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Predicting within-herd prevalence of infection with bovine leukemia virus using bulk-tank milk antibody levels

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ABSTRACT

Enzootic bovine leukosis (EBL) is an economically important infection of dairy cattle caused by bovine leukemia virus (BLV). Estimating the prevalence of BLV within dairy herds is a fundamental step towards pursuing efficient control programs. The objectives of this study were: (1) to determine the prevalence of BLV infection at the herd level using a bulk-tank milk (BTM) antibody ELISA in the Maritime region of Canada (3 provinces); and (2) to develop appropriate statistical models for predicting within-herd prevalence of BLV infection using BTM antibody ELISA titers.

During 2013, three monthly BTM samples were collected from all dairy farms in the Maritime region of Canada ($n = 623$) and tested for BLV milk antibodies using a commercial indirect ELISA. Based on the mean of the 3 BTM titers, 15 strata of herds (5 per province) were defined. From each stratum, 6 herds were randomly selected for a total of 90 farms. Within every selected herd, an additional BTM sample was taken (round 4), approximately 2 months after the third round. On the same day of BTM sampling, all cows that contributed milk to the fourth BTM sample were individually tested for BLV milk antibodies ($n = 6111$) to estimate the true within-herd prevalence for the 90 herds. The association between true within-herd prevalence of BLV and means of various combinations of the BTM titers was assessed using linear regression models, adjusting for the stratified random sampling design.

Herd level prevalence of BLV in the region was 90.8%. In the individual testing, 30.4% of cows were positive. True within-herd prevalences ranged from 0 to 94%. All linear regression models were able to predict the true within-herd prevalence of BLV reasonably well ($R^2 > 0.69$). Predictions from the models were particularly accurate for low-to-medium spectrums of the BTM titers. In general, as a greater number of the four repeated BTM titers were incorporated in the models, narrower confidence intervals around the prediction lines were achieved. The model including all 4 BTM tests as the predictor had the best fit, although the models using 2 and 3 BTM tests provided similar results to 4 repeated tests. Therefore, testing two or three BTM samples with approximately two-month intervals would provide relatively precise estimates for the potential number of infected cows in a herd. The developed models in this study could be applied to control and eradication programs for BLV as cost-effective tools.

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1. Introduction

Enzootic bovine leukosis (EBL) is an important infection of dairy cattle worldwide which is caused by bovine leukemia virus (BLV). The virus is transmitted through infected blood lymphocytes (Gillet et al., 2013). Premature culling, death, and condemnation of carcasses at slaughter due to lymphoma, impaired immune function, as well as restrictions on international trade of infected cattle and their products are among the most significant economic losses attributed to the disease (Sandev et al., 2000; Bartlett et al., 2014).

Many European countries, including the UK, France, Germany, Spain, the Scandinavian countries, Belgium, and the Netherlands, are officially free from EBL (Annual EU report, 2013). Some other countries, such as Japan, the United States, and Argentina, have actively been working on addressing their BLV problems in recent years in order to develop cost-effective programs for their dairy industries (Ott et al., 2003; Monti et al., 2007; Murakami, 2009; Rodríguez et al., 2011).

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In different provinces of Canada, there have been a number of serological studies which have estimated prevalence and impact of BLV infection. In 1980, the national prevalence of BLV infection in Canadian dairy herds was estimated at 40.5%, while only 9.3% of tested cattle were positive (Samagh and Kellar, 1982). However, 15–20 years later, infection levels appeared to have substantially increased. Sargeant et al. (1997) indicated 69.6% of the 102 tested dairy herds, and 23% of the 1330 tested cows in Ontario were positive to BLV. VanLeeuwen et al. (2001) reported that 70% of herds in the Maritime region of Canada (including provinces of Prince Edward Island (PE), New Brunswick (NB), and Nova Scotia (NS)) had at least one infected cow, while the prevalence of infection at the cow level was estimated at 20.8%. Similar studies have revealed a high prevalence of BLV infection across the country (VanLeeuwen et al., 2005, 2006; Scott et al., 2006). Nevertheless, there is still no broad-based program for controlling EBL in Canada.

Control of EBL at the national level usually consists of one or more of the following approaches: management interventions; test and segregation; and test and slaughter. Selection and success of each strategy are heavily dependent on having a reliable estimate of the within-herd prevalence (Bartlett et al., 2014). Attaining a reasonably valid estimate of the within-herd prevalence would be a fundamental step towards pursuing efficient control and eradication programs for BLV in every dairy herd. Individual serum or milk sampling from all cows on a dairy farm would provide an accurate measure of BLV infection prevalence; however, it would demand a great deal of time, labour, and cost. Therefore, in order to motivate farmers and veterinarians to maximum participation in future comprehensive control programs, a cost-effective screening or monitoring tool for BLV at the herd level would be desirable.

Using bulk-tank milk (BTM) samples, collected by the dairy herd improvement (DHI) companies, has become one of the most convenient and economically efficient mechanisms for screening for important infectious diseases in dairy cattle (Houe et al., 1995; Attalla et al., 2010; Sorge et al., 2011). For instance, BTM ELISA has frequently been applied to surveillance of EBL, Johne's disease, and bovine viral diarrhea (Niskanen, 1993; Bitsch and Ronsholt, 1995; Reber et al., 2012; Nielsen and Toft, 2014). Once cattle become infected with BLV, they remain infected for life and generate a continuous antibody response. This characteristic adds to the credibility of antibody-based diagnostic techniques for BLV (Radostits et al., 2006; Monti et al., 2007). Among the available commercial tests for detection of antibodies against BLV, milk ELISA is a desirable method in large-scale herd surveillance, which has often been used for classification of herds as infected or non-infected (Erskine et al., 2012). However, there has been no evaluation of the predictive ability of BTM ELISA tests for within-herd prevalence of BLV.

The objectives of this study were: (1) to determine the prevalence of BLV infection at the herd level using a BTM antibody ELISA in the Maritime region of Canada; and (2) to develop applied statistical models for predicting within-herd prevalence of BLV infection using BTM antibody ELISA titers.

2. Materials and methods

2.1. Herd selection for determining herd level prevalence, and sample collection (a census)

All dairy farms in the Maritime region of Canada ($n = 644$, in 2013) were the target and source populations for the herd level prevalence part of the study. Permission was granted from the governing bodies of dairy producers of the three Maritime provinces to obtain BTM samples collected by these bodies for regulatory purposes in order to conduct our study. Therefore, all dairy farms in the Maritime region were the study population.

During 2013, three bulk-tank milk samples (30 ml each), taken at one-month intervals, were obtained by the milk truck drivers on their routine milk pick-ups from the Maritime dairy farms. The drivers followed the standard procedures used for collection of samples for regulatory and payment purposes and, therefore, samples were well mixed.

The milk samples were kept at 4 °C until BLV laboratory testing commenced. All samples were tested for BLV antibodies using an indirect ELISA test (see Section 2.3). The test results were reported as percent positivity (PP), and the cut-off value for a positive result on the test kit for pooled (bulk tank) milk samples was ≥5, according to the manufacturer's specifications.

2.2. Herd and animal selection for predicting within-herd prevalence, and sample collection

From the study herds in the census, a subset of herds ($n = 90$) was selected to evaluate the association between the BTM titers and within-herd prevalence of BLV infection. Two steps were taken to create pools of eligible farms with a broad spectrum of within-herd prevalences for the stratified random sampling strategy used in this part of the study: (1) creation of five pools (strata) per province of increasing BTM-based herd level prevalence; and (2) identification of farms subscribing to monthly DHI testing.

For step 1, the arithmetic mean of the three monthly ELISA titers for each farm was calculated. Based on the test cut-off, and the distribution of the test means (Fig. 1), all study farms were assigned into one of five strata within each of the three study provinces (a total of 15 strata), as follows:

- Potentially uninfected or very low-prevalence farms (mean < 5)
- Expected low-prevalence farms (5 ≤ mean < 40)
- Expected medium-prevalence farms (40 ≤ mean < 55)
- Expected high-prevalence farms (55 ≤ mean < 70)
- Expected very high-prevalence farms (70 ≤ mean)

The reason for this stratification was to ensure that we would get BTM percent positivity titers across the range of possible ELISA test values (e.g., 0–100). This will lead to the most precise and representative estimates for the coefficients in our final regression models.

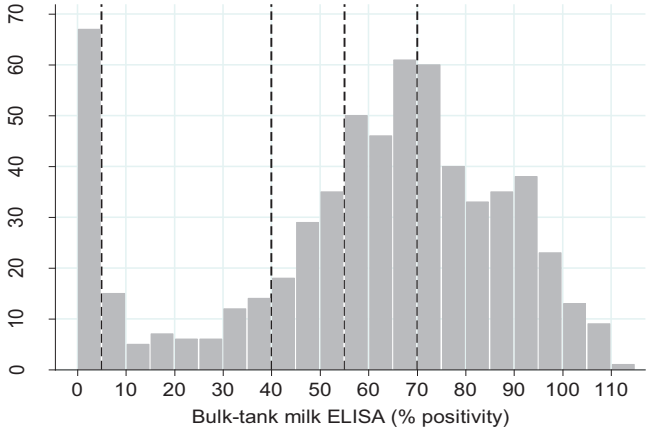

Fig. 1. Frequency distribution of the mean of three monthly bulk-tank milk ELISA results (percent positivity) for infection with bovine leukemia virus in 623 dairy farms from the Maritime region of Canada (2013). Four dashed lines indicate the cut-points for categorizing the herds into the five prevalence-level groups. ELISA values > 100 could represent the herds with expected very high prevalence of infection.

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