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Economic losses associated with mastitis due to bovine leukemia virus infection

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ABSTRACT

Bovine leukemia virus (BLV), which causes enzootic bovine leukosis and immunosuppression, is widely prevalent on Japanese dairy farms. However, in the absence of a national eradication scheme with compensation programs, it is important to estimate BLV-associated economic losses to raise farmers' awareness. Mastitis (includes both clinical and subclinical) is a common disease in the dairy industry and the most common reason for culling. We hypothesized that immunosuppression due to BLV predisposes subclinical mastitis. A retrospective cohort study was conducted to trace Holstein cows at 9 commercial dairy farms in the Nemuro and Kushiro regions of Hokkaido Prefecture, Japan, where monitoring of BLV proviral load is routine. Information regarding Dairy Herd Improvement data, parity number, and delivery day was collected at each farm. Cows with no confirmed infection with BLV during lactation were defined as non-infected. Low-proviral-load and high-proviral-load (H-PVL) cows were defined as those in which proviral load was below and over 2,465 copies/50 ng of DNA, respectively, or 56,765 copies/10^5 cells, respectively, throughout the lactation period. Survival analysis was performed using the frailty model to estimate the hazard ratio of subclinical mastitis for BLV infection status using data from 1,034 dairy cows after adjusting for parity number and delivery season as confounding factors. Kaplan–Meier survivor curves demonstrated that half of the H-PVL cows developed subclinical mastitis within 52 d after calving. The hazard ratio of subclinical mastitis for H-PVL cows was 2.61 times higher than that of non-infected cows. In 2017, there were 264,443 clinical mastitis cases in Hokkaido. Using field and published data, annual economic losses were estimated using Monte Carlo simulation. The economic loss due to mastitis associated with BLV infection per H-PVL cow was $418.59 (¥43,952), with the annual economic loss in Hokkaido Prefecture due to mastitis caused by BLV infection estimated at $6,097,225 (¥640,208,633). In summary, H-PVL cows were more susceptible to subclinical mastitis than non-infected and low-proviral-load cows, and mastitis due to BLV infection was projected to cause significant economic losses.

Key words: bovine leukemia virus, mastitis, survival analysis, economic loss

INTRODUCTION

Bovine leukemia virus (**BLV**) belongs to the genus *Deltaretrovirus* of the family Retroviridae and is related to several clinically important viruses, such as human T-cell lymphotropic virus type 1 and 2 and simian T-cell lymphotropic virus type 1 and 2 (El Hajj et al., 2012). Bovine leukemia virus causes enzootic bovine leukosis (**EBL**) in cattle, leading to significant economic losses (Rodríguez et al., 2011; Bartlett et al., 2020). Bovine leukemia virus is prevalent worldwide, except in Western Europe (OIE, 2018). Most infections are subclinical, but a proportion of cattle (<30%) over 3 yr old develop persistent lymphocytosis, and less than 5% of infected cattle develop lymphosarcomas in various visceral tissues (EFSA, 2015). Clinical signs and symptoms depend on tumor site and include digestive disturbances, inappetence, weight loss, weakness, general debility, and sometimes neurologic manifestations (OIE, 2018).

The BLV infection has a significant economic impact on the dairy industry due to trade restrictions, replacement costs, reduced milk production, and immunosuppression resulting in increased disease susceptibility (EFSA, 2015; OIE, 2018). Only a few studies have investigated economic losses associated with the effects of BLV infection on dairy production in Asia (Yang et al., 2016). We reported that carcass weight loss of

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dairy cattle due to infection with BLV causes annual economic loss of $1,391,649 in Hokkaido Prefecture, Japan (Nakada et al., 2022).

Mastitis (includes both clinical and subclinical) is the most common clinical disease in US dairy cows (NAHMS, 2014). In the United States, the annual economic loss due to mastitis was estimated at between $1.7 and $2.0 billion (Kvapilík et al., 2015), and that in Canada was 400 million Canadian dollars (Carson et al., 2017). Nutrition, host resistance, environmental conditions, milking equipment, milking technique, and hygiene are associated with the risk of mastitis (Lam et al., 2013). Bovine leukemia virus infection induces abnormal immune function (Frie and Coussens, 2015). A negative association between herd-level milk production and BLV positivity (Ott et al., 2003; Erskine et al., 2012) has been reported. At the individual animal level, 305-d mature equivalent yield (Norby et al., 2016) and milk production in the early and middle stages (Yang et al., 2016) in older cows were associated with BLV positivity. A higher SCS in the early and middle stages in BLV-positive cows than BLV-negative in older cows has been reported (Yang et al., 2016). However, the causality has not been proven.

The objectives of the present study were to estimate the association between BLV infection status and occurrence of subclinical mastitis and extrapolate losses due to mastitis caused by BLV infection in Hokkaido Prefecture, the main dairy production area of Japan.

MATERIALS AND METHODS

Study Design and Field Investigation

A retrospective cohort study involving 9 commercial dairy farms with BLV-infected cows was conducted. These farms, located in the Nemuro and Kushiro regions of Hokkaido Prefecture, Japan, are routinely surveyed once or twice per year to determine the BLV infection of cows based on either detection of anti-BLV antibody or BLV provirus using blood samples under veterinary clinical services. The study was conducted between April 2015 and March 2018. No cows infected with BLV were reported with malignant lymphoma-EBL within the 9 herds during the study period. The study was conducted in accordance with Strengthening the Reporting of Observational Studies in Epidemiology—Veterinary (STROBE-Vet; Sargeant et al., 2016).

Quantification of BLV Proviral Load

Genomic DNA was isolated from whole blood samples using a Wizard Genomic DNA Purification kit (Promega). The proviral load (**PVL**) was measured using one of 2 different real-time quantitative PCR (**qPCR**) assays, namely, the BLV-CoCoMo-qPCR (Riken Genesis) or Cycleave BLV qPCR (Takara) assay. Both qPCR methods have been reported to have high sensitivity (100% for 1.56 provirus per 10^5 peripheral blood mononuclear cells, 3 of 3 samples each; Jimba et al., 2012). The high specificity of BLV-CoCoMo-qPCR, which did not amplify long-terminal repeat of other various retroviruses in experiments, has been reported (Jimba et al., 2010). The field veterinarians in charge decided using either the BLV-CoCoMo-qPCR or Cycleave BLV qPCR assay. Good agreement between the results of these assays was described in our previous study (Nakada et al., 2022). Entire details on the quantification of BLV-PVL both BLV-CoCoMo-qPCR and Cycleave BLV qPCR assay have been published elsewhere (Nakada et al., 2022).

Quantification of BLV-PVL using the BLV-CoCoMo-qPCR assay was performed at the Agricultural Research Department of the Hokkaido Research Organization Animal Research Center, and that using the Cycleave BLV qPCR assay was performed at the Hokkaido Higashi Agriculture Mutual Aid Association clinical laboratory (Nakashibetsu, Japan) or the Research Institute for Animal Science in Biochemistry and Toxicology (Sagamihara, Japan).

The field veterinarians decided on the use of qPCR assays employing either BLV-CoCoMo-qPCR or the Cycleave BLV qPCR, depending on access to the corresponding laboratories.

Classification of BLV-PVL

Bovine leukemia virus-infected cows were classified into the following 2 groups according to PVL: low PVL (**L-PVL**) and high PVL (**H-PVL**). According to our previous research, the L-PVL and H-PVL cut-off thresholds as determined using the BLV-CoCoMo-qPCR and Cycleave BLV qPCR BLV assays were 2,465 copies/50 ng of DNA, and 56,765 copies/10^5 cells, respectively. Complete details on the classification of the level of BLV-PVL have been published elsewhere (Nakada et al., 2022). Low-PVL and H-PVL cows were defined as those in which the PVL was below and above the indicated cut-off thresholds throughout the lactation period, respectively. Based on our previous studies (Nakada et al., 2018, 2022), the cows continuously having H-PVL more than 2 times were considered to have persistent lymphocytosis. Cows in which the BLV-PVL fluctuated with values above and below the cut-off threshold during lactation were excluded from the study. The ELISA-positive cows with PVL below detection limit were categorized into L-PVL.

Data Collection and Management

Herd-level information such as the total number of cows and animal-level information such as breed, parity, and BLV test results and Dairy Herd Improvement (**DHI**) records was collected by field veterinarians in charge of the study farms via interviews with farm owners and checking farm records. Within the lactation period, event (subclinical mastitis) time was defined as the number of days from delivery until the first occurrence of subclinical mastitis. Dates of the first occurrence of subclinical mastitis were collected from DHI records of the 9 herds. Subclinical mastitis was defined as a linear SCC score of ≥5 (283,000 SCC/mL) in the DHI records. The cut-off value, 283,000 SCC/mL, was adapted based on a report of positive predictive value of 80% for dairy herds with 30% prevalence of IMI (Reneau, 1986). A high positive predictive value, 94.4%, has been reported from Belgium recently, by using 250,000 SCC/mL cut-off for IMI (note that positive predictive value is high when a prevalence is low) (Jashari et al., 2016). Cows with missing DHI records during the lactation period were excluded from the study.

Parity was categorized as 1st, 2nd and 3rd, 4th and 5th, or 6th and over. Delivery season was defined as follows: from January to March as winter, April to June as spring, July to September as summer, and October to December as fall. All data were digitized and handled using commercially available spreadsheet software (Excel 2013; Microsoft Corp.).

Statistical Analysis

According to the DHI data across Hokkaido Prefecture in 2017, 15% of milking cows recorded a liner score of ≥5 (Hokkaido Dairy Milk Recording and Testing Association, 2017). The sample size was calculated to detect the difference in time to event endpoint, for the 1.5 times increased rate of subclinical mastitis due to BLV infection, using the Freedman formula (Abel et al., 2015).

The frailty model is a random effect survival model that allows for unobserved heterogeneity or statistical dependence between observed survival data, and random effects are treated as continuous variables that describe excess risk or frailty (Rondeau et al., 2006). Frailties are useful in modeling correlations in multivariate survival and event history data, including recurrent events such as mastitis or lameness, in which an individual cow's frailty affects the occurrence of events, and community trials, in which different events within a community involve a common frailty (or shared frailty) shared by each individual within the community (Hanagal, 2011). Based on our hypothesis, a 2-level hierarchical causal web was constructed to illustrate the relationships between the explanatory and outcome variables (Figure 1). Therefore, Cox regression model nested frailty was applied.

To test our hypothesis based on the data, a Cox regression model with 2 nested frailties (herd and cow levels) was considered. Frailty is typically defined as a clustering effect in survival analyses (Dohoo et al., 2009). The 2 nested frailties Cox model (Elghafghuf et al., 2014a, b) can be written as follows:

$$h_{ij}\left(t \mid w_i w_{ij}, \mathbf{Z}\right) = w_i w_{ij} \exp\beta(t)\mathbf{Z}h_0(t), \qquad [1]$$

where h_{ij} represents for hazard of cow j in herd i, $h_0(t)$ represents for baseline hazard in the regression model, $\mathbf{Z}$ denotes the covariate vector, $\beta(t)$ represents the corresponding vector of the regression parameter, and w_i and w_{ij} represent unobserved random effects common to all observations from cow j in herd i, conditional on the 2 nested frailties. Hazards must be positive in the Cox model, so w follows a log-normal distribution. Equation 1 can be transformed into random effects context as follows:

$$h_{ij}\left(t \mid u_i u_{ij}, \mathbf{Z}\right) = \exp u_i + u_{ij} + \beta(t)\mathbf{Z}h_0(t), \qquad [2]$$

where $u_i = \log(w_i)$ and $u_{ij} = \log(w_{ij})$ indicate nested random effects with zero means and variances σ_i^2 and σ_{ij}^2 for the herd and cow levels, respectively.

As shown in Figure 1, the explanatory variable of interest was BLV infection status (X). We had 2 potential confounders, including greater parity number (C_1), which can be associated with higher probabilities of BLV infection (and progression) and subclinical mastitis occurrence, whereas season of delivery (C_2) affects only subclinical mastitis occurrence.

Descriptive analyses were carried out for explanatory variables aggregated in the data set, and distributions and collinearities among variables were assessed. Univariable analyses of explanatory variables for the hazard of subclinical mastitis were performed using a standard Cox regression model with the Efron method for ties. The proportional hazards assumption was appraised for every predictor using Schoenfeld residuals (Dohoo et al., 2009).

Potentially important predictors based on the unconditional analysis results were then included in a multivariable model. Statistical associations were then inferred based on the causal web.

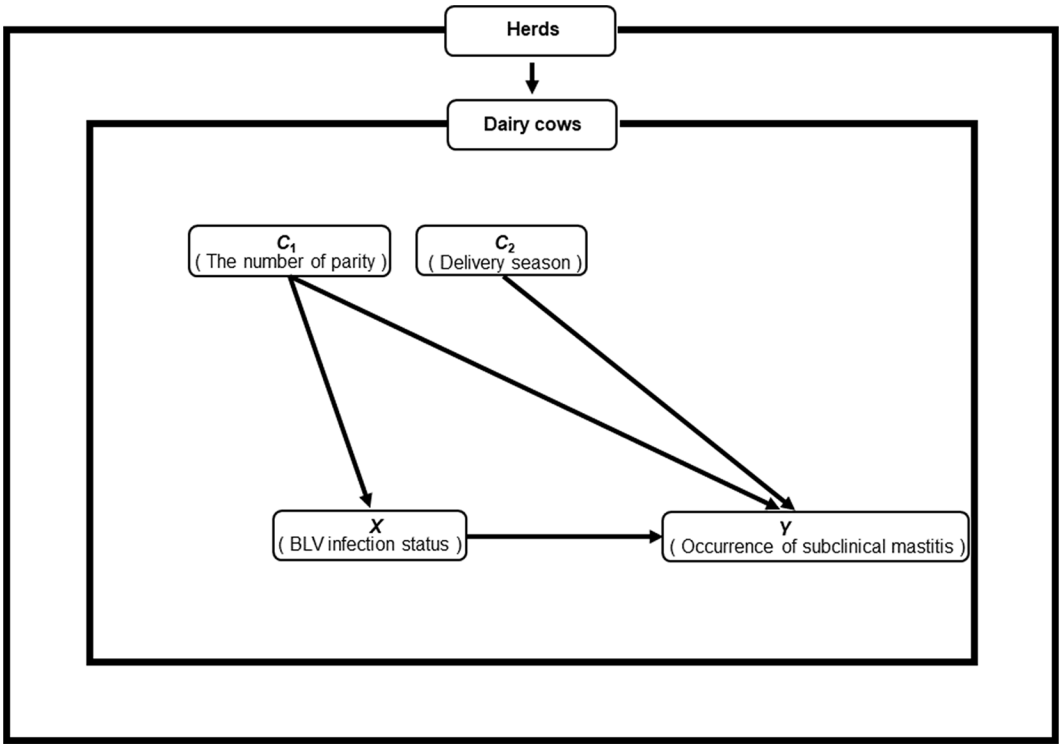

Figure 1. Theoretical causal web for the occurrence of subclinical mastitis due to bovine leukemia virus (BLV) infection in 9 Japanese dairy herds. Two-level hierarchy affects survivorship from mastitis. The factor of interest is X, and the outcome is Y. C_1 and C_2 are potential confounders.

Estimation of Economic Loss

Economic loss due to BLV infection was defined as an increase in economic loss due to the increment of clinical and subclinical mastitis occurrences caused by BLV infection in a year, compared with cows not infected with BLV. The definition applied to losses associated with both individual cows and Hokkaido Prefecture. Economic loss was categorized into 2 types as follows:

(1) Costs of discarded milk and intramammary antimicrobial agents associated with the treatment of BLV-induced clinical (veterinarian-treated) mastitis.
(2) Reduction in milk productivity due to BLV-induced subclinical mastitis.

In this analysis, secondary economic losses, such as increased work load, mental burden, and culling of mastitis cows, were not included.

Economic loss due to BLV-associated mastitis (includes both clinical and subclinical) across Hokkaido Prefecture was estimated. These data were used to calculate the loss per individual cow for both H-PVL and non-H-PVL cows (including L-PVL cows and the cows not infected with BLV by ELISA test, as the hazards of subclinical mastitis do not differ). In the estimation process, Monte Carlo simulations were used to calculate some parameters, and an interval estimate approach was selected for the final estimations.

Increased economic loss due to BLV infection across Hokkaido Prefecture was estimated as follows. First, the economic loss due to clinical mastitis in H-PVL cows was estimated ($\text{Losscmas}_{\text{HPVL}}$, i). Second, the loss due to reduced milk production caused by subclinical mastitis in H-PVL cows was estimated ($\text{Lossred}_{\text{HPVL}}$, ii). The loss associated with L-PVL was not included in these analyses as the loss from BLV infection because the hazard of subclinical mastitis in L-PVL cows was not significantly different from that of non-infected cows. Third, these 2 types of losses were summed ($\text{Loss}_{\text{HPVL}}$, iii = i + ii). Fourth, baseline losses due to clinical ($\text{Base}_{\text{cmas}}$, iv) and subclinical mastitis ($\text{Base}_{\text{scmas}}$, v), if these H-PVL cows were not infected with BLV, were calculated (Base, vi = iv + v). Finally, the baseline economic loss (vi) was subtracted from the economic loss for H-PVL cows (iii) to calculate the increased economic loss in Hokkaido Prefecture due to mastitis resulting from BLV infection (Eloss, vii = iii − vi).

To estimate the economic loss due to clinical mastitis in H-PVL cows in Hokkaido Prefecture (i), the number of H-PVL clinical mastitis cases ($Ncmas_{HPVL}$) was first estimated by multiplying the number of clinical mastitis cases reported in Hokkaido Prefecture in 2017 (Ncmas), 264,443 (Hokkaido-NOSAI, 2017), with the estimated proportion of H-PVL cows among clinical mastitis cases in the prefecture ($PHPVL_{cmas}$), which is as follows:

$$Ncmas_{HPVL} = Ncmas \times PHPVL_{cmas}. \quad [3]$$

The proportion of H-PVL cows among clinical mastitis cases, $PHPVL_{cmas}$, was estimated using the proportion of H-PVL cows among subclinical mastitis infections ($PHPVL_{scmas}$), assuming their similarity. The variable $PHPVL_{scmas}$ was estimated, as follows, by calculating the proportion of H-PVL cows among subclinical mastitis cows in Hokkaido Prefecture:

$$PHPVL_{scmas} = Nscmas_{HPVL} / (Nscmas_{HPVL} + Nscmas_{nonHPVL}). \quad [4]$$

The number of subclinical mastitis infections among H-PVL cows ($Nscmas_{HPVL}$) was estimated by multiplying the number of dairy cows in Hokkaido Prefecture (Ncows) as of 2017 (n = 496,400; MAFF, 2018a) with the following parameters: animal-level BLV prevalence (PrevBLV), 24.1%, as used in our previous study (Nakada et al., 2022); the proportion of H-PVL cows among BLV-infected cows in this study ($PHPVL_{overall}$), 19.7%; and the mean probability of subclinical mastitis occurrence among H-PVL cows ($Pscmas_{HPVL}$). The number of subclinical mastitis infections among H-PVL cows was calculated as follows:

$$Nscmas_{HPVL} = Ncows \times PrevBLV \times PHPVL_{overall} \times Pscmas_{HPVL}. \quad [5]$$

Similarly, the number of subclinical mastitis infections among non-H-PVL cows (L-PVL cows and cows not infected with BLV: $Nscmas_{nonHPVL}$) was estimated as follows using the number of non-H-PVL cows ($Ncows_{nonHPVL}$) and the mean probability of subclinical mastitis occurrence among non-H-PVL cows ($Pscmas_{nonHPVL}$):

$$Nscmas_{nonHPVL} = Ncows_{nonHPVL} \times Pscmas_{nonHPVL}, \quad [6]$$

where $Ncows_{nonHPVL}$ represents the value resulting from subtracting the number of H-PVL cows (Ncows × PrevBLV × $PHPVL_{overall}$ in Equation 5) from Ncows.

To estimate the probabilities of subclinical mastitis for H-PVL ($Pscmas_{HPVL}$) and non-H-PVL ($Pscmas_{nonHPVL}$) cows, respectively, the proportion of subclinical mastitis cows was randomly sampled based on Kaplan–Meier survival curves for subclinical mastitis in H-PVL and non-H-PVL cows at 310 d postcalving; therefore, these proportions represent the prevalence of subclinical mastitis at any time point. The Monte Carlo simulations were iterated 5,000 times for both H-PVL and non-H-PVL cows. We selected 310 d postcalving because in 2017, the calving interval mode for dairy cows in Hokkaido Prefecture was 357 d, and the dry period was approximately 60 d (LIAJ, 2017), resulting in 297 d in milking. Considering cows for which the calving interval was not calculated due to replacement, the milking period was set longer.

To estimate the cost of clinical mastitis per cow ($Losscmas_{cow}$), it was assumed that milk was discarded (i.e., wasted) for 7 d (Day_{waste}) for a single clinical mastitis treatment. Based on an average amount of 305-d milk of 9,626 kg in Hokkaido Prefecture in November 2017 (LIAJ, 2017), the average daily milk production per head was 31.6 kg (Vol_{day}). The raw milk unit was assumed to be ¥100/kg ($Price_{milk}$), and the cost of intramammary antimicrobials used to treat mastitis was ¥100 (equivalent to $0.95 based on the November 24, 2021, exchange rate of ¥105.0, $Price_{AM}$) per day. Clinical mastitis treatment was assumed to be intramammary antibiotic therapy for 3 d (Day_{treat}). The cost of clinical mastitis per cow was calculated as follows:

$$Losscmas_{cow} = Vol_{day} \times Day_{waste} \times Price_{milk} + Day_{treat} \times Price_{AM}. \quad [7]$$

Finally, the economic loss due to clinical mastitis in H-PVL-cows in Hokkaido Prefecture ($Losscmas_{HPVL}$, i) was calculated, as follows, from the product of the number of clinical mastitis cases in H-PVL cows ($Ncmas_{HPVL}$) and the unit cost ($Losscmas_{cow}$):

$$Losscmas_{HPVL} = Ncmas_{HPVL} \times Losscmas_{cow}. \quad [8]$$

To estimate the loss due to reduced milk production caused by subclinical mastitis associated with BLV infection ($Lossred_{HPVL}$, ii), the mean decline in milk production of a cow with subclinical mastitis was multiplied with the total number of milking days under subclinical mastitis conditions of affected cows in Hok-

kaido Prefecture (Equation 9). For this calculation, the mean number of milking days per cow in a year (Day_{milk}) was first estimated by randomly sampling 5,000 times under the scenario of the number of days milked by the end of the year (December 31) for a series of 365 cows with different delivery days starting January 1 and continuing through December 31, assuming that all of the cows were milked for 310 d. This process indicated that on average, a single cow in a farm (with or without subclinical mastitis) is milked 178.0 d per year. Second, the number of days milked under subclinical mastitis conditions (Day_{scmas}) was estimated by multiplying the number of milking days per year with the proportion of days milked under subclinical mastitis conditions for H-PVL-cows ($Pdayscmas_{HPVL}$). The $Pdayscmas_{HPVL}$ variable was calculated from the time since delivery at the median survival proportion for subclinical mastitis among H-PVL cows. Half of subclinical mastitis infections occur before 30 d, meaning that cows have subclinical mastitis or are in the recovery stages for 90.3% [(310 − 30 d)/310 d] of the lactation period. The rate of reduction in milk production among cows with subclinical mastitis (Red_{scmas}) was estimated by taking the complement to 1 of the ratio of the average milk yield over 305 d for cows with a linear score of ≥5 to that of healthy cows. The Red_{scmas} was 5.0%, indicating a reduction per cow per day of 1.678 kg [305-day milk yield in DHI data of cows with linear score ≥5 was 9,807.8 kg, and that of cows with linear score ≤4 was 10,319.7 kg; (10,319 − 9,807.8)/305 = 1.678 kg]. The following calculation assumed that the reduction in milk production continues even after mastitis, and this may be a strong assumption:

$$\begin{aligned} Lossred_{HPVL} &= Nscmas_{HPVL} \times Vol_{day} \times Price_{milk} \\ &\times Day_{milk} \times Pdayscmas_{HPVL} \times (1 - Red_{scmas}). \end{aligned} \quad [9]$$

The baseline loss due to clinical mastitis ($Base_{cmas}$, iv) among H-PVL cows (the loss if these cows did not exhibit H-PVL status) was estimated by applying the incidence rate of clinical mastitis in non-H-PVL-cows to the estimated H-PVL bovine population ($Ncmas_{HPVL}$).

The baseline loss due to subclinical mastitis ($Base_{scmas}$, v) among H-PVL cows (again, the loss if these cows did not exhibit H-PVL status) was estimated by applying the proportion of subclinical mastitis cows among non-H-PVL cows described above ($Pscmas_{nonHPVL}$) and the median number of days under subclinical mastitis conditions among non-H-PVL cows to the H-PVL bovine population ($Ncmas_{HPVL}$; Equation 10). The number of days under subclinical mastitis conditions among non-H-PVL cows was estimated using the same approach with H-PVL cows. Non-H-PVL cows had subclinical mastitis or were in the post-treatment state for 64.2% of the lactation period ($Pdayscmas_{nonHPVL}$). The baseline loss due to subclinical mastitis was calculated as follows:

$$\begin{aligned} Base_{scmas} &= Ncmas_{HPVL} \times Vol_{day} \times Price_{milk} \\ &\times Day_{milk} \times Pdayscmas_{nonHPVL} \times (1 - Red_{scmas}). \end{aligned} \quad [10]$$

The economic loss due to BLV-associated mastitis per H-PVL cow ($Loss_{HPVLcow}$)was estimated as follows by dividing the loss in Hokkaido Prefecture ($Loss_{HPVL}$) by the estimated number of H-PVL cows:

$$Loss_{HPVLcow} = Loss_{HPVL} / (Ncows \times PrevBLV \times PHPVL_{overall}). \quad [11]$$

For non-H-PVL cows, the economic losses associated with clinical and subclinical mastitis were separately estimated for Hokkaido Prefecture and then divided by the number of non-H-PVL cows. The economic loss due to clinical mastitis in non-H-PVL cows in Hokkaido Prefecture ($Losscmas_{nonHPVL}$) was estimated, as follows, by multiplying the number of clinical mastitis cases among non-H-PVL cows ($Ncmas_{nonHPVL}$, estimated using Equation 12) with the unit loss per cow ($Losscmas_{cow}$) estimated using Equation 7 (Equation 13):

$$Ncmas_{nonHPVL} = Ncmas - Ncmas_{HPVL}, \quad [12]$$

$$Losscmas_{nonHPVL} = Ncmas_{nonHPVL} \times Losscmas_{cow}. \quad [13]$$

The economic loss in non-H-PVL cows due to subclinical mastitis in Hokkaido Prefecture ($Lossred_{nonHPVL}$) was estimated by multiplying the number of subclinical mastitis cases in non-PVL cows ($Nscmas_{nonHPVL}$) estimated in Equation 6 with the unit loss for non-H-PVL cows. This loss was calculated as follows:

$$\begin{aligned} Lossred_{nonHPVL} &= Nscmas_{nonHPVL} \times Vol_{day} \times Price_{milk} \\ &\times Day_{milk} \times Pdayscmas_{nonHPVL} \times (1 - Red_{scmas}). \end{aligned} \quad [14]$$

The baseline economic loss due to mastitis per non-H-PVL cow ($Loss_{nonHPVLcow}$) was estimated as follows by dividing the sum of the 2 abovementioned losses by the number of non-H-PVL cows in Hokkaido Prefecture:

$$\begin{aligned} &Loss_{nonHPVLcow} \\ &= (Losscmas_{nonHPVL} + Lossred_{nonHPVL}) / Ncmas_{nonHPVL}. \end{aligned} \quad [15]$$

Table 1. Descriptive summary of cows and farms studied for bovine leukemia virus (BLV) infection and subclinical mastitis between April 2015 and March 2018

Variable	Total number of cows	Proportion of cows diagnosed with mastitis (%)
BLV infection status[1]		
Non-infected	868	40.7
Low-proviral load	135	45.9
High-proviral load	31	80.6
Parity		
1st	366	29.6
2nd and 3rd	431	44.8
4th and 5th	170	56.5
6th and over	67	64.2
Delivery season		
Spring	259	45.9
Summer	307	45.6
Fall	237	39.2
Winter	231	38.2
Herd		
A	23	56.5
B	99	48.5
C	186	53.8
D	127	18.9
E	83	48.2
F	69	40.6
G	72	19.7
H	304	48.7
I	71	35.2

[1]Low-proviral-load and high-proviral-load cows were defined as those in which the proviral load was below and above the cut-off threshold throughout the lactation period, respectively.

Using the proportion of H-PVL cows among BLV-infected cows in our dataset (i.e., 8.2%), the difference in economic loss between H-PVL and L-PVL cows was weighted, and the averaged loss per BLV-infected cow ($Loss_{BLVcow}$) was calculated. Finally, the increased cost of mastitis associated with H-PVL status at the individual cow level ($Eloss_{cow}$) was estimated using Equation 16, which is as follows:

$$Eloss_{cow} = Loss_{HPVLcow} - Loss_{nonHPVLcow}. \quad [16]$$

All statistical analyses were performed using R software, version 3.6.0, and R studio, version 1.2.5042 (RStudio Team, 2015; R Core Team, 2018). The R package coxme, version 2.2–16, was used for survival analyses (Therneau, 2020).

RESULTS

Descriptive Statistics

The farms included in the study kept between 57 and 284 cows, with a mean and median of 133 and 87 cows, respectively. Between April 2015 and March 2018, the proportion of cows within a herd diagnosed with subclinical mastitis ranged between 18.8 and 56.5%, with a mean and median of 41.1 and 48.2%, respectively. The total number of cows studied was 1,034, which included 868 cows not infected with BLV, 135 L-PVL cows, and 31 H-PVL cows. The total number of cows, 1,034, does not include the 35 cows excluded (35/1,069, 3.3%) due to the change of infection status between L-PVL and H-PVL (including H-PVL to L-PVL at a border of the cut-off value) during the study period. The overall proportion of cows diagnosed with subclinical mastitis during the study period in the 9 herds examined was 42.6% (440/1,034), and the proportions of cows diagnosed with subclinical mastitis within 50, 110, and 210 postpartum days were 15.0, 25.0, and 36.7%, respectively. The median day of subclinical mastitis diagnosis and censoring day were 92 (25 and 75 percentiles: 33 and 197.75) and 263.5, respectively.

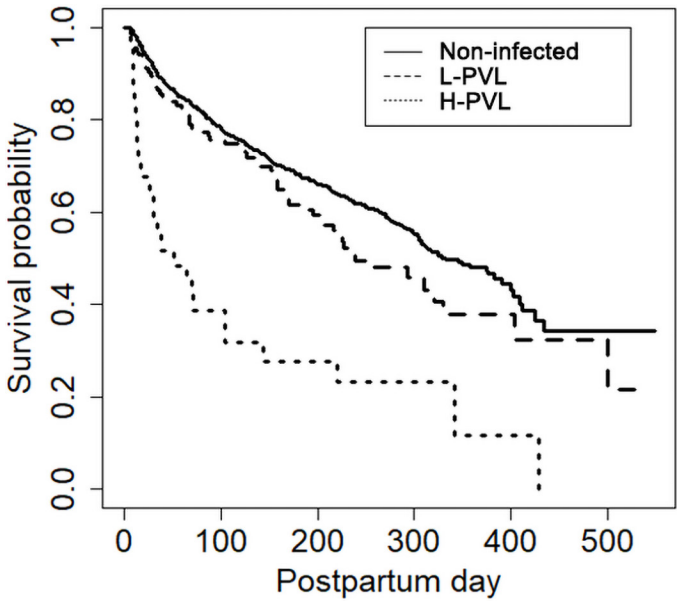

Figure 2. A comparison of Kaplan–Meier survivor curves for subclinical mastitis that occurred between April 2015 and March 2018 according to bovine leukemia virus infection status. L-PVL = low proviral load; H-PVL = high proviral load.

The descriptive statistics for cow-level predictor variables included in the analysis are shown in Table 1. Kaplan–Meier survival curves for subclinical mastitis events by BLV infection status (non-infected, L-PVL, and H-PVL), parity (1st, 2nd and 3rd, 4th and 5th, and 6th and over), and delivery season (spring, summer, fall, or winter) are shown in Figures 2, 3, and 4, respectively. The survival curve of L-PVL remained lower than that of non-infected cows, but significant difference was not observed between the 2 lines. In contrast, the survival curve of H-PVL decreased earlier than the other 2 lines

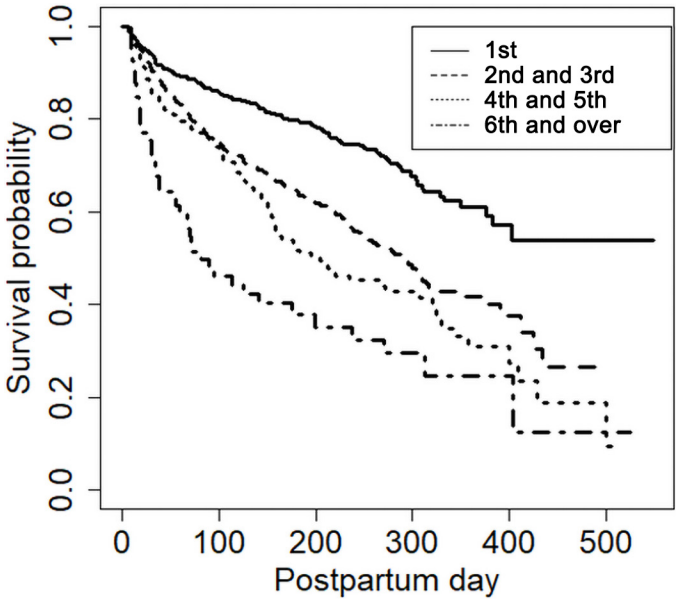

Figure 3. A comparison of Kaplan–Meier survivor curves for subclinical mastitis that occurred between April 2015 and March 2018 according to numbers of parity.

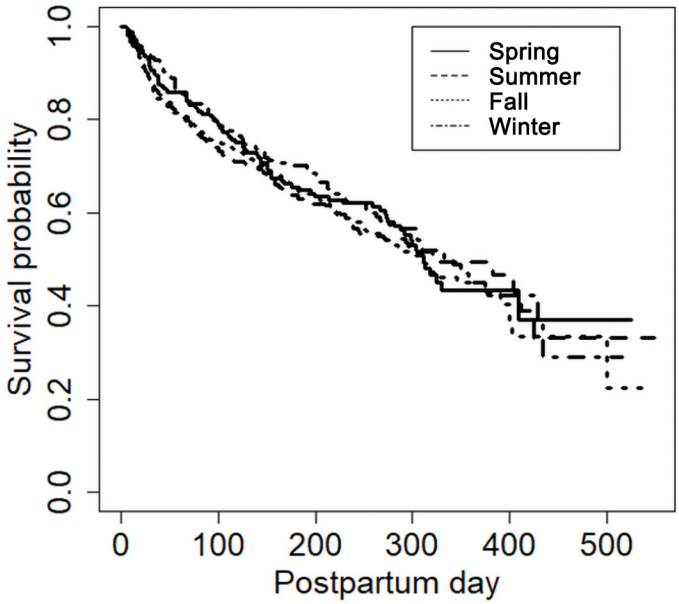

Figure 4. A comparison of Kaplan–Meier survivor curves for subclinical mastitis that occurred between April 2015 and March 2018 according to seasons.

(Figure 2). The survival probability decreased as parity increased (Figure 3), but was not different between delivery seasons (Figure 4).

In log-rank tests for multi-collinearity, the *P*-values for 3 predictors (BLV infection status, parity, and delivery season) were < 0.2, suggesting no collinearity.

Table 2. Results of multivariable survival analysis for subclinical mastitis among 9 herds between April 2015 and March 2018

Predictor	Hazard ratio	SE	*P*-value
Fixed effect			
BLV infection status[1]			
Non-infected	Reference		
Low-proviral load	0.79	0.18	0.18
High-proviral load	2.61	0.28	<0.01
Parity			
1st	Reference		
2nd and 3rd	2.10	0.13	<0.01
4th and 5th	2.81	0.17	<0.01
6th and over	5.45	0.22	<0.01
Delivery season			
Spring	Reference		
Summer	1.05	0.14	0.72
Fall	0.90	0.16	0.50
Winter	0.86	0.16	0.34
Random effect	Variance	SD	
Between-cow variance	0.37	0.61	
Between-herd variance	0.26	0.51	

[1]BLV = bovine leukemia virus; low-proviral-load and high-proviral-load cows were defined as those in which the proviral load was below and above the cut-off threshold throughout the lactation period, respectively.

Multivariable Analysis

The sample size required for a standard Cox regression analysis was 448 cows, and the number of cows studied exceeded the requirement. A multivariable analysis was performed based on BLV infection status (non-infected, L-PVL, and H-PVL), parity (1st, 2nd and 3rd, 4th and 5th, and 6th and over), and delivery season (spring, summer, fall, or winter). Figure 1 suggests the possibility of an interaction between X and C_1. However, Kaplan–Meier plots did not suggest an existing interaction, and the multivariable model did not include the interaction term. Results of the multivariable model are tabulated in Table 2. The hazard ratio (**HR**) for subclinical mastitis for H-PVL cows was 2.61 times higher than that for cows not infected with BLV. The HR of subclinical mastitis increased with parity number. Delivery season was not associated with the HR of subclinical mastitis.

Estimation of Annual Economic Losses

The estimated numbers of H-PVL and non-H-PVL cows in Hokkaido Prefecture were 24,119 [95% credible interval: 16,492–32,849] and 472,281 (95% credible interval: 463,413–480,073), respectively. The estimated prevalences of subclinical mastitis in H-PVL and non-H-PVL (including cows not infected with BLV by ELISA test) cows were 64.1% (95% credible interval: 60.5–67.3%) and 27.2% (95% credible interval: 24.9–

29.6%), respectively. Based on the prevalence data, the estimated numbers of subclinical mastitis H-PVL and non-H-PVL cows at any given time were 15,447 (95% credible interval: 14,582–16,223) and 128,603 (95% credible interval: 116,735–139,966), respectively. Based on these data, the estimated proportion of H-PVL cows among subclinical mastitis cases was 10.7%. Finally, the estimated annual incidences of clinical mastitis in H-PVL and non-H-PVL cows in Hokkaido Prefecture were 28,398 (95% credible interval: 25,929–31,232) and 236,057 (95% credible interval: 233,210–238,503) cases, respectively.

Table 3 summarizes the economic loss due to mastitis (includes both clinical and subclinical) associated with BLV infection. The annual economic loss due to mastitis in H-PVL cows in Hokkaido Prefecture (iii in Table 3) was almost $9.7 million. By subtracting the baseline loss—the loss, which would have occurred even in the absence of BLV infection—in these cows (vi), the estimated increased loss due to BLV-associated mastitis in Hokkaido Prefecture (vii) was $6.1 million.

At the individual cow level, the annual loss due to mastitis per H-PVL cow ($418.59) was 2.73 times greater than that per non-H-PVL cow ($152.96). The increased cost of BLV-associated mastitis per cow ($265.63) demonstrates the magnitude of economic impact; for example, it was even greater than the baseline loss due to mastitis per non-H-PVL cow ($265.63).

DISCUSSION

Mastitis (including both clinical and subclinical) is an inflammation of the mammary gland caused primarily by bacterial infection. Typically, 72.8% of cows with clinical mastitis recover and remain in the herd, whereas 24% are removed or sold (USDA 2014). Risk factors for mastitis have been widely investigated (Green et al., 2007; Lam et al., 2013). However, the relationship between BLV infection and the occurrence of mastitis remains unclear. To our knowledge, this study is the first to report that BLV infection in H-PVL cows is associated with the occurrence of mastitis.

We hypothesized that BLV suppresses immune function, leading to bacterial IMI, and consequently increasing incidence of subclinical mastitis. Numerous researchers have reported that BLV infection reduces milk productivity (Ott et al., 2003; Erskine et al., 2012; Bartlett et al., 2013; Nekouei et al., 2016; Norby et al., 2016), but the mechanism has not been elucidated. The Kaplan–Meier survivor curves constructed in the present study demonstrated that a half of H-PVL cows suffered from subclinical mastitis within 52 d after calving, and among the subclinical mastitis cases observed within 310 d after delivery, one-half occurred within 30 d. Milk fever and ketosis, which frequently occur during the postpartum period until peak lactation, are associated with nutritional management (Goff, 2006). Negative energy balance from the postpartum period to peak lactation inhibits immune function (Sordillo, 2016). In addition to these known factors, our survival analysis results identified H-PVL status as a significant risk factor for subclinical mastitis, which reduces milk productivity. Several previous studies reported the mechanism of immunosuppression in BLV infection. Bovine leukemia virus is harbored in the mammary glands of BLV-infected cows with subclinical mastitis (Yoshikawa et al., 1997), where it can cause immunosuppression. A higher percentage of $CD5^-/CD11b^-$ B cells in the milk of BLV-infected cows with persistent lymphocytosis compared with non-infected or BLV-infected aleukemic cows has been reported, indicating dysfunction of milk neutrophils (Della Libera et al., 2015). Moreover, the concentration in milk of lingual antimicrobial peptide, a natural immunity factor that is indicative of immune function in the mammary gland, is lower in H-PVL cows than L-PVL cows (Watanabe et al., 2019). This study demonstrated that subclinical mastitis associated with BLV infection may be induced by immunosuppression when H-PVL status (European Community's key for lymphocytic status) occurs, rather than infection with BLV.

The survival analysis of the present study identified another significant factor associated with subclinical mastitis, which is parity number. The incidence of mastitis reportedly increases with the increment parity number (Hiitiö et al., 2017; Zeryehun and Abera, 2017). In contrast, unlike previous studies (Olde Riekerink et al., 2007; Kurjogi and Kaliwal, 2014), delivery season was not associated with the occurrence of subclinical mastitis in our study. The study area was located near 43° north latitude, which is characterized by a cool climate. Cows on the farms studied are therefore less likely to undergo heat stress during the summer, thus reducing the likelihood of seasonal subclinical mastitis.

Several farm-level factors, such as farm dairy hygiene (Fujimoto et al., 2020; Miyama et al., 2020), and individual animal-level factors, such as sensitivity to mastitis (Martin et al., 2018), are known to affect the occurrence of mastitis. However, our study employed a frailty model to adequately control for such clustering effects (Hanagal, 2011), in showing the pure effect of BLV infection on subclinical mastitis. This method can be applied to other countries and be recommended in quantifying economic loss from mastitis due to BLV infection.

This was the first economic study of BLV-associated mastitis in Japan as far as we know. The estimated annual economic loss in a BLV-infected cow was $206.8.

Table 3. Estimation of annual economic losses due to mastitis associated with bovine leukemia virus (BLV) infection in Hokkaido Prefecture (95% credible interval)

Notation[1]	Description	Value (USD[2])	Value (JPY[3])
$Losscmas_{HPVL}$ (i)	Loss due to clinical mastitis in H-PVL cows in Hokkaido Prefecture	6,063,647 (5,538,324 to 6,639,604)	636,682,961 (581,523,977 to 697,158,397)
$Lossred_{HPVL}$ (ii)	Loss due to reduced milk production caused by subclinical mastitis in H-PVL cows in Hokkaido Prefecture	3,722,651 (3,514,271 to 3,909,753)	390,878,34 (368,998,484 to 410,524,098)
$Loss_{HPVL}$ (iii): (i) + (ii)	Loss due to mastitis (includes both clinical and subclinical) in H-PVL cows in Hokkaido Prefecture	9,786,298 (9,052,595 to 10,549,357)	1,027,561,309 (950,522,461 to 1,107,682,495)
$Base_{cmas}$ (iv)	Baseline loss due to clinical mastitis among H-PVL cows (the loss if these cows did not exhibit H-PVL status) in Hokkaido Prefecture	2,573,893 (1,755,727 to 3,510,385)	270,258,805 (184,351,328 to 368,590,374)
$Base_{scmas}$ (v)	Baseline loss due to subclinical mastitis among H-PVL cows (above mentioned) in Hokkaido Prefecture	1,115,180 (762,545 to 1,518,848)	117,093,871 (80,067,252 to 159,479,091)
Base (vi): (iv) + (v)	Baseline loss due to mastitis among H-PVL cows (above mentioned) in Hokkaido Prefecture	3,689,073 (2,518,272 to 5,029,233)	387,352,676 (264,418,581 to 528,069,465)
Eloss (vii): (iii) − (vi)	Elevated loss associated with mastitis due to BLV infection in Hokkaido Prefecture	6,097,225 (5,520,124 to 6,534,323)	640,208,633 (579,613,029 to 686,103,880)
$Loss_{HPVLcow}$	Loss due to mastitis (includes both clinical and subclinical) per H-PVL cow	418.59 (295.52 to 599.68)	43,952 (31,030 to 62,966)
$Loss_{BLVcow}$	Loss due to mastitis per BLV-infected cow	206.77 (184.04 to 240.85)	21,711 (19,324 to 25,289)
$Loss_{nonHPVLcow}$	Baseline loss due to mastitis per non-H-PVL cow	152.96 (150.90 to 155.23)	16,061 (15,845 to 16,299)
$Eloss_{cow}$	Elevated cost of mastitis associated with BLV infection per cow	265.63 (144.62 to 444.45)	27,891 (15,185 to 46,667)

[1]H-PVL = high-proviral load.

[2]USD = US dollars.

[3]JPY = Japanese yen.

According to a study in the United States, BLV-seropositive cows produced $59 less annually than non-infected cows (Ott et al., 2003). The methods for calculating economic losses were different in these studies, but considering the difference in raw milk unit prices between the United States (raw milk unit price per kilogram in the United States was $0.41 as of 2021 September) and Japan ($1.00 per kilogram), our estimate of the annual economic loss in BLV-infected cows appears reasonable. In considering the magnitude of economic burden in dairy production in Hokkaido Prefecture, the elevated loss associated with mastitis due to BLV infection accounted for 0.17% of total raw milk production ($6.1 million/$3.5 billion in 2017; MAFF, 2018b). This may sound a trivial damage; however, our study pointed out the economic importance of H-PVL cows. Additionally, only about 10% of study cows had H-PVL and the economic effects were only noted in these cattle. The annual profit per milking cow in Hokkaido Prefecture in 2017 was $2,853 (MAFF, 2018c), and the annual loss due to mastitis per H-PVL cow ($418.59, 14.7% of the profit) is not negligible. The reasons of such a large economic impact of H-PVL cows were high prevalence of subclinical mastitis (64.1%) and development of subclinical mastitis during early lactation. The estimated loss due to early lactation clinical mastitis in a previous study was $444 (Rollin et al., 2015), approximately the same as the loss in H-PVL cows in the present study.

An economic study of animal disease is a powerful tool to develop control measures for chronic animal disease with the consent from stakeholders (Isoda et al., 2019). The annual economic loss in H-PVL cows was 2.73 times higher than that in non-H-PVL cows ($418.59/$152.96), suggesting that there is economic merit in removing H-PVL cows from the herd. Isolation and culling of H-PVL cows have been recommended as effective methods for decreasing BLV infection because these cows are particularly infectious (Ruggiero et al., 2019). In Japan, however, in the absence of a national eradication scheme with compensation programs, dairy farmers are reluctant to cull H-PVL cows at their cost. The messages from our study should provide clear incentive for owners to cull H-PVL cows earlier and thus prevent the spread of BLV infection on their dairy farms. Moreover, the number of EBL notifications has tripled compared with a decade ago (MAFF, 2020), and an increase in infection BLV prevalence reduced milk production (Ott et al., 2003; Erskine et al., 2012). This suggests that the damage to the Hokkaido dairy industry will be even greater than the estimation in this study, if control measure is not taken now. From the viewpoint of efficient milk production and prevention of the spread of BLV infection, the government should consider some kind of compensation for the culling of H-PVL cows.

This research has 4 limitations. First, we only assumed that clinical mastitis was treated using intramammary antimicrobial agents; however, treatment of clinical mastitis included veterinary care. The cost of veterinary services was not calculated because the system in Japan involves publicly funded livestock insurance. Therefore, the economic losses estimated in this study may be underestimated. Second, mastitis was the second most common reason for dairy cow removal in Hokkaido Prefecture in 2017, as approximately 10% of mastitis-affected cows were culled (LIAJ 2017). However, this economic study did not assume the cost of replacing culled cows, which could have led to further underestimation of economic losses. Third, the definition of subclinical mastitis relied on the cut-off value of linear score, and the estimation was not adjusted for the sensitivity and specificity as they are not available. Fourth, the reduction in milk productivity was modeled to begin at the occurrence of subclinical mastitis and continue throughout the lactation period; thus, these losses could be slightly overestimated. Actually, the reduction of milk may be a function of SCC and type of responsible bacteria, but detailed modeling was avoided as this problem is too complex for a population-wise estimation. The economic analysis was conducted based on several assumptions that the sampled data can be extrapolated to entire Hokkaido Prefecture. Occurrence of EBL is observed in entire Hokkaido Prefecture, and the study site was one of the intensive dairy production areas. Therefore, the economic loss calculated in this study can represent Hokkaido Prefecture. However, there can be some areas where voluntary BLV control is weak. In such areas, the proportion of H-PVL cows may be higher than the present study. There are such data gaps in estimating the economic loss due to BLV infection, and uncertainties in the parameters were presented using probability distributions. The economic loss of mastitis may share a large part of the loss due to BLV infection. However, the entire picture of economic loss due to BLV infection has not been quantified. Integrative assessment of the economic loss is needed in future.

In conclusion, this study revealed that BLV-infected H-PVL cows exhibit a higher HR for subclinical mastitis after adjustment for parity number, delivery season, and clustering effects at the farm and individual cow levels. BLV-infected H-PVL cows are associated with significant economic losses in Hokkaido Prefecture. Priority removal of BLV-infected H-PVL cows is recommended in terms of both BLV infection control and economics.

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