



Incidence of Bovine Leukemia Virus in Cattle from the Different Districts of Sindh, Pakistan

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Abstract

The goal of this study was to determine the epidemiology of BLV infection in three breeds of cattle in different districts (Tando Muhammad Khan, Thatta and Hyderabad) of Sindh, Pakistan's. Blood samples n=750 were obtained from three breeds (Holstein Friesian, Jersey and Red Sindhi) each breed n=250 and treated to indirect ELISA to determine BLV incidence by breed, location, and sex. Out of 750 cattle, 20.13% were positive for BLV. There were considerable variances between the breeds and research regions. Holstein Friesian cattle had the highest incidence 29.2%, followed by Jersey 21.6% and Red Sindhi cattle 9.6%. Males and females of the Friesian breed had the highest prevalence $P < 0.05$. This research implies that BLV is widespread in the area, and that the virus may have been brought in exotic breeds or sperm and spread locally. Furthermore, exotic varieties and crossbred cattle are more susceptible to BLV than native breeds.

Keywords: Bovine Leukemia Virus; Cattle; ELISA; Enzootic Bovine Leucosis; Sindh

Introduction

The etiological agent of the economically important bovine disease, enzootic bovine leucosis (EBL), has been identified as bovine

leukaemia virus (BLV), a B-Lymphotropic oncogenic retrovirus of the Retroviridae family. BLV and human T-Lymphotropic virus type 1 (HTLV-1) are closely related, and BLV's oncogenic features

may cause pathogenicity in humans, primarily in farm worker who consume raw milk [1]. Human breast tissues have been found to have BLV-positive cells. EBL is one of the most serious economic diseases affecting cattle, resulting in significant losses in terms of management and eradication costs. It also causes export embargoes on cattle and their products, thus the World Organization for Animal Health has classified it as a disease of economic importance to international trade (OIE). It also prohibits the importation of unusual sperm and animals. BLV infection has a significant impact on the dairy industry since it can diminish fertility, raise heifer replacement costs, reduce milk production, and result in economic loss due to animal culling and international trade restrictions [2]. The majority of BLV-infected animals are carriers, meaning they show no clinical indications and are asymptomatic. Infections with BLV cause immunological deregulation, resulting in reduced milk supply, a high prevalence of infectious diseases, and decreased reproductive efficiency [3]. BLV has been recorded in 51 nations in recent years, with varying levels of frequency from country to country. The United States had the highest prevalence of 84%, while Canada had 26% [3]. Herd prevalence has continued to rise in nations where no eradication campaign has been implemented. This infection has been eradicated and properly controlled in several European countries since the 1960s, thanks to the implementation of eradication efforts [4]. However, there is a dearth of published information on the prevalence of BLV in Pakistani cattle. As a result, the goal of this study was to determine the incidence of BLV in three cow breeds in different districts of Sindh, Pakistan.

Materials and Methods

The current research was carried out in the main portions of Pakistan's Sindh province. A total of n=750 blood samples from three distinct breeds of cattle were obtained at random, including 250 samples from each of the Holstein Friesian, Jersey and Red Sindhi breeds. To separate serum, a blood sample from a cattle's jugular vein was put into gel clot activator tubes and centrifuged at 3000 rpm for 5 minutes. The serum was transferred to an Eppendorf tube and kept at -20°C until ELISA processing. Antibodies to BLV were detected using an ELISA assay, which followed the instructions of a commercially available kit (IDEXX Leukosis Serum X2, IDEXX, Switzerland). In a nutshell, 90 µL of sample diluent was poured into each well of a BLV antigen-coated plate. Then, in duplicate wells, 10 µL of each undiluted positive control (PC) and

negative control (NC) were added. At 37°Celsius, the plates were covered and incubated for 60 minutes. Following washing, 100 µL of the conjugate was added to each well and incubated for 60 minutes at 37°C. Using an ELISA reader, the colour changes were noticed (MR-96 Microplate Reader, CLINDIAG SYSTEMS, Belgium). Peroxidase converted the substrate into a blue molecule if the immune-complex was present. After that, each well was filled with 100 µL of stop solution, which turned yellow after blocking. The optical density (OD) of colour development was measured at 450 nm using an ELISA reader. The number of antibodies present in the serum sample to be analyzed and the intensity of colour were calculated. The results were interpreted as follows: if the S/P % of a sample was equal to or greater than 40, the animal was positive for BLV antibodies; if the S/P % of a sample was greater or equal to 30 but less than 40, it was considered suspect; and if the S/P % of a sample was less than 30, it was recorded as negative for BLV antibodies. S/P % was calculated using the following formula:

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

SP denotes seropositive, NCx denotes negative control, and PCx denotes positive control. The positive OD values ranged from 0.420 to 2.796. Different families and farm animals were used to acquire cross-sectional data. IBM SPSS Statistics version 20 was used to conduct the analysis, which included proper statistical tests (Regression Binary Logistic Model).

Results, Discussion and Conclusion

Using an ELISA test, 20.13% of the 750 cattle tested were seropositive for BLV. The highest prevalence was found in Friesian cattle 29.2% followed by Jersey cattle 21.6% and Red Sindhi cattle 9.6% (Table 1).

Breed	Total Sample	Positive (n)	(%)
Friesian	250	73	29.2
Jersey	250	54	21.6
Red Sindhi	250	24	9.6
Total	750	151	20.13

Table 1: Breed wise prevalence of bovine leukemia virus (BLV).

Breed-wise prevalence of BLV in the three cattle breeds showed highly significant variations (P < 0.001). In addition, the district

Tando Muhammad Khan has the highest BLV prevalence 26.4%, followed by district Thatta 20.4%, and Hyderabad 13.6% (Table 2).

District	Total Sample	Positive (n)	(%)
Tando Muhammad Khan	250	66	26.4
Thatta	250	51	20.4
Hyderabad	250	34	13.6
Total	750	151	20.13

Table 2: District wise prevalence of bovine leukemia virus (BLV).

The results of area-wise prevalence [5] found 0.8 % BLV antibodies in buffalos in a prior study conducted in Pakistan, although all examined animals n=76 were negative. This shows that BLV is becoming more common in Pakistan. In China [6], Iran [7], and Iraq, the prevalence of BLV was reported to be 21, 25, and 8%, respectively [8]. Pakistan has a lower prevalence of BLV infection than other countries, with 78% prevalence recorded in Canada [9], 84% and 94% prevalence in US herds, respectively [3,10]. The variance in prevalence could be due to management issues and the lack of control or eradication initiatives for this deadly infection. The use of the same needle for injectable, as well as the same sleeves and gloves for rectal palpations, is common in Pakistan's rural areas. Furthermore, imported cattle and purchased heifers are not serologically tested for BLV, making it impossible to distinguish between infected and non-infected animals. Interestingly, despite sharing the same diet and habitat, the local indigenous Red Sindhi breed showed little prevalence of BLV. This could be due to the area cattle's limited production capacity, as EBL is thought to be a disease of high-producing animals, as well as genetic and epigenetic variables linked to EBL resistance [2]. The sex-wise distribution of BLV in several cow breeds is shown in (Table 3).

Breed	Sex	Total sample	Positive (n)	(%)
Friesian	Male	77	20	25.97
	Female	173	53	30.64
Jersey	Male	67	16	23.88
	Female	183	37	20.22
Red Sindhi	Male	62	5	8.06
	Female	188	20	10.64
Total		750	151	20.13

Table 3: Sex wise prevalence of bovine leukemia virus (BLV).

Overall, Female cattle 20.22% had a greater prevalence than male cattle 19.90% in all three breeds. Male Friesian cattle had the largest prevalence 25.97%, followed by Jersey 23.88% and Red Sindhi 8.06% cattle. Similarly, female cattle with the highest prevalence were Friesian 30.64 %, Jersey 20.22%, and Red Sindhi 10.64%). Geographical conditions, cattle types and sizes, and the specificity and sensitivity of detection technologies are all elements that can influence the epidemiology of a disease. BLV is prevalent in the area, according to this study, and the virus may have been brought in exotic breeds or sperm and distributed locally. Furthermore, exotic varieties and crossbred cattle are more susceptible to BLV than native breeds. The drinking of raw milk from BLV-infected cows could put people's health at risk.

Conflict of Interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article.

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