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Original Article

Quantifying the enzootic leukosis virus threat: how prevalent is the natural infection in Crioulo Lageano cattle in southern Brazil?

Quantificando a ameaça do vírus da leucose enzoótica: quanto prevalente é a infecção natural em bovinos Crioulo Lageano no sul do Brasil?

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Abstract

Enzootic Bovine Leukosis (EBL), is infectious disease affecting especially older animals, although animals of all ages can be infected with the Bovine Leukemia Virus (BLV). Crioula Lageana is a locally adapted, rustic and long-lived bovine breed, important as a genetic resource. However, little is known about its health status, making it difficult to preserve the breed. In this sense, this study aims to assess the prevalence of EBL/BLV in different animal categories of Crioula Lageana cattle. Then, blood samples were collected from 308 animals from different categories: bulls, cows, heifers and male and female calves, whereby these animals were derived from six farms located in nuclei of *in situ* conservation for the Crioula Lageana breed in Planalto Serrano de Santa Catarina, Brazil and the blood samples were analyzed with hemogram, ELISA and PCR tests. There was a prevalence of 6.82% (21/308) for the ELISA test and 36.69% (113/308) for the PCR technique. There was no association between categories and natural infection or BLV positivity in PCR by the Chi-square test (P<0.05). Leukocytosis with lymphocytosis was observed in 11% (18/113) of PCR-positive animals. None of the evaluated animals presented bovine lymphosarcoma. We conclude that the BLV is present in all animal categories of the Crioula Lageana cattle, requiring the establishment of prevention and control measures to avoid the spread of the disease within herds.

Keywords: ELISA, PCR, creole cattle, enzootic bovine leukosis, bovine leukaemia virus.

Resumo

A Leucose Enzoótica Bovina (LEB), é uma doença infecciosa que afeta especialmente animais mais velhos, embora animais de todas as idades possam ser infectados com o Vírus da Leucemia Bovina (BLV). A Crioula Lageana é uma raça bovina adaptada localmente, rústica e longeva, importante como recurso genético. No entanto, pouco se sabe sobre seu estado de saúde, dificultando a preservação da raça. Nesse sentido, este estudo tem como objetivo avaliar a prevalência de EBL/BLV em diferentes categorias animais do gado Crioula Lageana. Foram coletadas amostras de sangue de 308 animais de diferentes categorias: touros, vacas, novilhas e bezerros machos e fêmeas, sendo estes animais provenientes de seis fazendas localizadas em núcleos de conservação *in situ* para a raça Crioula Lageana no Planalto Serrano de Santa Catarina, Brasil e as amostras de sangue foram analisadas por hemograma, ELISA e PCR. Houve prevalência de 6,82% (21/308) para o teste ELISA e 36,69% (113/308) para a técnica PCR. Não houve associação entre as categorias e infecção natural ou positividade do BLV na PCR pelo teste Qui-quadrado (P<0,05). Leucocitose com linfocitose foi observada em 11% (18/113) dos animais PCR-positivos. Nenhum animal avaliado apresentou linfossarcoma bovino. Concluímos que o BLV está presente em bovinos Crioula Lageana de todas as categorias animais avaliadas, sendo necessária a instituição de medidas de prevenção e controle para evitar a disseminação da doença dentro dos rebanhos.

Palavras-chave: ELISA, PCR, bovino crioulo, leucose enzoótica bovina, vírus da leucemia bovina.

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1. Introduction

The Crioula Lageana bovine breed is known for its rusticity and high productivity in the hostile climate of the Serrano Plateau within the state of Santa Catarina, Brazil; moreover, due to the genetic selection it has been subjected to for decades, there has been a recovery and maintenance of their genetic material (Martins, 2021). In this way, the horned and polled varieties, which have been raised for beef and dairy (Schade et al., 2016), were developed. The breed has proven its high genetic variability and association of alleles with disease resistance, such as babesiosis (Casa et al., 2023). The continuity of this breed represents the existence of an unrivalled bank of genetic resources for the bovine species. However, the breed was recently listed on the extinction risk list of the Food and Agriculture Organization (FAO) and still has a small number of individuals, currently constituting a population of 1,316 animals in 2017, according to the last FAO evaluation (FAO, 2022).

Among various other infectious diseases, EBL is extremely critical to both dairy and beef cattle. EBL, also called bovine lymphosarcoma, is a worldwide infectious and contagious disease that induces natural infection of cattle via the enzootic bovine leukosis virus. BLV belongs to the retroviridae family, which integrates into the DNA of the host genome, forming a provirus that allows for viral replication (Willems et al., 2000). BLV has a tropism for leukocytes, particularly B-lymphocytes, causing several immunological dysfunctions, varying in intensity according to the viral load and infection time (Kuczewski et al., 2021). After initial infection, BLV replicates, causing persistent lymphocytosis however, this does not result in major health impacts. After approximately seven years of infection, the B-lymphocytes can begin to replicate irregularly, giving rise to malignant lymphocytic tumors, being either lymphoma or lymphosarcoma, that can affect different organs and eventually result in the death of the animal (Kuczewski et al., 2021; Tsutsui et al., 2016).

In addition, due to the involvement of B-lymphocytes, EBL causes immunodeficiency which compromises the health of the animal mainly during the peripartum period and contributes to the occurrence of other diseases like mastitis (Nascimento et al., 2023). EBL presents in a subclinical form most of the time, causing enormous economic losses that include, in addition to the costs of veterinary services, a reduction in milk production, reproductive efficiency, and productive life, as well as an increase in animal deaths, and restrictions on the sale of cattle. Due to the decline of milk production alone, EBL led to an estimated loss of 525 million USD in 2003 (Ott et al., 2003). EBL also compromises public health via its association with breast cancer (Gao et al., 2020). The virus was identified in human-derived breast cancer tissue, making it possible to correlate the consumption of meat and/or milk with a higher frequency of disease development (Schwingel et al., 2019; Khalilian et al., 2019), as the virus has been identified in the bovine-derived milk and meat (De Quadros et al., 2023). However, another study did not find any BLV proviral DNA or antibodies in human tissue (Yamanaka et al., 2022).

EBL can be transmitted in different ways, including horizontal transmission, animal-to-animal contact, iatrogenic, arthropods, and vertically or congenitally, from mother to calf, in utero or during the peripartum period, as well as through suckling, either through colostrum or milk (Ott et al., 2003). Due to these multiple routes of transmission, EBL is widespread throughout the world, including in Brazil, which represents an increase in risk for an already endangered species such as the Crioula Lageana breed (FAO, 2022).

In Brazil, serological prevalence research has been conducted in both beef and dairy cattle across all regions of the country, ranging from 9.2% (Carneiro et al., 2003) to 79.7% (Rajão et al., 2014). The only study on enzootic leukosis prevalence in the state of Santa Catarina was carried out by Rodakiewicz et al. (2018) in Holstein, Jersey and crossbred dairy cattle. The prevalence was 42.1% (191/454) in the Agar Gel Immunodiffusion Assay (AGID).

Furthermore, since there is currently no cure or commercially available vaccine (WOAH, 2021), it is necessary to adopt prevention and control measures through the identification and segregation of positive animals. Identifying the positive animals is the first step in controlling and eradicating BLV among a population (WOAH, 2021), which enables the segregation of positive and negative animals to create an EBL-free herd.

Due to the importance of the Crioula Lageana breed as a genetic resource, especially regarding disease resistance, and the fact that many individuals of this breed have a long life span, which allows greater contact with infectious agents such as the LEB virus, associated with the fact that there are no previous studies, this work is important as an effort to preservation of this breed. The objective of this study was to determine the prevalence of natural BLV infections using two different diagnostic methods, PCR and ELISA, in Crioula Lageana cattle of different animal categories, in the Planalto Serrano of Santa Catarina, Brazil.

2. Material and Methods

2.1. Sample size determination

For the determination of the prevalence of natural infection by the enzootic bovine leukosis virus (EBL) in Crioulo Lageano cattle population, first the number of animals to be sampled was calculated using the Formulas 1 and 2 proposed by the Panamerican Health Organization (OPAS, 1979), as follows:

$$n_0 = \frac{1.96^2\left[p(1-p)\right]}{(d)^2} \quad (1)$$

wherein: n_0 represents the number of samples, p is the expected prevalence, and d symbolizes the margin of error.

Assuming a 50% prevalence of positive samples, a 95% confidence interval, and a 5% margin of error, a sample size of 384 animals was estimated. However, since this is a finite population, further calculations were performed:

$$n = \frac{Nxn_0}{N + n_0} \quad (2)$$

wherein: *N* is the total number of animals in the population, which comprises 1500 animals of the Crioula Lageana breed.

These calculations resulted in the amount of 306 animals to be sampled.

2.2. Animals and ethics

Project approved by nº2461171115 of the Committee for Ethics in Animal Experimentation (CEUA) from Universidade do Estado de Santa Catarina (UDESC). The animals used were registered at the Crioula Lageana Cattle Breeders Association (ABCCL). Cattle of the Crioula Lageana breed (Figure 1) were used, coming from conservation nucleus properties *in situ*, located in four different towns (Figure 2): Lages (Latitude: -27.8167°, Longitude: -50.3264 27° 49' 0" S, 50° 19' 35" W, Altitude 930 m) two farms; Painel (Latitude: 27° 55' 30" S, Longitude: 50° 6' 12" W, Altitude 1.101 m) one farm; Ponte Alta (Latitude: 27° 29' 03" S, Longitude: 50° 22' 49" W, Altitude: 856 m) two farms; and Curitibanos (Latitude: 27° 16' 58" S, Longitude: 50° 35' 04" W, Altitude 987 m) one farm.

Blood samples were obtained from 308 animals, including the five available categories, 32 bulls, 140 cows, 65 heifers, 30 male calves, and 41 female calves. Blood samples were collected via venipuncture from the external jugular vein into vacuum tubes, with anticoagulant (10% EDTA) for hemogram and PCR, and without for ELISA assays. Samples without anticoagulant, after phase separation of the serum, were stored in a freezer at -20 °C until the ELISA test. The samples with EDTA were kept at -20 °C until DNA extraction and PCR assay.



Figure 1. Specimens of Crioula Lageana cattle (a), reared on native pasture, from an *in situ* conservation nucleus. Important to observe the variety in hair colour and the long horns. Municipality of Painel, Santa Catarina, Brazil (b).

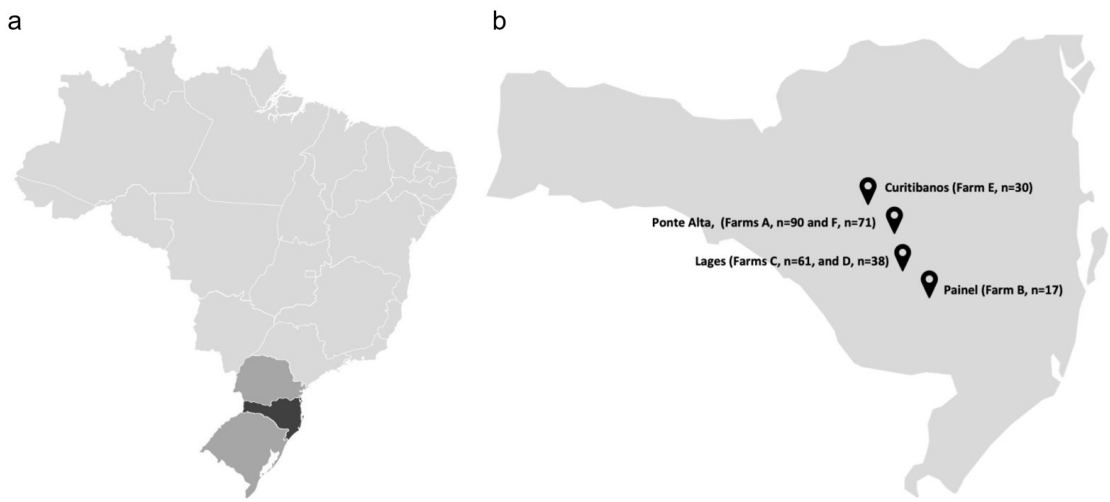


Figure 2. Highlighted (a), is Brazil's Southern region, emphasizing the state of Santa Catarina, where the experiment took place, in the darkest shade of gray; The four municipalities where six in situ conservation sites for the Crioula Lageana cattle breed are located, within the state of Santa Catarina, Brazil (b).

2.3. Hematology

The automated blood cell counter SDH3 Cleaner (Labtest, Lagoa Santa, Brazil) was used to measure the total number of leukocytes. A leukocyte differential count was performed using microscopic analysis of blood smears stained with Romanowsky-type stains (Fast Panoptic) (Jain and Schalm, 1993). After PCR and leukograms results, the animals were divided into four different groups: negatives, composed of animals with negative PCR and lacking hematological alterations; positives without leukocytosis, composed of animals with BLV-positives in PCR and not presenting leukocytosis; positives leukocytic non-lymphocytic: composed of animals with BLV-positives in PCR and presenting a non-lymphocytic leukocytosis; and positives leukocytic with lymphocytosis, composed of animals with BLV-positives in PCR and presenting a leukocytosis with lymphocytosis. Leukocytosis was considered when the total leukocyte count was greater than $15x10^3$/µL, and lymphocytosis was considered when the total lymphocyte count was greater than $10x10^3$/µL (Brenner et al., 2007).

2.4. Enzyme-linked immunosorbent assay (ELISA)

To carry out the enzyme immunoassays, the serum samples were thawed and subsequently used for the detection of antibodies against EBL using commercial ELISA reagents (Bovine Leukaemia Virus Antibody Test Kit, Veterinary Medical Research and Development, VMRD, Pullman, WA, EUA).

2.5. Polymerase chain reaction (PCR)

Cellular fraction samples were used for DNA extraction and subsequent PCR analysis. DNA extraction was performed using the ReliaPrep™ gDNA Miniprep System (Promega, WI, USA) according to the manufacturer's instructions and used for amplification of the 440 pb fragment of the EBL virus *env* gene. The PCR reaction took place in a final volume of 25 µL of reaction, containing 1 U of Taq Polymerase enzyme GoTaq® Hot Start Polymerase, (Promega, WI, USA); 8.5 pmoles of each primer: OBLV1A (5'-CTTTGTGTGCCAAGTCTCCCAGATACA-3') and OBLV6A (5'-CCAACATATAGCACAGTCTGGGAAGGC-3') [24], 0.2mM nucleotides (dNTPs); 3.5mM magnesium chloride; 5µL of 5 X Green GoTaq® Flexi Buffer (Promega, WI, USA); 3 µL of DNA (concentration between 20 and 100ng/µL) and ultra-pure water to complete the volume. For each reaction positive and negative controls were used.

The temperature conditions applied in the thermal cycler Biocycler® (Biosystems, Brazil) were: 94 °C for five minutes, followed by five cycles of 94 °C for 45 seconds, 60 °C for 1 minute, and 72 °C for 90 seconds, followed by 35 cycles of 94 °C for 45 seconds, 59 °C for 60 seconds, and 72 °C for 90 seconds, and the last cycle of final extension at 72 °C for 7 minutes. The electrophoresis of the amplification products was carried out in a horizontal tank in a 2% agarose gel with the addition of Unisafe Dye 20,000x (Uniscience, Brazil). A 100 pb molecular weight marker was used as a standard to determine the size of the sample bands. The electrical source conditions were 140 Volts and 400 mA for 40 minutes, and visualization was achieved by exposure to ultraviolet light. Bands with a size close to 440 pb were considered positive for BLV.

2.6. Statistical analysis

To calculate the prevalence, a descriptive statistical analysis of the data was carried out, with the calculation of the prevalence rate in the sample population by property and by category. The categories were separated by sex and age group, males over two years old, cows over two years old, heifers between one and two years old, and calves and heifers up to one year old. The categories were compared using the Chi-square test ($P<0.05$) between positives and negatives for the natural infection with BLV. The leukogram results were compared among groups using the Kruskal-Wallis test ($P<0.05$) after being assessed by the Shapiro-Wilk Normality Test ($P<0.05$).

3. Results

3.1. Prevalence of Natural BLV Infection in Crioulo Lageano

The prevalence of EBL was evaluated in 308 bovine animals of the Crioulo Lageano breed on six different farms using ELISA and PCR assays. We found an overall prevalence of 6.82% (21/308) for the ELISA test (Table 1) and 36.68% (113/308) for PCR (Table 2).

Table 1. Seroprevalence of enzootic bovine leukosis (EBL) in 308 Crioula Lageana cattle using the ELISA test in each different animal category and farms in Planalto Serrano, Santa Catarina, Brazil.

Farm	Bulls	Cows	Heifers	Calves		Total
				Males	Females	
A	1/4 (25.00%)	4/15 (26.67%)	2/40 (5.00%)	0/6 (0.00%)	0/25 (0.00%)	7/90 (07.38%)
B	0/0 (00.00%)	0/7 (00.00%)	0/6 (00.00%)	0/2 (0.00%)	0/2 (0.00%)	0/17 (00.00%)
C	1/5 (20.00%)	3/39 (07.67%)	0/3 (00.00%)	0/13 (00.00%)	0/1 (00.00%)	4/61 (06.56%)
D	0/3 (00.00%)	3/19 (15.79%)	0/8 (00.00%)	0/1 (00.00%)	0/7 (00.00%)	3/38 (07.89%)
E	0/0 (00.00%)	2/22 (09.09%)	2/7 (28.57%)	0/1 (00.00%)	0/0 (00.00%)	4/30 (13.33%)
F	1/20 (05.00%)	1/38 (02.93%)	0/1 (00.00%)	1/7 (14.28%)	0/6 (00.00%)	3/72 (04.16%)
Total	**03/32 (09.37%)**	**13/140 (09.27%)**	**04/65 (06.15%)**	**01/30 (03.33%)**	**00/41 (00.00%)**	**21/308 (06.82%)**

Table 2. Prevalence of enzootic bovine leukosis virus (BLV) in 308 Crioula Lageana cattle using PCR, in each different animal category and farms in Planalto Serrano, SC, Brazil.

Farm	Bulls	Cows	Heifers	Calves		Total
				Males	Females	
A	0/4 (00.00%)	5/15 (33.33%)	17/40 (42.50%)	0/6 (00.00%)	8/25 (32.00%)	30/90 (33.33%)
B	0/0 (00.00%)	0/7 (00.00%)	0/6 (00.00%)	0/2 (00.00%)	0/2 (00.00%)	0/17 (00.00%)
C	2/5 (40.00%)	25/39 (64.10%)	0/3 (00.00%)	6/13 (46.15%)	0/1 (00.00%)	33/61 (54.1%)
D	2/3 (66.67%)	1/19 (05.26%)	5/8 (62.50%)	0/1 (00.00%)	4/7 (54.14%)	12/38 (31.58%)
E	0/0 (00.00%)	5/22 (22.72%)	1/7 (14.28%)	0/1 (00.00%)	0/0 (00.00%)	6/30 (20.00%)
F	6/20 (05.00%)	21/38 (55.26%)	0/1 (00.00%)	4/7 (54.14%)	1/6 (16.66%)	32/72 (44.44%)
Total	**10/32 (31.25%)**	**65/140 (40.71%)**	**23/65 (35.38%)**	**10/30 (33.33%)**	**13/41 (31.71%)**	**113/308 (36.68%)**

Table 3. Prevalence of the bovine enzootic leukosis virus (BLV) in 308 Crioula Lageana cattle using PCR, in the different classes and associations using the Chi-Square test (χ^2), in six farms in Planalto Serrano, Santa Catarina, Brazil.

Categories		BLV positives	Chi-squared			
			Bulls	Cows	Heifers	Female calves
Bulls		31% (10/32)	----	----	---	---
Cows		40% (57/140)	P=0.321	---	---	---
Heifers		35% (23/65)	P=0.686	P=0.490	---	---
Calves	**Females**	32% (13/41)	P=0.967	P=0.313	P=0.697	---
	Males	33% (10/30)	P=0.861	P=0.470	P=0.885	P=0.831

3.2. Prevalence of natural BLV infection in Crioula Lageano cattle of different ages

For the ELISA test, the seroprevalence of BLV among different animal categories was 9.37% (03/32) for bulls; 9.28% (13/140) for cows, 6.15% (04/65) for heifers and 3.33% (01/30) for male calves. None of the evaluated female calves showed BLV serum reactivity (Figure 3). When PCR was used, the positive detection of BLV was 31.25% (10/32) for bulls; 40.71% (57/140) for cows; 35.38% (23/65) for heifers (10/30); 33.33% (10/30) for male calves; and 31.71% (13/41) for female calves (Figure 3). There was no difference between sex and categories by Chi-square Test between positives and negatives for the natural infection with BLV (P>0.05) (Table 3).

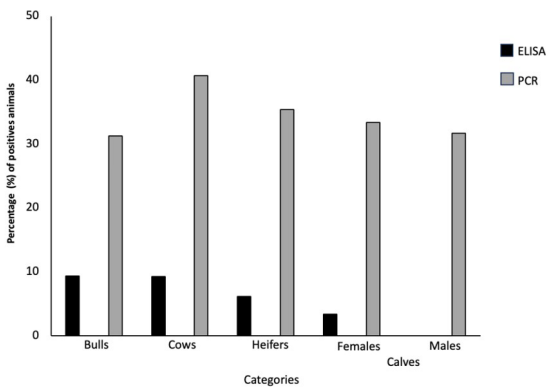


Figure 3. Prevalence of enzootic bovine leukosis (EBL) detected by ELISA and PCR tests in different categories of 308 Crioula Lageana cattle Planalto Serrano, Santa Catarina, Brazil.

3.3. Prevalence of BLV in Crioulo Lageano cattle in different farms

The seroprevalence for BLV among the different farms was also compared, showing farm A 7.78% (7/90); B 0% (0/17); C 6.56% (4/61); D 7.89% (3/38); E 13.33% (4/30); and F 4.16% (3/72) (Figure 4), with no differences (P<0.05) found for ELISA. The prevalence of BLV in PCR among the different farms was also compared with farm A 33.33% (30/90); B 0% (0/17); C 54.1% (33/61); D 31.58% (12/38); E 20% (6/30); and F 44.44% (32/72) (Figure 4) with no difference (P<0.05).

3.4. Hematology of BLV in Crioulo Lageano

The blood analysis showed that all animals with a BLV-negative result for PCR also were non-leukocytic (Table 4). The results for each group were: group negative 63.31% (195/308); group positive without leukocytosis 29.54% (91/308); group positive with leukocytosis without lymphocytosis 1.29% (4/308); and group positive with leukocytosis with lymphocytosis 5.84% (18/308) (Table 5). The prevalence of positive animals with leukocytosis and lymphocytosis was 11% (18/113) as evaluated with PCR. Among the animals presenting lymphocytosis, the results for each category were: bull 3.12% (1/32), cows 2.14%

Table 4. Mean values and standard deviations (x±sd) of the leukocyte count, total number of leukocytes, rod neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils of 308 Crioula Lageana cattle from the Planalto Serrano, Santa Catarina, Brazil, evaluated regarding EBL through polymerase chain reaction (PCR).

Cell types (x10³/μL)	Negatives (n=195)	Positives without leukocytosis (n=91)	Positives leukocytic non-lymphocytic (n=4)	Positives leukocytic lymphocytic (n=18)
Leukocytes	12.80±4.91^{bc}	10.71±2.93^{d}	17.35±2.3^{ac}	21.80±6.20^{a}
Band neutrophils	0.03±0.08^{a}	0.02±0.07^{a}	0.04±0.08^{a}	0.06±0.13^{a}
Segmented neutrophils	3.40±2.55^{ac}	2.76±1.55^{bc}	6.58±3.32^{a}	5.84±5.27^{a}
Lymphocytes	8.06±3.52^{c}	6.75±2.54^{b}	8.41±1.53^{bc}	14.43±3.94^{a}
Monocytes	0.39±0.34^{b}	0.41±0.39^{b}	0.68±0.09^{ab}	0.83±0.81^{a}
Eosinophiles	0.70±0.71^{a}	0.65±0.54^{a}	1.64±1.68^{a}	0.62±0.81^{a}
Basophiles	0.01±0.04^{a}	0.01±0.04^{a}	0.00±0.00^{a}	0.01±0.05^{a}

Different letters indicated statistical differences (P<0.05).

Table 5. Percentage and number over total of animals in each category negative for BLV in PCR and not presenting leukocytosis, positive without leukocytosis, positive with non-lymphocytic leukocytosis and with lymphocytosis in 308 Crioula Lageana cattle in the Planalto Serrano, Santa Catarina, Brazil.

Categories		Groups			
		Negatives	Positives without leukocytosis	Positives leukocytic non-lymphocytic	Positives leukocytic lymphocytic
Bulls		68.75% (22/32)	28.12% (9/32)	0% (0/32)	3.12% (1/32)
Cows		59.28% (83/140)	36.42% (51/140)	1.42% (2/140)	2.14% (3/140)
Heifers		63.07% (41/65)	30.76% (20/65)	1.53% (1/65)	4.61% (3/65)
Calves	**Females**	66.66% (20/30)	6.66% (2/30)	0% (0/30)	26.66% (8/30)
	Males	65.85% (27/41)	24.39% (10/41)	2.43% (1/41)	7.31% (3/41)
Average		**63.31% (195/308)**	**29.54% (91/308)**	**1.29% (4/308)**	**5.84% (18/308)**

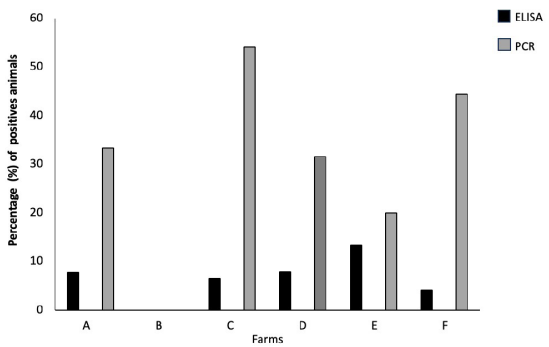


Figure 4. Prevalence of enzootic bovine leukosis (EBL) detected by ELISA and PCR tests in 308 Crioula Lageana cattle of six different farms in the Planalto Serrano, Santa Catarina, Brazil.

(3/140), heifers 2.14% (3/140), male calves 26.66% (8/30), and female calves 7.31% (3/41).

4. Discussion

In this study, we used two assays, ELISA and PCR, which are the most common methods to detect BLV (Gao et al., 2020), to assess the prevalence of BLV in six different herds. According to the World Organization for Animal Health (WOAH, 2021), both ELISA and PCR are recommended tests for the identification of EBL. The serological test, however, may present a false negative if it targets antibodies against gp51 because the animals may not have enough antibodies when the blood is collected, particularly if they are recently infected, pregnant, or if humoral immune failures occur (WOAH, 2021). As for the PCR, we used it to identify the presence of the *env* gene, as it is one of the most used in PCR tests (Gao et al., 2020; Lee et al., 2016) due to encoding viral proteins (gp51) that determine BLV infectivity (Zhao and Buehring, 2007).

The prevalence was of 6.82% (21/308) for the ELISA test and 36.69% (113/308) for the PCR technique. Our data indicate a low EBL seroprevalence based on ELISA tests 6.82% (21/308), similar to some studies. For example, in Turkey, which presented a 2% prevalence in dairy cattle (Şevik et al., 2015), 7% of a mixed herd of Friesian/native cattle crossbred with pure native cattle was EBL positive in Iraq (Khudhair et al., 2016), and 11% in Holstein and Brown Swiss in Chile (Uysal et al., 1998). Although, other studies showed a great prevalence of EBL. In Colombia, in a dairy cattle study, 19.8% of ELISA-tested animals were positive for BLV (Benavides et al., 2013), while in

the USA, 33.6% and 47.6% of beef and dairy cattle were BLV positive, respectively, with an average prevalence of 38.6% (Bauermann et al., 2017). Even higher prevalence was presented in dairy cattle in Venezuela (60.83%) (Nava et al., 2011), and Iran (81.9%) (Morovati et al., 2012). Other locally adapted or creole breeds have also been subjected to prevalence studies for EBL. Curraleiro Pé-Duro cattle from Brazil showed an average serum reactivity of 21.1% on the IGDA test (Juliano et al., 2014). In Hartón Del Valle Creole cattle from Colombia, a seroprevalence of 30.3% was identified (Hernandez et al., 2016). Both breeds showed a higher prevalence than what was detected and presented for Crioula Lageana cattle in the present study.

Regarding the PCR characterization of BLV infection, we found that Crioula Lageana cattle presented a prevalence of 36.69%, with no difference among the different categories. Our results are similar to those found in Hartón Del Valle Creole cattle; 38.3% of the animals tested positive for PCR (Hernandez et al., 2016), but higher than in a native Iraqi breed, in which 8.3% were PCR-positive for BLV (Şevik et al., 2015). In a study of Pyar Sein breeds, Friesian, Jersey, and Pyar Ni breeds from several ages, ranging from three months old to 11 years old, in Myanmar, 9.1% of animals were BLV-positive in PCR; however, the prevalence for each breed was not presented (Polat et al., 2017).

In this study, the prevalence of EBL detected by PCR, when compared among animal categories, ranges from zero in female calves to 9.37% for bulls, the category with the highest prevalence, followed by cows, with a similar prevalence of 9.28%. However, there was no difference ($P<0.05$) among the categories. This result differs from those that indicate that adult animals show a higher prevalence due to BLV (Juliano et al., 2014). The increase in the frequency of positivity as age advances may be related to a higher likelihood of contact between infected and non-infected animals, due to their longer tenure within the herd (Juliano et al., 2014).

When analyzing infection among farms, 83.33% (5/6) of farms had positive animals, regardless of whether by ELISA or PCR. This occurred because in order to obtain positive results using the ELISA test, the animal needs to present antibodies, which in turn depends on the viral load and incubation time (Gillet et al., 2007). Once the virus has infected this animal, its DNA will be present and therefore will be identified at any time, which justifies the disparity of results when comparing both tests used. PCR techniques do not replace serological tests; however, they are complementary methods that effectively identify the presence of early infection when the production of antibodies is still lacking (WOAH, 2021). One of the analyzed farms tested negative for both ELISA and PCR, which is likely the result of the adoption of handling techniques to prevent the entry of BLV since the handling methods used influence the prevalence of EBL in the herd, as shown by other studies (Benavides et al., 2013; Porta et al., 2023), or the absence of contact between the cattle and virus on this farm.

Interestingly, despite the lower or higher prevalence, no animal presented symptoms of the disease, i.e. lymphosarcoma, during this study. However, 11% (18/113) of PCR-evaluated BLV-positive animals showed leukocytosis with lymphocytosis. Lymphocytosis can be found in 30 to 70% of infected cattle, often accompanied by lymphocytic atypia; BLV primarily infects B lymphocytes, altering cellular morphology and modulating the apoptotic process (Spinola et al., 2013). This indicates that the animals are exposed to the virus, and are infected, and few animals have developed leukocytosis with lymphocytosis. None of the cattle developed the clinical form of lymphosarcoma. However, despite the absence of clinical signals, once the disease is present in the herd, the infected animals could be a source of infection for other animals on the farm.

In our study, due to logistical issues, a second blood sample collection to characterize persistent lymphocytosis was not performed. Persistent lymphocytosis can be confirmed by repeating the hemogram after 72 days (Spinola et al., 2013). Another limitation was not characterizing the viral-load in the samples. Despite the importance of this type of analysis, we were not able to performing a quantitative-PCR due to technical difficulties. However, we hope to be able to implement this technique in the future and include it in our next studies.

5. Conclusion

This is the first study on the prevalence of EBL/BLV in the Crioula Lageana breed. We found that EBL/BLV is uniformly present in Crioula Lageana cattle in both animal categories evaluated using both diagnostic methods. All animals were considered asymptomatic at the time of collection, however, they could be a source of infection for other animals on the farm or in other herds. Therefore, it is recommended that measures be taken to prevent and control the disease within the herds. Based on this knowledge, it will also be possible to carry out studies on the resistance or susceptibility of this breed in relation to EBL/BLV, as has already been done for other diseases.

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