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Estimation of economic loss by carcass weight reduction of Japanese dairy cows due to infection with bovine leukemia virus

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ABSTRACT

Bovine leukemia virus (BLV) infection is endemic in Japanese dairy farms. To promote the participation of farmers in BLV infection control in Japan, it is important to provide estimates of the economic losses caused by this infection. We hypothesized that decreased immune function due to BLV infection would increase visceral abnormalities, in turn reducing carcass weight. We employed mediation analysis to estimate the annual economic loss due to carcass weight reduction caused by BLV infection.

Culled Holstein cows from 12 commercial dairy farms in the Nemuro and Kushiro regions of Hokkaido, Japan, were traced. Information on age and the last delivery day were collected. A non-infected culled cow was defined as a cow from which BLV provirus was not detected. A high-proviral-load (H-PVL) cow was defined as a cow whose PVL titer was above 2465 copies/50 ng DNA or 56,765 copies/10^5 cells. A BLV-infected cow with PVL titer lower than the thresholds was categorized as low-proviral load (L-PVL). Post-mortem examination results for culled cows were collected from a meat inspection center.

The hypothesis was tested by three models, using data from 222 culled dairy cows. Model 1, a generalized linear mixed-effects model, selected carcass weight as an outcome variable, BLV status and the potential confounders (lactation stage and age) as explanatory variables, and herd as a random effect. Model 2 additionally included the number of abnormal findings in the post-mortem examination (AFPE) as an explanatory variable. Model 3 applied a Bayesian generalized linear mixed model, which employed a mediator separately modeled for AFPE, to estimate the amount of direct, indirect, and total carcass weight loss with adjustment for known confounding factors.

Compared to the mean carcass weight for the non-infected culled cows, the carcass weight for H-PVL culled cows was significantly decreased by 30.4 kg on average. For each increase of one in the number of AFPE, the mean carcass weight was decreased by 8.6 kg. Only the indirect effect of BLV H-PVL status on carcass weight loss through AFPE was significant, accounting for 21.6 % of the total effect on carcass weight reduction. In 2017, 73,650 culled dairy cows were slaughtered in Hokkaido, and the economic loss due to carcass weight loss caused by BLV infection that year was estimated to be US $1,391,649. In summary, unlike L-PVL cows, H-PVL status was associated with carcass weight reduction, which was partially mediated by an increase in the number of visceral abnormalities.

1. Introduction

Bovine leukemia virus (BLV) is B-lymphotropic oncogenic member of the family Retroviridae (OIE, 2012) that is a causative agent of enzootic bovine leukosis (EBL) in cattle. Although animals can be infected with BLV at any age, tumors (lymphosarcomas) are typically seen in infected animals older than 3 years of age. Infections usually are subclinical or aleukemic (AL); about 30 % of infected cows have persistent lymphocytosis (PL), and 2–5 % of the infected cows develop tumors (EFSA, 2015).

National sero-epidemiological surveys have confirmed that BLV-infected cattle are distributed across all continents, with the exception

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of Western Europe (Rodríguez et al., 2011). High herd-level prevalence has been reported in endemic countries: 84 % in Argentina (Trono et al., 2001), 78.3 % in Canada for pooled data between 1998 and 2003 (Nekouei et al., 2015), 94.2 % in the USA (LaDronka et al., 2018), and 68.1 % in Japan (Murakami et al., 2011). Despite such high prevalence, because of the long period taken until the development of a tumor, BLV is not prioritized for national eradication schemes with compensation programs in these endemic countries. In the absence of a national eradication scheme, the perception of bovine leukemia among dairy farmers is key to farmer participation in local voluntary disease control programs. However, given subclinical nature of the disease, the motivation for BLV control is generally low among Japanese dairy farmers, as also reported in the USA (LaDronka et al., 2018).

Several studies have described three types of significant economic losses from this disease. First, BLV-infected cows have shorter lifespans than non-infected cows (Bartlett et al., 2013; Nekouei et al., 2016). Second, BLV infection is associated with decreased milk production, both at the herd (Erskine et al., 2012), and animal levels (Norby et al., 2016), though some studies failed to detect a significant difference in milk production between BLV-infected and non-infected animals (Kale et al., 2007; Tiwari et al., 2007; Sorge et al., 2011). Third, the carcasses of BLV-infected dairy cows are condemned at higher rates than those of non-infected animals, due to the presence of malignant lymphoma (White and Moore, 2009; Amirpour Haredasht et al., 2018). The carcasses of dairy cows are an important source of beef (Rezac et al., 2014); in Japan these carcasses accounted for 10.8 % of the total amount of beef sold in 2017 (MAFF, 2018a). The BLV incidence in Hokkaido prefecture is rapidly increasing from 288 in 2010 to 745 in 2020 (MAFF, 2020) while cattle population has not changed, and this affected the efficiency of beef production in Japan.

In addition to these three types of economic losses, we hypothesized that reduction in carcass weight due to degraded immunity of infected cows (Frie and Coussens, 2015) to be another factor to economic loss due to this disease. The chronic retroviral infection causes alternations in cytokine expression and it may contribute to disease progression (Kabeya et al., 2001). Cytokine expression profiles of BLV-infected cows differ depending on the stage of the disease (Farias et al., 2016). Infection with BLV decreases the production of TNF-α in cows (Ohira et al., 2016). Decreased TNF-α causes weight loss due to decreased insulin resistance (Nieto-Vazquez et al., 2008), and chronic inflammation (Feghali and Wright, 1997), which induces tissue damage. There is a report that H-PVL was positively associated with the severity of bovine mastitis, and negatively associated with the level of a lingual antimicrobial peptide in milk, which exhibits nonspecific activity against many kinds of bacteria and viruses (Watanabe et al., 2019). This suggests that H-PVL reduces the level of immunity through several mechanisms and causes damages in potentially many organs. Meat from all cattle undergoes veterinary meat inspection for ensuring food safety, according to the legislations of Japan, USA, and EU (MHLW, 1953; White and Moore, 2009). Meat inspection is a valuable tool for surveillance of animal health and welfare conditions (Nielsen et al., 2017), and biological abnormalities caused by BLV-induced alternations in cytokine expression may be recorded in visceral abnormal findings during post-mortem examination (AFPE).

While the number of EBL cattle condemned at meat inspection in slaughterhouses has been increasing in Japan due to the increase in the prevalence of BLV (Somura et al., 2014), carcasses of BLV-infected cows at the subclinical stage continue to be sold for consumption. Even in the latest review on EBL control, the economic loss due to decreased carcass loss was not described (Bartlett et al., 2020). To our knowledge, no previous study has quantified the economic loss associated with the reduced carcass weight of BLV-infected dairy cows.

The objectives of the present study were to infer the theoretical causal relationship between infection with BLV, pathology potentially caused by BLV-induced immunodeficiency, and carcass weight reduction; and to estimate the loss of carcass weight in the dairy industry in Hokkaido due to the BLV infection. This study was conducted as a part of the economic assessment of loss due to BLV infection in the dairy industry, and the findings will be integrated in the future work.

2. Materials and methods

2.1. Study design

A prospective cohort study was used, selecting an exposed group as BLV-infected cows and a non-exposed group as BLV non-infected cows, to measure the effect of BLV infection on carcass weight. To infer the causal relationship between BLV infection and carcass weight loss in more detail, the role of BLV-induced immunodeficiency as a mediator was investigated using the number of AFPE.

2.2. Study area, and farm and cow selection

This study involved 12 BLV-infected commercial dairy farms in the Nemuro and Kushiro regions of Hokkaido, Japan. The BLV infection status of cows at these farms has been monitored by routine testing, performed one or two times per year. All these farms participated in the voluntary BLV control program. The number of cows raised on these farms ranged between 57 and 435, with mean and median numbers of 163.2 and 125 cows, respectively. In terms of the production scale, these farms did not deviate from dairy farms in the area. Within-farm prevalence of BLV infection ranged between 6.9 % and 55.2 %, with mean and median prevalences of 26.0 % and 24.2 %, respectively. Cows of known BLV status that were slaughtered within a week after removal from the farms between April 2015 and March 2018 were studied. The culled cows included in the present study were 2 years of age or older, and had previously given birth. BLV-infected cows in this study were defined as those cows that were diagnosed as being infected in the two most-recent consecutive blood tests (explained below) preceding slaughtering; non-infected cows were defined as those cows that were diagnosed as non-infected by the same tests.

2.3. Quantification of BLV PVL

Genomic DNA was isolated from whole blood samples using the Wizard Genomic DNA Purification Kit (Promega, Tokyo, Japan). The proviral load (PVL) was measured using either one of two real-time quantitative PCR assays, namely BLV-CoCoMo-qPCR (Riken Genesis, Tokyo, Japan) or Cycleave BLV qPCR (Takara, Shiga, Japan).

BLV-CoCoMo-qPCR, which was used to ascertain BLV infection status and to gauge the PVL, was performed as described in our previous study (Nakada et al., 2018). The quantification of BLV PVL was performed at the Agricultural Research Department of Hokkaido Research Organization Animal Research Center.

The Cycleave BLV qPCR (Takara, Shiga, Japan) amplifies the tax region of BLV, and PVL assess by this test was expressed as the number of proviral copies per 50 ng of DNA. The quantification of BLV PVL was performed at the Hokkaido Higashi Agriculture Mutual Aid Association clinical laboratory or at the Research Institute for Animal Science in Biochemistry and Toxicology.

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

2.4. Classification of the level of BLV PVL

The BLV-infected cows were classified into two groups according to the PVL: low PVL (L-PVL) and high PVL (H-PVL). The cut-off thresholds used to classify the PVL levels for both PCR methods were determined using receiver operating characteristic curve analysis (Schisterman et al., 2005) and employing the European Community (EC)'s key for lymphocytic status, which is based on the absolute lymphocyte count and age of a cow (Mekata et al., 2018). Specifically, blood samples were collected from 69 apparently healthy but BLV-infected Holstein cows from a commercial dairy farm in the Kushiro region, and lymphocyte count (Lym), and BLV PVL quantifications were performed. Using the age and Lym information, these cows were classified as lymphocytic or not based on the EC's key. The optimal cut-off thresholds of PVL measurement to classify infected cows as H-PVL or L-PVL were calculated as 56,767 copies/10^5 cells (for BLV-CoCoMo-qPCR; area under the curve (AUC) = 0.96) and 2,464.8 copies/50 ng DNA (for Cycleave BLV qPCR; AUC = 0.97), using the pROC software package (Robin et al., 2011), in the context of classification based on the EC's key. The high AUCs endorsed the high sensitivity and specificity when the cut-off thresholds were selected in both PCR methods. The BLV-CoCoMo-qPCR and Cycleave BLV qPCR viral quantification results were highly correlated (Spearman's rank correlation coefficient = 0.97, $p < 0.01$) using serum samples of 60 BLV-infected cows from other region of Hokkaido prefecture.

L-PVL and H-PVL cows were defined as those cows whose PVLs were below and above (respectively) the cut-off thresholds in the two most-recent consecutive tests before slaughtering. Cows with contradictory results for classification as either L-PVL or H-PVL in the two most-recent consecutive tests were excluded from the present study.

2.5. Data collection and management

Herd-level information (such as a total number of cows and the number of culled cows) and animal-level information (such as age, parity, and BLV test results of culled cows and the postpartum days at slaughtering) were collected by the field veterinarians in charge of the study farms through interviews with the farm owners and checking the farm records.

Cow ages were categorized as 2, 3, 4, or 5 and over 5 years old. None of the 2-year-old culled cows were infected with BLV; animals in this age range were excluded from all the statistical analyses. The median calving interval for dairy cows in Hokkaido was 407 days (Livestock Improvement Association of Japan, 2016), and cows culled at more than 1000 days from the last delivery may be outliers. Therefore, culled cows with periods of greater than 1000 days since calving were excluded from the analysis. The lactation stages were assigned to 4 categories based on time since delivery: early lactation, 0–49 postpartum days; peak lactation, 50–109 days; mid-lactation, 110–209 days; and late lactation, 210 days or longer.

The destinations of culled cows were traced using the database of the Japan National Livestock Breeding Center. All culled cows used for this study were confirmed not to have exhibited any clinical symptom suggestive of EBL from the clinical records. The carcass weight data of culled cows were collected from meat processing wholesalers after obtaining informed consent from the farm owners.

The post-mortem examination records of the culled dairy cows (Supplementary Fig. 1) were collected from the Department of Health and Welfare, the Hokkaido Prefectural Government, after obtaining informed consent from the farm owners. The uterine abnormal findings included pregnancy uterus, postpartum uterus, and endometritis. Postpartum uterus and pregnant uterus were excluded from this study because they were not pathological abnormalities. Animals whose whole carcasses were condemned due to sepsis, edema, jaundice, and uremia also were excluded, because these condemnations were due to a systemic symptom, and the primary lesion could not be identified. In the case of malignant lymphoma due to BLV infection, the whole carcass is condemned. Therefore lymphoma due to BLV is not included in AFPE in this study.

For the purposes of analysis, the number of AFPE was counted for all the culled dairy cows studied. All the data were digitized and handled using commercially available spreadsheet software (Excel 2013; Microsoft Corp., Redmond, WA, USA).

2.6. Statistical analysis

Descriptive statistics were performed by calculating the mean, median, interquartile range, and 2.5 and 97.5 percentiles for the variables collected. The data of carcass weight were right-skewed, and the logarithms of these data exhibited normality as assessed by the Shapiro-Wilk test.

For univariable analyses, generalized linear mixed-effects models (GLMMs) with log-link gamma errors were performed, selecting carcass weight as an outcome variable, identification of herds as a random effect, and BLV infection status, the number of AFPE, age, and lactation stage as explanatory variables. In addition to the analyses, the relationship between AFPE and carcass weight was analyzed using a generalized linear model (GLM) with log-link gamma errors.

For the multivariable causal inference of the association between BLV infection and carcass weight loss; and for measurements of direct, indirect, and total carcass weight losses, three models were used: (1) prediction of carcass weight loss by BLV infection status (Model 1), (2) prediction by BLV infection status and AFPE in a linear model (Model 2), and (3) prediction by BLV infection status mediated by AFPE (Model 3). Multi-collinearity between variables was diagnosed by the generalized variance inflation factor (GVIF): when GVIF is 2 or higher (Fox and Monette, 1992), the variables are considered to have multi-collinearity.

For Model 1 (Eq. (1), Fig. 1a), a GLMM with log-link gamma errors was performed.

$$E(Y_i) = \nu + \varphi_1 B_i + \varphi_2 C_i + H_j + e_{ij} \tag{1}$$

where Y_i is the carcass weight for ith culled cow ($i = 1, 2, \ldots, 83$), ν is overall mean, B_i is BLV infection status (non-infected, L-PVL and H-PVL), C_i represents indicator variables for the known confounders (age and lactation stage) related with B_i and Y_i, φ_1 is the total effect of B_i, and φ_2 is a regression coefficient for C_i. H_j is herd identification as a random effect ($j = 1, 2, \ldots, 12$); e_{ij} expresses an error term for the carcass weight for ith culled cow in jth herd.

Model 2 additionally included AFPE as an explanatory variable (Eq. (2)).

$$E(Y_i) = \nu + \varphi_1 B_i + \theta_2 A_i + \varphi_2 C_i + H_j + e_{ij} \tag{2}$$

where A_i is the number of AFPE. The degree of interaction of the AFPE on the effect of BLV infection status on the reduction of carcass body weight, as an indirect effect, was measured using the difference method of mediation analysis (VanderWeele, 2016). Mediation analysis evaluates the relative magnitude of different pathways and mechanisms by which exposure may affect an outcome (VanderWeele, 2016). The change of the estimates for BLV infection status between Model 1 and Model 2 was calculated by dividing the differences in the estimates for L-PVL and H-PVL by the respective estimates in Model 1. Since the GLMMs applied a log function, the carcass weight loss was calculated by exponential transformation. This statistical analysis was applied using R package lem4 version 1.1-23 (Bates et al., 2015).

After checking the indirect effect of the AFPE on the carcass body weight, more accurate measurements of the direct effect by the BLV status and of the indirect effect by the AFPE on carcass body weight were conducted using Bayesian mediation analysis (Model 3, Fig. 1b). Model 3 consisted of two equations: the Bayesian GLMM with gamma errors (Eq. (3)), which includes the mediator (M_i); and the M_i, Bayesian GLMM with Poisson errors (Eq. (4)).

$$E(Y_i) = \mu + \theta_1 B_i + \theta_2 M_i + \theta_3 C'_i + H_j + e_{ij} \tag{3}$$

$$E(M_i) = \lambda + \sigma_1 B_i + \sigma_2 C''_i + H_j + e_{ij} \tag{4}$$

where M_i is the number of AFPE for ith culled cow; μ and λ are the overall means; C'_i represents confounders (age and lactation stage) related with BLV infection status and carcass weight for the culled cow; C''_i

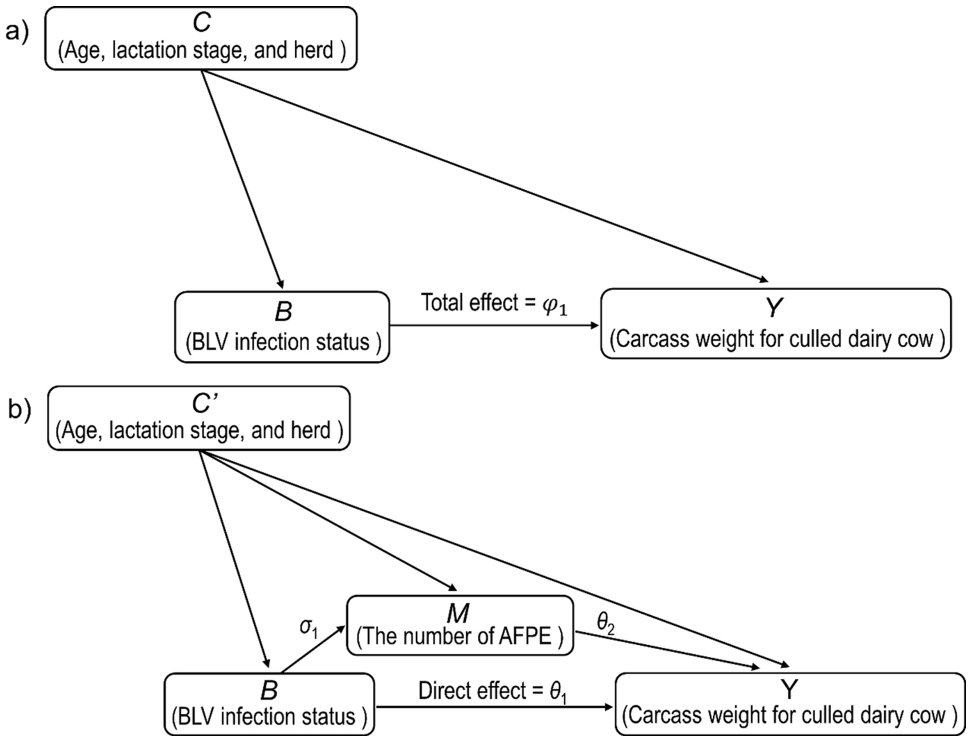

Fig. 1. Causal webs for carcass weight loss. Panel (a) shows Model 1, which describes the total effect of BLV infection status on carcass weight, with potential confounders. Panel (b) shows Model 3, the mediation analysis model. θ_1 indicates the strength of the direct effect of B (BLV infection) on Y (carcass weight), and θ_2 and σ_1 indicate the strengths of the effects of B on M (AFPE), and M on Y, respectively. The indirect effect is calculated in panels (a) and (b) as: $\varphi_1 - \theta_1 = \sigma_1 \times \theta_2$. The parameters φ_1, φ_3, θ_1, θ_2, θ_3, σ_1, and σ_3 are the regression coefficients relating to Eqs. (1)–(4) in the main text.

represents a confounder, age, related with BLV infection status and the AFPE (it is unlikely that a particular lactation stage increases the AFPE); and θ and σ are the regression coefficients (Fig. 1b). θ_1 is the direct effect of BLV infection status, and the product of θ_2 and σ_1 is the indirect effect.

Model 3 was constructed via Markov Chain Monte Carlo (MCMC) using the R package brms 2.12.0 (Bürkner, 2018), rstan 2.21.2 (Stan Development Team, 2020), and sjstats 0.17.9 (Lüdecke, 2020). Uniform distribution was selected for the prior distributions; the sample size and length of burn-in were decided based on visual inspections of trace plots. Samples were derived by the MCMC algorithm, using 4 chains with 10, 000 iterations (1000 warm-up samples for each chain) and 36,000 samples. Unstandardized effect estimates and 95 % credible intervals (CreI) were reported. All statistical analyses were performed using R version 3.2.2 and R studio 1.2.5042 (R Core Team, 2015; RStudio Team, 2020).

2.7. Estimation of economic loss

The annual economic loss of carcass weight due to BLV infection in Hokkaido Prefecture in 2017 was calculated by multiplying the total carcass weight loss posterior distributions for H-PVL and L-PVL cows (estimated using Bayesian mediation analysis) with the estimated number of BLV-infected cows culled in a year, and by the unit price of one kilogram of meat for a culled dairy cow, using a Monte Carlo simulation. The information on the number of culled dairy cows slaughtered in Hokkaido in 2017 was collected from the Hokkaido Prefectural Government Department of Health and Welfare. The proportion of BLV-infected cows among culled dairy cows in Hokkaido was estimated as follows. The prevalence of BLV in dairy cows in Hokkaido has not been updated since 2009–2011 data (11.5 %, 95 % CI: 8.9–14.1 %, $Prev_{2010}$) (Murakami et al., 2013). Using the public report of annual confirmed cases of EBL, prevalence of BLV in dairy cows in Hokkaido prefecture in 2017 ($Prev_{2017}$) can be estimated as Eqs. (5) and (6).

$$\frac{IR_{2010}}{Prev_{2010}} = \frac{IR_{2017}}{Prev_{2017}}, \tag{5}$$

$$Prev_{2017} = \frac{IR_{2017}}{IR_{2010}} \times Prev_{2010} \tag{6}$$

where IR_{2010} and IR_{2017} are incidence rates of BLV in 2010 and 2017, respectively. The incidence report included unknown number of EBL in beef cattle. However, as EBL occurs predominantly in dairy cows, incident rates in dairy cows in Hokkaido were estimated using the incidences.

The proportion of H-PVL cows relative to the cows infected with BLV (11/43, 25.6 %) may be lower than the general Japanese dairy population, as all the studied farms participated in the BLV control program. A point estimate, 33.3 % was used for the proportion of H-PVL relative to total number of cows in Hokkaido based on a literature (Ruggiero et al., 2019). The numbers of L-PVL and H-PVL dairy cows slaughtered in Hokkaido in 2017 were estimated using the proportion. Actually, the proportion of H-PVL cows in the previous study using slightly different cutoff threshold was 39.8 % (Ruggiero et al., 2019). A sensitivity analysis of the proportion of H-PVL cows was conducted using the proportion in our study (25.6 %, see Results) and the higher value reported by Ruggiero et al. (39.8 %).

The unit price per carcass for culled dairy cows was 560 yen/ kg (which is 5.3 US dollars based on the 2020 September 6 exchange rate), based on a survey published by the Ministry of Agriculture, Forestry and Fisheries (MAFF, 2018b).

3. Results

3.1. Descriptive statistics

Out of the 330 head of Holstein cows culled at the 12 study farms, 222 culled cows were enrolled after the exclusions mentioned above. According to the BLV tests, 11 cows were H-PVL, 32 were L-PVL, and 179 were not infected with BLV.

Fig. 2 shows the distribution of carcass weight, and the mean, median, and interquartile range were 299.2, 284.0, and 113.5 kg, respectively. The descriptive statistics for carcass weight, age, the number of AFPE, and lactation stage by BLV infection status are shown in Table 1.

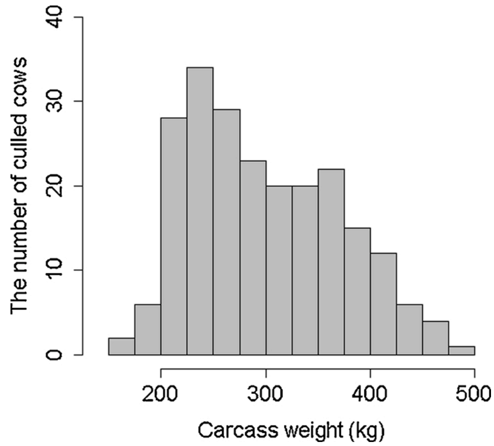

Fig. 2. Histogram of the carcass weight of culled dairy cows (n = 222).

Table 1
Medians with 2.5 and 97.5 percentiles of carcass weight, age, and abnormal findings, and the numbers of cows by lactation stage for the culled dairy cows studied, according to the BLV infection status.

Variables	Non-infected	Low proviral load	High proviral load
Carcass weight	285 (196.8−450.3)	290 (202.0−419.2)	264 (192.0−391.1)
Age	5.2 (3.1−11.9)	6.6 (3.4−10.8)	5.6 (3.6−8.2)
AFPE[a]	1 (0−5)	1.5 (0−6)	3 (0−5.8)
The number of cows			
Early lactation	8 (4.5 %)	3 (9.4 %)	1 (9.1 %)
Peak lactation	19 (10.6 %)	3 (9.4 %)	1 (9.1 %)
Mid-lactation	38 (21.2 %)	4 (12.5 %)	0 (0 %)
Late lactation	114 (63.7 %)	22 (68.7 %)	9 (81.8 %)
Total	179 (100 %)	32 (100 %)	11 (100 %)

[a] Abnormal findings in the post-mortem examination.

The mean AFPE showed an increasing trend over the progression of BLV infection status. The most frequent condemned organs among BLV-infected cows were liver, heart, and ruminant stomach: forestomach and abomasum; the proportions, were 67.4 % (95 % confidence interval (ConfI): 51.3 %, 80.5 %), 27.9 % (95 % ConfI: 15.8 %, 43.9 %), and 16.3 % (95 % ConfI: 7.3 %, 31.3 %), respectively.

Table 2
Univariable analysis results for the factors associated with carcass body weight of culled dairy cows among categorical variables.

Variable	Median carcass weight	2.5−97.5 percentiles	Number of cows	*p*-value
BLV infection status				
Non-infected	285.0	238.5–355.5	179	Reference
Low proviral load	290.0	261.8–354.0	32	0.89
High proviral load	264.0	239.1–308.7	11	0.30
Age				
3–4	242.3	217.8–311.3	40	Reference
4–5	280.0	228.8–356.0	46	0.03
Over 5 years	299.0	256.0–357.5	136	<0.01
Lactation stage				
Early	239.6	226.8–268.0	12	Reference
Peak	239.0	223.5–263.0	22	0.41
Middle	258.0	229.0–300.5	43	0.88
Late	322.0	266.0–369.0	145	<0.01

3.2. Univariable analysis

Table 2 shows the univariable analysis results for the factors associated with the carcass body weight of culled dairy cows, among categorical variables. Age and lactation stage were associated with carcass weight, whereas BLV infection status was not.

The number of AFPE was negatively associated with carcass weight (slope = −0.047, standard error (se) = 0.01, p < 0.01).

3.3. Measurement of degree of interaction of AFPE with effect of BLV infection status on reduction of carcass body weight analysis

GVIFs of Model 1 and Model 2 were close to 1, and multi-collinearity was not observed between variables (GVIF < 2). The result of Model 1 (Table 3) showed that a mean carcass weight for H-PVL culled cows was significantly lighter, by 30.2 kg, than that of non-infected cows (estimate = −0.14, p = 0.03) (total effect). The mean carcass weight of late-lactation culled cows was significantly heavier, by 47.5 kg, than that of the early-lactation culled cows (estimate = 0.19, p < 0.01). The mean carcass weight for culled cows aged 5 years or older was significantly heavier, by 35.0 kg, than that of culled cows aged 3 and 4 years old (estimate = 0.14, p < 0.01).

The results of Model 2 (Table 4) indicated that when AFPE increased by 1, carcass weight decreased by 8.55 kg (estimate = −0.04, p < 0.01). The difference in mean carcass weights for culled early- and late-stage lactation cows was slightly smaller (42.0 kg, estimate = 0.16, p < 0.01) than that seen in Model 1 (estimate = 0.19, Table 3). BLV infection status was not significantly associated with carcass weight in this model. Age remained a significant factor for carcass weight.

The change of the estimates for H-PVL cows between Models 1 and 2 was 42.9 % (from −0.14 to −0.08, {−0.14 – (−0.08)}/−0.14), and that for L-PVL cows was 0 % (−0.06 in both models), suggesting the existence of the indirect effect of H-PVL, through AFPE, on carcass weight loss.

3.4. Mediation analysis for the estimation of precise effects

Table 5 shows the results of the mediation analysis. The distributions of direct effects of BLV infection on carcass weight included areas with different signs for both H-PVL and L-PVL, though the negative medians suggested some effects in reducing carcass weight. The AFPE significantly reduced carcass weight (median = −0.04, 95 % credible interval [CreI]: −0.06 to −0.03). BLV H-PVL significantly increased AFPE (0.48, 95 % CreI: 0.06–0.88).

The distributions of indirect effects of BLV infection on carcass weight through AFPE, calculated as the product of the effect of AFPE on

Table 3
Statistical relationships between carcass weight of culled dairy cows and BLV infection status with confounders (Model 1).

Variable	Estimate	Standard error	*P*-value
Fixed effects			
Intercept	5.44	0.08	<**0.01**
BLV infection status			
Non-infected	Reference		
Low proviral load	−0.06	0.04	0.12
High proviral load	−0.14	0.06	**0.03**
Age			
3–4	Reference		
4–5	0.06	0.04	0.10
Over 5 years	0.14	0.03	<**0.01**
Lactation stage			
Early	Reference		
Peak	−0.07	0.07	0.27
Middle	0.00	0.06	0.97
Late	0.19	0.06	<**0.01**
Random effect	Variance	SD	ICC%
Herd	0.01	0.09	14.9

Table 4
Statistical relationships between carcass weight and BLV infection status with confounders adjusted by the number of AFPE (Model 2).

Variable	Estimate	Standard error	*P*-value
Fixed effect			
Intercept	5.5	0.07	<**0.01**
BLV infection status			
Non-infected	Reference		
Low proviral load	−0.06	0.04	0.17
High proviral load	−0.08	0.06	0.17
Age			
3–4	Reference		
4–5	0.09	0.04	**0.02**
Over 5 years	0.16	0.03	<**0.01**
Lactation stage			
Early	Reference		
Peak	−0.07	0.06	0.23
Middle	−0.01	0.06	0.83
Late	0.16	0.05	<**0.01**
The number of AFPE[a]	−0.04	0.01	<**0.01**
Random effect	Variance	SD	ICC%
Herd	0.01	0.08	16.15

AFPE[a]: abnormal finding of the post-mortem examination.

Table 5
Bayesian mediation analysis results showing estimates (in logarithmic scale).

Variables	Median	Error	95 % credible interval
Carcass weight intercept	5.52	0.07	5.38, 5.67
AFPE intercept	0.14	0.18	−0.22, 0.48
Estimates for carcass weight			
BLV status: non-infected	Reference		
BLV status: low PVL	−0.05	0.04	−0.13, 0.03
BLV status: high PVL	−0.08	0.06	−0.21, 0.04
Age: 3 years old	Reference		
Age: 4–5 years old	0.09	0.04	−0.13. 0.03
Age: over 5 years old	0.16	0.03	0.10, 0.23
Lactation stage: Early	Reference		
Lactation stage: Peak	−0.07	0.06	−0.20, 0.05
Lactation stage: Middle	−0.01	0.06	−0.20, 0.05
Lactation stage: Late	0.16	0.05	0.05, 0.26
AFPE	−0.04	0.01	−0.06, −0.03
Estimates for AFPE			
BLV status: non-infected	Reference		
BLV status: low PVL	0.1	0.16	−0.21, 0.41
BLV status: high PVL	0.48	0.21	0.06, 0.88
Age: 3 years old	Reference		
Age: 4–5 years old	0.38	0.19	0.02, 0.76
Age: over 5 years old	0.36	0.16	0.06, 0.69
Standard deviation for carcass weight	0.14	0.04	0.08, 0.23
Standard deviation for AFPE	0.28	0.12	0.09, 0.57

carcass weight and the effect of BLV infection on AFPE, showed that the indirect effect was significant for H-PVL culled cows (credible interval did not include the areas with different signs; in 95 % credibility, increased AFPE which was induced by BLV infection reduced carcass weight), but was not significant for L-PVL culled cows (Table 6). Moreover, the total effects of BLV infection on carcass weight were not significant (Table 6). The proportion of the effect mediated by AFPE was

Table 6
Direct, indirect and total effects of BLV infection on carcass weight losses with 95 % credible intervals.

BLV infection status	Direct	Indirect	Total
Low proviral load	−0.05 (−0.13 to 0.03)	0 (−0.02 to 0.01)	−0.06 (−0.14 to 0.02)
High proviral load	−0.08 (−0.21 to 0.04)	−0.02 (−0.04 to −0.00)	−0.1 (−0.23 to 0.02)

21.56 % for H-PVL culled cows, a value that was much larger than the 8.74 % obtained for L-PVL culled cows. Fig. 3 shows the posterior distributions of the direct and indirect effects of BLV L-PVL and H-PVL on carcass weight.

3.5. *Estimation of the economic loss on carcass weight*

The estimated median annual direct loss of carcass weight due to BLV infection in Hokkaido Prefecture in 2017 was 262,575 (2.5 and 97.5 percentiles: 122,218–404,638) kg, corresponding to an annual economic loss of 147,042,168 (2.5 and 97.5 percentiles: 68,442,231–226,597,118) yen, or 1,391,649 (2.5 and 97.5 percentiles: 647,757–2,144,580) US dollars. According to the sensitivity analysis, when the highest option, 39.8 % was used for the proportion of H-PVL cows in an infected herd, the estimated economic loss in Hokkaido (USD 1,489,230) was almost USD 100,000 greater than the loss using our result (USD 1,391,649, Table 7).

4. Discussion

This study investigated the theoretical causal relationships among BLV infection status, the number of AFPE, and carcass weight of culled dairy cows (which contribute to the food chain), adjusted for age, lactation stage, and between-herd variability. In these tripartite relationships, we hypothesized that BLV infection caused carcass weight loss, mediated by an increased number of AFPE due to degraded immune status. Our results supported that mediation exists for H-PVL cows.

This work represents the first prospective cohort study investigating the influence of BLV infection on bovine carcass weight, using meat inspection records, to our knowledge. In other work, pre- and post-mortem inspection data have been utilized to ensure the safety of meat products and to plan control strategies for infectious diseases at the farm level (Thomas-Bachli et al., 2014; Adachi and Makita, 2015, 2017; MHLW, 2017). The present study is unique because this analysis further applied the meat inspection data for understanding multiple theoretical causations in economic loss due to BLV, using the information collected at farms.

Malignant lymphoma, which is the pathology most strongly associated with BLV infection, is the most common reasons for cow carcass condemnations in the US (Bartlett et al., 2020), and it would be easy to estimate the resulting loss in Japan as well. However, that aspect was beyond the scope of our study. The annual economic loss due to carcass weight loss in Hokkaido, which has not been determined previously, was not negligible (US $1,391,649); this value would be an important piece of information for farmers considering participation in voluntary BLV control programs. The farmers participated in this study were conducting BLV control, and the proportion of H-PVL cows might be underestimated. The sensitivity analysis suggested that actual economic loss in Hokkaido prefecture might be far greater than this figure.

The mediation analysis suggested the significant effect of immunosuppression on carcass weight, as the credible interval of indirect effect on carcass weight caused by AFPE did not include zero (or was marginal) for H-PVL cows. BLV causes immune deregulation after the primary infection stage (EFSA, 2015), and H-PVL cows become immunosuppressed (Frie et al., 2017). We hypothesize that the increased number of AFPE among H-PVL cows reflects immunosuppression due to BLV infection. According to the literature, most of the macroscopic lesions caused by BLV infection are found in the digestive organs such as omasum, abomasum, intestine, liver, and spleen; clinical signs depend on the site of formation of macroscopic lesions, leading to symptoms such as digestive disturbances, inappetence, weight loss, and weakness (OIE, 2012). Our results showed similar findings, suggesting that BLV-associated damage to the digestive organs negatively affected carcass weight.

Carcass weight loss also may be a direct result of BLV infection. Although the credible intervals of direct effects of L-PVL and H-PVL

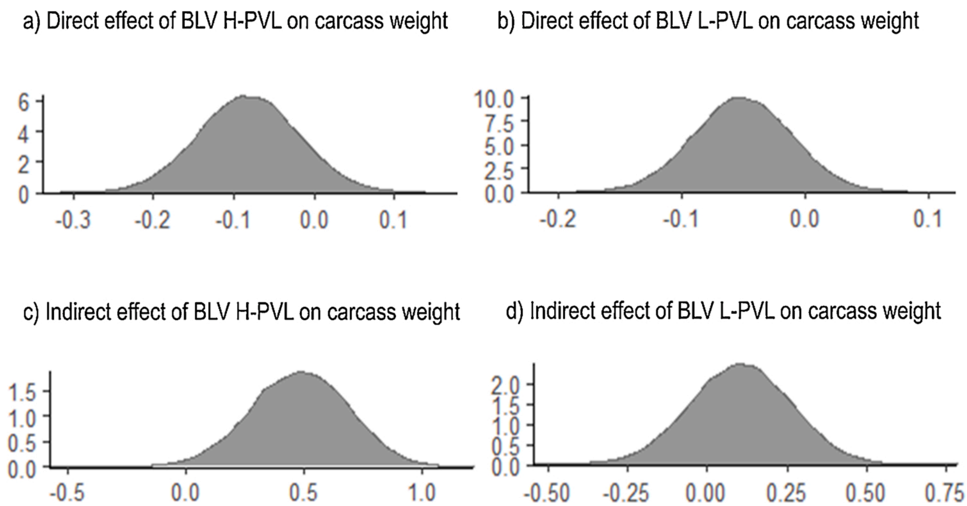

Fig. 3. Posterior distributions of precise direct and indirect effects of BLV infection on carcass weight, as estimated by the mediation analysis. The x-axes show the value spaces, and the y-axes show the probability densities. Panels (a) and (b) show the direct effects of BLV H-PVL and L-PVL on carcass weight, respectively; and panels (c) and (d) show the respective indirect effects.

Table 7
A sensitivity analysis result of the proportion of high-viral load (H-PVL) cows in an infected herd to the economic loss in Hokkaido prefecture estimated.

Proportion of H-PVL cows in an infected herd	Economic loss in US dollars	Economic loss in Japanese Yen
25.6 %	1,391,649	147,042,168
33.3 %	1,435,580	151,683,955
39.8 %	1,489,230	157,352,587

cows included zero, the distributions suggested that a direct effect may exist (Fig. 3). As bovine leukemia progresses, the numbers of both BLV-PVL copies per cell and lymphocyte increase (Nakada et al., 2018). The organs that incorporate large number of lymphocytes therefore harbor BLV at high copy number; for instance, in a previous study conducted in a slaughterhouse, lymph nodes and spleens from EBL cows had higher BLV PVL than those from asymptomatic BLV-infected cows (Somura et al., 2014). Immunodeficiency arises as BLV infection develops to the PL stage (Kabeya et al., 2001); thus, H-PVL may induce carcass weight loss through malfunction of organs that showed abnormal findings.

The largely calculated mediation resulted from carcass loss, which itself reflected increased AFPE caused by H-PVL; these losses confirm the importance of priority culling of H-PVL cows for controlling BLV infection (Ruggiero et al., 2019). In the dairy industry, the detection and culling of H-PVL cows are critical not only for BLV infection control but also for securing income from carcasses. Another strategy for preventing economic loss from BLV infection is to prevent the development of high BLV viremia in infected cows. Although it is in the research stage, two approaches can be explored. The first approach is the use of cattle bred to be tolerant to BLV. Specifically, bovine lymphocyte antigen class II haplotypes are associated with BLV PVL (Miyasaka et al., 2013), and cattle breeds lacking these haplotypes may be BLV tolerant. The second approach is the treatment of BLV using immunoinhibitory molecules, such as Programmed cell death protein 1 (PD-1) and its ligand (PD-L1), which have been shown to be effective against cows with high levels of viremia (Sajiki et al., 2019).

There is a limitation in this study related to field data. The sample size was small and there were only 11 HPVL cows. This may be because the 12 farmers were implementing BLV infection control practices and culling HPVL cows preferentially. In the absence of national control program of BLV, collection of longitudinal infection status data of BLV in a dairy farm is a big challenge. Moreover, acquiring consents of the access to meat inspection data from farmers, and collecting the data from the authority were additional challenges. The small number of H-PVL cows was taken into account by the large uncertainty of the economic loss, by using the standard error of GLMM result. Even with the larger uncertainty, the loss was found to be significant. The concern of possibly lower proportion of H-PVL cows in the study population due to the ongoing BLV control program was adequately addressed by the sensitivity analysis.

5. Conclusion

In conclusion, this study suggested that infection with BLV decreased the carcass weight of culled dairy cows and resulted in significant economic loss in Hokkaido. Our results indicated that persistent high BLV PVL causes a reduction in carcass weight due to an increased number of AFPE. Priority culling of high-viremia cows is a reasonable strategy in terms of both BLV infection control and economics.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2021.10

5528.

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