Acta Veterinaria-Beograd 2025, 75 (4), 448-466
UDK: 636.2.09:616.98-097(862)
DOI: 10.2478/acve-2025-0035

Research article

SEROPOSITIVITY OF THE BOVINE LEUKOSIS VIRUS AND ITS EFFECT ON THE PRESENCE OF SUBCLINICAL MASTITIS IN SPECIALIZED DAIRY HERDS IN THREE HIGH TROPICAL REGIONS OF ANTIOQUIA

Caterine LÓPEZ-SÁNCHEZ[1], Cristian C. RÚA-GIRALDO[2], Juan Pablo ARISMENDY MORALES[3], Cristina ÚSUGA-MONROY[4], Albeiro LÓPEZ-HERRERA[5*]

[1]Universidad Nacional de Colombia Medellín campus, Faculty of Agricultural Sciences, Grupo BIOGEM, Cra. 65 #59a-110, Medellin, Colombia; [2]Universidad Nacional de Colombia Medellín campus, Faculty of Agricultural Sciences, Grupo BIOGEM, Cra. 65 #59a-110, Medellin, Colombia; [3]Colanta, Cra. 64c #72-157e, Medellín, Colombia; [4]Corporación Universitaria Remington, Faculty of Veterinary Medicine, Grupo GINVER, Calle 51 #51 – 27, Medellin, Colombia; [5]Universidad Nacional de Colombia Medellín campus, Faculty of Agricultural Sciences, Grupo BIOGEM, Cra. 65 #59a-110, Medellin, Colombia.

(Received 22 April, Accepted 10 September 2025)

Bovine leukosis virus (BLV) negatively affects the immune health of cattle, increasing their susceptibility to diseases such as subclinical mastitis, a common condition in dairy cows that generates significant economic losses due to decreased milk production, treatment costs, and culling of animals. The aim of this study is to evaluate the relationship between BLV seropositivity and subclinical mastitis in 200 cows from 20 specialized herds in the Aburrá Valley, north and east of Antioquia, Colombia. Milk and blood analyses were performed using the California mastitis test (CMT), somatic cell count (SCC) by flow cytometry, and BLV detection by ELISA. The results showed a 68% seropositivity to BLV, an average SCC of 168.350/mL, and a CMT index of 0.42. A high positive correlation (90%) was found between SCC and CMT, indicating the effectiveness of CMT as a diagnostic tool to assess mammary health. Furthermore, the negative correlation (-20%) between SCC and milk production evidences the impact of mastitis on productivity. The significant relationship between BLV seropositivity and increased SCC (P=0.00129) confirms the immunosuppressive effect of BLV, which predisposes cows to subclinical mastitis. In conclusion, bovine leukosis increases the susceptibility of cows to develop subclinical mastitis by weakening their immune system, compromising the general health of herds, and generating economic losses, highlighting the importance of the CMT as an efficient, rapid, and economical method for its diagnosis.

Keywords: compositional quality, diagnosis, infection, milk, production, profitability

*Corresponding author: e-mail: alherrera@unal.edu.co

INTRODUCTION

Colombian specialized dairy farming plays a fundamental role in agricultural development. This activity not only provides staple food such as milk but is also a sector that generates employment and contributes significantly to the economy of the country [1]. Its importance is reflected in the diversity of areas that stand out for their dairy production, including the Altiplano Cundiboyacense, Nariño, and Antioquia regions. The high tropics of Antioquia mainly comprise three subregions, north, east, and the Aburrá Valley, that are widely recognized for their specialized dairy production [2]. However, the efficiency of production systems can be affected by the presence of infectious agents that can harm the productive and reproductive behavior of animals, making these systems less efficient and profitable [3].

Bovine leukosis virus (BLV) is a retrovirus that primarily affects cattle, causing enzootic bovine leukosis (EBL) disease [4]. BLV is widely distributed worldwide, with twelve genotypes spread across all geographic regions [5], with some areas severely affected and others where eradication has been achieved, such as Europe [4]. In studies carried out in Colombia, a molecular prevalence of 63% was found in cattle, with the presence of genotypes 1, 3, and 6 within the national territory [6]. The virus has been found to have the ability to infect B lymphocytes of bovines and other species, including humans [4]. It is an enveloped virus and has little resistance to adverse environmental conditions. Transmission occurs horizontally through the transfer of infected cells from one individual to another through fluids such as blood, saliva, milk, or colostrum, facilitating the dissemination of the virus from infected to uninfected bovines; poor handling of animals also contributes to the spread of BLV among them through fomites [7]. Recently, BLV DNA has been detected in raw milk and fresh meat intended for human consumption, suggesting a potential zoonotic risk, although effective transmission to humans and its clinical relevance remain under debate [8]. These findings raise public health concerns, particularly in regions where proper pasteurization or adequate cooking of animal products is not guaranteed.

BLV infection has also been associated with increased somatic cell count (SCC) in milk, especially evident in cows with more than four lactations. This occurs because BLV causes a reduction in the response capacity of the immune system, making infected animals more susceptible to other diseases of infectious origin, such as mastitis [4]. The increase in somatic cell count is an indicator of subclinical mastitis, and if not effectively treated, it becomes clinical and, therefore, is associated with lower milk production, milk with higher colony forming unit (CFU) values, a lower percentage of total solids, lower production yield and quality of cheeses, and shorter shelf life of dairy products [9].

Subclinical mastitis is a form of bovine mastitis characterized by inflammation of the mammary gland without visible signs, such as the presence of pus in the milk or changes in texture or color [10]. It is a common disease that affects cows in specialized dairies around the world, causing significant economic losses for producers since it

causes a decrease of up to 30% in milk production. Further, the costs associated with the use of supplies increase due to the treatments that must be implemented; especially the use of antibiotics can increase costs by up to 12.9% [11] and, additionally can lead to the generation of bacterial populations resistant to antibiotics. For example, *Staphylococcus aureus* can be resistant to most antibiotic treatments such as penicillin, macrolides, lincomycin, cephalosporins, tetracyclines, chloramphenicol, and methicillin [12], leading to: i) an increase in the discard of unproductive cows due to the loss of glandular quarters of the udder and therefore, an increase in the expense associated with the entry of new replacements, ii) a decrease in milk payment associated with lower sanitary quality, and iii) the elimination or discarding of milk during the withdrawal period to avoid the presence of antibiotic residues in the milk [10].

Unlike clinical mastitis, subclinical mastitis does not show visible clinical signs, so it is crucial to have diagnostic techniques that allow its identification before it causes more significant losses [13]. The California mastitis test (CMT) and somatic cell count (SCC) are methods used to diagnose subclinical mastitis in dairy cows. These methods allow the evaluation of udder health and the detection of mammary infections before visible symptoms appear in the milk or the udder of the animal [14]. The CMT is a rapid and inexpensive field test based on the chemical reaction between the somatic cells in the milk and a detergent solution (sodium duodecyl sulfate or SDS). This test subjectively classifies mastitis into different degrees of severity, providing a preliminary indication of the presence of the disease. On the other hand, SCC is an objective, more accurate, and quantitative method that counts the number of somatic cells present in a milk sample by flow cytometry. An elevated somatic cell count indicates the presence of subclinical mastitis, which may require several additional management measures, such as monitoring and treating affected cows and implementing appropriate management practices [15].

Accordingly, the aim of this work was to evaluate BLV seropositivity and its effect on udder health, measured by the presence of subclinical mastitis. BLV can potentially compromise the immune system, which can increase the susceptibility of cows to udder infections, such as subclinical mastitis. Furthermore, compare the efficiency of CMT and SCC in detecting subclinical mastitis. The proper use of either of these methods allows producers to take corrective actions that decrease the prevalence of the disease and improve herd health. These types of studies are essential to improve the health management of dairy production systems, optimizing the performance and quality of the final product.

MATERIALS AND METHODS

Ethical considerations

The endorsement of the ethics committee was approved by the Institutional Committee for the Care and Use of Animals (CICUA, for its Spanish acronym) of Universidad Nacional de Colombia, Medellín campus, through Act CICUA-39-2023.

Study population and sampling

The sample size was calculated at convenience in 200 cows under production from specialized dairy systems located in the Aburrá Valley and the north and east regions of Antioquia, Colombia. These were taken from 20 herds belonging to the Colanta milk control program, selecting the same number of animals per herd (10 animals per herd), regardless of their size and composition, and chosen randomly. The list of selected cattle was taken to each herd. Two samples were taken per animal (CMT and a milk sample for SCC, and hygienic and compositional quality) with a separation of two months between each sampling (two samplings in total) to increase the reliability of the results obtained in terms of the determination of subclinical mastitis.

California mastitis test (CMT)

For the CMT test, the following protocol was carried out: The CMT paddle was washed with clean water; the udder was cleaned, and the four teats were milked, discarding the first milk letdown into a dark-bottomed container to determine the presence of abnormalities in the milk (clumps or blood). The paddle was placed under the udder and two letdowns (approximately 4 mL) were milked from each teat into its corresponding well; the same amount of the NOCAR Mastitis Diagnostic Reagent (Composition: Each 100 mL contains 4 g of sodium lauryl ether sulfate at 27.5%) was placed in each well for the California mastitis test; the reagent was mixed with the milk by moving the paddle in a circular motion for ten seconds. The result was interpreted immediately, according to the supplier's instructions and according to the intensity of the reaction found in each well. The results were classified as negative (zero crosses), traces (one cross +), positive (two crosses ++), and positive (three crosses +++).

The results of each sample were recorded for each mammary quarter evaluated, and when any of the quarters were not functional or presented clinical mastitis, this was registered. The data were stored in a database for later analysis.

Colony-forming unit (CFU) assay

Representative samples of the milking production were taken in sterile polypropylene bottles with a safety seal cap and a capacity of 50 mL to analyze the hygienic quality of the milk from each cow. The milk sample for each animal was taken directly from

the udder, milking the same amount of milk per teat and depositing it in the container without any preservative. The samples were stored in a portable cooler at 4°C to avoid bacterial growth before analysis and were sent to the Colanta Diagnostic Laboratory to quantify the CFU present in 1 mL of sample.

Somatic cell count (SCC) and compositional quality

For the compositional quality analysis (SCC), percentage of fat (%FAT), and percentage of protein (%PROT) of the milk per cow, representative samples of the production of one milking were taken in sterile polypropylene bottles with a security seal cap and a capacity of 50 mL. The milk sample for each animal was taken directly from the udder, milking the same amount of milk per teat and depositing it in a bottle to which bronopol was previously added as a preservative to inhibit bacterial growth during transport and until analysis. The samples were sent to the Colanta Diagnostic Laboratory to quantify SCC automatically by flow cytometry and milk composition (%FAT, %PROT). Somatic cell count (SCC) in milk was performed using the automated Fossomatic method, which is based on flow cytometry. Cells were stained with ethidium bromide, and the light emitted by each cell as it passed through a flow cell was measured, allowing for precise cell counting [16]. Milk fat (%FAT) and protein (%PROT) contents were determined using near-infrared spectroscopy (MilkoScan) [17].

Blood and plasma sampling

Blood samples were taken from the middle coccygeal vein using an 18-gauge needle with a vacutainer vacuum system (VACUETTE®) and EDTA as an anticoagulant to obtain plasma. All samples were homogenized by inversion and transported to the laboratory under refrigerated conditions (4°C). Blood samples were placed in a 15-mL conical tube without anticoagulant and centrifuged at 3,000 rpm for 10 min at 10 °C. Plasma was recovered and stored at – 20°C until use.

Serological test for BLV

Serological analysis was performed using a commercial ELISA kit (SVANOVIR® BLV gp51-Ab). An amount of 4 µl of the positive control and 4 µl of the negative control (both included in the kit) were added to the control wells, while 100 µl of the dilution buffer and 4 µl of serum from each of the samples were added to the other wells. The plate was covered with a sealing film and incubated at 37 °C for 1 h, after which it was washed with the PBS-Tween washing solution, dried thoroughly, and 100 µl of the conjugate (horseradish peroxidase-conjugated with anti-bovine IgG monoclonal antibodies) was added to each well and incubated at 37 °C for 1 h. Subsequently, the plate was rewashed and dried, and 100 µl of substrate solution (tetramethylbenzidine in substrate buffer with H_2O_2) was added and incubated for 10 min at 25 °C. Finally, 50 µl of the stop solution (2 M sulfuric acid) was added, and the optical density (OD)

of the controls and samples was measured at 450 nm in an ELISA reader (BioTek® ELx800) [18].

Data analysis for CMT

A cow was considered to have subclinical mastitis when the CMT result showed two (++) or three (+++) crosses, and for none of the analyses, the lost quarters (non-functional) or those with clinical mastitis were considered. For the mastitis test carried out in the field, the CMT index and the low risk–high risk (LR–HR) index [14] were calculated to quantify the results obtained and thus make them comparable with the results obtained by SCC. For this, the following equations were used.

$$CMT\ index = \frac{Sum\ of\ the\ crosses\ of\ each\ quarter}{Number\ of\ quarters}$$

$$LR\ /HR\ index = \frac{Number\ of\ quarters\ showing\ 0\ and\ 1\ cross}{Number\ of\ quarters\ showing\ 2\ and\ 3\ cross}$$

For calculations, a negative result is associated with the number 0, one cross (+) with the number 1, two crosses (++) with the number 2, and three crosses (+++) with the number 3.

Additionally, the data were processed according to the following epidemiological equations to evaluate the mammary health of each herd [14].

$$Prevalence\ in\ cows\ (P) = \left(\frac{Number\ of\ positive\ cows}{Total\ number\ of\ cows}\right) * 100$$

Prevalence in total mammary quarters (PTMQ)

$$= \left(\frac{Number\ of\ positive\ quarters}{Total\ number\ of\ quarters}\right) * 100$$

Prevalence in individual mammary quarters (PIMQ)

$$= \left(\frac{Number\ of\ positive\ quarters\ by\ position}{Total\ number\ of\ quarters\ by\ position}\right) * 100$$

Proportion of mammary quarters affected (PMQA)

$$= \left(\frac{Number\ of\ positive\ quarters\ by\ position}{Total\ number\ of\ positive\ quarters}\right) * 100$$

$$\textit{Reaction intensity (RI)} = \left(\frac{\textit{Number of cases by degree of reaction}}{\textit{Total number of cases or total number of quarters}}\right) * 100$$

Data analysis for SCC

A cow was determined to have subclinical mastitis when there were more than 250,000 somatic cells per milliliter of milk.

Statistical analysis

Data collected during herd visits were integrated into a single Excel database. The first step for statistical analysis was data exploration through descriptive statistics (mean and median), which gives an overview of the distribution and behavior of the variables in the database (CMT Index, Low-Risk – High-Risk Index, SCC, milk production, %PROT, %FAT, CFU, and %BLV seropositivity). Because outliers can significantly influence the mean, the Pearson correlation analysis results showed that the median was used as a data-cleaning tool. The median was also used to detect and handle outliers in the variables SCC, %FAT, %PROT, and CFU. Data analysis was performed in the R Software V4.3.3.

Correlation analysis between SCC and CMT

A correlation study was performed using the Pearson method in the R software V4.3.3 between SCC and the CMT index to evaluate both tests against the best diagnosis. The Pearson coefficient measures the linear relationship between these two variables. This value ranges between – 1 and 1.

If:

$r = 1$, there is a perfect positive correlation (when the CMT increases, the SCC also increases proportionally).

$r = -1$, there is a perfect negative correlation (when the CMT increases, the SCC decreases).

$r = 0$, there is no linear correlation between the two variables.

The results of the correlation analyses were statistically significant if $p<0.05$, with a confidence level of 95%.

Correlation analysis for milk quality and subclinical mastitis

A correlation was established between each diagnostic method (SCC and CMT) and the productive (kg of milk), compositional (%FAT and %PROT), and hygienic (CFU/mL) characteristics of milk. The Pearson coefficient was calculated to evaluate the

linear relationship between each method (SCC and CMT) and each variable (kg of milk, %FAT, %PROT, and CFU/mL). The correlation analysis results were statistically significant if $p<0.05$, with a confidence level of 95%.

Association analysis between diagnostic methods for mastitis and BLV infection in cows

To validate the assumptions of the analysis of variance, normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) were assessed The Shapiro–Wilk test yielded a p-value of 0.01696 ($W = 0.98307$), indicating a slight deviation from normality, which was considered acceptable given the sample size and the robustness of ANOVA. Levene's test resulted in a p-value of 0.08004, indicating no statistically significant evidence of heteroscedasticity. Hypothesis tests were performed to determine whether the observed differences between groups or conditions were statistically significant. To compare the means, the tests used were ANOVA and Tukey, where the ANOVA compares the means of two or more groups and determines whether there are significant differences ($p<0.05$). If the ANOVA indicated significant differences, the Tukey HSD test identifies which groups differ from the mean. The ANOVA test was used to compare the SCC means quantified by the CMT index and the SCC test based on two groups of BLV animals (positive and negative animals to BLV). A p-value ≤ 0.05 indicates significant differences in the CMT and SCC levels between the BLV positive and negative groups. The Tukey test allowed to identify how both groups differ from each other, and which group (positive and negative) has a higher mean (CMT and SCC). In addition, effect sizes were calculated using Cohen's *d* coefficient.

RESULTS

BLV seropositivity was detected in 68% of the cows (136 out of 200). Among the 20 herds evaluated, three herds (1.2.1, 1.2.2, and 1.5.6) showed a seroprevalence of 100%, while only one herd (2.2.1) recorded a seroprevalence of 0%, meaning that all sampled animals in this herd tested negative by ELISA (Table 1).

Table 1 shows that the general SCC is within the desirable parameters with a value of 168,350 cells per mL of milk, but five of them have a high somatic cell count; the herd that presents the lowest count corresponds to herd 2.2.1, the same herd that obtained a BLV seropositivity of 0%.

Regarding the CMT test performed in the field, 17 herds have a CMT index lower than 1 and an LR–HR index higher than 3, which is ideal; the other three herds are outside the desirable parameters. Two of the herds do not have any high-risk quarters in any of the 10 animals, and only one herd tested negative in all four quarters of each animal evaluated, this being the only herd that also presented a negative result in the serological analysis for bovine leukosis in all the animals that underwent this test (2.2.1) (Table 1).

Table 1. Characterization of the bovine leukosis virus (BLV) presence, udder health status, and productive parameters in the analyzed herds.

Herd code	% BLV seroprevalence	SCC/mL (x1000)	Indices		Coefficient of correlation CMT/SCC	CFU/ mL (x1000)	Production (kg/ milking)	Milk quality	
			CMT	LR–HR				Fat (%)	Protein (%)
1.1.1	60	41.5	0.18	19.00	0.94	36.00	7.82	3.83	3.13
1.1.2	80	252.0	0.48	9.00	-0.07	10.10	12.56	3.52	3.16
1.1.3	30	63.5	0.10	No HR exists	0.31	7.30	10.36	4.18	3.84
1.1.5	90	90.0	0.54	5.50	0.15	8.20	14.92	3.16	2.95
1.2.1	100	75.5	0.36	6.80	0.38	14.50	12.60	3.15	3.09
1.2.2	100	112.0	0.48	5.67	0.82	41.80	9.66	3.64	3.22
1.3.2	80	158.5	0.26	8.75	0.65	387.00	12.96	2.84	3.21
1.3.3	80	663.5	1.63	0.67	0.23	247.40	10.56	3.60	3.28
1.4.2	80	46.0	0.18	38.00	0.74	177.50	7.30	3.37	3.40
1.4.3	50	340.0	0.70	7.00	0.08	8.11	6.81	3.58	3.30
1.5.2	20	65.5	0.23	19.00	0.50	4.20	8.24	4.27	3.66
1.5.4	70	463.0	1.10	2.08	0.80	225.30	5.16	4.99	3.76
1.5.5	80	293.5	1.13	1.79	-0.05	3.50	11.52	3.33	3.08
1.5.6	100	154.5	0.30	39.00	0.56	6.00	14.04	3.11	3.15
2.1.1	90	38.5	0.15	19.00	0.84	192.80	13.88	3.04	3.02
2.2.1	0	18.0	0.00	No HR exists	-	2.80	10.72	3.54	3.49
2.3.1	50	67.0	0.25	19.00	0.55	15.40	7.74	4.79	3.32
2.4.1	50	158.5	0.13	19.00	0.85	19.10	11.86	4.12	3.70
3.1.1	60	53.0	0.13	18.5	0.94	93.70	10.88	3.89	3.39
3.2.1	90	213.0	0.15	19.00	-0.05	8.38	14.18	2.92	3.24
General	68	168.35	0.42	14.26	0.90	75.45	10.69	3.64	3.32

SCC: somatic cell count; **CMT:** California mastitis test; **LR:** low risk; **HR:** high risk; **CFU:** colony-forming unit.

The correlation obtained between the CMT and SCC methods to determine the presence of subclinical mastitis is high (R= 90%), which suggests that either of the two methods for diagnosing subclinical mastitis provides a reliable result. Table 1 also shows that CFU are generally within ideal parameters with a value of 75,450 per mL of milk, but when observing by herd, five herds have a high count (>100,000 per mL of milk), and two of these also have a high SCC, a very high CMT index and a very low

proportion of low-risk quarters compared to high-risk quarters. Finally, herd 1.5.4 is one of the herds with the highest prevalence of subclinical mastitis. Furthermore, it is the herd with the lowest milk production, with a value of 5.16 kg of milk per milking.

Prevalence of subclinical mastitis

An average prevalence of subclinical mastitis of 46.00% was obtained in all herds in sampling 1, indicating a moderate prevalence, suggesting that a significant proportion of the animals are affected by subclinical mastitis. The prevalence of subclinical mastitis in the total number of mammary quarters obtained was 24.56%, which indicates that a small to medium proportion of the evaluated mammary quarters are affected by subclinical mastitis. Additionally, the prevalence in the mammary quarters was calculated individually (PIMQ), which resulted in values for the anterior right quarter (ARQ) of 24.24%, 24.50% for the anterior left quarter (ALQ), 22.34% for the posterior right quarter (PRQ), and 27.14% for the posterior left quarter (PLQ). Regarding the proportion of affected mammary quarters, the result obtained was ARQ = 6.05%, ALQ = 6.17%, PRQ = 5.54%, and PLQ = 6.80%. No significant differences were observed in these two parameters. Finally, the intensity of the reaction was calculated, obtaining as a result that of the 100% of the cases evaluated, 75.44% had no reaction, that is, the test was negative, 12.34% had one cross (+), 6.80% had two crosses (++) and only 5.42% of the quarters evaluated registered three crosses (+++). These results and the prevalence in the second sampling are shown in Table 2.

Table 2. Estimation of the prevalence of subclinical mastitis using the California mastitis test in both samplings.

Sampling	**Sampling 1**				**Sampling 2**			
Prevalence (%)	46				56.25			
PTMQ (%)	24.56				30.28			
	ARQ	**ALQ**	**PRQ**	**PLQ**	**ARQ**	**ALQ**	**PRQ**	**PLQ**
PIMQ (%)	24.2	24.5	22.3	27.1	31.1	27.6	29	33.5
PMQA (%)	6.05	6.17	5.54	6.80	7.43	6.68	6.93	8.06
RI (%)	N	+	++	+++	N	+	++	+++
	74.40	12.30	6.80	5.42	67.00	14.90	7.56	6.68

PTMQ: prevalence in total mammary quarters; **PIMQ:** prevalence in individual mammary quarters; **PMQA:** proportion of mammary quarters affected; **RI:** reaction intensity; **ARQ:** anterior right quarter, **ALQ:** anterior left quarter, **PRQ:** posterior right quarter, **PLQ:** posterior left quarter, **N:** negative, **+, ++ and +++:** positive.

The hygienic and sanitary quality of milk measured in CFU and the CMT and SCC indices are positively correlated, with a medium-high correlation of 39% and 60%, respectively. Milk production shows a negative correlation with the mastitis results using both methods: 20% with the CMT and 18% with the SCC. The correlation between the compositional quality of milk and the CMT and SCC indices is very low

and, therefore, is not considered significant, except for protein, which shows a negative correlation of 11% in relation to the mastitis results obtained by CMT (Table 3).

Table 3. Correlations between the California mastitis test (CMT) and somatic cell count (SCC) with productive characteristics, milk quality, and colony-forming unit (CFU).

Parameters	CFU	Production	Fat	Protein
CMT index	0.39	-0.20	0.03	-0.11
SCC	0.60	-0.18	0.02	-0.01

A significant statistical difference (p=0.00129) was found in the mean SCC of BLV-positive animals (136 cows) compared to the mean SCC of negative animals (64 cows), with a higher count in positive animals. Additionally, the mean SCC and the mean CMT index were higher when the animals were BLV-positive. The effect size, measured using Cohen's *d*, was 0.29, indicating a moderate effect of BLV on the presence of subclinical mastitis as determined by both methods (Table 4).

Table 4. Somatic cell count (SCC) and California mastitis test (CMT) in bovine leukosis virus (BLV)-positive and BLV-negative cows.

BLV	Sampling 1		Sampling 2	
	SCC/mL (x1000)	CMT index	SCC/mL (x1000)	CMT index
Positive cows (n=136)	571.64	0.45	518.93	0.54
Negative cows (n=64)	241.80	0.38	379.07	0.48
P-value	0.00129		0.0077	
Cohen's d	0.29		0.29	

DISCUSSION

The results obtained in this study indicate a high exposure to BLV in the evaluated bovine population, with a seropositivity of 68% (136/200 cows). This high seroprevalence suggests a circulation of BLV in the dairy herds analyzed, especially in the northern region of the department of Antioquia (57.1%). The above is consistent with previous studies that have reported the wide dissemination of this virus in several regions of the department [19, 20]. Notably, three of the 20 herds evaluated presented a seroprevalence of 100%, indicating an efficient transmission of BLV within these herds. Olarte-Quintero et al. (2024) reported that the molecular prevalence of BLV exceeds 70% of infection in herds from different dairy regions of Antioquia [19]. However, notably, in the current study, herd 2.2.1 presented 0% seropositivity to BLV; the above is related to the control and biosecurity measures of this herd to avoid the introduction and spread of the virus; these strategies include the control of animal entry through the purchase in BLV-free herds, diagnostic tests, use of individual needles and gloves for each animal, disinfection of equipment and personnel, vector

control and proper handling of colostrum. Risk factors such as inadequate practices and high animal density favor transmission, while the implementation of good livestock practices, biosecurity, regular testing, and colostrum management protect the herd from BLV [21,22]. Factors such as reusing needles and palpation gloves, failure to disinfect surgical equipment, the presence of haemoparasites, the grazing system, and artificial insemination (with semen from bulls not tested to be BLV-free) have been reported to be risk factors for BLV infection [23,24], while feeding colostrum from negative mothers, the use of nutritional supplements, and manual milking are protective factors [24,25], since the lack of hygiene of mechanical systems where BLV can remain on the machine and potentially infect the next cow compared with manual milking that is a more hygienic practice if the hands of the milking personnel are disinfected between cows [25]. Implementing virus control and prevention measures in production systems, as done in the United States, has shown to be an effective strategy for reducing BLV prevalence when programs are rigorously followed [23].

The contrast found between animals seropositive to BLV and SCC (whose ideal parameter is SCC/mL (x1000) < 250) allowed the establishment of an association ($p = 0.00129$) between BLV seropositivity and SCC as an indicator of the presence of subclinical mastitis, finding that the average of SCC and CMT was higher in positive animals (n=136) compared to negative animals (n=64). This finding is consistent with the hypothesis that BLV compromises the immune system of cows, increasing susceptibility to intramammary infections. The absence of direct microbiological analysis of milk in this study limits the ability to conclusively attribute elevated somatic cell counts (SCC) solely to BLV infection, as other intramammary pathogens—undetected in this context—may also contribute to subclinical mastitis. Nevertheless, the statistical association observed through ANOVA (Table 4) suggests a relationship between BLV seropositivity and increased SCC levels ($p<0.05$), supporting a potential link between BLV and subclinical mastitis.

Cows that are BLV-positive have a lower capacity for macrophage-mediated immune response. Ortiz et al. determined that macrophages obtained from milk samples from cows with BLV infection have a lower capacity to phagocytose *Staphylococcus aureus* [26]. BLV infection is associated with a high prevalence of subclinical mastitis since the immunosuppression caused by BLV facilitates the appearance of opportunistic infections. This virus impacts the immunity of the mammary glands by affecting macrophages that act as the first line of defense against pathogens [26,27]. In cows infected with BLV, the capacity of macrophages to phagocytose *Staphylococcus aureus* is reduced, allowing this pathogen to multiply and establish infections in the mammary glands. As demonstrated by Lima et al. (2021) [27], BLV-positive cows are more susceptible to subclinical infections by *S. aureus*, the most common bacteria in mammary gland diseases in dairy cows. It is an infectious and opportunistic pathogen responsible for approximately one-third of clinical and subclinical mastitis cases. Moreover, this disease causes significant economic losses in the dairy industry worldwide [12]. The results support the hypothesis that BLV-seropositive cows have

a higher predisposition to developing subclinical mastitis, likely due to virus-induced immunosuppression. Although this study did not directly assess milk microbiota, the findings are indirectly aligned with those reported by Lv et al. (2021), who observed dynamic changes in milk microbial composition across different stages of lactation [28],. Given that immunosuppressed animals are more susceptible to intramammary infections and microbial imbalances, future research should incorporate microbiota analysis to better understand the role of BLV in altering the mammary environment and its potential association with mastitis.

Additionally, the results show that both the average SCC and the average CMT are higher in BLV-positive animals, although in the case of the CMT index, it remains within the ideal range (<1). This trend suggests that the presence of BLV can intensify the indicators of subclinical mastitis, even when the values have not yet reached clinically critical levels, highlighting the importance of considering BLV infection as a significant risk factor for mammary health [26] and dairy productivity that is related to the economic sustainability of productive herds. Shinozuka et al. [29] determined that the economic losses due to mastitis associated with BLV infection per cow with a high proviral load were 418 USD annually, and for the Hokkaido prefecture, it was estimated at 6,097,225 USD annually.

The CMT and SCC subclinical mastitis diagnostic methods were found to have a 90% correlation, meaning that both methods have a high concordance and are equally reliable for detecting subclinical mastitis in dairy cows. This finding is relevant for producers as it allows flexibility in the choice of diagnostic method depending on financial resources and the availability of tests.

In the samplings carried out, of the 20 herds tested, 17 presented a CMT index lower than 1 and a low risk–high risk (LR–HR) index higher than 3, which is considered ideal for maintaining good mammary health and high milk productivity. This reflects that most of the herds are within the desirable parameters, evidencing adequate management of their animals and, consequently, a good health status. Nakada et al. (2023) indicate that measures to maintain good mammary health include SCC control, adequate nutrition, animal management, and complete and hygienic milking [30]. However, in the current study, three herds (1.3.3, 1.5.4, and 1.5.5) were recorded as not meeting the ideal parameters, which is why they require special attention and corrective measures based on biosecurity to improve their situation [21, 22].

Herds 1.1.3 and 2.2.1 did not record any high risk in any mammary quarter of the animals evaluated, which is a positive indication of udder health in these herds. Furthermore, the finding of herd 2.2.1 with negative results in all four quarters of each animal evaluated, which in turn also tested negative in the BLV serological analysis, is an indication of good health status and can be related to the health control and management plans at the herd level [21,22].

The results of this study show that there is a medium-high positive correlation between CFU (whose ideal parameter is CFU/mL (x1000) < 100) and CMT and

SCC, with correlation values of 39% and 60%, respectively. This suggests that as the indicators of subclinical mastitis increase, the bacterial load in the milk produced also increases, affecting its hygienic and sanitary quality [31] and the economic income of the producer, emphasizing the importance of intervening and controlling in time the cases of subclinical mastitis to improve the microbiological quality of milk.

Milk production showed a negative correlation with mastitis results by both methods, with 20% in the case of CMT and 18% in the case of SCC, meaning that the presence of subclinical mastitis is associated with a reduction in milk production. Azooz et al. [32] obtained a statistically negative correlation ($P<0.0001$) between the level of mammary inflammation and milk production ($r = – 0.59$), as well as milk protein ($r = – 0.55$), lactose ($r = – 0.51$), and fat ($r = – 0.46$) content. Additionally, subclinical mastitis during the pre-breeding period was associated with a 14% increase in the probability of having a higher number of artificial inseminations per conception [33], increasing the lactation days in the last third, where animals consume the same amount of feed, but produce less milk per day; the above reflects very significant economic losses caused by this disease.

Regarding the compositional quality of milk, the correlation with the CMT and SCC indices is very low and is not considered significant ($p>0.6$) in most of the variables evaluated (SCC vs. %PROT, SCC vs. %FAT and CMT vs. %FAT). The only exception is the protein percentage, which presents a negative correlation of 11% against the mastitis results obtained by CMT. Although this correlation is low, it suggests that subclinical mastitis could impact negatively on the protein content of milk. Azooz et al. [32] also reported a reduction of one percentage unit for protein in cattle with greater mammary gland inflammation.

The prevalence of subclinical mastitis in all herds was, on average, 46.00%, showing that a significant proportion of animals are affected by subclinical mastitis, which has a negative impact on both herd milk production and milk quality. This moderate prevalence highlights the need to implement more effective control strategies to reduce the incidence of this disease [29]. The prevalence of subclinical mastitis in the total mammary quarters evaluated was 24.56%, indicating that a considerable proportion of udder quarters are affected. These data are important as they suggest that although the disease is not widely disseminated in all mammary quarters, it remains a relevant problem that must be addressed to prevent its spread and minimize adverse effects. The individual mammary quarter prevalence analysis (PIMQ) showed that the values were similar between the different quarters: ARQ= 24.24%, ALQ= 24.50%, PRQ= 22.34%, and PLQ= 27.14%. These figures reflect a relatively uniform distribution of subclinical mastitis in different quarters of the mammary gland. Regarding the proportion of mammary quarters affected, the results were: ARQ= 6.05%, ALQ= 6.17%, PRQ= 5.54%, and PLQ= 6.80%, indicating no marked preference for any specific quarter. The intensity of the reaction to the test showed that of the 100% of the cases evaluated, 75.44% had no reaction, that is, the test was negative; 12.34% had one cross (+), 6.80% had two crosses (++), and only 5.42% of the quarters evaluated

showed three crosses (+++). These data indicate that most cases of subclinical mastitis are mild, although there is a significant percentage that shows a more intense reaction, which requires more specific attention and treatment [34].

This study demonstrates a significant association between bovine leukosis virus (BLV) seropositivity and increased somatic cell counts (SCC) ($p<0.05$), supporting the hypothesis that BLV infection compromises the immune system and increases the susceptibility of dairy cows to subclinical mastitis. The high seroprevalence of BLV (68%) observed among the herds evaluated in the Aburrá Valley highlights the presence of the virus in this region and its potential role in compromising udder health and milk productivity. The strong positive correlation between SCC and CMT (90%) further validates the California Mastitis Test as a practical, cost-effective screening tool for early detection of subclinical mastitis under field conditions. However, some limitations must be considered. The absence of microbiological analysis of milk restricts the ability to attribute elevated SCC exclusively to BLV infection, since other intramammary pathogens not assessed in this study may also contribute to mastitis. And the exploratory design, based primarily on univariate and bivariate analyses, did not control confounding variables such as age, parity, or herd management practices. Future research should include broader and more balanced sampling in terms of the number of herds and cattle with varying levels of BLV prevalence, incorporate microbiological analysis of milk, and apply multivariate statistical approaches to control for potential confounding factors. These improvements would enhance the understanding of the causal mechanisms underlying the relationship between BLV infection and subclinical mastitis and inform more effective control strategies to reduce economic losses in the dairy industry.

Acknowledgements

The authors thank the specialized dairy producers of the Department of Antioquia, who allowed the collection of samples and the use of data from their herds to carry out this research. Finally, the authors thank the Technical Assistance Department of Colanta for its essential support in executing this project.

Authors' contributions

CUM and ALH contributed to the conceptualization of the study. CLS, CCRG, CUM, and ALH developed the methodology and performed the formal analysis. CLS, CCRG, JPAM, CUM, and ALH conducted the investigation. CUM and ALH prepared the original draft of the manuscript, while CLS, CCRG, CUM, JPAM, and ALH contributed to its writing, review, and editing. CUM was responsible for visualization. ALH supervised the study and administered the project. JPAM, ALH, and CUM acquired the funding. All authors have read and agreed to the published version of the manuscript.

Declaration of interest statement

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This research was funded by the "National Call to strengthen training through the support for research, artistic creation, and innovation projects of Universidad Nacional de Colombia 2022-2024" with Hermes code 57626 and Colanta. The funders had no role in study design, data collection and analysis, publication decisions, or manuscript preparation.

Data availability

All data analyzed during the current study are available from the corresponding author upon request.

ORCID iDs

Caterine López-Sánchez https://orcid.org/0009-0005-6937-1082
Cristian C. Rúa-Giraldo https://orcid.org/0000-0003-1389-7462
Juan Pablo Arismendy Morales https://orcid.org/0000-0002-0524-3162
Cristina Úsuga-Monroy https://orcid.org/0000-0001-6101-2994
Albeiro López-Herrera https://orcid.org/0000-0003-1444-3470

REFERENCES

1. Fedegán: Cifras de referencia del sector ganadero. Fedegán, Bogotá; 2017.
2. Secretaría de Agricultura y Desarrollo Rural: Anuario Estadístico del Sector Agropecuario en el Departamento de Antioquia 2022. Gobernación de Antioquia, Medellín; 2022.
3. Úsuga-Monroy C, Zuluaga J, López Herrera A: El virus de la leucosis bovina disminuye la producción y calidad de leche en Holstein. Archivos de Zootecnia 2018, 67(258):254–259.
4. Gutiérrez SE, Lützelschwab CM, Barrios CN, Juliarena MA: Leucosis bovina. Rev Invest Vet Perú 2020, 31(3): e16913. https://doi.org/10.15381/rivep.v31i3.16913
5. Le DT, Yamashita-Kawanishi N, Okamoto M, Nguyen SV, Nguyen NH, Sugiura K, Miura T, Haga T: Detection and genotyping of bovine leukemia virus (BLV) in Vietnamese cattle. J Vet Med Sci 2020, 82(7):1042–1050.
6. Corredor-Figueroa AP, Salas S, Olaya-Galán NN, Quintero JS, Fajardo Á, Soñora M, Moreno P, Cristina J, Sánchez A, Tobón J, Ortiz D, Gutiérrez MF: Prevalence and molecular epidemiology of bovine leukemia virus in Colombian cattle. Infect Genet Evol 2020, 80.
7. Villegas V: *Leucosis Bovina Enzoótica.* Bachelor Thesis, Facultad de Ciencias Agropecuarias, Medicina Veterinaria, Universidad de la Salle. 2015. https://ciencia.lasalle.edu.co/cgi/viewcontent.cgi?article=1006&context=medicina_veterinaria Accessed 17 January 2025.
8. Olaya-Galán NN, Corredor-Figueroa AP, Guzmán-Garzón TC, Ríos-Hernandez KS, Salas-Cárdenas SP, Patarroyo MA, & Gutiérrez MF: ADN del virus de la leucemia bovina en

leche fresca y carne cruda para consumo humano. Epidemiología e infección. 2017, 145 (15), 3125-3130.

9. Olaya-Galán NN, Corredor-Figueroa AP, Guzmán-Garzón TC, Ríos-Hernandez KS, Salas-Cárdenas SP, Patarroyo MA, & Gutiérrez MF: ADN del virus de la leucemia bovina en leche fresca y carne cruda para consumo humano. Epidemiología e infección. 2017, 145 (15), 3125-3130.
10. Reyes JMH, Cedeño JLCB: *Importancia del conteo de células somáticas en la calidad de la leche*. REDVET. Revista Electrónica de Veterinaria 2008, vol. IX, núm. pp. 1-34. Veterinaria Organización. Málaga, España. https://www.redalyc.org/pdf/636/63617329004.pdf Accessed 17 January 2025.
11. DANE, MinAgricultura, SIPSA: La mastitis bovina, enfermedad infecciosa de gran impacto en la producción lechera. Boletín mensual Insumos y factores asociados a la producción agropecuaria. 2014. https://www.dane.gov.co/files/investigaciones/agropecuario/sipsa/insumos_factores_de_produccion_ago_2014.pdf Accessed 17 January 2025.
12. Richardet M, Solari HG, Cabrera VE, Vissio C, Agüero D, Bartolomé JA, Larriestra AJ: The Economic Evaluation of Mastitis Control Strategies in Holstein-Friesian Dairy Herds. Animals 2023, 13(10):1701.
13. Akhtar M, Naqvi SUAS, Liu Q, Pan H, Ma Z, Kong N, Liu H: Short chain fatty acids (SCFAs) are the potential immunomodulatory metabolites in controlling Staphylococcus aureus-mediated mastitis. Nutrients 2022, 14(18):3687.
14. Ruiz-García LF, Sandoval-Monzón RS: Diagnóstico de mastitis subclínica de vacunos lecheros mediante el conteo de células somáticas empleando dos métodos de diagnóstico. Universidad del Zulia. Rev Científica 2018, 28(2):129–135.
15. Gómez-Quispe OE, Santivañez-Ballón CS, Villar FA, Flores OHE, Meza JM: Criterios de Interpretación para California Mastitis Test en el Diagnóstico de Mastitis Subclínica en Bovinos. Rev Invest Vet Perú 2015, 26(1):86.
16. Zuluaga J, Jaramillo M, Restrepo L: Evaluación comparativa de dos metodologías de diagnóstico de mastitis en un hato lechero del Departamento de Antioquia. Rev. Lasallista Investig Caldas 2010, 7(1).
17. Macías PDM: El efecto y la importancia del recuento de células somáticas en la industria láctea y los métodos rápidos para la detección de la mastitis bovina. Recuento de células somáticas. Colegio Mayor de Antioquia, Programa de Mastitis COLANTA. s.f.
18. Henao Restrepo M: Avances en la calidad de leche en Colanta. Medellín: Cooperativa COLANTA; s.f.
19. Olarte-Quintero M, Úsuga-Monroy C, Lopez-Herrera A: Relación entre el BLV y problemas sanitarios en un hato de lechería especializada de Antioquia. Rev Med Vet 2024. https://doi.org/10.19052/mv.vol1.iss48.16.
20. Castillo-Rey D, López-Herrera A, Úsuga-Monroy C: Molecular prevalence of Bovine Leukemia Virus in specialized dairies in the department of Antioquia, Colombia. Rev Fac Nac Agron Medellín 2023, 76(2):10393–10401.
21. Rúa Giraldo CC, López Herrera A, Ruiz-Cortés T: Bovine leukosis virus, bovine viral diarrhea, and bovine neosporosis seroprevalence in specialized dairy herds in Antioquia-Colombia. Trop Anim Health Prod 2023, 55:294.
22. Kuczewski A, Hogeveen H, Orsel K, Wolf R, Thompson J, Spackman E, van der Meer F: Economic evaluation of 4 bovine leukemia virus control strategies for Alberta dairy farms. J Dairy Sci 2019, 102(3):2578–2592.

23. Kuczewski A, Orsel K, Barkema HW, Mason S, Erskine R, van der Meer F: Invited review: Bovine leukemia virus—Transmission, control, and eradication. J Dairy Sci 2021, 104(6):6358–6375.
24. Bartlett P C, Sordillo LM, Byrem TM, Norby B, Grooms DL, Swenson CL, Zalucha J, Erskine R.J: Options for the control of bovine leukemia virus in dairy cattle. J Am Vet Med A 2014, 244(8):914–922.
25. Ohno A, Takeshima SN, Matsumoto Y, Aida Y: Risk factors associated with increased bovine leukemia virus proviral load in infected cattle in Japan from 2012 to 2014. Virus Res 2015, 210:283–290.
26. Ortiz D, Sanchez A, Tobon J, Chaparro Y, Cortes S, Gutierrez MF: Seroprevalence and risk factors associated with bovine leukemia virus in Colombia. J Vet Med Animal Health 2016, 8(5):35–43.
27. Lima EDS, Blagitz MG, Batista CF, Alves AJ, Fernandes ACDC, Ramos Sanchez EM, de Souza FN: Milk macrophage function in Bovine leukemia virus-infected dairy cows. Front Vet Sci 2021, 8:650021.
28. Lv G, Wang H, Wang J, Lian S, Wu R: Effect of BLV infection on the immune function of polymorphonuclear neutrophil in dairy cows. Front Vet Sci 2021, 8:737608.
29. Shinozuka Y, Suzuki N, Kitsukawa M, Hayashi M, Suenaga N, Shimizu Y & Kawai K: Longitudinal and cross-sectional studies to evaluate changes in cow milk microbiota over the lactation stages. Acta Veterinaria-Beograd, 2024, 74(2), 236-245.
30. Nakada S, Fujimoto Y, Kohara J, Makita K: Economic losses associated with mastitis due to bovine leukemia virus infection. J Dairy Sci 2023, 106(1):576–588.
31. Zigo F, Vasil' M, Ondrašovičová S, Výrostková J, Bujok J, Pecka-Kielb E: Maintaining optimal mammary gland health and prevention of mastitis. Front Vet Sci 2021, 8:607311.
32. Azooz MF, El-Wakeel SA, Yousef HM: Risk Factor Analysis of Salmonella Typhimurium, Staphylococcus aureus, Standard Plate Count and Somatic Cell Count in Bulk Tank Milk in Cattle Dairies. World's Vet J 2020, (3):338–361.
33. Harjanti DW, Sambodho P: Effects of mastitis on milk production and composition in dairy cows. In: IOP Publishing. IOP Conference Series: Earth and Environmental Science 2020, 518(1):012032.
34. Ranasinghe RMSBK, Deshapriya RMC, Abeygunawardana DI, Rahularaj R, Dematawewa CMB: Subclinical mastitis in dairy cows in major milk-producing areas of Sri Lanka: prevalence, associated risk factors, and effects on reproduction. J Dairy Sci 2021, 104(12):12900–12911.
35. McDougall S, Clausen LM, Hussein HM, Compton CW: Therapy of subclinical mastitis during lactation. Antibiotics 2022, 11(2):209.

SEROPOZITIVNOST NA VIRUS LEUKOZE GOVEDA I NJEGOV UTICAJ NA PRISUSTVO SUPKLINIČKOG MASTITISA KOD SPECIJALIZOVANIH MLEČNIH STADA U TRI VISOKA TROPSKA REGIONA ANTIOKIJE

Caterine LÓPEZ-SÁNCHEZ, Cristian C. RÚA-GIRALDO,
Juan Pablo ARISMENDY MORALES, Cristina ÚSUGA-MONROY,
Albeiro LÓPEZ-HERRERA

Virus leukoze goveda (eng. *Bovine Leukemia Virus*-BLV) negativno utiče na imunski sistem goveda, povećavajući njihovu podložnost bolestima kao što je supklinički mastitis, često stanje kod mlečnih krava koje generiše značajne ekonomske gubitke zbog smanjene proizvodnje mleka, troškova lečenja i klanja životinja. Cilj ove studije je da se proceni veza između seropozitivnosti na BLV i subkliničkog mastitisa kod 200 krava iz 20 specijalizovanih stada u dolini Abura, severno i istočno od Antiokije, Kolumbija. Analize mleka i krvi su sprovedene korišćenjem Kalifornija mastitis testa (CMT), broja somatskih ćelija (BSC) protočnom citometrijom i detekcije BLV, ELISA testom. Rezultati su pokazali 68% seropozitivnosti na BLV, prosečan BSC od 168,350/ml i indeks CMT od 0,42. Utvrđena je visoka pozitivna korelacija (90%) između BSC i CMT, što ukazuje na efikasnost CMT kao dijagnostičkog alata za procenu zdravlja mlečnih žlezda. Štaviše, negativna korelacija (-20%) između BSC i proizvodnje mleka dokazuje uticaj mastitisa na produktivnost. Značajna veza između seropozitivnosti na BLV i povećanog broja somatskih ćelija (P=0,00129) potvrđuje imunosupresivni efekat BLV, koji predisponira krave za subklinički mastitis. Zaključno, goveđa leukoza povećava podložnost krava razvoju supkliničkog mastitisa slabljenjem njihovog imunskog sistema, ugrožavanjem opšteg zdravlja stada i generisanjem ekonomskih gubitaka, što ističe važnost CMT-a kao efikasne, brze i ekonomične metode za njegovu dijagnozu.