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*Short Communication*

# Detection and identification of Enzootic Bovine Leukosis (EBL) in Calves in Iran

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**ABSTRACT**

Enzootic bovine leukosis (EBL) is known as bovine lymphosarcoma and normally affects the old cattle. EBL is caused by bovine leukemia virus (BLV), which is generally spread all around the world. This virus is transmitted via bovine blood products within and between cattle herds. Glycoprotein GP51 in the blood is responsible for cattle immune responses to BLV. This virus has been previously detected in cattle and even in the calf. However, to the best of my knowledge, this is the first short communication reporting the detection of EBL in calves in Iran. The samples of the present study were obtained from two calves of different mothers within the same dairy farm of 2000 cattle, located near Tehran, Iran. General clinical signs of the two calves, such as heart rate, respiratory rate, and body temperature were recorded. The clinical observations were confirmed by hematological tests, histopathological examination, enzyme-linked immunosorbent assay, polymerase chain reaction, and phylogenetic analyses. The originality of the detected virus was assessed by blasting against the other strains of BLV available on the NCBI web page. Regarding the clinical symptoms, bulging eyes were noticeable. Moreover, atypical and malignant lymphocytes were detected in the circulatory system and periorbital tissue. It should be noted that the presence and expression of GP51 in both calves and cattle was similar to the previously detected ones in Korea and Japan. The latter result confirms the originality of retrovirus structural subunit GP51. Similar observations were reported in a six-month follow-up.

**Keywords:** Enzootic bovine Leukosis, Leukaemia virus, Calf, Clinical

**Détection et identification de la Leucose Bovine Enzootique (LBE) chez des veaux en Iran**

**Résumé:** La leucose bovine enzootique (EBL) est connue sous le nom de lymphosarcome bovin et affecte normalement les vieux bétails. La LBE est causée par le virus de la leucémie bovine (BLV), généralement répandu dans le monde entier. Ce virus est transmis par le biais de produits à base de sang bovin au sein d'un même troupeau et entre différents troupeaux de bétails. La glycoprotéine GP51 présente dans le sang est responsable de la réponse immunitaire du bétail au BLV. Cependant, à notre connaissance, il s'agit du premier rapport relatant la détection de la leucémie à lisboline chez des veaux en Iran. Pour cette étude, les échantillons ont été obtenus à partir de deux veaux de mères différentes appartenant à la même ferme laitière de 2000 bovins située près de Téhéran, en Iran. Les signes cliniques généraux des deux veaux, tels que la fréquence cardiaque, la fréquence respiratoire et la température corporelle ont été enregistrés. Les observations cliniques ont été confirmées par des tests hématologiques, un examen histopathologique, un test ELISA, une réaction en chaîne de la polymérase (PCR) et des analyses phylogénétiques. L'originalité du virus détecté a été évaluée par dynamitage par rapport aux autres souches de BLV disponibles sur le site Web du NCBI. Les veaux montraient également des symptômes cliniques de la maladie et des yeux exorbités étaient perceptibles. De plus, des

lymphocytes atypiques et malins ont été détectés dans le système circulatoire et le tissu péri-orbitaire. Il convient de noter que la présence et l'expression de GP51 chez les veaux et les bovins étaient similaires à celles détectées précédemment en Corée et au Japon. Ce dernier résultat confirme l'originalité de la sous-unité structurale rétrovirale GP51. Des observations similaires ont été rapportées tout au long d'un suivi de six mois.
**Mots-clés:** Leucose bovine enzootique, virus de la leucémie, veau, clinique

## INTRODUCTION

Generally, the prevalence of Enzootic bovine leukosis (EBL) has increased in old cattle. EBL is defined as the tumor of lymphatic tissue or bovine lymphosarcoma caused by bovine leukemia virus (BLV). This virus is categorized as a retrovirus and the viral infection has been distributed around the world (Murakami et al., 2013; Nagy, 2014). The manifestation of BLV pathogenesis is categorized into persistent infection with no sign and those with continuous lymphocytosis developing lymphosarcoma (Nagy, 2014; Guzel et al., 2018). One of the main routes of BLV transmission is the transfusion of the blood products of infected cattle to naive animals. Consequently, the immune system of the naive cattle responds mainly to the glycoprotein GP51 received from the infected sample (Nagy, 2014). Env is a regulatory gene encoding GP51 and GP30 (Rola-Luszczak et al., 2013; Polat et al., 2017). To the best of my knowledge, this short communication is the first record of EBL occurrence in calf in Iran. This case was initially diagnosed by bulging eyes as the clinical sign. Afterwards, the animal was followed up and confirmed as EBL by laboratory methods and molecular tests.

## MATERIAL AND METHODS

This case study was conducted on a dairy farm containing 2000 cattle, located near Tehran, Iran. The samples were obtained from two calves and two cows of the same dairy farm. The two calves were the offspring of the two cows.

**Sampling and Clinical Pathology.** Blood samples were taken from the calves and their mothers. The tissue specimens were collected from the periorbital area of the calves, fixed in %10 buffered formalin solution, and stained by hematoxylin and eosin (H and E). Complete blood count and manual differential count were carried out in order to determine the count of lymphocytes. Furthermore, enzyme-linked immunosorbent assay (ELISA) was completed to detect the antibody against glycoprotein GP51 in the blood samples using the IDEXX Leukosis Blocking Ab Test (IDEXX laboratories, Inc., USA).

**DNA Extraction and Nested PCR.** DNA was extracted according to the guidelines of the rapid genomic DNA isolation kit. The kit was provided by molecular biological system (MBST) research institute, Tehran, Iran. Polymerase chain reaction (PCR) was performed as previously described by Licursi et al. (2003) and the purified PCR products were sequenced (Sequencing service, Bioneer, Republic of Korea).

**Further Molecular Analysis.** The investigation of the virus was continued by blasting test in the NCBI website, and the phylogenetic tree was outlined by the CLC sequence viewer (CLC bio, Qaigen).

**Follow-up Tests.** All the aforementioned experiments were repeated after six months.

## RESULTS AND DISCUSSION

**Clinical Observations.** Bulging eyes as demonstrated in Figure 1 and normal-sized superficial lymph nodes were observed in both calves. The 117-day-old calf had a body temperature of 37.6 °C, in addition to the respiratory and heart rates of 30 and 120, respectively.

Moreover, regarding the 112-day-old calf the body temperature, respiratory rate, and heart rate were reported as 38.9 °C, 24, and 120, respectively. The body temperature of the two cows was 38.2, 38.4 °C. In addition, their respiratory rates were revealed as 24 and 23. The heart rate of the two cases was 120 and 119. The slightly enlarged superficial lymph nodes of the prescapular and prefemoral regions were palpated in only one of the cows. On the other hand, the rectal touch revealed slightly large visceral lymph nodes in both cows. It should be mentioned that the body condition score of both cows was normal.

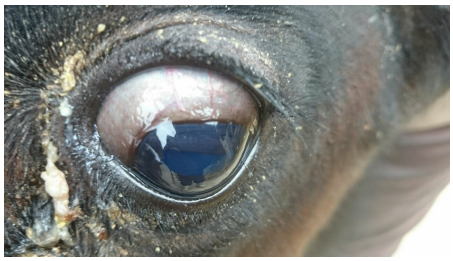

**Figure 1.** Bulging eyes observed in the calves of this study as a clinical sign of bovine leukemia virus.

**Hematological Test.** As indicated in Table 1, the findings of this study showed that lymphocytes and neutrophils were the predominant types of white blood cells in the calves and cows. With a lower percentage, the atypical lymphocytes were observed in the blood samples of the subjects. Monocytes were detected in the blood of one of the calves and one of the cows, and eosinophils were detected in that of the two calves and one cow, while no basophil was observed in the differential count of the samples.

**ELISA.** The kit of the IDEXX Leukosis Blocking Ab Test showed positive results regarding the presence of an antibody against GP51 glycoprotein in the blood samples.

**Nested PCR.** According to Figure 2, amplification indicated the presence of glycoprotein GP51 envelopes in the samples of the two calves and their mothers in the studied farm. This glycoprotein is the biomarker for confirming the presence of BLV (Rola-Luszczak et al., 2013). The size of our PCR product was found to be 444 base pairs (Licursi et al., 2003).

**Table1.** The outcome of hematological test on blood sample. Calf No.1 is offspring of Cow No.2 and Calf No.3 is offspring of Cow No.4. The differential population of WBCs were individually calculated. In which, 1% atypical lymphocyte was detected through CBC trial in both calves and cows. Abbreviation list in Table 1, as follow, N.S: Neutrophil Segmented, N.B: Neutrophil Band, L: Lymphocyte, E: Eosinophil, B: Basophil, M: Monocyte, AL: Atypical Lymphocyte.

| Sample | Total WBC (/µl) | Differential WBC Type | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | N.S (%) | N.B (%) | L (%) | AL (%) | B (%) | M (%) | E (%) |
| Calf NO.1 | 18,800 | 25 | 0 | 67 | 3 | 0 | 1 | 4 |
| Cow NO.2 | 23,600 | 14 | 0 | 82 | 2 | 0 | 2 | 0 |
| Calf NO.3 | 27,400 | 23 | 1 | 73 | 1 | 0 | 0 | 1 |
| Cow NO.4 | 24,800 | 27 | 0 | 71 | 1 | 0 | 0 | 1 |

**Phylogenetic Analysis.** As demonstrated in Figure 3, the isolated virus had a 100% similarity between the two calves and two cows investigated in the current study. Moreover, the isolated virus in our samples was quite similar to the ones detected in Japan and Korea (Lee et al., 2015; Oguma et al., 2017).

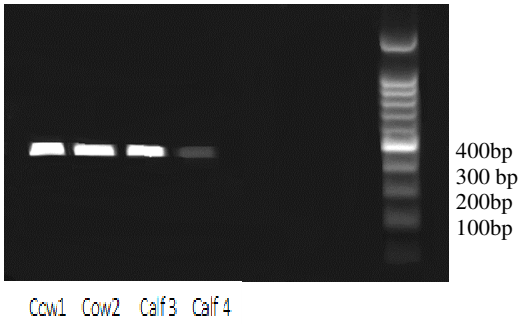


**Figure 2.** PCR proof of bovine leukemia virus. DNA of blood sample was extracted from cows1, 2 and calf 3, 4. Nested PCR was performed to detect env gene and PCR products were run on agarose gel electrophoresis. The PCR bands represented similar position in terms of amplicon size, which was 444 base pair (bp).

**Histopathology.** According to Figure 4, large to medium-sized lymphocytes were observed in the H and E stained histopathological sections of the periorbital tissue.

**Follow-up.** The repetition of serological tests, as well as PCR after six months, revealed no alteration in the initially obtained results noted in figures 1-3.

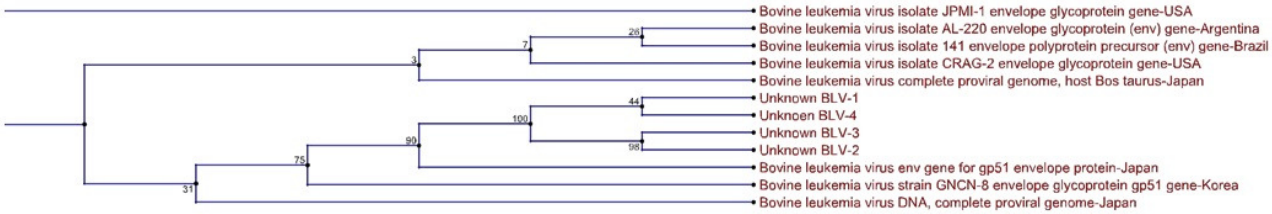


**Figure 3.** Phylogeny of bovine leukemia virus. The phylogenetic tree of nucleotide sequence shows that the isolated virus in calves and cows has 100% similarity with each other. And, it has 90% and 75% similarity with ones reported in Japan and Korea. The unknown BLV1-4 are the isolated viruses in Iran in this study.

According to the literature, EBL occurs in cattle and the virus has spread throughout the world (Nagy, 2014). Nekoei et al. (2015) investigated the prevalence of BLV in Iran and improved our understanding regarding the condition of this virus in Iran.

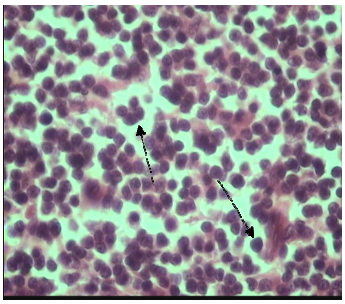

**Figure 4:** Histopathological evidence of bovine leukemia virus. This H&E section of calf periorbital tissue illustrates the infiltration of medium to large- sized malignant lymphocytes (arrows). The scale of microscopic magnification was 400X.

Few studies in Iran have detected BLV in cattle and proved the presence of this virus by genetic evidence, such as PCR and phylogenetic tree (Hemmatzadeh, 2007; Nekoei et al., 2015). Hemmatzadeh et al. (2007) showed the similarity of the identified strains with the ones found in Europe and Australia. Another study in Korea was performed in terms of molecular analyses on PCR amplification and sequencing of the env gene, in addition to the assessment of the phylogenetic tree in cattle (Lee et al., 2015). Furthermore, BLV has been detected in a two-month calf through molecular examinations and observed clinical symptoms, such as lymph node enlargement in Japan (Oguma et al., 2017). In agreement with the aforementioned studies, this short communication reveals the clinical sign of EBL along with the molecular evidence of virus presence in calves in Iran. For instance, bulging eye in calves were observed in the present study and atypical lymphocytosis was identified in the circulatory system. Furthermore, env gene was detected and a high similarity was found in nucleotide sequencing with BLV strain, which is consistent with the previous reports from Korea and Japan (Lee et al., 2015; Oguma et al., 2017). In other words, the present investigation recognized a similarity between the genome sequences of viruses identified in two dams, two offspring, and the cases evaluated in the other parts of the world by phylogenetic clustering (Figure 3). Moreover, the NCBI alignment of calves and cows in this study showed the highest similarity in the identity and query. Furthermore, virus presence after six months was convincing concerning that both calves and dams had persistent lymphocytosis.

In conclusion, the current case report provides molecular evidence to prove the presence of glycoprotein GP51 as a possible biomarker of BLV. Further investigations are required to elaborate more on the molecular epidemiologic overview of this report.

## Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

## Acknowledgment

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