Original Article

# Assessment of Hematological Parameters and Carcass Weight in Bovine Leukemia Virus Infection in Slaughtered Beef Cattle

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(Received 25 Apr, 2018/Accepted 15 Jun, 2018)

**Summary**

Most bovine leukemia virus (BLV)–infected cattle do not have clinical signs (aleukemic AL), but some develop persistent lymphocytosis (PL) and B-cell lymphosarcoma (enzootic bovine leucosis [EBL]). BLV infection is a well-known cause of chronic wasting disease, which is associated with a reduction in milk productivity and immunity in dairy cattle. However, the effect of BLV infection on beef cattle is not clear. The objective of this study was to investigate the effect of BLV infection on the productivity of slaughtered beef cattle. A total of 997 blood samples were collected from cattle in 2 slaughterhouses in Miyazaki prefecture, Japan. BLV-antibodies were tested in these cattle's blood samples using enzyme-linked immunosorbent assay (ELISA), to identify BLV-infected cattle. We compared blood parameters and carcass weight between BLV ELISA-positive and ELISA-negative cattle in two age groups : young ($\leq$ 60 months) and elder (> 60 months) groups. The results showed that the proportion of ELISA-positive cattle in the young and elder groups were 22.8% and 24.9%, respectively. The number of white blood cells (WBCs) and lymphocytes in ELISA-positive cattle was significantly higher than that in ELISA-negative cattle in the young group. In addition to the number of lymphocytes, the number of monocytes and neutrophils were also significantly higher in BLV ELISA-positive cattle than in ELISA-negative cattle in the elder group. There was no significant difference in the carcass weight between ELISA-positive and ELISA-negative cattle in both groups. The results of this study suggest that BLV infection has an effect on the host immune response in beef cattle.

Keywords : Bovine leukemia virus, ELISA, Beef cattle, Carcass weight, Blood parameters

## 1. Introduction

Bovine leukemia virus (BLV), which belongs to the genus *Deltaretrovirus* of the family *Retroviridae*, is the causative agent of enzootic bovine leukosis (EBL)[5]. Most BLV-infected cattle remain healthy, but some develop a disease known as persistent lymphocytosis (PL); rarely, the infection can result in B-cell lymphoma[9].

BLV infection has a worldwide distribution, and EBL has been listed by the World Organization for Animal Health (OIE) as a disease that can have a significant impact on international trade[22].

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According to previous studies, the cattle-level prevalence is 38.6% in the United States of America[2], 42.3% in Peru, 27.9% in Chile, 30.7% in Bolivia, 54.7% in Paraguay, 77.4% in Argentine[26], 0.04% in Italy[16] and 9.1% in Mongolia[21]. In Japan, EBL is a notifiable disease and has been subject to passive surveillance since 1997[17]. The prevalence of BLV infection increased yearly and reached 35.2% in 2009–2011[19].

BLV infection causes direct productivity losses in the affected farms. Death or culling of cows due to EBL is a direct cost associated with infection[6, 25]. Milk production is reduced by about 2.5 % in herds classified as BLV-infected compared with the production in BLV-free herds[8]. The decline in annual milk production associated with each percentage-point increase in BLV-seropositivity is 4.7 kg per cow[24]. The interaction term between BLV-status and longevity of the cows has been observed to be highly significant, which indicates BLV infection affects milk production in dairy cattle[20]. It has also been shown that the infection is associated with a slight increase in the interval from calving to last service and in the risk of cystic ovaries[8]. The spontaneous recovery rate from ringworm (*Trichophyton verrucosum*) in BLV-negative cows is significantly higher than that in BLV-positive cows[4]. These findings explain why the life expectancy of BLV-positive cows is significantly less than that of BLV-negative cows[4, 8, 20]. However, to the best of our knowledge, few studies have reported the effects of BLV infection on the productivity of beef cattle. Our objective was to investigate the association between BLV infection and hematological parameters and carcass weight in slaughtered beef cattle.

## 2. Materials and Methods

### 2.1. Sample collection

This study was conducted in 2 slaughterhouses (slaughterhouse A and B) in Miyazaki prefecture, which is located in Kyushu region, southern Japan, between 31°21′ and 32°50′ N latitude and between 130°42′ and 131°53′ E longitude. Sample collection was performed from December 2015 to June 2016 and from August 2016 to September 2016 in slaughterhouse A and B, respectively. The samples comprised of the blood discharged from the neck during the slaughtering process, and it was collected in EDTA tubes by veterinarians. The blood samples were collected from all slaughtered beef cattle during each slaughtering day. The information about all the slaughtered beef cattle, including sex, age, breed, and carcass weight, was obtained from the meat inspection office of each slaughterhouse.

### 2.2. Blood test

Hematological parameters were measured in the blood samples at Health Sciences Research Institute., Inc., Miyazaki, Japan. The measured parameters were red blood cell (RBC) count, hemoglobin level, hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and white blood cell (WBC), basophil, eosinophil, neutrophil, lymphocyte, and monocyte counts, which were analyzed by XE-2100 (Sysmex, Kobe, Japan).

### 2.3. BLV ELISA test

The blood in the EDTA tubes was centrifuged at 1500 *g* for 5 minutes to obtain plasma, which was then stored at −20℃ in the laboratory of the University of Miyazaki. Each plasma sample was tested using a commercial enzyme-linked immunosorbent assay (ELISA) kit (JNC Co., Ltd., Tokyo, Japan). The procedures were performed according to the manufacturer's instructions.

### 2.4. Statistical analysis

The F-test was used if the variances in the two groups were equal. The independent Student t-test was used for equal variances, and the Welch's t-test was used for unequal variances to assess the difference in each parameter between BLV ELISA-positive cattle and ELISA-negative cattle. P values $<0.05$ were considered statistically significant. All statistical analyses were performed using the R software (version 3.4.0; R development core team, Vienna, Austria).

### 2.5. Ethical approval

The study protocol was reviewed by the Cattle Ethics Committee of the University of Miyazaki's Faculty of Agriculture.

## 3. Results

A total of 997 blood samples were collected from the cattle; 457 and 540 samples were collected in slaughterhouse A and B, respectively. All the cattle were healthy. Thirty-six samples were excluded from the analysis because the data for carcass weight data was missing. Out of the 961 samples, 228 (23.7%) tested positive in the ELISA test (Table 1). The study population was divided into two categories : young and elder groups. Fattening and breeding cattle were mainly categorized into the young and elder group, respectively. The cut-off value for age categorization was 60 months (Table 2). With respect to hematological parameters, the number of WBCs and lymphocytes were significantly higher in the ELISA-positive cattle than in the ELISA-negative cattle in the young group (Table 3). The hematocrit in BLV ELISA-positive cattle was significantly lower than that in ELISA-negative cattle in the young group. In the elder group, the number of WBCs, neutrophils, lymphocytes, and monocytes were significantly higher in ELISA-positive cattle than in ELISA-negative cattle (Table 3). There was no significant difference in the carcass weight between ELISA-positive and ELISA-negative cattle in both groups (Table 3).

## 4. Discussion

This is the first report of assessment of the effects of BLV infection on hematological parameters and carcass weight in slaughtered beef cattle. The results of this study showed there were significant differences between BLV ELISA-positive and ELISA-negative beef cattle with respect to hematological parameters (hematocrit and WBC and lymphocyte counts in the young

Table 1 Proportion of BLV ELISA positive cattle depending on sex and breed

| | | ELISA positive N (heads) | ELISA negative N (heads) | Total N (heads) | Proportion of ELISA positive animals (%) |
|---|---|---|---|---|---|
| Sex | Male | 93 | 268 | 361 | 25.8 |
| | Female | 135 | 465 | 600 | 22.5 |
| Breed | Japanese Black | 206 | 707 | 913 | 22.6 |
| | Holstein | 11 | 5 | 16 | 68.8 |
| | F1 | 11 | 21 | 32 | 34.4 |
| Total | | 228 | 733 | 961 | 23.7 |

Table 2 Descriptive statistics values of cattle age by cut off

| Statistics for Age (months) | Young group | Elder group |
|---|---|---|
| Minimum | 15.2 | 62.3 |
| 25th percenitile | 27.6 | 117.0 |
| Median | 28.7 | 148.0 |
| 75th percentile | 30.2 | 173.0 |
| Maximum | 54.4 | 256.0 |
| Mean | 29.2 | 145.9 |
| Number of ELISA positive animals (proportion) | 122 (22.8%) | 106 (24.9%) |

Table 3 Comparison of hematologic parameters and carcass weight between BLV ELISA positive and negative cattle

| | Young group | | | | | Elder group | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ELISA positive | | ELISA negative | | P-value | ELISA positive | | ELISA negative | | P-value |
| Hematologic parameters | Mean | SE | Mean | SE | | Mean | SE | Mean | SE | |
| RBC ($\times 10^4/\mu L$) | 810.83 | 116.50 | 828.84 | 107.86 | NS | 641.43 | 191.88 | 646.55 | 177.80 | NS |
| Hemoglobin (g/dL) | 14.08 | 2.04 | 14.46 | 1.87 | NS | 12.12 | 3.99 | 12.16 | 3.56 | NS |
| Hematocrit (%) | 41.00 | 5.67 | 42.22 | 5.05 | 0.024 | 36.37 | 10.97 | 36.61 | 10.15 | NS |
| MCV (fL) | 50.84 | 4.37 | 51.21 | 3.71 | NS | 57.04 | 6.12 | 56.86 | 5.12 | NS |
| MCH (pg) | 17.41 | 1.36 | 17.48 | 1.13 | NS | 18.82 | 1.88 | 18.79 | 1.60 | NS |
| MCHC (%) | 34.31 | 1.22 | 34.21 | 1.20 | NS | 33.08 | 1.80 | 33.11 | 1.52 | NS |
| WBC ($/\mu L$) | 7818.03 | 2922.78 | 6754.11 | 2192.02 | <0.001 | 6656.60 | 3596.02 | 5107.84 | 1813.85 | <0.001 |
| Basophil ($/\mu L$) | 5.63 | 21.01 | 3.78 | 15.89 | NS | 3.57 | 12.69 | 5.32 | 17.70 | NS |
| Eosinophil ($/\mu L$) | 219.19 | 255.58 | 194.55 | 225.01 | NS | 223.45 | 296.48 | 192.76 | 285.66 | NS |
| Neutrophil($/\mu L$) | 4488.35 | 1970.12 | 4335.08 | 1901.66 | NS | 3018.02 | 1825.98 | 2614.08 | 1300.33 | 0.038 |
| Lymphocyte ($/\mu L$) | 2789.57 | 1500.06 | 1918.94 | 824.07 | <0.001 | 3078.20 | 2422.00 | 2028.70 | 1022.20 | <0.001 |
| Monocyte ($/\mu L$) | 315.29 | 229.04 | 301.76 | 207.85 | NS | 333.37 | 253.03 | 266.97 | 158.20 | 0.013 |
| Carcass weight (kg) | 463.67 | 68.28 | 459.19 | 53.72 | NS | 333.83 | 51.27 | 338.59 | 52.34 | NS |

group; and WBC, neutrophil, lymphocyte and monocyte counts in the elder group). These parameters (except for hematocrit) were significantly higher in BLV ELISA-positive cattle than in BLV ELISA-negative cattle. On the other hand, a significant difference in carcass weight between BLV ELISA-positive and ELISA-negative cattle was not observed.

B lymphocytes are the major target cells of BLV[18]. The BLV provirus encodes a series of accessory genes that modulate viral and/or cellular gene expression in B lymphocytes. The function of the tax gene, which is one of the accessory genes, is the activation of viral transcription and transformation of BLV-infected B lymphocytes[1, 9]. Hematological clues based on the number of lymphocytes and the BLV proviral load provide the initial means of identifying cattle at risk for developing EBL[23]. As expected, the numbers of WBCs and lymphocytes in BLV ELISA-positive cattle were significantly higher than those in ELISA-negative cattle in both the young and elder groups. In addition to the number of lymphocytes, the numbers of monocytes and neutrophils in BLV ELISA-positive cattle were also significantly higher than those in ELISA-negative cattle in the elder group, but they were not significantly different in the young group. On the other hand, the hematocrit in BLV ELISA-positive cattle was significantly lower than that in ELISA-negative cattle in the young group, but it was not significantly different in the elder group. These differences in the effects between the young and elder group were considered to be due to the animal's age and farm management practices. Hematological parameters are influenced by age, nutrition, physical activity and environmental conditions[3, 7, 15]. The young group was comprised of fattening cattle, and the elder group was comprised of breeding cattle in this study. The immune response is strongly related to the farming system[11, 13]. However, there is little evidence to support this explanation because immunological analysis was not conducted in this study. Further studies are needed to understand the effects of BLV infection on the immune system.

In general, the stimulation of the host immune response is associated with a proportional decline in growth and cellular proliferation, which leads to a decrease in body size, with resource allocation shifting toward survival and away from nonessential processes, such as growth[14]. Some of these effects are difficult to be prove, and they may not always be statistically significant although it has been observed there are losses in potential production[10]. Although the carcass weight in BLV ELISA-positive slaughtered beef cattle was not significantly different compared to that in ELISA-negative slaughtered beef cattle in this study, it is difficult to conclude that BLV infection has no effect on carcass weight. We compared the carcass weight between BLV ELISA-positive and ELISA-negative cattle without considering the viral load in the BLV-infected cattle. An increase in the proviral load correlates with disease progression in BLV-infected cattle[12]. It is still possible that the progression of BLV infection may have negative effects on the productivity of fattening cattle. Further studies would be required to clarify the effects of BLV infection on carcass weight.

In conclusion, BLV infection significantly affects hematological parameters in slaughtered beef cattle. This finding has important implications for understanding host immune response to BLV infection in beef cattle.

## 5. Acknowledgement

This work was supported by the Ito Foundation. The funders had no role in the study design, data collection, analysis and interpretation of data, writing of the report, or in the decision to submit the article for publication.

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This manuscript has not been published and is not under consideration for publication elsewhere. All the authors have read the manuscript and have approved this submission.

## 原　著

# 牛白血病ウイルス感染症がと畜牛の血液性状と枝肉重量に与える影響の評価

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## 要旨

牛白血病ウイルス（BLV）に感染した牛の大部分は，無症候感染（AL）であるが，一部の BLV 感染牛は持続性リンパ球増多症や B 細胞性のリンパ腫である地方病性牛白血病を引き起こす。BLV は AL 牛においても乳量の低下や免疫力の低下などの生産性に影響を及ぼす慢性消耗性疾患であることが知られているが，肉用牛における影響は明らかではなかった。そこで本研究は，と畜牛における BLV 感染症の影響を評価することを目的とした。2015 年から 2016 年にかけて宮崎県内のと畜場 2 か所において，997 頭のと畜牛の血液を採取した。BLV に対する抗体を検出する ELISA を用いて BLV 感染の有無を調べた。主に肥育牛が含まれる 60 か月齢以下の若齢グループと，繁殖母牛が含まれる 60 か月齢超の老齢グループに分けて，BLV 抗体陽性牛と陰性牛の間の血液性状と枝肉重量を比較した。その結果，ELISA に陽性を示した割合は若齢グループと老齢グループそれぞれ 22.8% と 24.9% であった。若齢グループにおいて，ELISA 陽性牛の方が陰性牛に比べて白血球数およびリンパ球数が有意に高かった。また老齢グループにおいて ELISA 陽性牛の方が陰性牛に比べて白血球数，好中球数，リンパ球数および単球数が有意に高かった。枝肉重量には有意差が見られなかった。本研究の結果は，BLV 感染が肉用牛の宿主免疫応答に影響を与える重要な知見となり得る。

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