The prevalence of the enzootic bovine leukosis in cattle in Ardahan region

ABSTRACT

Enzootic bovine leucosis (EBL) is a retroviral infection which is common all over the world. EBL causes huge economic losses due to milk loss, yield loss and death. The aim of the present study was to investigate the presence of EBL by ELISA method from blood samples taken from cattle which were determined to be healthy because of clinical examination in Ardahan province and its counties in March-April 2021. For this, 500 cattle of different breeds and sexes between 1-10 years of age in different enterprises were sampled and the antibody response against EBL in the blood serum samples obtained was investigated with a commercial ELISA test kit. All 500 blood serum samples tested by ELISA were negative for EBL. In conclusion In conclusion, EBL seropositivity was determined as 0% in Ardahan region as the studied area and time period.

Keywords: Ardahan, Bovine leukaemia virus, ELISA

INTRODUCTION

The Ardahan region has ideal geographical features in terms of cattle breeding with its rich meadows, pastures, grasslands and plateaus, and is among the top 10 provinces of Turkey in terms of cattle presence (Ayvazoğlu & Demir, 2020). Therefore, cattle breeding creates an important economic income for the people of the region. Both sporadic and enzootic viral infections in cattle breeding Enzootic bovine leukosis, EBL; infectious bovine rhinotracheitis, IBR; bovine viral diarrhea, BVD; blue tongue, BT; etc., causes multifaceted and great economic losses such as loss of productivity in animals, loss of product quality, Change to mortality and disease control expenses (Şimşek et al., 2017). The causative agent of the disease, BVL, is transmitted through blood (surgical procedures, infected needles, insects, etc.) (Foil et al., 1989; Van Der Maaten & Miller, 1990). Direct contamination can also be seen in intensive cattle breeding (Acar & Gür, 2013).

Enzootic bovine leukosis is a tumoral disease characterised by neoplastic cell infiltrations caused by a retrovirus called bovine leukosis virus (Bovine Leukaemia Virus, BVL) (Otlu et al., 2001). EBL emerges every year and causes great economic losses in the cattle industry due to productivity loss, loss of milk, and loss of genetic material (Gillet et al., 2007; Hooshmand et al., 2020). Cattle of all age groups are susceptible to the virus (Bordunova et al., 2021). The prevalence of the disease varies from country to country, from region to region, and even in different farms in the same region (Yıldırım et al., 2008).

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Research Article

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It has been reported that the annual prevalence of lymphoma is 1-2% in flocks infected with EBL for a long time (EFSA, 2015). Studies show that the prevalence of EBL varies between 0-59% in countries with cattle management (Savir et al., 1987; Klintevall, 1997; Zaghawa et al., 2002; Usui et al., 2003; Felmer et al., 2009; Murakami et al., 2013; de Almeida, 2021). The presence of the disease was reported in 51 countries in 2012 (EFSA, 2015). It is seen that the infection rate in small businesses varies between 0-60% in Turkey (Acar & Gür, 2013; Şimşek et al., 2017).

Enzootic bovine leukosis usually progresses clinically as asymptomatically (Chen et al., 2020; Hajj et al., 2012; Bordunova et al., 2021). It may show different symptoms depending on the organ where the infection is located. These symptoms are general symptoms such as fever, weight loss, weakness, and decreased milk yield (Yılmaz et al., 1995; Otlu et al., 2001). The significant increase in the number of circulating lymphocytes is observed in animals with tumour formation. Enlargements in lymph nodes can also be detected on rectal or vaginal palpation. The spleen, heart, kidney and uterus are among the affected organs along with lymph nodes. It should also be considered that animals have clinically neurological disorders or paralysis may have malfunctions in the brain and spinal cord (Holmes et al., 1989; Van Der Maaten & Miller, 1990; Dimmock et al., 1991; Sparling et al., 2000; Braun et al., 2007).

Agar gel immunodiffusion (AGID), radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) tests are used for the diagnosis of enzootic bovine leukosis (Otlu et al., 2001). However, in recent years, the most commonly used method to perform antigen-antibody reaction is ELISA (Çoşkun et al., 2016; Acar & Gür, 2013). ELISA sensitivity and specificity was reported to be about 97.2% in a study conducted in cattle in Argentina (Trono et al., 2001).

The aim of the present study was to determine the prevalence of EBL in cattle raised in Ardahan region by ELISA method.

MATERIAL and METHOD

Animal Material

The animal material used in the study was included bovine blood serums from 500 different breeds (Simental, Brown Swiss cattle, Holstein and habitant) and gender (442 Females, 48 Males) were sampled from 38 different focal points (Table 1) belonging to Ardahan province and its districts between March and April 2021 by random sampling method. The animals used in the study were selected from clinically healthy cattle, between the ages of 1-10 and subjected to the similar care and nutrition. The blood samples taken from the vena jugularis of the cattle were centrifuged at 3000 g for 10 minutes, and were stored at -20 °C until analysis.

Detection of Antibodies against Bovine Leukaemia Virus by ELISA

BLV antibodies in bovine blood serum samples were investigated by commercial ELISA kit (Enzootic Bovine Leukosis Virus (BLV) Antibody Test Kit, Idexx, USA). The assay was performed according to the manufacturer's instructions. The absorbance values of the serum samples were measured in spectrophotometer at a wavelength of 450 nm (A (450)). The presence/absence of BLV specific antibodies in serum samples was determined by using the optical density (OD) values of the sample, with the "%S/P" formula calculated separately for each sample. The following formulas were used to calculate %S/P values.

Negative control mean:

$$\mathrm{NC}(\bar{x}) = \frac{\mathrm{NC1\ A(450)} + \mathrm{NC2\ A(450)}}{2}$$

Positive control mean:

$$\mathrm{PC}(\bar{x}) = \frac{\mathrm{PC1\ A(450)} + \mathrm{PC2\ A(450)}}{2}$$

Calculation of S/P% mean:

$$S/P\ \% = 100x\frac{\text{Sample A}(450) - \text{NC}(\bar{x})}{\text{PC}(\bar{x}) - \text{NC}(\bar{x})}$$

Evaluation:

Samples with "S/P% ≤ 60" were considered as "NEGATIVE" and samples with "S/P% > 60" were evaluated as "POSITIVE".

Statistical Analysis

Data analysis was performed using statistical software (SPSS 20.00 for windows). The data obtained were presented in the form of a table.

RESULTS

The focal points and the number of animals used in the study are presented in Table 1. In the study, 351 Simmental, 122 Brown Swiss Cattle, 10 Holstein and 17 Native breed cattle were used. 156 of these animals were selected by random sampling from Ardahan Centre, 49 from Çıldır, 188 from Göle, 35 from Damal, 25 from Hanak and 47 from Posof and their affiliated villages.

Clinical Findings

Clinical findings (enlargements of lymph nodes, parasites etc.) related to the EPL of 500 cattle enrolled in the study were normal, which were raised in Ardahan and its region and sampled within the scope of the study.

Serological Findings

The blood serum samples of 500 cattle of different races and genders collected from 28 focal points were examined by ELISA method and no antibodies were detected against BVL as a result of the analysis.

Table 1. The focal points and number of animals studied in Ardahan region

Line	Focal Point	Breeds			
		Simental	Brown Swiss	Holstein	Native
1	**Centre/Centre**	17	8		5
2	**Centre/Gürçayır**	15	3	2	
3	**Centre/Sulakyurt**	9	14		3
4	**Centre/Tunçoluk**	19		3	
5	**Centre/Sugöze**	15			1
6	**Centre/Hacıali**	12	6		
7	**Centre/Hasköy**	8	3		
8	**Centre/Bağdeşen**	11			
9	**Çıldır/Centre**	10	2		2
10	**Çıldır/Öncül**	7			
11	**Çıldır/Sazlısu**	10	5		
12	**Çıldır/Âşık Şenlik**	12			1
13	**Göle/ Centre**	19	9		
14	**Göle/Demirkapı**	17	7		
15	**Göle/Sürgülen**	12	8		

16	**Göle/Tahtakıran**	14	4		
17	**Göle/Yanatlı**	11	6		
18	**Göle/Dengeli**	9	7		
19	**Göle/Dölekçayır**	11	4		
20	**Göle/Dedeşen**	17	9	3	4
21	**Göle/Molla Hasan**	13	4		
22	**Damal/Centre**	11	14		
23	**Damal/Dereköy**	10			
24	**Hanak/Centre**	13			
25	**Hanak/Binbaşak**	9	3		
26	**Posof/Aşık Zülali**	18	4	2	1
27	**Posof/Alabalık**	9			
28	**Posof/Aşık Üzeyir**	11	2		
Total		**351**	**122**	**10**	**17**

Clinical Findings

Clinical findings (enlargements of lymph nodes, parasites etc.) related to the EPL of 500 cattle enrolled in the study were normal, which were raised in Ardahan and its region and sampled within the scope of the study.

Serological Findings

The blood serum samples of 500 cattle of different races and genders collected from 28 focal points were examined by ELISA method and no antibodies were detected against BVL as a result of the analysis.

DISCUSSION

Malignant BVL, which is seen all over the world, causes systemic infections in cattle and causes great economic losses due to death, loss of productivity, treatment costs, and control/eradication programs (Otlu et al., 2001; Gillet et al., 2007; Hooshmand et al., 2020). The objective of the study was to determine the prevalence of EBL in Ardahan and its region in this study.

Studies have been carried out in many countries for the eradication of the disease and successful results have been gained (EFSA, 2015). The most appropriate way to control the disease is to eradicate sick animals (Acar & Gür, 2013). It has been reported that positivity is between 3-20% in offspring born from BVL infected cows (Ferrer & Piper 1981).

Enzootic Bovine Leukosis was reported by 51 countries and/or regions including 3 African, 6 Asian, 18 European and 21 American countries, as well as 1 region in Australia and Oceania in 2012 (OIE, 2015; EFSA, 2015). A study in Japan has been reported that BLV increased 10 times between 1980 and 2011 and reached 35.2% in a study on cattle (Murakami et al., 2013). The prevalence of the disease was found to be 59% in Chile (Felmer et al., 2009).

Studies in Turkey were found that prevalence (Tables 1 and 2). However, increases in the number of the case positive cattle in Turkey was reported (Acar & Gür, 2013). Our findings showed that the prevalence of EBL was negative in Ardahan province. There was similarity between our findings and results of the studies

performed in Turkey (Otlu et al., 2001; Yıldırım et al., 2008). It was concluded that this result was caused by the closed system breeding in Ardahan and its region (the arrival of a very small amount of animals from outside and the sale of especially meat breeds outside the city) and that EBL does not pose a risk for this region.

Table 2. EBL prevalence in different studies in Turkey according to provinces

Line No	Province	Prevalence	Reference
1	Aydın	0,3%	Tan *et al.*, 2006
2	Isparta	20%	Avcı *et al.*, 2013
3	Afyon	15,45%	Acar & Gür, 2013
4	Elazığ-Malatya	2,6%	Gülaçtı *et al.*, 2004
5	Diyarbakır	1,83%	Şimşek *et al.*, 2017
6	Kars	0%	Otlu *et al.*, 2001
7	Bursa	3,06%	Şen *et al.*, 1995
8	Trakya	10,63%	Uysal *et al.*, 1995

Table 3. EBL prevalence in different studies in Turkey according to regions

Line No	Region	Prevalence	Referance
1	The Northern Anatolia Region	1,58%	Yıldırım & Burgu, 2005
2	The South Marmara Region	9,62%	Batmaz *et al.*, 1999
3	The Eastern Anatolia Region	3%	Çabalar *et al.*, 2001
4	The South-eastern Anatolia Region	0,27%	Özgünlük *et al.*, 2005

CONCLUSION

In conclusion, EBL was determined as 0% as a result of the ELISA test performed on cattle of various breeds, ages and genders raised in Ardahan and its districts. However, both sporadic and enzootic viral infections in cattle breeding cause economic losses such as loss of yield, loss of product quality, animal deaths and disease control expenses. Although the rate of EBL positivity is very low in Turkey, we think that it is important to control this infection.

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Ethical approval: The study was conducted with the decision of Ardahan University Scientific Publication and Ethics Committee dated 04.03.2021 and numbered E-29486769-622.01-1150133 and the permission of Ardahan Provincial Directorate of Agriculture and Forestry dated 09.04.2021 and numbered E-29486769-622.01-1150133

Conflict of interest: The authors declare that there is no conflict of interest for this study.

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