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## Original Article

# A Serological Study on Bovine Leukemia Virus Infection in Ten Provinces of Iran between 2010 and 2012

**Kazemimanesh M[1], Madadgar O[1*], Mahzoonieh MR[2], Zahraei-Salehi T[1], Steinbach F[3]**

1. Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran.
3. Department of Virology, Institute of Veterinary Laboratories Agency, New Haw, Addlestone Surrey KT153NB, United Kingdom.

## Abstract

**Background and Aims:** Bovine leukaemia virus (BLV) is an oncogenic member of the genus *Deltaretrovirus* of the family *Retroviridae.* BLV is the causative agent of enzootic bovine leukaemia and infects cattle worldwide, imposing economic impact on the dairy cattle industry. The purpose of this study was to estimate the seroprevalence of BLV in cattle in some provinces of Iran.

**Materials and Methods:** A total of 280 cows over 2 years old from 10 provinces of Iran in different regions and environments from industrial and less industrial herds were used in the study. Blood samples from all cows were taken both with and without EDTA. Serum separation for the ELISA test and leukocyte count, were performed upon receipt without delay. Cattle without fever that had lymphocyte numbers of more than 9,000/µl were suspected to have persistent lymphocytosis (PL). Sera samples were examined for antibodies against BLV by blocking ELISA.

**Results:** The seroprevalence of BLV among animals was 32.8% and among provinces was 80%. Seropositive cattle had higher total leukocyte and lymphocyte count and lower neutrophil count than seronegative cattle ($P<0.001$). Among BLV seropositive animals, the rate of PL was 36.9%. None of the seronegative animals showed lymphocytosis.

**Conclusion:** Comparing the data with previous studies on seroprevalence of BLV in different localities in Iran, the prevalence of the infection has been raised. These results suggest that promoting control programs in Iran are very important. Furthermore, it will be essential to conduct nationwide surveillance program and determine the major risk factors.

**Keywords:** Bovine leukaemia virus (BLV); Seroprevalence; persistent lymphocytosis (PL); Iran

## Introduction

Bovine leukaemia virus (BLV) is an oncogenic member of the genus *Deltaretrovirus* of the family *Retroviridae* which also includes human T lymphotropic virus type 1 (HTLV-1) and simian T lymphotropic viruses (STLV-1, -2, and -3). BLV is the causative agent of enzootic bovine leukosis (EBL) (1). BLV is lifelong infection and most BLV infections are asymptomatic and are recognized only by serological testing. Among infected cattle, about 30% develop persistent lymphocytosis (PL), characterized by a benign polyclonal proliferation of B-cells, and less than 5% of infected animals develop lymphosarcoma (1, 2). Despite the low incidence of diseases

***Corresponding author:** Omid Madadgar, Ph.D. Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
Email: omadadgar@ut.ac.ir


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associated with BLV, the infection does cause significant economic losses that associated with the costs of control and eradication programs (3) which makes BLV an OIE notifiable disease (4). The obvious economic losses include the culling of cattle with lymphosarcoma, shortening of lifespan, loss of production potential and restrictions on export of cattle, semen and embryos to countries that maintain BLV control programs. Besides an impact on survival, BLV infection also may impair the immune system leading to opportunistic infections (1, 2).

Disease transmission between cattle is considered to occur via exposure to infected lymphocytes in blood from parturition, contaminated surgical instruments, rectal palpation and bloodsucking insects (2, 3, 5). Several authors have shown that it is possible to establish BLV-free herds by identifying seropositive animals and eliminating them from the herds (6-8). Calves could have BLV antibodies due to maternal antibodies from the colostrum or through parturition (1, 9, 10). All breeds of cattle are susceptible to BLV infection. It occurs rarely in animals less than 2 years of age and increases the incidence by age. The prevalence of infection is higher in large herds than in smaller herds (2). Previous studies have found no relationship between sex and EBL infection (11).

Enzyme immunoassay, agar gel diffusion, and syncytium-inhibition assays are used for serological diagnosis of BLV infection (1). There are several ELISA kits commercially available to detect antibodies against the virus, mainly the glycoprotein gp51, which appear early in the course of immune response (12). ELISA was used in this study to test cattle serum samples. Additionally, a hematological study was carried out to compare the results from the serology and to observe changes in blood parameters. Bovine leukemia virus infection occurs worldwide, but varies in prevalence between countries (13). The aim of the present study was to estimate the seroprevalence of BLV in certain areas of Iran.

## Methods

### Sample collection and preparation

A total of 280cows over 2 years old from 10 provinces of Iran were used in the study (Fig. 1). Animals were selected randomly mostly from the industrial and less industrial herds. Peripheral blood was aseptically obtained from jugular vein with and without EDTA. Samples were transported to the laboratory at 4°C. For serum collection, blood without EDTA was kept cool to allow clotting and tubes were centrifuged at 1500×g for 15 minutes. Serum was collected and stored at -20$^{o}$C until used. Whole blood samples for leukocyte count were used within 24 hours of sampling.

### Leukocyte count

The blood samples with anticoagulant were analyzed for total leukocyte count using an automated method. Lymphocytes, monocytes, basophils, neutrophils and eosinophils were determined in blood smears stained with Giemsa stain. Since cows with PL were seropositive for BLV and had a lymphocyte count of greater than 8,000 cells/µl which persisted for more than 3 months (14), seropositive cattle that had lymphocyte numbers of more than 9,000/µl without having fever and apparently healthy, were suspected to have PL.

### Serological study

All serum samples were analyzed for antibodies against BLV using blocking enzyme-linked immune sorbent assay (ELISA) kit, according to the instruction of the manufacturer, (ELISA Leukosis Blocking/BLV gp51 antibody test kit; Institut Pourquier, Montpellier, France). The sensitivity and specificity of the ELISA test were 99.0 and 99.6%, respectively (13). Both positive and negative control samples were provided in the kit.



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**Fig. 1.** Location of different areas of Iran considered in this study: (1) Yazd (0%); (2) Markazi (53.3%); (3) Qom (57%); (4) Alborz (45%); (5) Tehran (88.8%); (6) Razavi Khorasan (2.3%); (7) East Azerbaijan (50%); (8) Khuzestan (0%); (9) Gilan (100%); (10) Ardabil (9.5%).

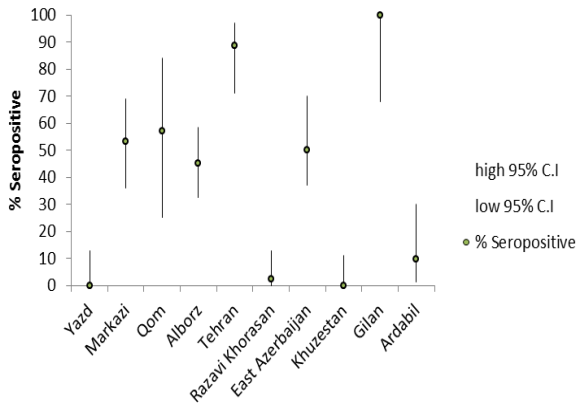


**Fig. 2.** Prevalence of BLV infection in 10 provinces of Iran. High and low confidence intervals of proportions are shown.

**Statistical analysis**

animals among the tested animals), herd prevalence (the proportion of farms with one or more positive animals among tested herds) and provinces prevalence (the proportion of positive animals on each seropositive province) were statistically examined and 95% confident limits were calculated. Student's*t*-test was used to compare the specific characteristic of the hematological profiles between seropositive and seronegative cattles. Data were represented as mean±SEM. For all the analyses, a value of $P< 0.05$ was considered significant.



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**Table 1**. Epidemiological data concerning the cows over 2 years old (n = 280) from 10 province of Iran in different regions and environments, Seropositivity rates of BLV and the rate of PL.

| Numbers contained in fig.1 | Province | Number of animals sampled | Location of sampling (number of samples per farm or slaughterhous) | No. of seropositive | % seropositive | 95% Confidence interval (CI) | No. of Persistent Lymphocytosis (PL) | % PL |
|---|---|---|---|---|---|---|---|---|
| 1 | Yazd | 30 | 1 farm | 0 | 0 | 0_13 | 0 | 0 |
| 2 | Markazi | 30 | 1 farm | 16 | 53.3 | 36_69 | 3 | 18.7 |
| 3 | Qom | 7 | 1 farm | 4 | 57 | 25_84 | 0 | 0 |
| 4 | Alborz | 53 | 1 farm | 24 | 45 | 32.6_58.5 | 9 | 37.5 |
| 5 | Tehran | 27 | 3 farms (12, 9, 6) | 24 | 88.8 | 71_97 | 13 | 54 |
| 6 | Razavi Khorasan | 43 | 3 slaughterhouses (17, 16, 10) | 1 | 2.3 | 0.01_13 | 0 | 0 |
| 7 | East Azerbaijan | 22 | 2 farms (16, 6) | 11 | 50 | 37_70 | 5 | 45.4 |
| 8 | Khuzestan | 37 | 2 farms (19, 18) | 0 | 0 | 0_11 | 0 | 0 |
| 9 | Gilan | 10 | 1 farm | 10 | 100 | 68_100 | 4 | 40 |
| 10 | Ardabil | 21 | 1 slaughterhouse | 2 | 9.5 | 1.4_30 | 0 | 0 |
| | Total | 280 | 12 farm & 4 slaughterhouses | 92 | 32.8 | 27.6_38.5 | 34 | 36.9 |

## Results

Among the 280 cattle sampled, 92 (32.8%, 95% confidence interval [C.I]: 27.6_ 38.5%) were seropositive to BLV (Table 1).

Seropositive animals were found in 8 of 10 provinces of Iran (Fig. 1). Prevalence rate of BLV infection among different regions of Iran was 80% (95% C.I: 48_ 95%) and infection rates among the areas were 0–100% which include Yazd 0% (95% C.I: 0_13% ); Markazi 53.3% (95% C.I: 36_69%); Qom 57% (95% C.I: 25_84%); Alborz 45% (95% C.I: 32.6_58.5%); Tehran 88.8% (95% C.I: 71_97%); Razavi Khorasan 2.3% (95% C.I: 0.01_13%); East Azerbaijan 50% (95% C.I: 37_70%); Khuzestan 0% (95% C.I: 0_11%); Gilan 100% (95% C.I: 68_100%) and Ardabil 9.5%(95% C.I: 1.4_30%) (Table 1, Fig. 1and Fig. 2).

A significant increase in total leukocyte count of BLV cattle was detected (P<0.001) (Table 2). Lymphocyte count of BLV-positive cattle was higher than that of negatives (P<0.001) (Table 2) and therefore neutrophil count of BLV-positive cattle was lower than that of negatives (P<0.001) (Table 2). There was no significant difference in eosinophil, monocyte, and basophil count between the BLV-positive and BLV-negative cattle (Table 2).

Among BLV seropositive animals, the rate of PL was 36.9 %. None of the seronegative animals were PL. PL rates among the seropositive areas were 0-54% which include Markazi 18.7%;Qom 0%; Alborz 37.5%; Tehran 54%; Razavi Khorasan 0%; East Azerbaijan 45.4%; Gilan 40% and Ardabil 0% (Table 1).

## Discussion

In this study 280 cattle from 10 provinces of Iran were sampled that the total prevalence rate of BLV infection was 32.8% (95% C.I: 27.6_ 38.5%) and infection rates among the areas were 0–100% (Table1).The ELISA method used in this study has high sensitivity and specificity of 99 and 99.6 percent respectively, thus providing a reliable and adequate method of testing (15).

Serological surveys in cattle in the United States indicate prevalence rates within herds ranging from 0-100%. Infection with the virus is estimated to be at least 20% in the adult dairy cow population of the United States, 6-11% in Canada, 27% in France, 37% in Venezuela; in the United Kingdom the prevalence of infection is low. In New Zealand, it is estimated that about 6.5% of the dairy herds have infected cattle, with an estimated within herd prevalence of 3.7% (2).



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**Table 2**. Hematological profiles of all cattle tested for bovine leukemia virus (BLV) in 10 provinces of Iran between 2010 and 2012.

| Parameter | BLV-seronegative (n=188) | | BLV-seropositive (n=92) | |
|---|---|---|---|---|
| | mean | SEM | mean | SEM |
| **Leukocyte** | 8874.167 | 444.4595 | 14375.15 | 1067.626 [a] |
| **Lymphocyte** | 5209.313 | 290.329 | 10433.95 | 1295.602 [a] |
| **Neutrophil** | 3369.346 | 252.4885 | 4953.282 | 352.4308 [a] |
| **Monocyte** | 79.85625 | 15.60815 | 57.26212 | 17.77649 [b] |
| **Eosinophil** | 198.875 | 27.93483 | 330.3909 | 56.38859 [c] |
| **Basophil** | 9.5625 | 6.08363 | 1.272727 | 1.272727 [d] |

[a] P<0.001
[b] P=0.363
[c] P=0.064
[d] P=0.127

The prevalence can be related to management or sanitary practices. The high density of animals on a farm where infected and uninfected animals are in continuous contact or multiple uses of injection needles during vaccinations or treatments and also the same gloves and sleeves for rectal palpations may have contributed to the transmission in the herd (16). Furthermore, presence of vector insects and climatic conditions can affect the prevalence (17, 18).

Previous studies have reported that the seroprevalences of 6% in 1996 (19), 22.3% in 2009 (20), 16.8% in 2010 (21), in Tehran, but results of the present study showed a significant higher seroprevalence of 45% (95% C.I: 32.6_58.5%) in Alborz and 88.8% (95% C.I: 71_97%) in Tehran. Furthermore, the rate of BLV infection have been reported only 3% in Markazi in 1999 (22), whereas we observed 53.3% (95% C.I: 36_69%). In addition seroprevalences of 6% in 1996 in East Azerbaijan(19) while, in present study has been 50% (95% C.I: 37_70%) although in neighboring province Ardabil has been 9.5% (95% C.I: 1.4_30%). These data suggest that the prevalence of the infection in Iran has been raised. Reason may be derived from increasing industrial cattle in Iran in recent years and there is no established control program in our farms.

Our data also demonstrates no seroprevalence (95% C.I: 0_11%) in Khuzestan. Similar prevalence of BLV infection were reported from Khuzestan including 0% in 1996 (19) and 0.5% in 2005. The data highlights low prevalence of BLV infection in Khuzestan area in comparison with other provinces. It may due to less industrial in Khuzestan or hot climate of there. Totally, our data confirmed prevalence rate of BLV infection in industrial herds is significantly more than less industrial herds.

In contrast to earlier observations of seroprevalences of 41.3% in Khorasan area in 2012 (23), results of the present study showed a significant lower seroprevalence in Razavi Khorasan Province: 2.3% (95% C.I: 0.01_13%). The reason may be derived from the different sampling that previous study was performed on bulk tank milk, while in this study blood Samples were obtained from slaughterhouse. The bulk tank milk ELISA is useful for identification of herds which are negative for BLV infection. Since the specificity of the ELISA test for milk was moderately low, herds identified as positive by the ELISA would require further testing at the individual or herd level to definitively establish their BLV status (24). The sensitivity and specificity of the milk ELISA is estimated to be adequate until the prevalence of BLV-infected individuals in the country is less than 1% (2).

Our data support seropositive cattle had a higher leukocyte, lymphocyte count and a lower neutrophil count than seronegative cattle (P<0.001). As in previous studies, the rate of PL in this study was approximately 30%.

In a spreadsheet analysis of dairy herds in Canada, total annual costs for an average, infected 50 cow herd were $806.00 for EBL (25). The association between EBL infection and annual value of production on dairy herds in the United States found that compared to



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herds with no test-positive cows, herds with test positive cows produced 218 kg less milk per cow. The average reduction in average value of production was $59.00 per cow relative to test-negative herds (26). Economic losses resulting from the formation PL (with infection rate of 20% and PL rate of 4%) was estimated at 200 million rials in Chaharmahal Bakhtiary province in Iran (27).

In the absence of an effective vaccine, eradication strategies used in other countries have been based on a policy of serotesting followed by segregating or culling seropositive animals (28). The obtained results show not only BLV is cattle health problem in Iran, but also the growth of the infected population and resulting a lot of Economic losses. Because no nationwide control program has been established, there is a need to raise farmers' awareness of the infection in order to help curtail its spread. Further research is needed to determine the extent of the infection as well as calculations of the economic losses associated with the infection. Furthermore, it will be essential to conduct nationwide surveillance to more accurately estimate BLV prevalence and the major risk factors. Hence, preventive and control programs should be instituted to combat the disease.

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