Open Veterinary Journal, (2023), Vol. 13(8): 1012-1020
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)

Original Research
DOI: *10.5455/OVJ.2023.v13.i8.7*

Submitted: 02/05/2023 Accepted: 25/07/2023 Published: 31/08/2023

Risk factors associated with enzootic bovine leukosis in Boyacá and Cundinamarca municipalities, Colombia

Deisy J. Lancheros-Buitrago[1], Diana M. Bulla-Castañeda[1], Julio C. Giraldo-Forero[2,3] and Martin Orlando Pulido-Medellin[1*]

[1]*Grupo de Investigación en Medicina Veterinaria y Zootecnia—GIDIMEVETZ, Universidad Pedagógica y Tecnológica de Colombia, Tunja, Colombia*
[2]*Grupo de Investigación en Parasitología y Microbiología Tropical, Programa de Biología, Universidad INCCA de Colombia, Bogotá, Colombia*
[3]*Grupo de investigación en Ecoepidemiología y Salud Colectiva, Facultad de Medicina y Ciencias de la Salud, Universidad Militar Nueva Granada, Bogotá, Colombia*

Abstract

Background: Enzootic bovine leukosis (EBL) is a lymphoproliferative disorder caused by the bovine leukemia virus (BLV), a virus of the Retroviridae family. The infection is distributed worldwide, and a high percentage of animals infected by the BLV are asymptomatic and act as carriers of the virus in many cattle populations.
Aim: To identify the risk factors associated with EBL in the municipalities of Boyacá and Cundinamarca (Colombia).
Methods: A simple descriptive cross-sectional study with random sampling was conducted. A total of 1,140 blood samples were taken from cattle (females and males) from the municipalities of Chiquinquirá, Ubaté, and San Miguel de Sema of different breeds and age groups. The samples were processed using the commercial ELISA SERELISA® BLV Ab Mono Blocking kit (sensitivity 97%, specificity 98%). The data were processed with the statistical programs WinEpi and Epi Info® version 7.2.4.0, estimating the prevalence ratio, implementing the chi-square test ($p \leq 0.05$) and logistic regression.
Results: A true prevalence (TP) and apparent prevalence (AP) of 23.61% and 22.7% in Ubaté, 19.22% and 18.1% in Chiquinquirá, and 15.61% and 14.3% in San Miguel de Sema, respectively, were established. Bovines 2–4 years old were the most prevalent in Ubaté and Chiquinquirá (37.5% and 21.21%, respectively), while in San Miguel de Sema individuals >4 years had the highest percentage of antibodies (18.3%). The Holstein breed had a higher prevalence in Ubaté and San Miguel de Sema (26.02% and 19.67%), and crossbreeds were more BLV-seroprevalence in Chiquinquirá (20.20%). In Ubaté, re-use of needles was identified as a risk factor, contaminated blood in needles is considered one of the main routes of transmission. On the other hand, manual milking was identified as a risk factor in San Miguel de Sema.
Conclusion: The non-implementation of an individual needle per animal in Ubaté; the Holstein breed and manual milking in San Miguel de Sema were identified as risk factors for the presence of antibodies against the disease. EBL prevention and control plans should be established that focus on the implementation of management and sanitary practices based on herd biosecurity.
Keywords: Cattle diseases, ELISA, Leukosis, Risk factors.

Introduction

Enzootic bovine leukosis (EBL) is a chronic lymphoproliferative disorder caused by bovine leukemia virus (BLV), of the genus *Deltaretrovirus* and family Retroviridae (Benitez *et al.*, 2019, 2020; Kobayashi *et al.*, 2020; Bulla-Castañeda *et al.*, 2021; Cândida Ramalho *et al.*, 2021; Nakada *et al.*, 2023). BLV generates a chronic, progressive, and contagious disease characterized by malignant lymphoid cell hyperplasia and systemic lymphadenopathy, which affects almost all countries and regions where cattle are raised (Ma *et al.*, 2021). The disease is considered an economically important infection of dairy cattle and the common mode of virus spread is horizontal or iatrogenic transmission, through direct and indirect exposure of susceptible animals to infected lymphocytes from blood, milk, colostrum, and by natural mating (Benitez *et al.*, 2019; Kuczewski *et al.*, 2021; Jiménez Sánchez *et al.*, 2022).

Dairy and beef cattle breeds are susceptible to BLV infection; however, the disease is the most prevalent

***Corresponding Author:** Martin Orlando Pulido Medellin. Grupo de Investigación en Medicina Veterinaria y Zootecnia—GIDIMEVETZ, Universidad Pedagógica y Tecnológica de Colombia, Tunja, Colombia. Email: *martin.pulido@uptc.edu.co*

in dairy herds, mainly due to management practices (Gutiérrez *et al.*, 2020). Proximately 70% of animals appear clinically healthy for the first few years' post-infection, becoming asymptomatic carriers of the virus and developing persistent lymphocytosis (Benitez *et al.*, 2020; Kobayashi *et al.*, 2020; Jiménez Sánchez *et al.*, 2022). The main transmission via is the horizontal way, this occurs by direct contact with infected leukocytes. Transplacental transmission could occur in less than 10% of infections in cattle (Tsutsui *et al.*, 2016; Ruiz *et al.*, 2018).

BLV infection leads to decreased immune function, making animals more vulnerable to other diseases, which could shorten their productive life (Benitez *et al.*, 2020). On the other hand, could be a potential zoonotic disease, Ma *et al.* (2021) and Olaya-Galan *et al.* (2021) reported that BLV has been found in the blood and mammary tissues of women with breast cancer, and an association between this and the virus was confirmed in Colombia, as an intermediate risk factor; however, the link between BLV and human breast cancer is somewhat controversial. Moreover, there is no specific treatment for the disease or commercial vaccine that allows the prevention of the disease (Bulla-Castañeda *et al.*, 2018).

Consequently, in the absence of control measures, the virus spreads slowly among the animals in the herd, which is why it is considered necessary to prevent the spread of BLV to reduce economic losses related to cattle infection (Gutiérrez *et al.*, 2020; Cândida Ramalho *et al.*, 2021; Wu *et al.*, 2023). Until now, there have been few seroprevalences of BLV studies in Colombia (Corredor-Figueroa *et al.*, 2020). Therefore, the objective of this study is to determine the risk factors associated with EBL in the municipalities of Boyacá and Cundinamarca (Colombia).

Materials and Methods

Study area

The Altiplano Cundiboyacense comprises the valleys of Ubaté, Chiquinquirá, and the valleys of Tunja, Duitama, and Sogamoso. Villa de San Diego de Ubaté, Chiquinquirá, and San Miguel de Sema are municipalities localized in the valleys of Chiqunquirá and Ubaté (Castrillón Franco, 2014; ICA, 2016). The municipalities are part of the high tropic zones where most milk is produced, with 75,731 heads of cattle registered in the region (ICA, 2022).

Sample size

The municipalities of Chiquinquirá, San Miguel de Sema, and Villa de San Diego de Ubaté have 33,398, 23,057, and 19,276 heads of cattle, respectively (ICA, 2022). Based on the number of bovine animals in the municipalities, a sample size of 385, 378, and 377 cattle was established for each of the municipalities, correspondingly. The sample size for each testing municipality was determined by considering a 95% confidence interval (CI), an accepted error of 5%, and an expected prevalence of 50% since there are no studies in the area, implementing the WinEpi statistical program and following the following equation:

$$n = \left(\frac{Z_{\alpha/2}\sqrt{p(1-p)}}{E} \right) = \frac{Z^2\alpha/2 \cdot p(1-p)}{E^2}$$

where: n = sample size; E = accepted error; p = expected value of the proportion; α = tail probability.

Sample collection and preparation

Blood samples were taken from females and males of dairy cattle breeds belonging to different age groups (<2, 2–4, and >4 years). The biological material was obtained via coccygeal venipuncture, using a 16 and 18- gauge, 3-inch needle. Approximately, 14 ml of blood was extracted in tubes without anticoagulant, previously labeled. The samples were transported to the Veterinary Parasitology Laboratory of the Universidad Pedagógica y Tecnológica de Colombia for processing. The tubes were centrifuged at 2,500 revolutions per minute (rpm) for 10 minutes to separate the cells from the serum (Figueiredo Marques *et al.*, 2017). The serum samples were processed via an Enzyme-Linked Immunosorbent Assay (indirect ELISA) technique with the commercial ELISA kit SERELISA® BLV Ab Mono Blocking (sensitivity 97%, specificity 98%), according to the manufacturer's instructions.

Variables

An epidemiological survey was conducted in each cattle herd, and a descriptive form was filled out to propose possible risk factors associated with EBL seropositivity. Is a form that retrieves information from the farms and that was designed considering those variables that, according to the literature, have some relationship with the positivity of the virus. The information was taken from questions whose answers were based on yes/no. The variables to be evaluated were classified into four categories: a) reproductive variables: artificial insemination, natural mating, certified semen, uncertified semen, and shared bull; b) management variables: livestock owned by other owners, pasture leasing, re-use of needles, attendance to livestock expositions, mechanical milking, and manual milking; c) herd size: large herd, and small herd; and d) animal variables: gender, age group, and cattle breed.

Statistical analysis

The apparent prevalence (AP) and true prevalence (TP) were determined with the WinEpi statistical program, considering the sensitivity and specificity of the test. Subsequently, the database was consolidated in Excel and processed using the statistical program Epi Info® version 7.2.4.0. The proportion of animals and herds affected by EBL and exposed to the factors was evaluated with the same proportion of a population not exposed to that factor to estimate prevalence ratios (PR). This PR was used to measure the association between EBL and the hypothesized causal factors, as well as the significance of these associations using a Chi-square test (Thrusfield, 2005). PR values greater than 1 (lower 95% CI < 1) and with $p < 0.05$ were considered risk factors, while PR values less than 1 (upper 95% CI < 1) and with $p < 0.05$ were protective factors. Once these factors were established, a stratified logistic regression was performed to test for confounding variables and to identify the simultaneous interaction between variables significantly associated with EBL (Martin *et al.*, 1997).

Ethical approval

This study was conducted in accordance with Resolution 8,430 of the Colombian Ministry of Health and Social Protection and Law 84 of 1989. These establish the standards that are suitable for the welfare of the animals during the research. Written informed consent was obtained from cattle owners before sample blood collection.

Results

An AP and TP of 23.61% (89/377) and 22.7%, respectively, were determined in Ubaté, with a positive predictive value (PP+) of 93.5% and a negative predictive value (NP−) of 99.1%. In Chiquinquirá, the AP was 19.22% (74/385), while the TP was 18.1% (PV+ 91.5%; NP− 99.3%), and in San Miguel, AP of 15.61% (59/378) and TP of 14.3% (PV+ 89%; NP− 99.5%) were found. Females were more prevalent than males in the three municipalities (Table 1).

According to the study, individuals between 2 and 4 years had the highest seropositivity in Ubaté and Chiquinquirá with 37.5% and 21.21%, respectively, while in San Miguel de Sema bovines >4 years had the highest percentage of antibodies (18.3%) (Table 2). The Holstein breed had a higher prevalence in Ubaté and San Miguel de Sema (26.02% and 19.67%, respectively), although crossbreeds were more seroprevalent in Chiquinquirá (20.20%) (Table 2).

No significant statistical association was found between seropositivity to the disease and variables gender, age, and breed of cattle in the municipality of Chiquinquirá ($p \geq 0.05$). Nevertheless, statistical significance was found between antibodies presence and bovines 2–4 years old in Ubaté ($p = 0.04742422$), while in San Miguel de Sema the Holstein ($p = 0.02443112$) and Normande ($p = 0.00103089$) breeds presented significant statistical association with the presentation of BLV antibodies (Table 3).

In relation to management practices and reproductive variables evaluated in the epidemiological survey, the purchase of animals and natural mating was statistically significant in Chiquinquirá ($p \leq 0.05$). In Ubaté, livestock owned by other owners, pasture leasing, re-use of needles, attendance to livestock expositions, purchase of animals, and implementation of uncertified semen were statistically significantly correlated with antibodies presentation. Also, the variables shared bull, natural mating, manual and mechanical milking, purchase of animals, pasture leasing, and livestock owned by other owners were statistics significant to the seropositivity disease in San Miguel de Sema ($p \leq 0.05$) (Table 4).

The variables of natural mating; in Chiquiquirá, re-use of needles and uncertified semen in Ubaté; and Holstein breed and manual milking in San Miguel de Sema were established as possible risk factors for the antibodies presentation (Table 4). Re-use of needles in Ubaté, Holstein breed, and manual milking in San Miguel de Sema were identified as true risk factors for the disease in the mentioned municipalities (Table 5).

Discussion

EBL is the most frequent tumor disease in cattle. Even though a high percentage of animals infected by BLV are asymptomatic, the infection can go unnoticed when the viral load is relatively low (Gutiérrez *et al.*, 2020). Despite this, there are different investigations on EBL worldwide. Seroprevalences of 0.36% have been reported in the Zona Deprimida del Río Salado (Buenos Aires, Argentina) (Panei *et al.*, 2017), 2.27%

Table 1. Seroprevalence of EBL by gender in cattle in the municipalities of Chiquinquirá, Ubaté, and San Miguel de Sema.

Gender	*n*	Positives EBL	AP (%)	TP (%)	PP+ (%)	NP− (%)
Ubaté						
Female	358	86	24.02	23.2	93.6	99.1
Male	19	3	15.79	14.5	89.2	99.5
Chiquinquirá						
Female	371	72	19.41	18.3	91.6	99.3
Male	14	2	14.29	12.9	87.8	99.5
San Miguel de Sema						
Female	352	57	16.19	14.9	89.5	99.5
Male	26	2	7.69	6	75.5	99.8

AP: apparent prevalence; TP: true prevalence; PP+: positive predictive value; NP−: negative predictive value.

Table 2. Seroprevalence of EBL by age group and breed in cattle in the municipalities of Chiquinquirá, Ubaté, and San Miguel de Sema.

Category	*n*	Positives EBL	AP (%)	TP (%)	PP+ (%)	NP− (%)
Ubaté						
Age group						
<2 years	110	25	22.73	21.8	93.1	99.2
2–4 years	32	12	37.50	37.4	96.7	98.2
>4 years	235	52	22.13	21.2	92.9	99.2
Breed						
Ayrshire	76	13	17.11	15.9	90.2	99.4
Crossbreed	32	6	18.75	17.6	91.2	99.3
Holstein	269	70	26.02	25.3	94.3	99
Chiquinquirá						
Age group						
<2 years	99	16	16.16	14.9	89.5	99.5
2–4 years	99	21	21.21	20.2	92.5	99.2
>4 years	187	37	19.79	18.7	91.3	99.3
Breed						
Ayrshire	105	18	17.14	15.9	90.2	99.4
Crossbreed	99	20	20.20	19.2	92	93.4
Holstein	181	36	19.89	18.8	91.8	99.3
San Miguel de Sema						
Age group						
<2 years	105	12	11.43	9.9	84.2	99.7
2–4 years	113	18	15.93	14.7	89.3	99.5
>4 years	160	29	18.13	17	90.8	99.4
Breed						
Ayrshire	99	6	6.06	4.3	68.4	99.9
Crossbreed	96	17	17.71	15.8	90.2	99.4
Holstein	183	36	19.67	18.6	91.7	99.3

AP: apparent prevalence; TP: true prevalence; PP+: positive predictive value; NP−: negative predictive value.

Table 3. Analysis of breed and age groups as possible risk factors associated with BLV infections. Results are presented as PR and 95% CI.

Variable	Category	RP	95% CI	*p*-value
Ubaté				
Age group	<2 years	0.9839	0.8712–1.1112	0.45418303
	2–4 years	1.2429	0.9448–1.6351	**0.04742422**
	>4 years	0.9495	0.8430–1.0696	0.22739026
San Miguel de Sema				
Breed	Holstein	1.0981	1.0054–1.1993	**0.02443112**
	Normando	0.8623	0.7994–0.9301	**0.00103089**
	Crossbreed	1.0342	0.9313–1.1485	0.30605368

Bold value indicates statistical significance $p < 0.05$.

Table 4. Analysis of management practices and reproductive variables as possible risk factors associated with BLV infections. Results are presented as PR and 95% CI.

Variable	Category	RP	95% CI	*p*-value
Chiquinquirá				
Management practices	Purchase of animals	0.8734	0.7963–1.0580	**0.00485086**
Reproductive	Natural mating	1.1132	1.0077–1.2296	**0.04428535**
Ubaté				
Management practices	Livestock owned by other owners	0.8393	0.7544–1.0338	**0.00406659**
	Pasture leasing	0.8386	0.7483–1.0223	**0.00109153**
	Re-use of needles	1.215	1.0876–1.3574	**0.00688512**
	Attendance to livestock expositions	0.822	0.7331–1.0216	**0.01088442**
	Purchase of animals	0.8384	0.7507–1.0274	**0.00118748**
Reproductive	Uncertified semen	1.1502	1.0204–1.2965	**0.03622579**
San Miguel de Sema				
Management practices	Livestock owned by other owners	0.8512	0.7918–1.0151	**0.00049798**
	Pasture leasing	0.8976	0.8273–1.0742	**0.0131408**
	Purchase of animals	0.8537	0.7895–1.0231	**0.00022574**
	Manual milking	1.0901	1.0026–1.1852	**0.03965664**
	Mechanical milking	0.9173	0.8437–0.9974	**0.04967664**
Reproductive	Natural mating	0.8222	0.6937–1.0277	**0.00024296**
	Shared bull	0.8619	0.7988–1.0931	**0.00858792**

Bold value indicates statistical significance $p < 0.05$.

in three provinces of Ecuador (Vásconez-Hernández *et al.*, 2017), 10.8% in Paraíba (Brazil) (Ramalho *et al.*, 2021), 10% in China (Ma *et al.*, 2021), and 17.7% in Egypt (Selim *et al.*, 2020), with seropositivity below those found in the municipalities evaluated in the present study, except in Ubaté with a seroprevalence lower in contrast to Selim *et al.* (2020).

In Colombia, percentages of antibodies against BLV have been determined in 13.5% of cattle from Toca (Boyacá) using Göttingen hematological keys and ELISA (Pulido-Medellín *et al.*, 2017), in 21.8% of individuals from the department of Santander (Vargas-Niño *et al.*, 2018), in 79.17%, 53.74%, and 46.67% in the Oriente, Norte, and Valle de Aburrá subregions (Antioquia), respectively (Úsuga-Monroy *et al.*, 2018a), 31.1% in Sotaquirá (Boyacá) (Bulla-Castañeda *et al.*, 2021), 14.64% in Puerto Boyacá (Naranjo *et al.*, 2022), and 31.1% of the animals sampled in Paipa (Boyacá) (Jiménez Sánchez *et al.*, 2022). Variations occur due to the number of animals sampled, management practices, and production intensification, as well as the geographical location of the farms since it has been reported that there is a greater tendency to seropositivity to the disease in farms with temperate climate and higher altitude, that is mainly associated with sanitary and management practices such as re-use of needles, rectal palpation gloves, natural matting, among others (Vásconez-Hernández *et al.*, 2017).

As regards the age of the cattle evaluated, the highest seroprevalences were established in the older age groups in our study, this finding is similar to that reported by Ma *et al.* (2021); Bulla-Castañeda *et al.* (2021) and Jiménez Sánchez *et al.* (2022), who also found increased antibody percentages in animals in production, this may be due to the fact that the time of infection by the virus is an important factor in the pathogenesis of EBL at an early age since commonly BLV requires a long latency period to cause the disease, this is why bovine leukosis in calves is more likely to present as a sporadic form (Oguma *et al.*, 2017). Individuals 2–4 years of age in Ubaté had a significant statistical association with BLV seropositivity, which is related to that established by Jiménez Sánchez *et al.* (2022) who reported this association with animals 3–4 years old but differs from Bulla-Castañeda *et al.* (2021) because in their research the relationship was determined with the age groups of 1–2 and older than 4 years.

Taking this into account, it should be mentioned that the prevalence in cattle increases with age (Ma *et al.*, 2021). Research reports that adults are four times more

Table 5. Analysis of variables as possible risk factors associated with EBL infections.

Variable	OR	ICI (95%)	ICS (95%)	*p*-value
Chiquinquirá				
Natural mating	1.928	0.9416	3.9478	0.0726
Ubaté				
Re-use of needles	3.2426	1.2441	8.4516	**0.0161**
Uncertified semen	2.0897	0.9831	4.4419	0.0554
San Miguel de Sema				
Holstein	1.931	1.0887	3.425	**0.0244**
Manual milking	1.9537	1.022	3.735	**0.0428**

Bold value indicates statistical significance $p < 0.05$.

likely to be seropositive than heifers, leading to the virus being widely distributed in herds (Luciani *et al.*, 2022) and that the disease seropositivity was associated with the number of calvings, where there is an 80% higher probability of being BLV-positive when calvings are ≥5 (Selim *et al.*, 2020). So timely prevention and control during the calf period can reduce the spread of the disease, which is why it is recommended to strengthen detection in cattle older than 1 year and timely isolate those that are seropositive (Ma *et al.*, 2021).

Females were more prevalent than males in the three municipalities sampled, which agrees with Bulla-Castañeda *et al.* (2021), who also found that gender is a risk factor. On the other hand, the Holstein breed was the most seroprevalent in Ubaté and San Miguel de Sema, while the crossbreeds were more seropositive in Chiquinquirá, and Holstein and Normande individuals were statistically significant in San Miguel de Sema; dairy and beef cattle breeds are susceptible to virus infection; however, the disease is more prevalent in dairy production, mainly due to management practices (Gutiérrez *et al.*, 2020); hence, individuals of the Holstein breed have been considered a risk factor for antibodies against disease presentation.

Úsuga-Monroy *et al.* (2018b) mention that the infection percentage was 55.9% for the Holstein breed while for Creole and crossbred cattle this value was lower, which according to what the researcher indicates can be attributed to the presence of resistance genes in the Creole breed, they also report that the level of infection is lower in crossbred dairy cows than in the Holstein breed. Recent research establishes that BLV infection leads to a decrease in immune function, which makes animals more vulnerable to other diseases, which could shorten their productive life and increase economic losses in the cattle industry, also according to reports, BLV seropositive dairy cows are more likely to be culled earlier compared to other uninfected animals in the herd (Benitez *et al.*, 2020).

Regarding the management practices implemented in the evaluated farms in this study, a significant statistical association was established between the purchase of animals and the presence of animals from other farms. Although those variables were not considered as risk factors, that differs from Bulla-Castañeda *et al.* (2021) and Ramalho *et al.* (2021) who did report these variables as risk factors for the presence of antibodies against disease presentation. In addition, Nekouei *et al.* (2015) mention that herds that purchased animals with unknown infection status had a higher seroprevalence. Therefore, it is considered important to know the origin and health status of the animals prior to entering the farm. Besides, the re-use of needles had statistical significance in Ubaté, Hutchinson *et al.* (2020) report that the reuse of needles is associated with a higher incidence rate of the virus, this may be due to the fact that the main route of transmission of the virus is iatrogenic through the transfer of blood containing infected lymphocytes (Benitez *et al.*, 2019); hence, the re-use of needles was determined as a risk factor for disease.

Methods of milking had a significant statistical association with seropositivity disease in San Miguel de Sema. In this sense, it is important to mention that BLV proviral DNA has been identified from milk and colostrum (Benitez *et al.*, 2019). Hutchinson *et al.* (2020) mention that there is an association between milking frequency and increased incidence of EBL, suggesting a possible mode of transmission that has been largely overlooked, as BLV-infected lymphocytes are known to be present in the milk of seropositive cows, highlighting the link between increased milking frequency and BLV incidence (Ferrer *et al.*, 1981; Hutchinson *et al.*, 2020). In addition, if residual milk containing infected lymphocytes remains within the teat lining and a negative cow with minor teat damage is subsequently milked, it may be exposed to BLV-infected cells. Probably residual milk may reflux into the mammary gland of the next cow during mechanical or hand milking (Thompson and Miller, 1974; Hutchinson *et al.*, 2020). It has also been mentioned that among the factors associated with the prevalence of EBL at the herd level is the exclusive use of hand milking (Hutchinson *et al.*, 2020), which would explain why this practice is a risk factor for EBL in San Miguel de Sema.

Regarding the natural mating, the use of uncertified semen and shared bulls was significantly associated with the presentation of antibodies to BLEV ($p \leq 0.05$). However, Jiménez Sánchez *et al.* (2022) have reported no significant statistical association with these variables. Artificial insemination has been established as a risk factor for the presentation of EBL (Bulla-Castañeda *et al.*, 2021; Ramalho *et al.*, 2021) but this was not found in the herds evaluated; it should be kept in mind that proviral DNA of BLV has been identified in semen and smegma (Benitez *et al.*, 2019), so not knowing the origin and sanitary status of semen implemented on farms should be considered as a point to be taken into account for the design of LEB prevention and control plans.

BLV infection is present in Colombia, so the establishment of prevention and control plans should be implemented based on herd-level measures, focusing on good hygiene in manual and mechanical milking, sound practices and standards for artificial insemination and serological testing of animals prior to purchase (García and Olivera-Angel, 2019; Ramalho *et al.*, 2021). Additionally, it must be kept in mind that the eradication of BLV in herds should be based on the implementation of good livestock practices, where the importance of biosecurity measures such as the establishment of a closed herd is highlighted, thus avoiding the introduction of BLV in herds (Kuczewski *et al.*, 2021).

Conclusion

A moderate seroprevalence of EBL was found in the herds of the three municipalities evaluated. Nevertheless, general knowledge about the asymptomatic disease presentation, and identification of the health status of livestock, would allow the establishment of control and prevention plans for the implementation of biosecurity measures based on management practices. Adult cattle and dairy breeds were the most seroprevalent, which is mainly due to intensive herd management practices and prolonged exposure to the virus that older animals may have had. Non-implementation of individual needles, Holstein breed, and hand milking were determined as risk factors for EBL, which is related to the susceptibility of dairy breeds and the importance of implementing proper management practices to prevent iatrogenic transmission of the virus.

Acknowledgments

The authors would like to thank the Universidad Pedagógica y Tecnológica and the producers who participated in the study.

Conflict of interest

The authors declare that there is no conflict of interest.

Author´s contributions

Diana M. Bulla-Castañeda, Julio C- Giraldo-Forero and Martin O. Pulido-Medellin were responsible for the design of the study; Diana M. Bulla-Castañeda and Martin O. Pulido-Medellin performed the experiments; Diana M. Bulla-Castañeda analyzed the data; Deisy J. Lancheros- Buitrago and Diana M. Bulla-Castañeda wrote the manuscript; Deisy J. Lancheros-Buitrago, Diana M. Bulla-Castañeda, Julio C- Giraldo-Forero, and Martin O. Pulido-Medellin reviewed and editing the paper. All authors have read and agreed to the published version of the manuscript.

Funding

This investigation was funded by the call for proposal number 02-2023 of the Research Department of the Universidad Pedagógica y Tecnológica de Colombia (UPTC).

Data availability

All data supporting the findings of this study are available within the manuscript. Any extra data needed are available from the corresponding author upon reasonable request.

References

Benitez, O.J., Norby, B., Bartlett, P.C., Maeroff, J.E. and Grooms, D.L. 2020. Impact of bovine leukemia virus infection on beef cow longevity. Prev. Vet. Med. 181, 1–4.

Benitez, O.J., Roberts, J.N., Norby, B., Bartlett, P.C., Maeroff, J.E. and Grooms, D.L. 2019. Lack of Bovine leukemia virus transmission during natural breeding of cattle. Theriogenology 126, 187–190.

Bulla-Castañeda, D.M., Díaz-Anaya, A.M., Garcia-Corredor, D.J., Tobon-Torreglosa, J.C., Ortiz Ortega, D. and Pulido-Medellín, M.O. 2021. Seropositivity and risk factors associated with the presentation of bovine leukosis virus in Sotaquirá, Colombia. Vet. World 14(8), 2212–2218.

Bulla-Castañeda, D.M., García-Corredor, D.J. and Pulido-Medellín, M.O. 2018. Leusosis bovina enzoótica, revisión sistemática de literatura. Pensamiento Acción 25, 1–18.

Cândida Ramalho, G., Rodrigues Silva, M.L.C., Rodrigues Falcão, B.M., Limeira, C.H., Batista Nogueira, D., Martins dos Santos, A, Marinelli Martins, C., Alves, C.J., Clementino, I.J., de Sousa Américo Batista Santos, C. and Santos de Azevedo, S. 2021. High herd-level seroprevalence and associated factors for bovine leukemia virus in the semi-arid Paraíba state, Northeast Region of Brazil. Prev. Vet. Med. 190, 1–7.

Castrillón Franco, D. 2014. INFORME: Cuencas lecheras, motores de la producción nacional. Available via https://www.contextoganadero.com/ganaderia-sostenible/informe-cuencas-lecheras-motores-de-la-produccion-nacional

Corredor-Figueroa, A.P., Salas, S., Olaya-Galán, N.N., Quintero, J.S., Fajardo, Á., Soñora, M., Moreno, P., Cristina, J., Sánchez, A., Tobón, J., Ortiz, D. and Gutiérrez, M.F. 2020. Prevalence and molecular epidemiology of bovine leukemia virus in Colombian cattle. Infect. Genet. Evol. 80, 1–8.

Ferrer, J.F., Kenyon, S.J. and Gupta, P. 1981. Milk of dairy cows frequently contains a leukemogenic virus. Science 213, 1014–1016.

Figueiredo Marques, G., Augusto Pompei, J.C. and Martini, M. 2017. Manual Veterinario de Toma y Envío de Muestras 2017. Brazil: Panaftosa, Organización Panamericana de la Salud, pp: 112.

García, N.R. and Olivera-Angel, M. 2019. Frecuencia de anticuerpos contra el virus de la leucosis bovina (VLB) en leche de tanque. Rev MVZ Cordoba. 24(3), 7362–7365.

Gutiérrez, S.E., Lützelschwab, C.M., Barrios, C.N. and Juliarena, M.A. 2020. Leucosis bovina, una visión actualizada. Rev. Investig. Vet. Peru 31(3), 1–28.

Hutchinson, H.C., Norby, B., Erskine, R.J., Sporer, K.R.B. and Bartlett, P.C. 2020. Herd management practices associated with bovine leukemia virus incidence rate in Michigan dairy farms. Prev. Vet. Med. 182, 1–8.

ICA. 2016. El ICA trabaja con lecheros para certificar 1.200 predios como libres de brucelosis y tuberculosis en el altiplano Cundiboyacense. Available via https://www.ica.gov.co/noticias/todas/2016/el-ica-trabaja-con-lecheros-para-certificar-1-200 (Accessed 31 March 2023).

ICA. 2022. Censo Pecuario Nacional. Censo Bovino en Colombia. Available via https://www.ica.gov.co/areas/pecuaria/servicios/epidemiologia-veterinaria/censos-2016/censo-2018 (Accessed 31 March 2023).

Jiménez Sánchez, J.A., Bulla-Castañeda, D.M., Díaz-Anaya, A.M., Garcia-Corredor, D.J. and Pulido-Medellin, M.O. 2022. Serological determination of enzootic bovine leukosis virus (EBLV) in the municipality of Paipa, Boyacá (Colombia). Rev. Mex. Cienc. Pecu. 13(1), 200–210.

Kobayashi, T., Inagaki, Y., Ohnuki, N., Sato, R., Murakami, S. and Imakawa, K. 2020. Increasing bovine leukemia virus (BLV) proviral load is a risk factor for progression of Enzootic bovine leucosis: a prospective study in Japan. Prev. Vet. Med. 178, 104680.

Kuczewski, A., Orsel, K., Barkema, H.W., Mason, S., Erskine, R. and Van der Meer, F. 2021. Invited review: Bovine leukemia virus—transmission, control, and eradication. J. Dairy Sci. 104, 6358–6375.

Luciani, M.E., Gorordo, M.L., Margineda, C.A., Rüegger, M.J. and Magnano, G. 2022. Seroprevalencia del virus de la leucemia bovina en rodeos lecheros del Departamento Iriondo, Santa Fe, Argentina. Rev. Vet. 33(1), 29.

Ma, B.Y., Gong, Q.L., Sheng, C.Y., Liu, Y., Ge, G.Y., Li, D.L., Diao, N.C., Shi, K., Li, J.M., Sun, Z.B., Zong, Y., Leng, X. and Du, R. 2021. Prevalence of bovine leukemia in 1983–2019 in China: a systematic review and meta-analysis. Microb. Pathog. 150, 1–14.

Martin, S.W., Meek, A. and Willebreg, P. 1997. Veterinary epidemiology: principles and methods. Zaragoza, Spain: Editorial Acribia S.A.

Nakada, S., Fujimoto, Y., Kohara, J. and Makita, K. 2023. Economic losses associated with mastitis due to bovine leukemia virus infection. J. Dairy Sci. 106(1), 576–588.

Naranjo Guerrero, L.F., Rodríguez Colorado, N. and Mejía Araque, J. 2022. Prevalencia de diarrea viral bovina, neosporosis bovina, leucosis bovina enzoótica y paratuberculosis bovina en vacas de doble propósito en condiciones del trópico colombiano. Rev. Investig. Vet. Peru 33(2), 1–11.

Nekouei, O., VanLeeuwen, J., Sanchez, J., Kelton, D., Tiwari, A. and Keefe, G. 2015. Herd-level risk factors for infection with bovine leukemia virus in Canadian dairy herds. Prev. Vet. Med. 119, 105–113.

Oguma, K., Suzuki, M. and Sentsui, H. 2017. Enzootic bovine leukosis in a two-month-old calf. Virus Res. 233, 120–124.

Olaya-Galan, N.N., Salas-Cárdenas, S.P., Rodriguez-Sarmiento, J.L., Ibañez-Pinilla, M., Monroy, R., Corredor-Figueroa, A.P., Rubiano, W., De la Peña, J., Shen, H.M., Buehring, G.C., Patarroyo, M.A. and Gutierrez, M.F. 2021. Risk factor for breast cancer development under exposure to bovine leukemia virus in Colombian women: a case-control study. PLoS One 16(9), 1–18.

Panei, C.J., Pérez Aguirreburualde, M.S., Echeverría, M.G., Galosi, C.M., Torres, A. and Silva, H.J.E. 2017. Seroprevalencia de infección por el virus de leucosis bovina durante 2015 en rodeos de cría de la Zona Deprimida del Río Salado, provincia de Buenos Aires, Argentina. Analecta Vet. 37(1), 65–68.

Pulido-Medellín, M., González Ariza, W., Bayona, H. and Chavarro Tulcán, G. 2017. Determinación De Leucosis Enzoótica Bovina Mediante Las Claves Hematológicas De Göttingen Y Elisa En Boyacá, Colombia. Rev. Fac. Cs. Vets. 58(1), 10–16.

Ramalho, G.C., Rodrigues Silva, M.L.C., Rodrigues Falcão, B.M., Limeira, C.H., Batista Nogueira, D., Martins dos Santos, A., Marinelli Martins, C., Alves, C.J., Clementino, I.J., Américo Batista Santos, C. de S. and Santos de Azevedo, S. 2021. High herd-level seroprevalence and associated factors for bovine leukemia virus in the semi-arid Paraíba state, Northeast Region of Brazil. Prev. Vet. Med. 190, 1–7.

Ruiz, V., Porta, N.G., Lomónaco, M., Trono, K. and Alvarez I. 2018. Bovine leukemia virus infection in neonatal calves. Risk factors and control measures. Front. Vet. Sci., 5, 1–7.

Selim, A., Megahed, A.A., Kandeel, S. and Abdelhady, A. 2020. Risk factor analysis of bovine leukemia virus infection in dairy cattle in Egypt. Comp. Immunol. Microbiol. Infect. Dis. 72, 1–6.

Thompson, P.D. and Miller, R.H. 1974. Retrograde flow of milk within machine-milked teats. J. Dairy Sci. 57(12), 1489–1496.

Thrusfield, M. 2005. Veterinary epidemiology, 3rd ed. Oxford, MS: Editorial Blackwell Publishing.

Tsutsui, T., Kobayashi, S., Hayama, Y. and Yamamoto. 2016. Fraction of bovine leukemia virusinfected dairy cattle developing enzootic bovine leukosis. Prev. Vet. Med. 124, 96–101.

Úsuga-Monroy, C., Echeverri-Zuluaga, J.J. and López-Herrera, A. 2018a. Detección molecular y serológica del virus de la leucosis bovina en una población de vacas Holstein, de Colombia. Rev. Mex. Cienc. Pecu. 9(2), 387–399.

Úsuga-Monroy, C., Echeverri, J.J. and López Herrera, A. 2018b. El componente racial influencia la resistencia a la infección con el virus de la leucosis bovina. Rev. Med. Vet. Zoot. 65(2), 130–139.

Vargas-Niño, A., Jorge Vargas, R., Parra-Martin, J.A., María Vásquez, R., Agustín Góngora, O. and Mogollón-Waltero, E. 2018. Serological status of IBR, BVD, leucosis, Leptospira and Neospora caninum in bovine females of the department of Santander, Colombia. Rev. MVZ Cordoba. 23(2), 6671–6680.

Vásconez-Hernández, A., Sandoval-Valencia, P., Puga Torres, B.H. and De La Cueva-Jácome, F. 2017. Seroprevalencia De Leucosis Enzoótica Bovina En Animales Entre 6 a 24 Meses En Las Provincias De Manabí, Pichincha Y Chimborazo—Ecuador. La Granja Rev. Ciencias Vida 26(2), 131–141.

Wu, X., Notsu, K., Matsuura, Y., Mitoma, S., El Daous, H., Norimine, J. and Sekiguchi, S. 2023. Development of droplet digital PCR for quantification of bovine leukemia virus proviral load using unpurified genomic DNA. J. Virol. Methods 315, 114706.