Preventive Veterinary Medicine 133 (2016) 1–9

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

# Lifetime effects of infection with bovine leukemia virus on longevity and milk production of dairy cows

Omid Nekouei[a,*], John VanLeeuwen[b], Henrik Stryhn[b], David Kelton[c], Greg Keefe[b]

[a] Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB T2N 4Z6, Canada
[b] Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE C1A 4P3, Canada
[c] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada

ARTICLE INFO

*Article history:*
Received 25 January 2016
Received in revised form 26 June 2016
Accepted 12 September 2016

*Keywords:*
Bovine leukosis
Lifetime impacts
Longevity
Milk yield
Canada

ABSTRACT

Enzootic bovine leukosis (EBL) is an economically important disease of dairy cattle caused by bovine leukemia virus (BLV). The economic impacts of the infection have been debated in the literature. The present study was conducted to determine the lifetime effects of BLV infection on longevity and milk production of dairy cows in Canada.

The data were aggregated from a combination of two data sets: 1) BLV serum-ELISA test results from Canada-wide surveys of production limiting diseases, which took place between 1998 and 2003 in 8 provinces, and 2) longitudinal production data for all cows in the former study, extracted from the Canadian dairy herd improvement database. All participant cows had been culled or died by the onset of this study. A historical cohort study was designed, including cows which tested positive to BLV-antibodies in their first lactation (positive cohort, n = 1858) and cows which tested negative in their second or later lactations (negative cohort, n = 2194). To assess the impacts of infection with BLV on longevity (the number of lifetime lactations), a discrete-time survival analysis was carried out. The effect of BLV on the lifetime milk production (the sum of all life 305-day milk production) was evaluated using a multilevel linear regression model.

Overall, 4052 cows from 348 herds met the eligibility criteria and were enrolled in the study. In the longevity model, the interaction term between time (lactation number) and BLV-status was highly significant. Cows which were positive to BLV had consistently greater probabilities of being culled (or dying) than the test-negative cows. In the milk production model, the interaction term between BLV-status and longevity of the cows was highly significant; indicating that lifetime BLV effects on the total milk production was dependent on the lactation in which the study cows were culled/died. Infected cows with 2 and 3 lactations showed significantly lower life milk productions [−2554 kg (−3609 to −1500) and −1171 kg (−2051 to −292), respectively] compared with their negative counterparts with 2 and 3 lactations. As the cows lived longer (>3 lactations), the differences in life milk production between the two cohorts were no longer significant. Overall, it was predicted that the test-positive cows produced substantially lower milk compared to the test-negative cows throughout their study lifespans. With the high prevalence of BLV in Canadian dairy cows and its detrimental economic impacts, pursuing broad-based control programs in Canada should be evaluated.



## 1. Introduction

Enzootic bovine leukosis (EBL) is an economically important disease of dairy cattle across the world. The causative agent of EBL is a retrovirus, bovine leukemia virus (BLV). The virus is transmitted through infected blood lymphocytes (Gillet et al., 2013). Once cattle become infected with BLV, they remain infected for life and generate a continuous antibody response (Monti et al., 2007; Kobayashi et al., 2010). Clinical signs of the disease are not displayed by most infected cattle. Persistent lymphocytosis (PL) will occur in approximately 30% of infected cattle and fewer than 5% will eventually develop malignant lymphoma (Radostits et al., 2006).

The prevalence of BLV infection in North America has consistently been high over the past years (Bartlett et al., 2014; Nekouei et al., 2015a). For instance, in the Maritime region of Canada, herd-level prevalence of BLV infection was estimated at 70% in 1998

* Corresponding author.
*E-mail address:* onekouei@upei.ca (O. Nekouei).

http://dx.doi.org/10.1016/j.prevetmed.2016.09.011
0167-5877/

(VanLeeuwen et al., 2001) and at 90% in 2013 (Nekouei et al., 2015a). In the United States, as a part of the 2007 national dairy study (APHIS-USDA report, 2008), 83.9% of the tested herds were found to be positive.

Measured at the herd level, the direct production losses from EBL in the Canadian Maritimes were conservatively estimated at $806 per year in an average 50-cow herd (Chi et al., 2002). This did not include costs associated with lost sales of genetically superior purebred cattle which are likely more substantial than the direct production impacts. In another study, economic loss per case of lymphoma was estimated to be $412 (Rhodes et al., 2003). With respect to the economic impacts of BLV infection, some of the losses attributed to the disease (suggested by different literature) are: premature culling, death, and condemnation of carcasses at slaughter due to lymphoma, production loss, lower reproductive efficiency, impaired immune function, as well as trade restrictions imposed on infected cattle and their products (Sandev et al., 2000; Bartlett et al., 2014). However, reports on production and longevity effects of subclinical BLV in dairy cattle have been quite controversial in the literature. A number of studies could not demonstrate any statistically significant association between BLV infection and milk production in dairy cows and herds (Landston et al., 1978; Huber et al., 1981; Brenner et al., 1989; Da et al., 1993; Tiwari et al., 2007; Sorge et al., 2011). In contrast, detrimental effects of BLV infection on production have been documented by others (Emanuelson et al., 1992; Sargeant et al., 1997; D'Angelino et al., 1998; Ott et al., 2003; Erskine et al., 2012; Norby et al., 2016). A few studies failed to find any statistically significant association between BLV infection and the survival of dairy cows (Huber et al., 1981; Tiwari et al., 2005). On the other hand, others have reported negative effects of BLV infection on the longevity of dairy cows (Pollari et al., 1992; Emanuelson et al., 1992; Bartlett et al., 2013).

Some of the potential reasons for obtaining such inconsistent findings were: 1) the cross-sectional nature of the majority of these studies; in some studies, 2) limited numbers of study cows or herds; 3) the application of different methods for data analysis; and 4) the use of production data only in the lactations in which BLV testing was performed (therefore, not accounting for potential seroconversions and progression of the infection pathology). In addition, there has not been any study investigating the lifetime impacts of BLV infection, which could economically be more relevant with regards to the chronic nature and gradual progression of the infection. Therefore, this historical cohort study was conducted to determine the effects of BLV infection on 1) lifetime milk production, and 2) longevity of dairy cows in Canada.

## 2. Materials and methods

In order to assess the lifetime effects of BLV infection on cow longevity and milk production, the following steps were taken: 1) generating a master data set by merging available BLV test results (from a previous study) with longitudinal lifetime production data for the study cows; 2) defining a pool of eligible cows for selecting two comparable cohorts of cows (negative and positive to BLV, in a historical cohort setting); and 3) longitudinally evaluating the two cohorts of cows with respect to the longevity and milk production measures using multilevel mixed- effects regression analyses. The following subsections elaborate on the details of the 3 steps, respectively.

### 2.1. Data collection and management

The master data set used in this study was generated via combining two data sets, the historic BLV test results and the longitudinal production data. The BLV test results were extracted from the Canada-wide surveys of production limiting diseases (Nekouei et al., 2015b) that took place between 1998 and 2003 on randomly selected dairy farms in 8 (out of 10) provinces of Canada (i.e. source population), including Prince Edward Island (PE), New Brunswick (NB), Nova Scotia (NS), Quebec (QC), Ontario (ON), Manitoba (MB), Saskatchewan (SK), and Alberta (AB). One of the objectives of that project was to obtain estimates for the prevalence of infection with BLV in all participant provinces. Herd and animal selections in the surveys were based on a stratified two-stage random sampling procedure. From every randomly selected herd (n = 364), a median of 30 cows (range: 9–45) at different lactations were randomly selected for blood collection. The blood samples were tested for BLV antibodies by a commercial serum-ELISA (IDEXX Corporation, Westbrook, ME, USA; sensitivity = 0.985 and specificity = 0.999). A cow was considered to be infected with BLV if the serum-to-positive ratio was $\geq$0.5, as recommended by the manufacturer of the test kit. Every herd with at least one infected cow was defined as being positive (VanLeeuwen et al., 2001). Complete details on the surveys, including sample size calculation, sample collection, and laboratory procedures have been published elsewhere (Nekouei et al., 2015b).

In 2013, based on the available unique identification numbers for all participant herds and cows in the former broad surveys, production data (including 305-day milk yield (kg), 305-day fat and protein contents of milk (kg), days-in-milk (DIM), average somatic cell count (SCC)) for all lactations of a cow during her lifetime was extracted from the Canadian dairy herd improvement (DHI) database and combined with the BLV test results. All of the cows had been culled or died by the onset of the present study.

### 2.2. Study design

The eligible pool of cows for inclusion in this historical cohort study (i.e. sampling frame) consisted of Holstein cows (96% of all cows in the master data set- to eliminate breed as a potential confounder) with complete longitudinal data for 305-day lactation measurements (from their first lactation to their culling point, with no missing observations; 90% of the Holsteins). From this pool, two cohorts of cows (negative and positive to BLV) were selected as follows:

- Positive cohort: cows that tested positive to BLV in their first lactation. Thus, this group of cows was considered infected for all of their productive life.
- Negative cohort: cows that tested negative to BLV in their second or later lactations (i.e., disregarding the first lactation test-negative cows). Therefore, this group of cows was considered negative from birth to at least the beginning of their second lactation.

Due to the definition of the negative cohort, only cows with 2 lactations or more were included in the study. The reason for applying the outlined inclusion criteria was to reduce two major potential sources of bias in establishing the associations of interest (i.e. potential seroconversions after testing dates in test-negative cows and unknown onset of the infection in test-positive cows before testing dates; related details are presented in the Discussion Section).

### 2.3. Statistical analyses

All of the statistical analyses were carried out in Stata 14 (StataCorp, College Station, TX, USA).