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Clinical Staging and Hematological Evaluation of Enzootic Bovine Leukosis in American Friesian Cattle

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Bovine leukemia virus, Asymptomatic, Disease progression, Lymphocytosis, Lymphopenia complete blood count

ABSTRACT

Enzootic bovine leukosis (EBL) disturbs the immune system, increasing infection incidence. Clinical staging, along with hematological analysis is crucial for disease diagnosis and its progression. Our objective was to determine the clinical staging among the different age groups of BLV seropositive dairy cattle. Blood samples (n= 72) were collected from three age groups of American Friesian dairy cattle and 24 samples were collected from each group. The seroconversion against BLV was determined by using competitive ELISA, and the complete blood count was determined by using a Hematological analyzer. Hematological parameters were measured in all seropositive cattle and divided into three clinical stages based on lymphocyte and white blood cell counts. This study revealed a 37.5% seroprevalence of BLV in American Frisian dairy herd. During the progression of the disease, there was a significant change in white blood cells, lymphocytes, and monocytes. Lymphopenia developed in a later stage of the disease, with lower mean values of lymphocytes and white blood cells. The effect of age on disease occurrence and progression was significant, as evidenced by the higher seroprevalence in older animals. In conclusion, overall hematological alterations and clinical stages provide a clear visualization of the progression and management of the disease.

INTRODUCTION

Enzootic bovine leukosis (EBL) caused by the bovine leukemia virus (BLV) belongs to the *Retroviridae* family, *Orthoretrovirinae* subfamily and *Deltaretrovirus* genus which includes human T-cell leukemia virus types I and II, feline immunodeficiency virus, feline leukemia virus, and simian T-cell leukemia virus (Gillet *et al*., 2007; Frossard, 2022). BLV infection disrupts the cattle immune system, leading to poor resistance to infections and vaccinations (Emanuelson *et al*., 1992; Erskine *et al*., 2011). BLV-infected animals experience production loss, longevity, and trading restrictions, causing significant economic loss to the global cattle industry (Khudhair *et al*., 2016). The BLV-infected cattle's apparent prevalence 46.5% of US dairy herds, 84% of Argentina dairy herds, 40.9% of dairy cattle in Japan, and other countries, impacting the cattle industry remains a complex epidemiological issue (Trono *et al*., 2001; Murakami *et al*., 2013; LaDronka *et al*., 2018). Diagnostic testing for bovine leukosis has evolved with PCR and ELISA now commonly used, replacing the previously standard AGID (agar gel immuno-diffusion) test as the first serological method recommended by the OIE (Hoff-Jorgensen, 1989; González *et al*., 2007). ELISA is more efficient, sensitive, and accessible method than AGID (Simard *et al*., 2000). BLV is epidemiologically associated with malignancy in lymphocytes and a susceptibility to lymphoproliferative disorders. Based on disease progression, EBL is classified

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into aleukemic, persistent lymphocytosis (PL), and malignant stages (Trono *et al*., 2001; Radostits *et al*., 2006). Clinical staging includes determining normal and abnormal lymphocytic and leukocyte counts indicating the occurrence of proliferative disorders. BLV positive animals remained infected throughout their lives, with 30% of animals developing persistent lymphocytosis, and more likely to develop B cell tumors (Gillet *et al*., 2007). Hematological keys determined animals at risk for EBL, about half of tumor developing cattle exhibit persistent lymphocytosis, a subclinical infection stage (Mammerickx *et al*., 2010). BLV infected animals develop persistent leukocytes, lymphocytes, and neoplastic proliferation, which mostly influence later stages of age. About 1-5% of infected animals develop malignancy conditions involving B lymphocytes in spleen, abomasum, and mammary glands. This significantly reduces productive life and carcass value (Nakada *et al*., 2022; Benitez *et al*., 2022).

Clinical staging is crucial for understanding disease progression, prognosis, and treatment (Fatima *et al*., 2025). The European community leukosis key is a haematology technique used to detect BLV infection in dairy cattle by identifying persistent lymphocytosis through lymphocyte count and age (Akagami *et al*., 2019). Neoplastic disease often resulting abnormalities in blood (hematopoietic system), is challenging to diagnose because it has a latent period during the asymptomatic stage. However, disruptions in normal hematological parameters indicate disease severity and its progression. Hematological analysis is a reliable method for determining EBL stages, but there is limited information on specific changes. The study aimed to determine changes in haematology cellular distribution in BLV infected cattle, excluding the uninfected cattle, across EBL clinical stages.

MATERIALS AND METHODS

American Friesian dairy cattle (n=72) were selected on a random basis from different age groups at the dairy farm located in Punjab with a milking herd size of about 700. The milking herd was categorized into three different age groups A (<4 years), B (<6 years), and C (>6years), and 24 animals were randomly selected from each group. Blood samples were collected using the 6 ml EDTA-K3 containing tube (Vacuum tube, Bio Vac, USA) via a jugular vein. Samples were transported in an ice box for further processing to the University Diagnostic Lab, UVAS at 4˚C and processed within 48 h. A 3 ml EDTA-mixed whole blood sample aliquot was set aside for hematology analysis. Serum was separated from the remaining EDTA-mixed blood by centrifugation at 1500 rpm for 5 min at 10˚C and stored in 1.5 ml Eppendorf tubes at -20 ºC. Seroconversion against BLV has been determined by using a competitive ELISA to detect anti-gP51 antibodies (ID Screen® BLV Competition, USA) as per instructions provided by the manufacturer. The EDTA mixed whole blood aliquot of all seropositive samples was further evaluated to determine the complete blood count by using an automatic junior jet vet hematological analyzer (Abacus Junior Vet). Heamotological parameters (haemoglobin, red blood cell, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, white blood cell, lymphocyte, granulocyte, monocyte, and platelet count) of all seropositive animals were measured, and divided into 3 clinical stages (asymptomatic, persistent lymphocytosis, lymphopenia) based on lymphocyte and white blood cell count.

To assess the BLV seropositivity, a binomial test with a mixed effect model was conducted by using GLIMMIX in SAS software (SAS 9.4 version; SAS Institute Cary, NC) with a logit link, as Elisa seropositivity status was binary (0 = negative and 1 = positive). We have performed descriptive statistics to summarise the distribution of each hematological parameter within the infectious status of the animal and analysis of variance (ANOVA) to assess the overall significant changes in the hematological parameters across the clinical stages.

RESULTS AND DISCUSSION

In the current investigation, overall seroprevalence of BLV was found 37.5% (27/72) in American Frisian dairy cattle. Table I presents the mean and standard deviation of haemotological parameters in seropositive animals. In seropositive cattle, an earlier stage of disease was characterized as asymptomatic (AS) (n=10), during which all parameters were observed within the normal range. However, the mean values of granulocyte and monocyte count (6.55×10^9/L and 1.35×10^9/L) were observed higher than the reference values (0.6-4.0×10^9/L and 0.0-0.8×10^9/L). In the second stage of disease, referred to as persistent lymphocytosis (PL) (n=12), the mean values of white blood cell (WBC), lymphocyte (LYM), granulocyte (GRAN), and monocyte (MONO) count increased, while haemoglobin (HB), red blood cell (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and platelet count were all within normal range values. It was observed that lymphopenia typically emerged in later stages of the disease (n=5), characterized by lower mean values of WBC, LYM, and RBC count. During disease progression, disruptions were observed in various parameters; however, statistically significant alterations were found only in white blood cell, lymphocyte,

Table I. Hematological values (mean ± standard error) of BLV seropositive American Friesian cattle (n=27) across the clinical stages.

Hematological parameters[3]	Clinical stages[1]			P-value[2]
	AS	PL	L	
Haemoglobin (g/dl)	9.94 ± 1.46	10.11 ± 1.71	9.18 ± 1.73	0.56
Red blood cells (g/dl)	6.63 ± 1.00	6.80 ± 1.38	5.85 ± 1.38	0.37
Mean corpuscular haemoglobin (pg)	15.12 ± 0.42	14.97 ± 0.72	15.96 ± 1.53	0.09
Mean corpuscular haemoglobin concentration (g/dl)	36.85 ± 0.32	34.87 ± 6.07	37.60 ± 1.47	0.39
Mean corpuscular volume (fl)	40.84 ± 1.06	40.37 ± 2.64	42.28 ± 3.91	0.29
White blood cells (× 10^9/L)	11.21 ± 4.66	25.25 ± 14.53	5.78 ± 1.23	<0.01
Lymphocytes (× 10^9/L)	3.70 ± 1.04	18.40 ± 13.24	1.92 ± 0.27	<0.01
Granulocytes (× 10^9/L)	6.55 ± 3.60	6.76 ± 3.83	3.38 ± 0.95	0.17
Monocytes (× 10^9/L)	1.35 ± 0.35	1.75 ± 0.66	0.48 ± 0.08	<0.01
Platelets (× 10^9/L)	225.80 ± 72.54	217.75 ± 77.14	180.60 ± 51.64	0.51

[1]AS, asymptomatic; PL, persistent lymphocytosis; L, lymphopenia; [2]P value in the column represents the significant change in hematological parameters across the infection status (P <0.01). [3]pg, pictogram; fl, femtoliter; L, liter.

and monocyte count (P <0.01) as shown in Table I. There was a statistically significant relationship between the animal age and occurrence of BLV because both were directly linked (P <0.01). It was observed that Seropositivity and progression of disease were higher in group C (> 6 years) as compared to other groups (Fig. 1).

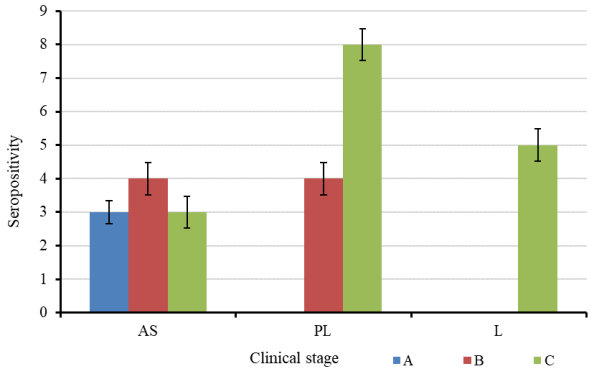

Fig. 1. Progression of disease significantly influenced by age. Age groups A (<4 years), B (<6 years), and C (>6 years) had 3 different clinical stages. AS, asymptomatic; PL, persistent lymphocytosis; L, lymphopenia in BLV ELISA positive American Friesian cattle based on lymphocyte count. Error bars indicate standard deviation in age groups according to stages.

Our objective was to evaluate the clinical staging among the BLV seropositive dairy cattle, and the impact of BLV infection on hematological parameters. BLV infection status in seropositive dairy cattle was determined by analyzing the alterations in blood cell counts. Clinical staging was classified into 3 categories based on the BLV infection status in seropositive cattle.

This study found a noteworthy association between the BLV infection and notable significant alterations in white blood cell, lymphocyte, and monocyte count (P <0.01) as the disease progressed in seropositive cattle. This suggests a higher level of infection severity. These hematological parameters play a crucial role in disease progression. This finding corroborates previous research by Williams *et al*. (1988); Nikolay *et al*. (2013); Chen *et al*. (2020), which also demonstrated significant associations between BLV infection and change in WBC, lymphocyte, and monocyte counts. While some studies, such as Petropavlovskiy *et al*. (2019) highlighted the significant impact by revealing increased leukocyte and lymphocyte numbers, but decreased monocytes.

During the asymptomatic stage (AS) of BLV infection, seropositive cattle exhibited significantly elevated WBCs, reaching the upper limit of the normal range (11.2 ×10^9\L compared to the 4-12×10^9\L normal range). Moreover, the mean values of granulocytes and monocytes were observed to be higher than the normal range. This deviation suggests that BLV infection can significantly alter the host's hematological profile. During this condition, the virus remained in latency, and cell-mediated immunity (CMI) suppresses disease progression. CMI is crucial for controlling BLV replication. However, its decline may accelerate infection (Kabeya *et al*., 2001). The alteration in WBC counts observed in this study may serve as an indicator of asymptomatic infection and provide insights into the host immune system.

The study outcomes revealed that BLV infection

tends to remain latent during the asymptomatic stage but may progress to persistent lymphocystosis (PL), which is characterized by a significant increase in mean of WBC, LYM, GRAN, and MONO counts. This elevation in WBC and LYM counts may indicate BLV infection and can be used to assess infection activation status (Chen *et al*., 2020). B lymphocytes are the preferentially target of BLV, with B-cell expansion and its impairment mainly impaired the host immune system, especially in PL according to Libera *et al*. (2012). Meanwhile, Chen *et al*. (2020) reported an increase in T and B lymphocytes. PL involves an elevated lymphocyte count exceeding 8000/µL that remains despite the absence of visible signs, as it consistently triggers the immune response (Okagawa *et al*., 2012; Khudhair *et al*., 2016). Evidence suggests that mean values of monocyte count were significantly elevated during the both AS and PL stages of disease. However, this finding, regarding the role of monocytes in BLV infection was mixed. While some studies, such as Heeney *et al*. (1992) have reported evidence of BLV infection in monocytes, suggesting a broader impact on the immune system. Mirsky *et al*. (1996) found no evidence of BLV provirus in monocytes, suggesting that they may not be a significant target of virus.

One critical transition was the shift from persistent lymphocytosis stage to lymphopenia stage of disease. This stage was marked by the decrease in the mean values of WBCs and LYM count. Lymphopenia is a concerning development, accompanied by lymphocyte exhaustion. T-cell exhaustion was driven by the increased expression of immunosuppressive factors which induced immune exhaustion, specifically T-cell, and diminishing cell proliferation (Barber *et al*., 2006; Velu *et al*., 2009; Okagawa *et al*., 2018). This study revealed a significant correlation between animal age and BLV occurrence in older animals showing higher BLV infection seroprevalence. According to previous studies, age has a significant influence on the progression of infection and may increase the risk of BLV infection (Barros Filho *et al*., 2010; Morovati *et al*., 2012; Selim *et al*., 2020). The study emphasizes the significance of monitoring blood parameters as a key indicator of BLV infection severity, and provides an understanding of disease progression.

CONCLUSIONS

Clinical staging is essential in evaluating the progression of disease in seropositive animals, as these animals may have different clinical presentations. During the asymptomatic stage, the virus latency period may not affect animal health. However, as the disease progresses in animals, it weakens the host immune system and becomes dormant. After disease diagnosis, clinical staging provides a clear visualization of disease extent and progression, which helps to develop effective disease control strategies.

DECLARATIONS

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Ethical statement

All the experimental procedures involving animals were approved by the Institutional Guidelines of the Ethical Review Committee (No. DR 325) of the University of Veterinary and Animal Sciences, Lahore.

Statement of conflict of interest

The authors have declared no conflict of interest.

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