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Seroprevalence of Bovine Leukemia Virus Infection in Cattle in Muş Province, Türkiye

Alaattin Sökmen[1,a], Ali Rıza Babaoğlu[2,b,*]

[1]*Department of Virology, Graduate School of Health Sciences, Van Yuzuncu Yil University, 65040 Van, Türkiye*
[2]*Department of Virology, Faculty of veterinary Medicine, Van Yuzuncu Yil University, 65040 Van, Türkiye*
[*]*Corresponding author*

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ABSTRACT

Bovine leukemia virus (BLV) is known as the causative agent of enzootic bovine leukosis (EBL), which is a worldwide distributed disease and has also been detected in marketed beef and dairy products. BLV causes significant economic losses due to the loss of milk and yield or the slaughter of animals without adequate development. It has been reported in epidemiological studies that this infection is common in Türkiye, especially in the western provinces. There is no data on the possible presence or prevalence of BLV infection and its seroepidemiology in Muş province. The aim of this study is to determine the possible presence and prevalence of the infection, its role in yield losses, and to obtain epidemiological data on cattle farming in the Muş district. For this purpose, 300 blood serum samples were collected from cattle aged six months and older in the province of Muş and its different districts. The blood serum samples taken were tested for the presence of BLV-specific antibodies by agar gel immunodiffusion (AGID) and competitive enzyme-linked immunosorbent assay (C-ELISA) methods. As a result of the study, all of the controlled districts were evaluated as negative in the AGID and C-ELISA tests for the presence of BLV-specific antibodies. In conclusion, for the first time, it was demonstrated that cattle farming in the Muş province were BLV-free during the sampling period. Although BLV seropositivity was not detected in the tested animals, it is emphasized that the control of infection and eradication program should not be ignored.

[a] *alaattinsokmen@gmail.com* *https://orcid.org/0000-0003-0024-8324* | [b] *arbabaoglu@yyu.edu.tr* *https://orcid.org/0000-0001-8023-3442*

Introduction

Bovine leukemia virus (BLV) is known as one of the important viruses of bovine lymphotropic virus infection and is the etiological agent of enzootic bovine leukosis (EBL), also known as bovine leucosis (Rovnak et al., 1991). BLV, directly and indirectly, causes economic losses in cattle farms, such as a decrease in animal productivity, early slaughter, and restrictions on the import of animals and animal products from BLV-infected areas. Dairy cattle are the natural hosts for BLV infection, but buffaloes and camels can also become naturally infected (Nishimori et al., 2016).

The etiological agent is BLV, an exogenous C-type oncovirus located in the *Retroviridae* family, in the *Deltavirus* genus. It is closely related to human T-lymphotropic virus types 1 and 2 (HTLV-1 and HTLV-2). This virus preferentially infects B lymphocytes but has also been detected in T-lymphocytes, monocytes, and granulocytes (Marawan et al., 2021). This virus, as an RNA virus, upon entry into a cell rapidly creates a DNA copy of its genome by the enzyme reverse transcriptase in the host cell, and this retrotranscribed DNA copy predominates in infected cells (Ruiz et al., 2018).

BLV infection is a worldwide-distributed retroviral disease and is highly prevalent in North and South America, Asia, and Eastern Europe. Although most European countries have become free of infection through an efficient eradication program, the prevalence of disease remains high worldwide. The prevalence of BLV in the Middle East was lower than in other districts of the world, except for Türkiye and Iran, with an average of 48.3% and 64.7%, respectively (Rodríguez et al., 2011). The disease is listed by the World Organization for Animal Health (OIE) as an important disease for international trade (Ruiz et al., 2018).

The most important sources for BLV infection are blood lymphocytes and other tissue products of infected cattle (Mekata et al., 2015). The infection is not only transmitted horizontally, such as by arthropods and

iatrogenic transmission, but can also be transmitted vertically by ingestion of colostrum from BLV-infected cows or in utero. Iatrogenic transmission may occur through surgical instruments or sleeve gloves contaminated with infected blood during rectal palpation (Esteban et al., 2009). Most BLV-infected cattle (approximately 70%) do not show any clinical symptoms, of which about 30% develop persistent lymphocytosis (PL), and 1-5% of infected cattle develop malignant B-cell lymphosarcoma causing EBL (Pandey et al., 2017). Lymphoma occurs in approximately 5-10% of BLV-infected cows, predominantly in animals older than 3-5 years (Gutiérrez et al., 2014).

In Türkiye, clinical and pathological cases of leukosis were detected in dairy cattle for the first time in 1942 (Burgu et al., 1990). In the following years, the presence of infection was reported in serum and milk samples taken from dairy cattle from different regions of Türkiye, with seroprevalence rates varying between 0 and 59.6% (Çabalar et al., 2001; Otlu et al., 2001; Gülaçti et al., 2004; Özgünlük et al., 2005; Kale et al., 2007; Avcı et al., 2013; Acar and Gür, 2013; Şimşek et al., 2017; Ayvazoğlu et al., 2021).

BLV infected cattle develop specific antibodies against the major core protein p24 and envelope (gp51) virion proteins in their serum and milk; therefore, antibody-based serological tests are widely used for the diagnosis and screening of BLV infection in cattle older than 6 months and are a good indicator of the disease (Constable et al., 2016). Among serological tests, ELISA and AGID are the reference techniques that are recommended by the OIE for the diagnosis of BLV infection through the detection of antibodies that are directed to BLV gp51 and p24 proteins. Since there is no effective vaccine against the disease, control of the disease is not possible. Therefore, detection and early diagnosis of the disease are of great importance in order to reduce the spreading and the economic losses it causes (Marawan et al., 2021). Eradication schemes for BLV infection were mainly based on serological diagnosis by the AGID test, followed by the separation or removal of infected animals. Although AGID is the gold standard, ELISAs have been frequently used due to their higher sensitivity. The AGID test is a specific but not very sensitive test for detecting antibodies in serum samples from individual animals. However, the relatively low sensitivity of AGID may result in the occurrence of low-titer BLV infections in clinically normal herds (Dolz and Moreno, 1999; OIE, 2012; Marawan et al. 2021). In retroviral infections, ELISA is a rapid method and is used as a diagnostic and screening test for BLV because of its high sensitivity and specificity (Mousavi et al. 2014; Elhaig et al., 2017).

Data on the seroprevalence of BLV infection in cattle are essential not only in countries that have been successful in eradication programs and have already made some progress in controlling the disease but also in countries that need to establish a strategy for eradication programs. Therefore, the present study was aimed to determine the seroprevalence of BLV antibodies in cattle in Türkiye based on the AGID test (IDVet, BLV AGID, France) and Bovine Leukosis Serum C-ELISA (ID Screen® BLV Competition, France).

Material and Methods

Ethical Statement

The study was approved by the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (approval date: 27/01/2022; decision no: 2022/01-02) and the Ministry of Agriculture and Forestry of the Republic of Türkiye (approval date and no: 17/01/2022-4144507).

Study Area and Sampling

The current study was carried out in the province of Muş and its five different districts located in the Eastern Anatolia region of Türkiye (Figure 1). A total of 300 serum samples were collected randomly from healthy-looking cattle over 6 months old from private livestock farms between March and October 2022 to determine the presence of BLV anti-gP51 antibodies (Figure 2). All serum samples of cattle collected into vacuum tubes were centrifuged at 2000 rpm, and the sera transferred to the stock tubes were inactivated at 56˚C for 30 minutes in order to inactivate indigenous complement and kept at -20˚C until testing. Sera samples were tested with an BLV-AGID kit and a competitive ELISA-Ab kit reported and validated in the previous study using the same commercial test kits to detect BLV anti-gP51 antibodies (OIE, 2012). The AGID and C-ELISA assays were performed according to the manufacturer's instructions.

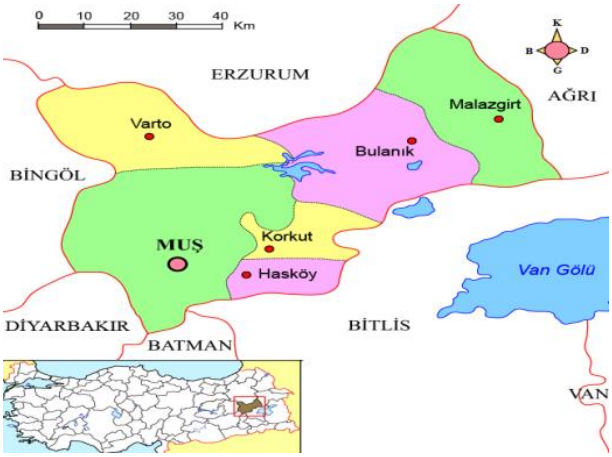

Figure 1. Geographical map of Muş province in Türkiye
The red circles show the location of Muş province and the districts where the cattle samples were collected

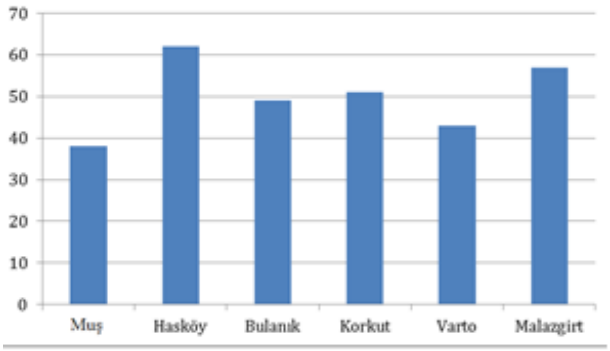

Figure 1. Distribution and number of serum samples collected in this study based on districts

Table 1. The number of samples based on districts and their serological results in AGID and C-ELISA methods

District	Number tested	BLV-AGID +	BLV-C-ELISA +
Muş (center)	38	-	-
Hasköy	62	-	-
Bulanık	49	-	-
Korkut	51	-	-
Varto	43	-	-
Malazgirt	57	-	-
Total	300	0%	0%

Results and Discussion

In the present study, antibodies against BLV infection were not detected in all 300 serum samples obtained from cattle from different districts of Muş province at ages ranging from 6 months to 10 years in either method by using BLV-AGID and BLV C-ELISA, as summarized in Table 1. The results of this study reveal that there was no virus circulation in cattle in Muş province during the sampling period.

Table 1. The number of samples based on districts and their serological results in AGID and C-ELISA methods

BLV infection is widespread all over the world except in western Europe (Rodríguez et al., 2011; Morovati et al., 2012). In Türkiye, infection was first reported in 1942 (Burgu et al., 1990) and studies on the seroepidemiology of the infection have been reported by many researchers using AGID and ELISA techniques in serum samples obtained from cattle (Otlu et al., 2001; Çabalar et al., 2001; Kale and Öztürk, 2004; Yildirim and Burgu, 2005; Özgünlük et al., 2005; Yavru et al., 2007; Yildirim et al., 2008; Acar and Gür, 2013; Ayvazoğlu et al., 2021). In the eastern neighboring countries, the prevalence rate of BLV infection has been reported to be between 0.5-25% in Iran (Morovati et al., 2012) and 7% in Iraq (Khudhair et al., 2016). Previous serological studies in Türkiye reported different seroprevalence rates for the presence of BLV antibodies.

The seropositivity of BLV infection was found to be 0% in cattle in the Kars region in 2001 (Otlu et al., 2001). In a study conducted in the Burdur region, the presence of BLV infection in dairy cows was found to be 4.9% in the AGID method and 19.18% in the ELISA method (Kale and Öztürk, 2004). In the Northeastern Anatolia region, BLV seropositivity rates were reported as 8% in Artvin, 4.87% in Erzurum, and 0% in Iğdır, Ağrı, Kars, Bayburt, Gümüşhane and Ardahan (Yildirim and Burgu, 2005). In the study carried out within the GAP (Southeast Anatolia Project), the AGID method was used on samples taken from cattle in nine provinces (Siirt, Diyarbakır, Batman, Adıyaman, Şanlıurfa, Gaziantep, Kilis, Mardin and Şırnak) in the Southeastern Anatolia region, 0.27% were positive for BLV antibodies (Özgünlük et al., 2005). The seroprevalence of BLV in culture-bred cattle owned by small-scale family farms in the Kars region has been reported as seronegative using AGID and ELISA methods (Yildirim et al., 2008). In the Afyonkarahisar in 2013, the rate of BLV positivity in cattle samples was determined to be 15.45%, and they reported that infection rates were low or even absent in small-scale family-type enterprises (Acar and Gür, 2013). In recent years, seropositivity has been reported as 0% in serum samples taken from cattle aged 1–10 years in Ardahan province (Ayvazoğlu et al., 2021). The data from these studies show that the seropositivity rates determined in private or public livestock where animal husbandry is carried out in large herds are much higher than the seropositivity rates in livestock in small family farms. In addition, the presence of BLV in cattle in the Eastern regions is lower and/or 0% compared to the Western and Central Anatolian regions of Türkiye.

In the current study, BLV seropositivity rate was found to be 0% in 300 blood serum samples collected randomly from cattle bred in different districts of Muş province by using AGID and ELISA. The results of this study are compatible with the results of previous studies conducted on BLV prevalence in the Eastern region of Türkiye (Otlu et al., 2001; Gülaçti et al., 2004; Özgünlük et al., 2005; Yildirim and Burgu, 2005; Yildirim et al., 2008; Acar and Gür, 2013; Şimşek et al., 2017; Ayvazoğlu et al., 2021). On the other side, the results of the current study showed compatibility with the results of previous studies that sampled animals on small family farms type in provinces close to Muş (Otlu et al., 2001; Özgünlük et al., 2005; Yıldırım and Burgu, 2005; Yıldırım et al., 2008; Ayvazoğlu et al., 2021).

Conclusion

Large animal livestock in the mentioned region is generally carried out in the style of small family farms for the purpose of livelihood. According to 2021 data, the total number of cattle in the region and its districts was reported as 328,207 heads. Therefore, it is important to reveal the epidemiological data and determine the control/eradication program based on the slow persistence and transmission routes of the infection. In this way, the status of BLV infection in cattle on small family farms in the region has been determined for the first time in terms of epidemiological dynamic information. In addition, although BLV seropositivity was not detected in the tested animals, it is thought to be useful to emphasize that a control/eradication program of BLV infection in animals bred on small family farms should be planned and initiated in advance.

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Conflict of Interest

The authors declared that they have no conflict of interest.

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