modified Poisson regression model

| | No. (%) | | *Salmonella* Dublin | |
|---|---|---|---|---|
| Variable | Herds | Samples | Prevalence Ratio (95% CI) | *P*-value |
| Region | | | | |
| North | 139 (28) | 543 (28) | | |
| Central | 196 (40) | 768 (39) | 0.60 (0.27 – 1.32) | 0.208 |
| South | 160 (32) | 632 (33) | 2.56 (1.23 – 5.32) | 0.012 |
| Herd Size | | | | |
| Hutterite Colony Herds | 134 (27) | 532 (27) | | |
| Small (≤3,600 L/day) | 81 (60) | 322 (61) | Reference | |
| Medium + large (>3,600 L/day) | 53 (40) | 210 (39) | 0.25 (0.05 – 1.16) | 0.077 |
| Non-colony Herds | 361 (73) | 1411 (73) | | |
| Small (≤3,600 L/day) | 165 (46) | 634 (45) | Reference | |
| Medium + large (>3,600 L/day) | 196 (54) | 777 (55) | 2.20 (1.14 – 4.26) | 0.019 |
| Herd Type | | | | |
| Small Herds | 246 (50) | 956 (49) | | |
| Non-colony herds | 165 (67) | 660 (67) | Reference | |
| Hutterite colony herds | 81 (33) | 324 (33) | 0.62 (0.23 – 1.68) | 0.347 |
| Medium + Large Herds | 249 (50) | 987 (51) | | |
| Non-colony herds | 196 (79) | 783 (79) | Reference | |
| Hutterite colony herds | 53 (21) | 212 (21) | 0.07 (0.02 – 0.27) | <0.001 |
| Milking System | | | | |
| Conventional | 354 (72) | 1388 (71) | Reference | |
| Automated milking system | 141 (28) | 555 (29) | 0.64 (0.32 – 1.26) | 0.197 |

manufacturer's recommended cut-off value (PP% ≥ 35) for a sample to be positive similar to other studies in Canada (BC Animal Health Centre, 2023; Perry et al., 2023). However, Um et al. (2022) used a lower cut-off value (PP% ≥ 15) in line with the cut-off value used by the Quebec provincial authorities to enhance sensitivity for the surveillance of this important zoonotic pathogen. If we were to use the lowered cut-off values in Alberta, overall apparent prevalence would increase from 15.5 to 28.3% whereas 10.5 versus 3.6% of herds would have been consistently positive. Using an ELISA test on BTM samples with cut-offs of ≥15 and ≥35, sensitivities were 40.6 and 16.3%, respectively and specificity was 91.9 and 97.5% (Um et al., 2022). It is also important to note that some cross-reactivity with non-Dublin *Salmonella* (such as *S.* Typhimurium) having O-antigen factors 1, 9 and 12 may occur with using this ELISA kit. Overall, herd-level *S.* Dublin prevalence in Alberta and the rest of Canada seemed lower than estimates from the UK (40%, Henderson et al., 2022) but higher than from New York State, USA (0.94%; Cummings et al., 2018).

Herds in the south of Alberta were more frequently positive for *S.* Dublin antibodies compared with the north. These findings were consistent with British Columbia (BC Animal Health Centre, 2023) where southern regions of Fraser valley (36%) and Creston valley (38%) had a higher prevalence compared with central regions of North Okanagan (19%) and north region of Bulkley valley (5%). Similarly, *S.* Dublin prevalence was higher in southwestern Ontario (Nobrega et al., 2024). These provinces border with the USA on the south and cross-border movement of animals and animal importation may be potential reasons to be explored for a higher prevalence in south regions. However, a lower *S.* Dublin prevalence (<1%) in New York State (Cummings et al., 2018) bordering with Ontario contradicts this argument; however, this study is based on a single round of BTM samples collected in 2013 and current estimates are likely to be higher in New York State as well. There are no prevalence estimates on *S.* Dublin available on northern states of the USA bordering with western Canadian provinces. Additionally, there may be other associated factors for a high *S.* Dublin prevalence in South Alberta region including climate, soil type or a higher concentration of beef and cow-calf operations in southern Alberta (Dimmell, 2021), which we did not explore in our study, and may contribute to the higher *S.* Dublin prevalence.

The higher *S.* Dublin prevalence in large versus small farms was in line with previous studies (Davison et al., 2006; Nielsen et al., 2007b; Nielsen and Dohoo, 2012, 2013) although others have not reported this association and they argue that some management practices on larger farms, e.g., calving in group pens and more animals per calving pen, might be associated with *S.* Dublin spread and increased prevalence on large farms (Ågren et al., 2017; Perry et al., 2023). In our study, a low prevalence was noted among Hutterite colony herds compared with non-Hutterite herds. This may again be associated with herd management practices at colony farms versus non-colony farms, which should be explored.

Twenty-eight percent of Alberta dairy herds were *N. caninum* antibodies positive. A study using animal level samples from 77 dairy herds (clients of veterinarians participating in a Johne's disease control program) in Alberta identified that all except 1, i.e., 99% herds had at least 1 antibody-positive animal (Scott et al., 2006). However, this study did not include a random sample. Also, they used individual-animal samples instead of BTM, which increases sensitivity. In Manitoba, Canada, 24 (60%) of the 40 dairy farms, included in the study, had at least 1 *N. caninum* seropositive animal, of which 15 (37%) had at least 2 positive animals (VanLeeuwen et al., 2006). Herd-level prevalence using BTM was 6.4% in 2004 and 10% in 2005, in Prince Edward Island, Canada (Wapenaar et al., 2007). Our study estimated comparatively higher prevalence than Prince Edward Island, consistent, with a study based on individual-animal samples (VanLeeuwen et al., 2010). In other countries, BTM-based prevalence of *N. caninum* positive herds ranged from 0.7% in Norway (Klevar et al., 2010) to 19% in Ireland (O'Doherty et al., 2013), 55% in Italy (Varcasia et al., 2006), and 60% in Spain (González-Warleta et al., 2011).

A threshold for *N. caninum* within-herd seroprevalence of ≥ 15% was estimated for a BTM to be positive using a cut-off value of ≥0.60 (Bartels et al., 2005; Wapenaar et al., 2007). Conversely, prevalence estimated in our study could have been underestimated where negative herds may still have a lower within-herd prevalence. In lieu of this, we used the standard manufacturer recommended individual sample cut-off value of ≥ 0.50 as used by others (Nasir et al., 2012), which improved sensitivity to 73 from 61% (while using a cut-off ≥ 0.60) at the expense of slightly lower specificity (89 vs. 92% using a cut-off ≥ 0.60) (Bartels et al., 2005). If we were to use the cut-off ≥ 0.60, the overall prevalence would be reduced to 20.5 from 27.9% and only 1.2% farms would be positive all 4 times instead of 3.0%. The variation of prevalence between time points in our study can be attributed to the changes in herd characteristics contributing to the bulk tank from 1 time point to the next as variation in antibodies against *N. caninum* is associated with milk production, stage of lactation, parity, etc. (Schares et al., 2004; Haddad et al., 2005; Bartels et al., 2007).

Similar to Scott et al. (2006), north Alberta had a higher prevalence of *N. caninum* positive herds compared with the central and southern regions in this study. In the absence of farm management data, we hypothesize that exposure of cattle to wild canids could be higher in

farms in the north than other regions (VanLeeuwen et al., 2010). North Alberta has more forest lands that support larger population of wild canids (coyotes, foxes etc.), increasing the likelihood of contact between cattle and wild canids (Scott et al., 2006).

Also, smaller farms had were more frequently positive compared with large farms, in line with a previous Canadian study (VanLeeuwen et al., 2010). An explanation to this association might be housing and management practices in small versus large farms. Large farms tend to have more free-stall than tie-stall housing and such farms are more likely to keep their animals indoors in summer (VanLeeuwen et al., 2010) leading to lower exposure to dogs or wild canids; however, Alberta does not have higher number of tie-stall farms, therefore, this perspective may not be valid in Alberta context. It can be hypothesized that dogs may have a higher likelihood to be in contact with aborted fetuses, carcasses, placenta, and uterine discharges in smaller versus larger farms (Corbellini et al., 2006). Moreover, larger farms may also benefit due to a higher average production and potential dilution effect (Bartels et al., 2007). Our study also indicated that Hutterite colony herds had a lower prevalence compared with non-Hutterite farms. This may have been due to herd management practices and potentially less exposure of animals with canids at colony farms that were not explored in this study.

This study was conducted using BTM samples from all Alberta dairy herds through Alberta Milk. The authors had no direct access to the farms, hence no farm-level data including management practices were obtained. Therefore, other potential factors associated with prevalence could not be identified. Understanding management practices and other associated factors in different regions and farm types (Hutterite colony herds vs. non-colony herds) are important for future studies. A more focused study using a subset of Alberta dairy herds based on random sampling method may be sufficient to identify risk factors. Furthermore, the ELISAs used in this study are not perfect and therefore a true prevalence may vary from the apparent prevalence reported in this study when test imperfections are taken into account. The estimation of true prevalence is challenging using traditionally used Rogan and Gladen method to account for test imperfections especially when estimated apparent prevalence is less than 1 – Specificity such as in case of *N. caninum* in this study. Moreover, test characteristics of BLV ELISA kit for BTM analysis are currently not reported. Alternatively, Bayesian latent class models (BLCM) could be utilized to estimate true prevalence for such imperfect scenarios, however, such models are not readily available for implementation especially considering repeated bulk tank milk testing as employed in this serial cross-sectional study. Therefore, to be consistent for all infections reported in this study, we decided to report apparent prevalence in this study while a future recommendation would be utilizing BLCM methods to estimate true prevalence of these infections using repeated quarterly BTM analysis. Finally, our study was limited to only the dairy herds in Alberta; however, given that Alberta has a substantial proportion (44%) of Canada's beef cattle, including cow-calf operations, exploring the burden of infections such as *S.* Dublin and *N. caninum* in these production types could yield important insights and implications for animal health in both the dairy and beef industries.

Up-to-date prevalence estimates generated in this study will be imperative in guiding future research, extension, and policy decisions to improve animal health and safeguard public health not only in western Canada but also in other regions of the world, especially North America. Moreover, similar surveillance programs using BTM can be initiated in other countries where these infections are deemed to be endemic. Finally, data analysis approach employed in this study can be applied in similar prevalence studies.

## CONCLUSIONS

Most herds in Alberta, Canada, were BLV-antibody positive and a substantial proportion of herds were *S.* Dublin and/or *N. caninum*-antibody positive. BLV and *S.* Dublin prevalence was higher in central and south Alberta, respectively, compared with northern Alberta, whereas prevalence of *N. caninum* positive herds was higher in the northern region compared with central and south regions. Similarly, BLV prevalence was higher on Hutterite colony farms whereas *S.* Dublin and *N. caninum* prevalence was higher on non-Hutterite farms. Large herds were more frequently positive for BLV and *S.* Dublin and less frequently positive for *N. caninum* compared with small herds. Moreover, *N. caninum* was higher on farms with conventional milking systems than farms with automated milking system. These results provide up-to-date information of the disease frequency that will inform investigations of within-herd prevalence of these infections and help in devising evidence-based disease control and eradication programs, not only in Alberta but also in other regions of North America and beyond.

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