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SHORT COMMUNICATION

Seroprevalence of Bovine Leukemia Virus (BLV) in Cattle from the North West of Pakistan

Muhammad Faraz Khan[1], Umar Siddique[1*], Asad Ali Shah[2], Ihsanullah Khan[2], Farhan Anwar[1], Ijaz Ahmad[1], Muhammad Tariq Zeb[3], Mohammad Farooque Hassan[4] and Tariq Ali[3]*

[1]Faculty of Animal Husbandry and Veterinary Sciences, University of Agriculture, Peshawar; [2]Livestock and Dairy Development Department (Extension) Peshawar, Pakistan; [3]Veterinary Research Institute, Peshawar, Khyber Pakhtunkhwa, Pakistan; [4]Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Pakistan
*Corresponding author: tariq.phd.18@gmail.com; tariqali@cau.edu.cn; druskhattak@aup.edu.pk; druskhattak@yahoo.com

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ABSTRACT

The aim of present study was to know seroepidemiology of BLV infection in four breeds of cattle in north-west of Pakistan. Blood samples were collected from four breeds (*n*=600) and subjected to indirect ELISA to investigate the breed-wise, area-wise and sex-wise seroprevalence of BLV. Overall, 20% cattle were seropositive for BLV out of total 600 cattle. There were significant differences in different breeds and areas under study. Highest prevalence was noted in Holstein Friesian (42%) in tehsil Tangi (38%), followed by Jersey (28%) and crossbred cattle (11%) in Shabqadar (16.5%) and Charsadda tehsils (6%). Highest seroprevalence ($P<0.05$) was also noted in males and females of Friesian breed. This study suggests that BLV is prevailing in the area and that the virus might be imported in exotic breeds or semen and locally disseminated. Additionally, exotic breeds and crossbred cattle are more prone to BLV as compared to local breed.

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INTRODUCTION

Bovine leukemia virus (BLV) is identified as the etiological agent of the economically important bovine disease, enzootic bovine leucosis (EBL), which is a B-Lymphotropic oncogenic retrovirus of family Retroviridae. BLV is closely related to human T-Lymphotropic virus type 1 (HTLV-1), and the oncogenic properties of BLV may also cause pathogenicity in humans, mostly in farm workers via drinking raw milk (Buehring *et al.*, 2014). BLV positive cells are also detected in human breast tissues. EBL is one of the important economic diseases of cattle associated with heavy losses in terms of cost of control and eradication programs. Besides, it is also causing export embargo on cattle and its products, thus it is enlisted as a disease of economic importance to international trades by the World Organization for Animal Health (OIE). It also poses a restriction on the import of exotic semen and cattle. BLV infection has crucial impacts on the dairy industries because it can cause reduce fertility, increase heifer replacement costs, decrease milk production, loss of income resulting from premature culling of animals and international trade restrictions (Moore *et al.*, 2009). Most of the BLV infected animals are carriers, showing no clinical signs and remain asymptomatic. The immunological deregulation are also noted in BLV infections, leading to low milk production, high incidence of infectious diseases and low reproductive efficiency (Bartlett *et al.*, 2014).

In the recent years, 51 countries have reported the presence of BLV with varied prevalence from country to country. The highest prevalence of 84% was noted in United State and 26% in Canada (Bartlett *et al.*, 2014). In countries without implementation of eradication program, the herd prevalence has continued to increase. Following the execution of eradication programs since 1960s, this disease has been eliminated and properly controlled in many countries of Europe (EFSA, 2015). However, there is scarcity of published data on the prevalence of BLV in cattle of Pakistan. Therefore, this study was designed to enumerate seroprevalence of BLV in four breeds of cattle.

MATERIALS AND METHODS

The current study was conducted in the central areas of Khyber Pakhtunkhwa province, located in the north west of Pakistan (34.2165° N, 71.7148° E). A total of 600

blood samples were randomly collected from four different breeds of cattle including 150 samples each from Holstein Friesian, Jersey, Crossbred and Achai breeds. The collected blood sample from jugular vein of cattle was transferred into gel clot activator tubes, centrifuged at 3000 rpm for 5 m to separate serum. The serum was transferred into Eppendorf tube and stored at -20°C till further processing for ELISA. The antibodies against BLV were identified by ELISA assay, using the recommendations of commercially available kit (IDEXX Leukosis Serum X2, Idexx, Switzerland). Briefly, the sample diluent was dispensed 90 µL into each well of BLV antigen-coated plate. Then 10 µL of each undiluted positive control (PC) and negative control (NC) was added into duplicate wells. The plates were covered and incubated for 60 m at 37ºC. After washing, 100 µL of the conjugate was added into each well and was incubated at 37°C for 60 m. The color changes were noted using ELISA reader (MR-96 Microplate Reader, CLINDIAG SYSTEMS, Belgium). If the immune-complex was present the peroxidase transformed the substrate into a blue compound. Then 100 µL stop solution was added into each well, it becoming yellow after blocking. The optical density (OD) of color development was read with the help of ELISA reader at 450 nm. The measurement of the intensity of color and the number of antibodies present in the serum sample to be tested were calculated. The results were interpreted according to the recommendations as; when the S/P% was equal or greater than 40, the animal was positive for BLV antibodies, and when the S/P% of a sample was greater or equal to 30 but less than 40 it was considered as suspected, and when S/P% was less than 30 then it was recorded as negative for BLV antibodies. Formula used to calculate S/P % was:

$$S/P\ \% = \frac{\text{OD value of sample (450 nm)} - \text{NCx}}{\text{PCx} - \text{NCx}} \times 100$$

Here, SP stands for seropositive, NCx is mean of negative control and PCx is mean of positive control. The OD values positivity range was 0.420 to 2.796. The cross sectional data were collected from different households and farm animals. The analyses were carried out through IBM SPSS Statistics version 20 using appropriate statistical tests (Regression Binary Logistic Model).

RESULTS AND DISCUSSION

Overall, 20% cattle were seropositive for BLV out of total 600 cattle using ELISA. Mean OD values of seropositive Holstein Friesian, Jersey and Cross breed cattle were 1.5928, 1.5649 and 1.5225, respectively. Thus, the highest prevalence was noted in Friesian breed (42%), followed by Jersey (28%) and crossbred cattle (11.3%) as shown in Table 1. However, no positive animal was detected in local indigenous breed of Achai cattle. Highly significant differences (P<0.001) were observed in breed-wise seroprevalence of BLV in the four breeds of cattle. In addition, highest seroprevalence of BLV was observed in tehsil Tangi (38.5%), followed by Shabqadar (17%) and Charsada (6%). The result of area-wise seroprevalence is presented in Table 2. In a previous study conducted in Pakistan, Meas *et al*. (2000) detected 0.8% BLV antibodies in buffalos, while all the screened cattle (*n*=76) were negative. This suggests that the prevalence of BLV is increasing in Pakistan. In the neighboring countries, prevalence of BLV was reported 21, 25 and 8% in China (Ma *et al.*, 2016), Iran (Mousavi *et al.*, 2014) and Iraq respectively (Khudhair *et al.*, 2016). The prevalence of BLV infection in Pakistan is lower than the other countries such as 78% prevalence is reported in Canada (Nekouei *et al.*, 2015), 84% and 94% of prevalence in the herds of USA (Bartlett *et al*., 2014; LaDronka *et al.*, 2018). The variation in the prevalence might be attributed to the managemental factors and nonexistent of control or eradication programs for this fatal disease. In most of rural areas of Pakistan, there are practices of using the same needle for injectable and also the same sleeves and gloves for rectal palpations. Furthermore, imported cattle and bought in heifer are not serologically tested for the BLV and thus, the infected and non-infected cattle could not be isolated. Interestingly, local indigenous Achai breed showed 0% seroprevalence of BLV although sharing the same feeding and habitat. This may be attributed to the low production potential of the local cattle and the EBL is considered to be the disease of high producing animals, along with genetics and epigenetic factors associated with resistance to EBL (Moore *et al.*, 2009). Table 3 shows sex-wise distribution of BLV in different cattle breeds. Overall, the seroprevalence was higher in male cattle (26.7%) as compared to female cattle (19.3) in the four breeds. The highest prevalence was noted in males of Friesian (34.8%) cattle, followed by Jersey (29%) and crossbred (31.1%) cattle. Similarly, in the female cattle, the highest seroprevalence was also observed in Friesian (43.3%) breed, followed by Jersey (27.7%) and crossbred (9.0%) cattle. Several factors may contribute in the epidemiology of a disease such as geographical conditions, the types and size of the cattle, and the specificity and sensitivity of the detection methods.

This study concludes that BLV is prevailing in the area and that the virus might be imported in exotic breeds or semen and locally disseminated. Additionally, exotic breeds and crossbred cattle are more prone to BLV in comparison to local breed. The consumption of raw milk from BLV infected animals may pose a risk to public health.

Table 1: Breed wise seroprevalence of bovine leukemia virus (BLV) in four breeds of cattle

Breed	Numbers	Positive (%)	Negative (%)	Suspected (%)
Friesian	150	63 (42.0)	85 (56.7)	2 (1.3)
Jersey	150	42 (28.0)	107 (71.3)	1 (0.7)
Cross	150	17 (11.3)	132 (88.0)	1 (0.7)
Achai	150	0 (00.0)	150 (100)	0 (0.0)
Total	600	122 (20.3)	474 (79.0)	4 (0.7)

Table 2: Area-wise seroprevalence of bovine leukemia virus (BLV) in the cattle

Tehsils	Numbers	Positive (%)	Negative (%)	Suspected (%)
Tangi	200	77 (38.5)	122 (61)	1 (0.5)
Shabqadar	200	33 (16.5)	164 (82)	3 (1.5)
Charsadda	200	12 (6.0)	188 (94)	0 (0.0)
Total	600	122 (20.3)	474 (79)	4 (2.0)

Table 3: Sex-wise seroprevalence of bovine leukemia virus (BLV) among four breeds of cattle

	Friesian		Jersey		Cross		Achai		Total	
Sex	*n*	Positive (%)	*n*	Positive (%)	*n*	Positive (%)	*n*	Positive (%)	*n*	Positive (%)
Males	23	8 (34.8)	31	9 (29)	16	5 (31.3)	12	0 (0)	82	22 (26.8)
Females	127	55 (43.3)	119	33 (27.7)	134	12 (9.0)	138	0 (0)	518	100 (19.3)

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Author's contribution: MFK, US and AAS designed and conceived the study. MFK, IK, MTZ and TA carried out the research. MFK, FA and IA analyzed the data. MFK, US and TA wrote the manuscript. TA and MFH critically reviewed and revised the manuscript.

REFERENCES

Bartlett PC, Sordillo LM, Byrem TM, *et al.*, 2014. Options for the control of bovine leukemia virus in dairy cattle. J Am Vet Med Assoc 244:914-22.

Buehring GC, Shen HM, Jensen HM *et al.*, 2014. Bovine leukemia virus DNA in human breast tissue. Emerg Infect Dis 20:772-82.

EFSA, 2015. Panel on Animal Health and Welfare (AHAW), Enzootic bovine leukosis. EFSA J 13:63.

Khudhair YI, Hasso SA, Yaseen NY, *et al.*, 2016. Serological and molecular detection of bovine leukemia virus in cattle in Iraq. Emerg Microbes Infect 5:e56.

LaDronka RM, Ainsworth S, Wilkins MJ, *et al.*, 2018. Prevalence of bovine leukemia virus antibodies in US dairy cattle. Vet Med Int 2018:5831278.

Ma JG, Zheng WB, Zhou DH, *et al.*, 2016. First report of bovine leukemia virus infection in yaks (*Bos mutus*) in China. BioMed Res Int 2016:9170167.

Meas S, Seto J, Sugimoto C, *et al.*, 2000. Infection of bovine immunodeficiency virus and bovine leukemia virus in water buffalo and cattle populations in Pakistan. J Vet Med Sci 62:329-31.

Moore D, Adaska J, Higginbotham G, *et al.*, 2009. Testing new dairy cattle for disease can boost herd health, cut costs. Calif Agr 63:29-34.

Mousavi S, Haghparast A and Mohammadi G, 2014. Prevalence of bovine leukemia virus (BLV) infection in the northeast of Iran. Vet Res Forum 5:135-9.

Nekouei O, VanLeeuwen J, Sanchez J, *et al.*, 2015. Herd-level risk factors for infection with bovine leukemia virus in Canadian dairy herds. Prev Vet Med 119:105-13.