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Molecular frequency of bovine leukemia virus in Creole cattle of Eastern Colombia

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ABSTRACT

Enzootic Bovine Leukosis (EBL), caused by the bovine leukosis virus (BLV), is a global infectious disease affecting livestock. This study focuses on studying the frequency and genetic traits of BLV in three Creole breeds including Chino Santandereano (Chino), Casanareño (CAS), and Sanmartinero (SM) in Eastern Colombia. We implemented a cross-sectional survey between 2019 and 2020 across four departments (Arauca, Casanare, Santander and Meta) in Eastern Colombia to assess the molecular characteristics of BLV infection in these breeds. A total of 253 cattle were analyzed, of which 42.6 %, 28.8 %, and 28.4 % belonged to the Chino, CAS, and SM breeds, respectively. BLV provirus was detected using nested polymerase chain reaction (n-PCR) targeting the conserved region of the *env* viral gene. Subsequently, the obtained amplicons were sequenced and subjected to phylogenetic analyses. The overall BLV infection frequency was 26.48 % (95 % CI: 21.01 – 31.98 %), with Chino exhibiting the highest frequency (35.1 %) following by SAM and CAS, respectively ($P < 0.05$). Other epidemiological variables associated with the infection included age, department, and season ($P < 0.05$). BLV-positive animals exhibited elevated levels of total serum proteins ($P < 0.05$), while molecular characterization revealed the exclusive circulation of BLV genotype 1 within these breeds. This study provides an updated assessment of BLV infection in Creole breeds from the eastern of Colombia, underscoring their lower infection frequency compared to introduced breeds and their reduced susceptibility to developing clinical signs. The epidemiological and molecular characteristics observed should be considered in developing control programs aimed at improving genetic resistance to BLV in Colombian cattle.

1. Introduction

Enzootic Bovine Leukosis (EBL) is a important worldwide infectious disease caused by the bovine leukosis virus (BLV), a retrovirus belonging to the genus Deltaretrovirus within the Retroviridae family (Barez et al., 2015). The genomic RNA of BLV spans approximately 9 kb and is flanked at both 5′ and 3′ ends by long terminal repeat (LTR) sequences (Pluta et al., 2018). Following reverse transcription, this genetic material integrates into the genome of diverse host cells, forming a provirus. The BLV provirus contains structural *gag, pol*, and *env* genes, as well as nonstructural *tax, rex, R3*, and *G4* genes (Chameettachal et al., 2023). Due to the crucial biological functions of the *env*-gp51 gene, it has been extensively utilized for BLV genotyping, phylogenetic, and epidemiological studies (Corredor-Figueroa et al., 2020). Phylogenetic analysis based on *env* sequences has revealed that BLV strains can be classified into twelve genotypes with varying distributions (Sultanov et al., 2022).

BLV can be transmitted through medical procedures (iatrogenesis), vertically via semen, or by blood-sucking insects (Order Diptera, Family Muscidae) (Chacón et al., 2023; Irimia et al., 2021; Ooshiro et al., 2013). In cattle, the clinical course of BLV infection unfolds in four distinct stages: Primary infection, persistent infection (PI), persistent lymphocytosis (PL), and lymphosarcoma (LS). The majority of cattle infected with BLV remain in the primary and PI stages, serving as asymptomatic carriers for a variable duration, ranging from a few months to several years (Aida et al., 2013). Subsequently, about 30 % of BLV-infected cattle enter the PL stage, characterized by an abnormal lymphocyte count, leading to disruptions in the immune system during several years. However, some experimental infections suggest that PL disease states

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might be achieved shortly after infection (Hutchinson et al., 2020). Tumor development occurs in a small fraction of infected animals (less than 5 %), predominantly in older cattle with a prolonged BLV infection history (Pandey et al., 2017).

While the majority of Western European Union member countries, along with New Zealand and Australia, have been declared free of BLV, there has been a concerning rise in BLV prevalence in certain American countries (Marawan et al., 2021). This increase has been particularly notable in areas from Canada and Argentina, resulting in significant economic losses (John et al., 2024; Porta et al., 2023). In the United States, approximately 47.1 % of dairy cattle are affected, and 90 % of US dairy herds harbor at least one infected animal (Benitez et al., 2022; LaDronka et al., 2018). Recent studies conducted across six different regions in Colombia have revealed prevalences ranging from 14 to 85 % among cattle, with BLV infection present in 75–100 % of the dairy and beef herds (Corredor-Figueroa et al., 2020).

Due to the absence of a worldwide vaccine, mainly due to the complexity of the BLV, along with the difficulty in implementing strategies for reducing prevalence, such as the diagnosis and elimination of BLV-positive animals, and control of hematophagous insects, the utilization of breeds resistant to the clinical course of BLV could be alternative approaches for disease control (Hernández-Herrera et al., 2014). Colombian Creole cattle and their crosses offer a viable alternative for meat and milk production in this country, due to their adaptability and resistance to ectoparasites and tolerance to infections by hemotropic agents such as *Anaplasma, Babesia*, and *Trypanosoma* (Jaimes-Dueñez et al., 2021,; 2024; Rocha et al., 2019; Rosero et al., 2012). Nonetheless, limited information exists regarding the infection rate, clinical manifestations, and circulating BLV genotypes in these breeds (Hernández--Herrera et al., 2011). This study aimed to assess the frequency and genetic traits of BLV in three Colombian Creole breeds i.e. Chino Santandereano (Chino), Casanareño (CAS), and Sanmartinero (SM), in order to gain a more thorough understanding of the current situation of BLV infection in these animals.

2. Materials and methods

2.1. Ethics statement

This study was approved and monitored by the Animal Ethics Committee of the University Cooperative of Colombia (Act No. BIO467 of 2023). All procedures were conducted in strict adherence to the applicable guidelines and regulations outlined in the Colombian Law 576 of 2000 regarding animal studies. Additionally, all participating farmers and associations provided informed consent, granting permission for the utilization of biological samples of animals in epidemiological studies over five years.

2.2. Sampling and study area

The blood samples used in this study were collected as part of a prior research conducted by our research group, aiming to investigate the epidemiological characteristics associated with hemotropic agents (Jaimes-Dueñez et al., 2021). Between June 2019 and March 2020, a total of 253 EDTA blood samples were randomly collected from a population of 422 Colombian Creole cattle across eleven farms in the departments of Santander (A-G), Casanare (H-I), Arauca (J), and Meta (K), Colombia (Table 1; Fig. 1). The number of animals sampled from each farm was proportional to the size of the farm's population relative to the total population. Within each farm, animals were randomly sampled, considering the proportion of the population under 1 year old and over 1 year old. Chino samples were obtained from seven farms in Santander. CAS samples were sourced from two farms in Casanare and one farm in Arauca. Whereas, SM samples were collected from a farm in Meta deparment (Table 1). In these farms, animals are primarily kept using extensive or semi-intensive feeding systems, where they are fed with improved grasses like *Brachiaria* spp., *Cynodon* spp., and *Panicum* spp., or native grasses such as *Axonopus* spp. and *Trachypogon* spp. These farms experience a dry season from December to the end of April and a rainy season from May to October, with the remaining months considered transitional between the two seasons.

From each animal, two blood samples of 5 mL each were collected from the jugular vein unsing a BD Serum and EDTA.K3 vacutainers (Improve, CN), respectively. Samples were stored at 4 °C until processing. During blood sampling, the rectal temperature of each animal was measured using a digital thermometer (iProven, NY, USA). Additionally, the body condition was assessed using a five-scale scoring system ranging from emaciated (very thin = 1) to obese (very fat = 5) (Ferguson et al., 1994).

Table 1
Environmental characteristics of the farms evaluated in the departments of Arauca, Casanare, Meta, and Santander Colombia.

Department	Municipality	Farm	Cordinates	Breed	Population	Sample	Altitude (m)	Annual average temperature (°C)	Annual precipitation (mm)	Season
Santander	Lebrija	A	7°06′47″N 73°13′08″W	Chino	38	20	1032	23.1	1189	Rain
	San Gil	B	6°33′33″N 73°8′6″W	Chino	35	20	1120	23.0	1274	Rain
	Barichara	C	6°38′17″N 73°12′53″W	Chino	27	20	1350	21.4	1474	Rain
	Pinchote	D	6°30′39″N 73°11′04″W	Chino	30	19	1151	22.2	1518	Rain
	Los Santos	E	6°45′12″N 73°06′06″W	Chino	12	6	1602	21.5	599	Transition
	Barrancabermeja	F	7°03′35″N 73°51′05″W	Chino	28	16	75	28.4	2836	Transition
	Los Santos	G	6°44′42″N 73°06′41″W	Chino	15	7	1600	21.5	599	Transition
Casanare	Paz de Ariporo	H	5°58′58″N 71°48′56″W	CAS	70	32	176	26.2	1216	Dry
	Yopal	I	5°19′58″N 72°23′27″W	CAS	5	4	350	26.3	3009	Dry
Arauca	Arauca	J	6°58′32″N 70°51′16″W	CAS	72	37	115	26.8	1868	Dry
Meta	San Martin	K	3°41′17″N 73°41′32″W	SM	90	72	420	25.0	2600	Dry
Total					422	253				

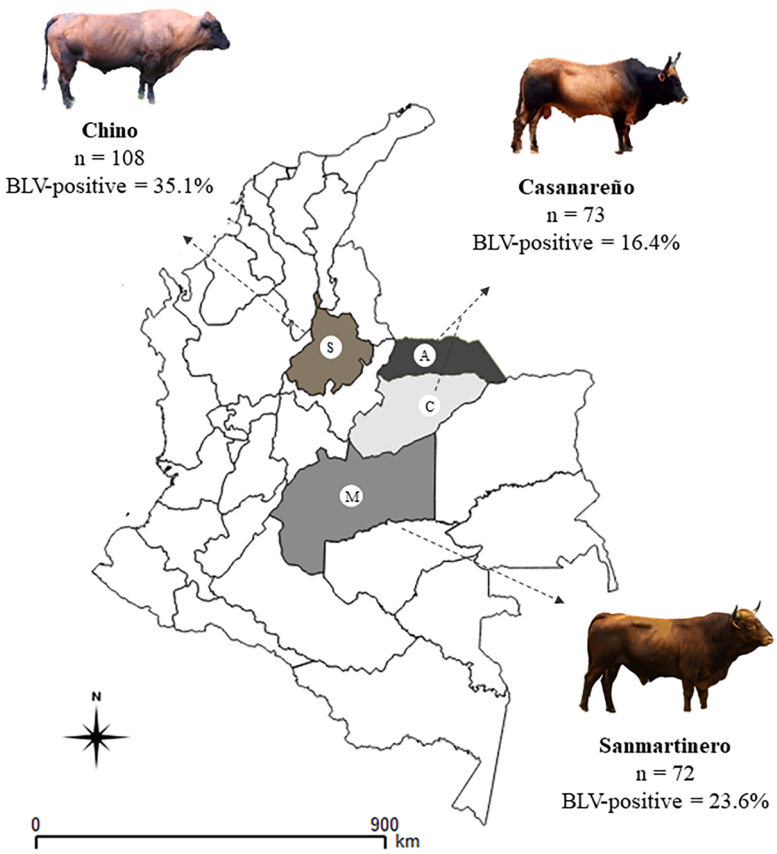

Fig. 1. Map of Colombia showing the departments of Arauca (1), Casanare (2), Meta (3), and Santander (4), and the frequency of BLV in Chino, CAS, and SM breeds.

2.3. Sample processing and molecular diagnosis

For each animal, samples in BD Serum vacutainers were centrifuged at 5000 x g for 10 min to extract the serum. To evaluate total serum proteins, 20 µl of serum were analyzed in a spectrophotometer (Thermo Fisher Scientific, USA) using a total serum protein kit AA, following the manufacturer's instructions (Wiener Lab, ARG). To evaluate packed cell volume (PCV), 50 µl of EDTA blood was transferred into a Glass Micro-Hematocrit Capillary Tube containing sodium heparin (80 IU/mL; Vitrex Medical, Herlev, Denmark). The tube was sealed at one end with Hawksley CristaSeal (Hawksley, UK). Capillary tubes were spun in a micro-hematocrit centrifuge (Thermo Fisher Scientific, USA) for 5 min at 5000 x g. PCV values were evaluated in a Micro-Hematocrit Capillary Tube Reader (Bacto, AU). Genomic DNA was extracted from 350 µl of blood containing EDTA, using a DNA purification Kit following the manufacturer's instructions (Corpogen, COL). The concentration of DNA was measured using 1 µL of sample in a Qubit™ 4 Fluorometer instrument from Thermo Fisher Scientific, USA.

For the detection of BLV provirus a conserved region of the *env* viral gene was amplified from the isolated DNA using a nested polymerase chain reaction (n-PCR) following the thermal profile (Beier et al., 2001). The first reaction was done in a 25 µl assay composed of DNA (500 ng), primers [1.25 mM] (F: TCTGTGCCAAGTCTCCCAGATA and R: AACAACAACCTCTGGGGAGGGT), each dNTP [0.2 mM] (Corpogen, COL), 1X reaction buffer [100 mM Tris–HCl, 50 mM KCl, pH 8.8], MgCl2 [1.5 mM] and Taq DNA polymerase (0.625 U) (Corpogen, COL). For the second reaction, 1 µl of the PCR product from the first reaction served as DNA template, with the same concentrations of the other components. The primers used were F: CCCACAAGGGCGGCGCCGGTTT and R: GCGAGGCCGGGTCCAGAGCTGG. The amplified PCR products were analyzed by 2 % agarose gel electrophoresis, stained with GelRed (Thermo Scientific, US), and visualized under UV light in an M-20 V lamp (Analytik-Jena, Germany). Samples were considered positive for the detection of BLV provirus when and a band of ~440 bp was observed in the electrophoresis. Positive PCR controls used in this study were derived from animals with clinical signs of EBL and confirmed as positive for BLV provirus through PCR assays.

2.4. Phylogenetic analysis

For each farm, a BLV-amplified PCR product was sent for purification and sequencing using the Sanger method at Macrogen Inc., Seoul, Korea (https://www.macrogen.com/en/main). The obtained sequences were edited, and consensus sequences were assembled using SeqMan PRO (DNASTAR Inc. Software, Madison, WI, USA) and confirmed by using the basic local alignment search tool (BLAST) at the NCBI (https://www.ncbi.nlm.nih.gov/). Datasets were constructed with sequences taken from the GenBank including relevant Colombian Sequences. The sequences were aligned using the CLUSTAL W and phylogenetic trees were constructed using the maximum likelihood (ML) method with a bootstrap value of 1000. The most appropriate nucleotide replacement model was selected considering the Akaike (AIC) and Bayesian (BIC) information criteria. All analyses were performed in MEGA v.7.0 software.

2.5. Data analysis

The frequency of infection was calculated along with a 95 % confidence interval (CI). To assess the epidemiological variables associated with the infection, a bivariate analysis (chi-squared test; χ^2) was conducted, considering seven independent variables (breed, age, sex, production system, altitude, state, and season), along with the results of the molecular analyses (BLV-positive or BLV-negative). Based on the criteria of Hosmer and Lemeshow (2005), variables with a P value < 0.25 in the

bivariate analysis were selected for testing in a multivariable logistic regression model. This model used a *Generalized Estimated Equation* (GEE) with a binomial distribution, a log link function, an exchangeable interclass correlation, and a stepwise method. In the analyses, the feature farm was included as the group variable. Results are expressed as prevalence ratio (PR) with 95 % CI. Associations were considered statistically significant if the *P* value was <0.05. To assess differences in PCV, total serum proteins, and body condition values between BLV-positive and BLV-negative, a *t*-test or Mann-Whitney test was conducted after performing the Kolmogorov-Smirnov normality test. These analyses were carried out using IBM SPSS Statistics software v.18.0 (IBM, Chicago, USA).

3. Results

3.1. Frequency of BLV provirus infection

Two hundred fifty-three cattle were analyzed by n-PCR, of which 42.6 %, 28.8 %, and 28.4 % belonged to the Chino, CAS, and SM breeds, respectively. In terms of gender, 82.2 % were females, while 73.5 % were animals older than one year. Regarding the BLV infection, 26.48 % (67/253; 95 % CI: 21.01 – 31.98 %) of the animals were positive for the *env* fragment across all farms. The highest frequency of infection was in Chino, SM, and CAS, respectively (Table 2; Fig. 1). Concerning the region, the highest frequency was detected in Santander, followed by Arauca, Meta, and Casanare, respectively. In terms of the season, the highest frequency was observed during the transition, the rainy season, and the dry season. For the other variables, the highest frequency was in animals older than one year, females, semi-extensive production systems, and altitudes higher than 1000 m (Table 2).

3.2. Epidemiological variables associated with the infection

In the bivariate analysis, all independent variables showed a significant ($P < 0.05$) association with the frequency of BLV infection, except for gender and altitude. However, when these variables were analyzed in the multivariable analysis only age, department, and season showed statistical significance ($P < 0.05$) with the frequency of infection. Specifically, for age, adult animals demonstrated an PR of 5.38 (95 % CI: 1.789–16.194) compared to animals under one year. Concerning geographical area, the departments of Arauca, Casanare, and Meta showed PRs of 0.272 (95 % CI: 0.098–0.755), 0.045 (95 % CI: 0.008–0.245), and 0.237 (95 % CI: 0.093–0.602), respectively, compared to the department of Santander. Finally, during the rainy season, there was an PR of 0.369 (95 % CI: 0.155–0.878) compared to the transition season (Fig. 2).

Table 2
Bivariate analysis between independent variables and Bovine Leukemia Virus (BLV) infection in Colombian Creole cattle populations.

Variable	n	BLV (%)	χ^2	*P*- value
Breed				
CAS	73	12 (16.4 %)	8.289	0.015*
Chino	108	38 (35.1 %)		
SM	72	17 (23.6 %)		
Age				
<1 year old	53	5 (9.4 %)	10.009	0.001*
>1 year old	200	62 (31 %)		
Sex				
Female	208	59 (28.3 %)	2.296	0.310
Male	45	8(17.7 %)		
Production system				
Semi-extensive	180	55 (30.5 %)	5.316	0.027*
Extensive	73	12 (16.4 %)		
Altitude				
≥1000 m	92	27 (29.3 %)	0.610	0.461
<1000 m	161	40(24.8 %)		
Department				
Arauca	38	10(26.3 %)	12.261	0.002*
Casanare	35	2 (5.7 %)		
Meta	72	17 (23.6 %)		
Santander	108	38 (35.1 %)		
Season				
Rain	79	23 (29.1 %)	12.901	0.003*
Transition	29	15 (51.7 %)		
Dry	145	29 (20 %)		

3.3. Clinical signs associated with the infection

A total of four clinical parameters were analyzed. In the case of total serum proteins, body condition, and PCV, higher values were observed in BLV-positive animals compared to negative ones, while for temperature, higher values were observed in the negative animals. However, significant differences were only observed in the total serum proteins ($P < 0.05$) (Table 3).

3.4. Phylogenetic analysis

BLAST analysis confirmed that all samples corresponded to the BLV. However, due to the sequence's low coverage, only two haplotypes derived from six samples collected from farms in Santander and Meta departments were included in the alignment. The analysis of the BLV best-fit model determined that General Time Reversable (GTR) + Gamma (G) + invariable sites (I) was the appropriate model for the analyzed partial sequences of the *env gene*. The Maximum Likelihood phylogenetic tree (Fig. 3) of the Colombian samples formed a Cluster with < 70 % bootstrap support inside genotype 1. Due to high homology (≥99 %), only three sequences were included in the final tree. Our sequences cluster with previously reported sequences from Colombia belonging to the Antioquia, Cundinamarca, and Boyacá departments. The twelve BVL genotypes described through the partial sequence of the *env gene* could be presented.

4. Discussion

The bovine leukemia virus is an important livestock pathogen that causes major economic losses worldwide, especially in dairy farms (Barez et al., 2015). Assessing the current status of BLV in Creole cattle breeds is crucial for determining the sanitary traits of these breeds and their suitability for integration into reproductive programs. In this study, we assessed the frequency of infection and conducted molecular characterization of BLV in three Creole breeds from the eastern region of Colombia. Our results show a low frequency of BLV infection without relevant clinical signs in positive animals, suggesting an adaptation of these breeds to the pathogen. More studies relate to BLV infection in these breeds, could emphasizing their potential role in initiatives aiming to reduce the impact of BLV on Colombian livestock.

In terms of infection frequency, our findings are lower than those detected in six departments of the country (62 %), where the evaluation focused on introduced breeds predominantly composed of Brahman and Holstein Friesian breeds and their crosses (Corredor-Figueroa et al., 2020). However, our results align with those observed in eight Colombian Creole cattle breeds in 2009 (26.7 %), using similar methods (Hernández-Herrera et al., 2011). The consistently low frequency of BLV in Colombian Creole breeds might be attributed to the high frequency of specific genotypes of the bovine leukocyte antigen (BoLA) DRB3.2 gene associated with BLV resistance in these breeds (Hernández-Herrera et al., 2014), indicating these genotypes could remain stable within these populations; however, depending on the genetic management practices, there is a possibility that the frequency of these resistant genotypes could decrease, potentially increasing susceptibility to BLV infection. Another explanation for the low frequency of infection detected here relates to diagnostic methods. It has been demonstrated that some BLV-positive cows (antibody-positive) have undetectable proviral loads and therefore may not be detected through a PCR test

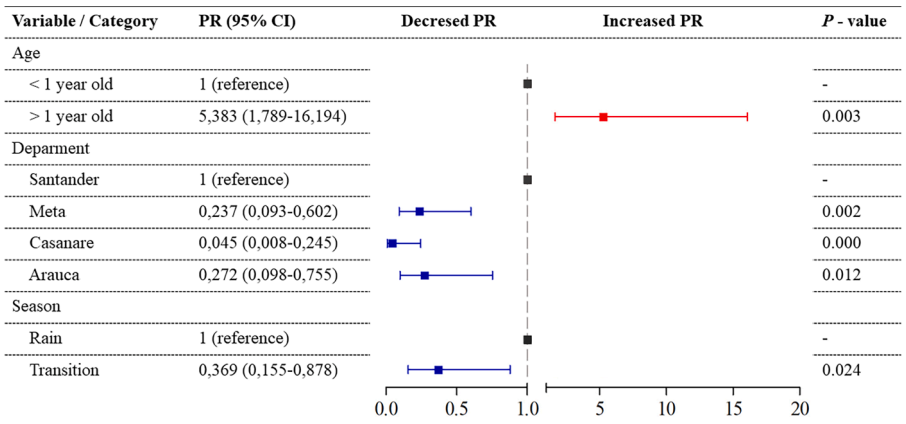

Fig. 2. Forest plot of prevalence ratios (PR) obtained from the multivariate analysis (GEE) of infection with BLV in Chino, CAS, and SM breeds.

Table 3
Clinical and hematological parameters based on Bovine Leukemia Virus (BLV) infection in Colombian Creole cattle populations.

	Reference value	Positive ± SD	Negative ± SD	P-value	Test
Temperature (°C)	36.7–39.3	39.05 ± 0.4	39.08 ± 0.59	0.470	MW
PCV (%)	24–46	44.07 ± 19.5	42.72 ± 18.84	0.411	MW
Total serum proteins	5.7–8.1	11.51 ± 8.18	9.60 ± 7.42	0.029*	MW
Body condition (1 to 5)	–	3.63 ± 0.71	3.48 ± 0.62	0.125	MW

MW = Mann - Whitney test.

(Jacobs et al., 1992; Reichel et al., 1998). It is possible that due to enzootic stability in the study area, Colombian Creole breeds may maintain a low proviral load of BLV, resulting in a lower frequency of infection compared to introduced breeds when analyzed by nested PCR (n-PCR). Future serological studies between these breeds are necessary to test these hypotheses.

Otherwise, while the frequency of BLV infection has been assessed in different regions of Colombia (Corredor-Figueroa et al., 2020; Hernández-Herrera et al., 2011,; 2014), there is limited information available regarding epidemiological variables associated to the infection. This study reveals that variables such as age, department, and season demonstrate statistically significant associations with BLV infection. Regarding age, similar trends have been observed in epidemiological studies from America and Asia (Lancheros-Buitrago et al., 2023; Selim et al., 2020,; 2021), suggesting that adult animals have a higher risk of infection compared to the young. Regarding the season, the high frequency of infection detected during the transition can be explained by the increased populations of vectors such as stable flies (*Stomoxys calcitrans*), horn flies (*Haematobia irritans*), and tabanids (Diptera: Tabanidae) during this period, similar to observations in other vector-borne diseases in these regions (Jaimes-Dueñez et al., 2018). Therefore, control strategies for BLV should be intensified during this period. At last, although the association of infection frequency with departments could be statistically linked to the presence of different breeds with varying degrees of susceptibility, the high infection frequency in Santander could be explained by the increased movement of animals in this department associated with exhibitions, trade, and livestock imports, compared to others one; however, future studies are necessary to confirm this hypothesis.

On the other hand, although we analyzed the temperature, PCV, total serum proteins, and body condition in each animal, only total serum proteins exhibited an association with BLV infection, showing an increase of this biomarker in the positive animals; The serum/plasma contains thousands of proteins produced by various tissues/cells of the body. Changes in the concentration, structure, and function of serum proteins suggest an abnormal pathophysiological state (Geyer et al., 2019). Given that approximately 50–60 % of proteins in cattle are albumins and 40 % are globulins (including 2–20 % immunoglobulins) (Alberghina et al., 2011), the rise in serum proteins in BLV-positive animals could be linked to an increase in immunoglobulin levels (specifically IgM and IgG antibodies) during the acute or chronic phases of infections, and explanation to asymptomatic cases observed here. Nevertheless, considering that the research areas are epidemic regions for other hemotropic agents (Jaimes-Dueñez et al., 2021), which could also produce an increase in total serum proteins, it is imperative to conduct future clinical studies to confirm this hypothesis.

Finally, our sequences allow us to confirm the presence of the BLV genotype-1 in Creole breeds from Colombia. Genotype 1 is distributed worldwide (Polat et al., 2017), and has been found in different regions of the Americas, including Brazil, Uruguay, Argentina, Peru, and Mexico (Camargos et al., 2002; Montero Machuca et al., 2022; Moratorio et al., 2010; Polat et al., 2016). In Colombia, previous phylogenetic analysis has shown a major circulation of the BLV Genotype 1 in different departments and mainly in dairy cows (Benavides et al., 2017; Corredor-Figueroa et al., 2020; Úsuga-Monroy et al., 2023). Considering the abundance of this genotype in several countries of the Americas and its presence in Colombian Creole breeds, it is plausible that this genotype corresponds to the first genotype introduced to the Americas. However, in selected samples, Genotypes 3 (Úsuga-Monroy et al., 2023) and 6 (Corredor-Figueroa et al., 2020) have been reported, which allows us to propose that noncommon genotypes could be present in Colombia. Further analysis including a wider sample size and samples from other regions of the country is needed to evaluate the possible presence of new genotypes in the Colombian Creole cattle population.

5. Conclusions

We provided an updated assessment of both the epidemiological and molecular aspects of BLV infection in three Creole breeds from the eastern region of Colombia. The findings evidence a lower frequency in Creole breeds when compared to introduced breeds. Epidemiological analyses highlighted a higher frequency in adults and animals from Santander, along with an increased likelihood of infection during the transition season. Molecular characterization revealed the exclusive circulation of genotype 1 of BLV in these breeds, indicating a limited genetic variability likely influenced by strong selective pressures by the immune system of these hosts. These observed epidemiological and molecular traits should be considered in the development of effective control and prevention programs for BLV in Colombian livestock.

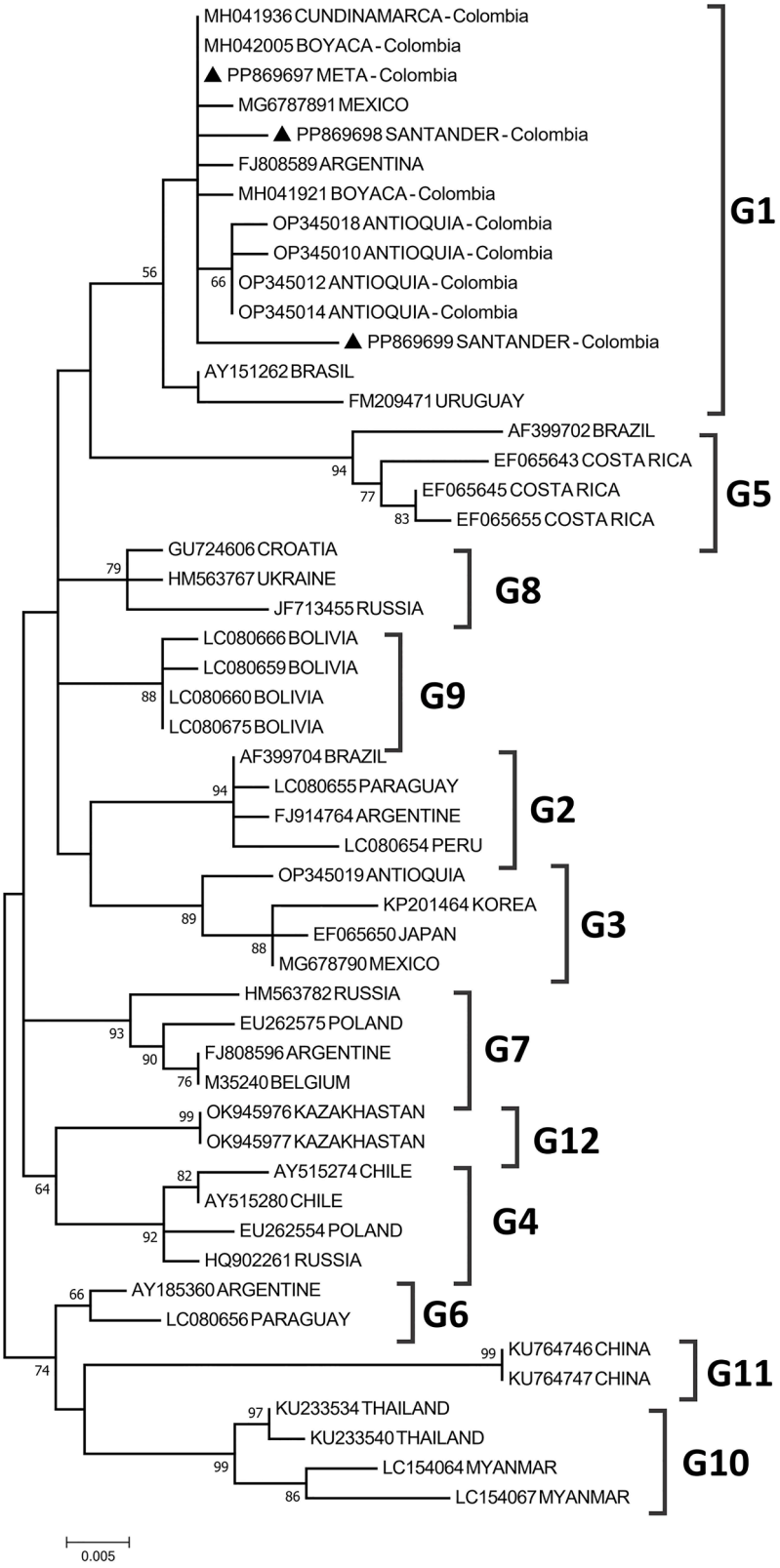

Fig. 3. Phylogenetic maximum likelihood tree of the partial BLV *env* gene. Brach support from 1000 bootstrap replicates. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Creole cattle BLV sequences are denoted in black triangles.

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Ethics statement

This study was approved and monitored by the Animal Ethics Committee of the University Cooperative of Colombia (Act No. BIO467

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CRediT authorship contribution statement

Jeiczon Jaimes-Dueñez: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Eyner Goyeneche-Ortiz:** Methodology. **Marisol Tique-Oviedo:** Investigation, Formal analysis. **Melissa C Ortiz-Pineda:** Methodology, Investigation. **Luis Cardenas-Pinto:** Methodology, Funding acquisition. **Angela Patricia Jimenez-Leaño:** Supervision, Resources, Methodology, Investigation, Formal analysis. **Julian Ruiz-Saenz:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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