

Diagnostic concordance and animal-level epidemiological patterns of bovine tuberculosis, paratuberculosis, and enzootic bovine leukemia in Colombian dairy cattle

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Abstract

Bovine tuberculosis (bTB), paratuberculosis (PTB), and enzootic bovine leukemia (EBL) are endemic infections that challenge dairy production and disease surveillance in Colombia. Differences in diagnostic performance across tests complicate infection detection and epidemiological interpretation under field conditions. A cross-sectional study was conducted with 371 cattle (366 females and 5 males) from 30 specialized dairy herds in the Ubaté Valley, Colombia. Animals were tested via the tuberculin skin test (bTB), ELISA (PTB and EBL), and PCR-based assays (PTB and EBL). The apparent prevalence and diagnostic concordance were estimated, and associations of animal- and herd-level characteristics with BLV outcomes were explored using logistic regression with herd-clustered standard errors. No animal was tuberculin positive (four doubtful reactors, 1.1%), whereas the apparent prevalence was 4.6% for PTB and 58.0% for EBL by ELISA. PCR detected BLV proviral DNA in 14.3% of the animals, whereas all the PCR assays for bTB and PTB were negative. The agreement between the BLV ELISA and PCR results was slight ($\kappa = 0.19$). BLV seropositivity was lower in the four higher-yielding herds, whereas PCR positivity was more frequent in older animals and in those herds. The marked difference between serological and molecular detection highlights the complexity of BLV diagnosis in endemic dairy systems. Animal-level epidemiological patterns differ according to diagnostic method, underscoring the need for integrated surveillance strategies and cautious interpretation of single-test results in field-based studies.

INTRODUCTION

Bovine tuberculosis (bTB), paratuberculosis (PTB), and enzootic bovine leukemia (EBL), caused by *Mycobacterium bovis*, *Mycobacterium avium* subsp. *paratuberculosis* (MAP), and bovine leukemia virus (BLV), respectively, are chronic infections of dairy cattle whose long subclinical periods hinder early diagnosis and favor silent within-herd transmission (Nielsen and Toft 2009; Waters et al. 2012; Porta et al. 2023). *M. bovis* is zoonotic (Thoen et al. 2006), MAP is associated with Crohn's disease in humans (Feller et al. 2007; Asgari et al. 2025), and the oncogenic potential of BLV in humans has been explored (Gillet et al. 2007; Buehring et al. 2015).

Diagnostic interpretation is complicated by heterogeneous immune responses, intermittent shedding, and variable test performance (Álvarez et al. 2008; Jurado-Martos et al. 2023). The *sensitivity* of the tuberculin test and serology for *M. bovis* varies with infection stage (de la Rua-Domenech 2006; Bezos et al. 2014); MAP serology has reduced sensitivity in subclinically infected animals (Whittington and Sergeant 2001; Logar et al. 2012), and BLV antibody and proviral DNA detection reflect different stages of infection (Nikbakht-Brujeni et al. 2023). Concordance between methods can therefore inform the interpretation of surveillance data (Steuer et al. 2015; Kelly et al. 2022).

In Colombia, livestock contributes more than 25% of the agricultural gross domestic product (FEDEGAN 2025), and milk production will reach 7.7 billion liters in 2024 (Bolsa Mercantil de Colombia 2025). EBL

and PTB are notifiable but are not subject to official control programs of the Colombian Agricultural Institute (ICA; ICA 2025).

This study aimed to assess diagnostic concordance and describe animal- and herd-level epidemiological patterns associated with bTB, PTB, and EBL detection in Colombian dairy cattle using serological and molecular methods. It was designed as a descriptive cross-sectional characterization rather than a formal evaluation of diagnostic accuracy, which would require Bayesian latent class analysis with multiple independent tests and populations.

MATERIALS AND METHODS

Study area and design

A cross-sectional study was conducted between November and December 2022 to evaluate diagnostic concordance and describe animal-level epidemiological patterns of bTB, PTB, and EBL in dairy cattle from 30 farms located in three municipalities of the Ubaté Valley, Central Colombia: Simijaca and Lenguazaque (Ubaté Province, Cundinamarca) and San Miguel de Sema (Boyacá). This valley (approximately 5°22'N, 73°45'W; altitude range: 2,500–2,900 m a.s.l.) is among the most important dairy-producing regions in the country. The selected farms were distributed across seven localities (i.e., Aposentos, Centro, Churnica, Don Lope, Hato Viejo, Pacaguita, and Pantano). All herds were operated under specialized dairy production systems based on pasture grazing supplemented with concentrate feeding. Herd-level management variables were not analyzed; daily milk yield, available as a single value per herd, was the only herd-level characteristic included in the analyses.

Sampling was performed by convenience. Within each farm, lactating cows and replacement heifers ≥ 6 months of age were the target population. However, the number of animals sampled per herd varied because the study was conducted during routine field visits, and the availability of eligible animals depended on milking schedules, grazing rotation, and the feasibility of safe handling on the day of sampling. Therefore, all eligible animals that were physically present and could be safely restrained were included, with no selection based on clinical status, production level, or other characteristics. This feasibility-based approach minimizes subjective selection and reflects operational conditions in specialized dairy systems in the region. Because sampling followed routine handling, the final dataset also included 12 calves younger than 6 months and 5 young males (≤ 2 years of age), which were retained in all analyses.

Each animal was individually identified to ensure accurate linkage between demographic, productive, and diagnostic data. Although specific farm coordinates cannot be disclosed owing to confidentiality agreements with the owners, the study herds reflected the main specialized dairy production system of the region.

No clinical examinations were performed, and concurrent conditions (e.g., mastitis or lameness) were not recorded.

This study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional studies.

Tuberculin test

The tuberculin skin test (TST) was performed by an inspection organization authorized by the ICA, following national guidelines for single intradermal tests using bovine purified protein derivative (PPD). Briefly, 0.1 mL of bovine PPD tuberculin (containing at least 2,000 IU, in accordance with WOA and ICA standards) was injected intradermally into the caudal fold, approximately 6–8 cm from the base of the tail, using a sterile 26-gauge tuberculin needle and ensuring the formation of a small intradermal bleb. The skin-thickness response was measured 72 ± 6 hours post-inoculation by the same accredited inspector, who applied the test and compared pre- and postinoculation thickness with a calibrated cutimeter. Animals showing inflammatory or local clinical signs at the injection site were also considered for interpretation. A reaction was classified as negative when the increase in skin thickness was ≤ 2 mm and when no clinical signs were present. Reactions between 2–4 mm without clinical signs were considered doubtful, whereas increases > 4 mm or the presence of local clinical signs were interpreted as positive. In this study, the final classification was based on the recorded skin thickness increments according to these criteria.

Sampling

Blood samples were collected via coccygeal or jugular venipuncture via Vacutainer® tubes (Becton Dickinson, Sparks, NV, USA). Tubes containing ethylenediaminetetraacetic acid (EDTA; purple cap) were used to collect whole blood, whereas plain tubes without anticoagulant (red cap) were used for serum collection. Each tube was labeled with the animal identification number and transported under refrigerated conditions (4–8°C) in polystyrene coolers to the Molecular Genetics Laboratory at the Tibaitatá Research Center (AGROSAVIA). Upon arrival, the serum samples were centrifuged at 1,300 rpm for 5 minutes; the supernatants were aliquoted and stored at – 80°C until analysis. Whole blood in EDTA tubes was kept refrigerated (4–8°C) until processing.

Fecal samples were collected directly from the rectum of each animal immediately after blood sampling, and individual disposable gloves were used to ensure sample-to-animal correspondence. Approximately 10–15 g of feces was placed into sterile, labeled polypropylene containers. The samples were refrigerated at 4–8°C during transport to the laboratory (within 4–6 hours of collection) and stored at – 20°C until DNA extraction. This procedure minimizes environmental contamination and bacterial cell wall degradation, both of which can reduce the sensitivity of MAP or *M. bovis* PCR detection.

Serological testing (ELISA)

Serum samples were analyzed via commercial indirect ELISA kits (Gold Standard Diagnostics, Spain) following the manufacturer's instructions. An INgezim BLV Compac 2.0® kit (Ref. R.12.BLV.K.3/5) was used for the detection of anti-p24 antibodies against BLV, and an INgezim Paratuberculosis® kit (Ref. R.12.PTB.K.1/5) was used for the detection of antibodies against MAP. Optical density (OD) was measured at 450 nm via a microplate reader, and the results were interpreted according to the cutoff values specified by the manufacturer. Each analytical run included the positive and negative control sera supplied by the manufacturer as part of the kits. The internal standards included in the plates were also used to verify the optical density ranges and plate-to-plate reproducibility, yielding a coefficient of variation < 5%.

DNA extraction

Fecal DNA for MAP detection was extracted with the ZR Fecal DNA Kit™ (Zymo Research, Irvine, CA, USA) after 5 min of homogenization and mechanical disruption (Disruptor Genie®, Thomas Scientific, Swedesboro, NJ, USA), as described previously (Leite et al. 2013). Genomic DNA for BLV and *M. bovis* PCR was extracted from EDTA whole blood using a commercial silica-membrane spin-column kit according to the manufacturer's instructions. DNA concentration and purity were measured with a NanoDrop 2000® spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and the DNA was stored at – 20°C until analysis.

PCR for BLV detection

BLV proviral DNA was detected by endpoint PCR targeting a 598-bp fragment of the env gene (gp51) with the primers env5032 (5'-TCTGTGCCAAGTCTCCCAGATA-3') and env5608 (5'-AACAAACACCTCTGGGAAGGGT-3') previously used in Colombian surveys (Corredor-Figueroa et al. 2020). Reactions (12.6 µL) contained 6.3 µL of MyTaq™ Red Mix 2X (Meridian Bioscience, London, UK), 0.5 µL of each primer (10 µM), and 2 µL of DNA. The samples were cycled at 95°C for 1 min, followed by 30 cycles of 95°C for 15 s, 60°C for 15 s, and 72°C for 10 s. The products were visualized on 1.8% agarose gels; samples were considered positive when a single band of the expected size was observed relative to a molecular weight marker, and runs were accepted only when the positive control (DNA from a confirmed proviral-positive animal) was amplified and the no-template control was not amplified. This env-PCR is not listed by WOAHP but has been widely used in regional studies.

PCR for *M. bovis* detection

M. bovis DNA was detected in whole-blood DNA with a BIOTK088 Bovine Tuberculosis real-time PCR kit, which was validated by the French National Reference Laboratory for Tuberculosis (ANSES, Maisons-Alfort, France), on a CFX96 system (Bio-Rad Laboratories, Hercules, CA, USA). The reactions used 5 µL of DNA, positive (MTBC DNA) and negative controls, an internal process control, and the manufacturer's cycling conditions and interpretation criteria.

qPCR for MAP detection

MAP DNA was detected in fecal DNA with an ADIAVET™ Paratb Real-Time kit (ADI045-100; Bio-X Diagnostics, Rochefort, Belgium), which targets IS900 and includes an internal control, on a CFX96 Touch™ system (Bio-Rad Laboratories) using 5 µL of DNA and the manufacturer's cycling conditions (45 cycles). Each run included extraction and amplification controls and a field MAP-positive control confirmed by Sanger sequencing. The samples were tested in duplicate, and those whose Ct values were ≤ 37 were considered positive.

Data collection and statistical analysis

For each animal, demographic variables (i.e., breed, sex, and age) were recorded, together with field and laboratory diagnostic results for EBL, PTB, and bTB. Daily milk yield was available as a single value per herd and was therefore treated as a herd-level variable, categorized according to the observed distribution as Q1 (≤ 12 L/day) or Q2 (> 12 L/day; all Q2 herds had a recorded yield of 14 L/day). Descriptive statistics (frequencies, percentages, means, standard deviations, and ranges) were generated to characterize the study population and diagnostic outcomes. Apparent prevalence rates and 95% confidence intervals (CIs) were calculated via the Wilson method for binomial proportions. Since the PCR results for bTB and PTB were uniformly negative, agreement analyses were performed only for EBL. Diagnostic concordance between ELISA and PCR for BLV detection was assessed by cross-tabulation and Cohen's kappa (κ) with 95% CI and interpreted via conventional benchmarks (slight 0.00–0.20; fair 0.21–0.40; moderate 0.41–0.60; substantial 0.61–0.80; almost perfect > 0.80) (Landis and Koch 1977). Associations between BLV infection status (for both ELISA and PCR outcomes) and breed, age, and herd milk yield were first explored with chi-square or Fisher's exact tests for categorical variables and Student's t tests for continuous variables, and crude odds ratios (ORs) with 95% CIs were estimated by univariable logistic regression. Variables with $p < 0.20$ in these analyses were included in the multivariable logistic regression models. Because PTB and bTB had very low prevalence rates and no PCR-confirmed coinfections with BLV were identified, these pathogens were not considered explanatory variables in the multivariable models. Separate models were constructed for ELISA and PCR outcomes, including breed (Holstein vs. other), age (≥ 48 vs. < 48 months), and herd milk yield category (Q2 vs. Q1) as explanatory variables. Because animals were clustered within herds and milk yield was measured at the herd level, all logistic regression models were fitted with cluster-robust standard errors using herd as the cluster variable; random-effects multilevel models were not fitted because of the small number of animals sampled in several herds. Model fit was assessed via the Pearson goodness-of-fit test across covariate patterns (the small number of covariate patterns precluded decile-based Hosmer–Lemeshow grouping) and the McFadden pseudo- R^2 , whereas collinearity was evaluated through variance inflation factors (VIFs < 5 acceptable). Statistical significance was defined as $p < 0.05$. All analyses were performed via Stata v19.5 (StataCorp LLC, College Station, TX, USA).

RESULTS

Descriptive analysis of the study population

A total of 371 cattle (366 females and 5 males) from 30 dairy farms were evaluated, with an average of 12.4 animals per herd (SD = 15.1; range = 2–63). Most animals were Holsteins (60.9%), followed by Jersey×Holstein crosses (13.7%), with other pure and crossbred breeds representing smaller proportions (Table 1). The mean age was 50.8 months (SD = 27.4; range = 2–140 months), and 63.6% were older than three years. The recorded herd daily milk yield ranged from 7 to 14 L; 26 herds (210 animals, 56.6%) were classified as Q1 (≤ 12 L/day), and four herds (161 animals, 43.4%) were classified as Q2 (14 L/day). The four Q2 herds were also the largest sampled herds (17–63 animals each vs. 2–34 in Q1 herds) and had a higher proportion of Holstein cattle (74.5% vs. 50.5%). All animals had been vaccinated against foot-and-mouth disease and *Brucella* (when applicable by age), and all tested negative for *Brucella*. None of the animals presented a history of abortion, retained placenta, decreased milk production, weak calves, enlarged superficial lymph nodes, orchitis, or arthritis. Individual sterile needles were reportedly used for all the injections across the farms.

Table 1
Descriptive characteristics of the bovine population included in the study

Variable	Category/Statistic	n (%) or mean $\pm$ SD (range)
Animals per herd	Mean $\pm$ SD (range)	12.4 $\pm$ 15.1 (2–63)
Breed	Holstein	226 (60.9)
	Jersey×Holstein	51 (13.7)
	Normando	19 (5.1)
	Jersey	18 (4.9)
	Ayrshire	16 (4.3)
	Normando×Holstein	13 (3.5)
	Other*	28 (7.6)
Age (in months)	Mean $\pm$ SD (range)	50.8 $\pm$ 27.4 (2–140)
Age group	≤ 1 year	28 (7.5)
	1–2 years	29 (7.8)
	2–3 years	78 (21)
	> 3 years	236 (63.6)
Herd daily milk yield (L)	Q1 (≤ 12 L/day)	210 (56.6)
	Q2 (14 L/day)	161 (43.4)

*Other breeds include Simmental, Ayrshire×Normando, Normando×Jersey, Gyrolando, Ayrshire×Holstein, Montpellier, Angus, Crossbred, Angus×Jersey, Jersey×Brown Swiss, Wayuu×Holstein, and Blanco Orejinegro. Sex: 366 females (98.7%) and 5 males (1.3%). Herd daily milk yield is a herd-level value assigned to all the animals in each herd. SD = standard deviation.

Breed and age categories were represented across most herds. Although Holstein animals predominated, crossbred and minority breeds were present on multiple farms rather than being confined to isolated herds. Similarly, the age distribution (youngstock, first-calf heifers, and adult cows) was observed across all the farms.

Diagnostic test results

According to the recorded skin-thickness increments, no animal met the criterion for a positive tuberculin reaction (0/371; 95% CI: 0.0–1.0%); four animals from two herds (1.1%; 95% CI: 0.4–2.7%) showed doubtful reactions (2.2–2.8 mm), and 367 (98.9%) were negative. Real-time PCR for *M. bovis* in blood samples yielded negative results for all the animals. For PTB, indirect ELISA detected antibodies against MAP in 17 animals (4.6%; 95% CI: 2.9–7.2%), whereas all MAP qPCR tests performed on fecal DNA were negative, indicating the absence of detectable MAP DNA in the feces. With respect to BLV, serological testing revealed 215 positive animals (58.0%; 95% CI: 52.9–62.9%) and 156 negative animals (42.0%), whereas PCR identified 53 positive animals (14.3%; 95% CI: 11.1–18.2%) and 318 negative animals (85.7%) (Table 2). The overall agreement between ELISA and PCR was slight ($\kappa = 0.19$; 95% CI: 0.13–0.25), indicating limited concordance between the serological and molecular detection of BLV (Table 3).

Table 2
Results of diagnostic tests performed in the bovine population

Test	Result	n (%)
Tuberculin test	Negative	367 (98.9)
	Doubtful	4 (1.1)
ELISA – Paratuberculosis	Negative	354 (95.4)
	Positive	17 (4.6)
ELISA – Enzootic bovine leukemia	Negative	156 (42.0)
	Positive	215 (58.0)
PCR – Enzootic bovine leukemia	Negative	318 (85.7)
	Positive	53 (14.3)

Tuberculin test: negative, ≤ 2 mm increase in skin thickness; doubtful, > 2 –4 mm; positive, > 4 mm or local clinical signs. No animal met the positive criterion.

Table 3
Cross-tabulation ELISA and PCR results for bovine leukosis in the study population

ELISA result	PCR negative (n, %)	PCR positive (n, %)
Negative	153 (98.1)	3 (1.9)
Positive	165 (76.7)	50 (23.3)

No animals were concurrently positive for BLV and PTB by PCR. Among the 17 MAP-seropositive animals, 10 were also BLV seropositive, and one was BLV PCR positive. Given the very low prevalence of PTB and bTB and the absence of PCR-confirmed coinfections, these pathogens were not included as covariates in the multivariable models describing BLV diagnostic outcomes.

Animal-level epidemiological patterns according to BLV diagnostic outcomes (ELISA and PCR)

When evaluated by ELISA, BLV seropositivity was frequent across most breeds, with higher proportions among Normando (78.9%) and Normando×Holstein (84.6%) cattle and lower proportions among Ayrshire (50.0%), Jersey (55.6%), and Holstein (51.3%) cattle (Online Resource 2, Table S1). Seropositivity did not vary by age group ($p = 0.90$) but was markedly higher in animals from Q1 herds (77.1%) than in those from the four Q2 herds (32.9%) (Online Resource 2, Tables S1 and S2). In univariable models with herd-clustered standard errors, animals from Q2 herds had lower odds of seropositivity (OR = 0.15; 95% CI: 0.05–0.44; $p = 0.001$), whereas the lower odds observed in Holstein cattle (OR = 0.49; 95% CI: 0.21–1.14; $p = 0.097$) and the association with age ($p = 0.666$) were not significant (Table 4).

Table 4
Univariate logistic regression of animal- and herd-level characteristics according to bovine leukemia virus ELISA results (herd-clustered standard errors)

Variable	Comparison	OR (95%CI)	<i>p</i> value
Breed	Holstein vs. Other	0.49 (0.21–1.14)	0.097
Age group	≥ 48 months vs. < 48 months	0.83 (0.37–1.90)	0.666
Herd daily milk yield	Q2 (14 L/day) vs. Q1 (≤ 12 L/day)	0.15 (0.05–0.44)	0.001

Animal-level epidemiological patterns according to BLV PCR detection

PCR positivity for BLV was relatively low across most breeds, with the highest proportions in Simmental (2/5), Montpellier (1/2), and Holstein (17.7%) cattle (Online Resource 2, Table S3). The proportion of positive animals increased with age, from 0% in calves ≤ 1 year to 16.9% in animals > 3 years, and was higher in animals from Q2 herds (19.9%) than in those from Q1 herds (10.0%) (Online Resource 2, Tables S3 and S4). In univariable models with herd-clustered standard errors, PCR positivity was associated

with Holstein breed (OR = 2.18; 95% CI: 1.18–4.03; $p = 0.013$), age ≥ 48 months (OR = 1.94; 95% CI: 1.19–3.16; $p = 0.008$), and Q2 herd milk yield (OR = 2.23; 95% CI: 1.25–3.98; $p = 0.007$) (Table 5).

Table 5
Univariate logistic regression of animal- and herd-level characteristics according to bovine leukemia virus PCR results (herd-clustered standard errors)

Variable	Comparison	OR (95%CI)	<i>p</i> value
Breed	Holstein vs. Other	2.18 (1.18–4.03)	0.013
Age group	≥ 48 months vs. < 48 months	1.94 (1.19–3.16)	0.008
Herd daily milk yield	Q2 (14 L/day) vs. Q1 (≤ 12 L/day)	2.23 (1.25–3.98)	0.007

In the multivariable model for ELISA results, breed and age group were not associated with seropositivity, whereas animals from Q2 herds had lower odds of BLV seropositivity (adjusted OR = 0.16; 95% CI: 0.05–0.48; $p = 0.001$). The goodness-of-fit test indicated a lack of fit (Pearson $\chi^2 = 16.73$, $df = 4$, $p = 0.002$; McFadden pseudo- $R^2 = 0.152$), which is consistent with between-herd heterogeneity not captured by the model (Table 6).

Table 6
Multivariable logistic regression of animal- and herd-level characteristics according to bovine leukemia virus ELISA results (herd-clustered standard errors)

Variable	Comparison	aOR (95%CI)	<i>p</i> value
Breed	Holstein vs. Other	0.72 (0.40–1.29)	0.270
Age group	≥ 48 months vs. < 48 months	0.95 (0.37–2.47)	0.922
Herd daily milk yield	Q2 (14 L/day) vs. Q1 (≤ 12 L/day)	0.16 (0.05–0.48)	0.001

N = 371 animals in 30 herds; 95% CIs and *p* values based on herd-clustered standard errors. Model fit: Pearson goodness-of-fit $\chi^2 = 16.73$, $df = 4$, $p = 0.002$; McFadden pseudo- $R^2 = 0.152$.

In the multivariable model for PCR results, age ≥ 48 months (adjusted OR = 1.87; 95% CI: 1.15–3.04; $p = 0.012$) and Q2 herd milk yield (adjusted OR = 1.89; 95% CI: 1.08–3.32; $p = 0.027$) were associated with higher odds of BLV PCR positivity, whereas the association with the Holstein breed was borderline (adjusted OR = 1.88; 95% CI: 0.98–3.59; $p = 0.056$). The model showed adequate fit but limited explanatory power (Pearson $\chi^2 = 2.89$, $df = 4$, $p = 0.577$; McFadden pseudo- $R^2 = 0.048$) (Table 7).

Table 7
Multivariable logistic regression of animal- and herd-level characteristics according to bovine leukemia virus PCR results (herd-clustered standard errors)

Variable	Comparison	aOR (95%CI)	p value
Breed	Holstein vs. Other	1.88 (0.98–3.59)	0.056
Age group	≥ 48 months vs. <48 months	1.87 (1.15–3.04)	0.012
Herd daily milk yield	Q2 (14 L/day) vs. Q1 (≤ 12 L/day)	1.89 (1.08–3.32)	0.027

N = 371 animals in 30 herds; 95% CIs and p values based on herd-clustered standard errors. Model fit: Pearson goodness-of-fit $\chi^2 = 2.89$, df = 4, p = 0.577; McFadden pseudo- $R^2 = 0.048$.

DISCUSSION

In this study, which was conducted in specialized dairy herds in Colombia, we observed a high frequency of BLV-positive diagnostic results accompanied by a marked discrepancy between serological and molecular detection methods. These findings highlight the diagnostic complexity of chronic bovine infections—namely, bTB, PTB, and EBL—in tropical production systems, where environmental pressures, management constraints, and endemic pathogen circulation converge (Motta-Delgado et al. 2019; Corredor-Figueroa et al. 2020; Castillo-Rey et al. 2023; Conde-Muñoz et al. 2023; Rúa Giraldo et al. 2023; Bonilla-Aldana et al. 2024; Jaimes-Dueñez et al. 2024; Mendoza et al. 2024). The Colombian dairy sector, which is experiencing strong growth and increasing relevance for food security, must contend with the sanitary and economic implications of these persistent infections.

Despite the widespread use of ELISA and PCR in bovine infectious disease surveillance, our results revealed substantial disagreement between these methods when BLVs were detected. This limited agreement is consistent with the well-described biological course of BLV, in which many seropositive animals carry low or undetectable proviral loads, resulting in PCR-negative results despite antibody positivity. This pattern is consistent with prior studies showing limited agreement between diagnostic modalities targeting distinct immunological or pathogen-detection windows (Kelly et al. 2022). Similar challenges have been documented for bTB, in which IFN- γ assays and TST show only “fair to moderate” concordance (Broughan et al. 2016). These discrepancies reinforce a key epidemiological principle: reliance on a single diagnostic test may lead to underestimation of the true prevalence, misclassify animals, and compromise control programs. Given the high BLV seroprevalence and the potential for subclinical shedding of MAP and *M. bovis*, combined or sequential testing strategies are particularly necessary for Colombian dairy systems. From a control perspective, this has direct operational implications. Recent evidence indicates that BLV-infected animals with persistently low proviral loads (LPL) are unlikely to be efficient transmitters under natural conditions, whereas high-proviral-load (HPL) animals act as the principal source of horizontal transmission within and between herds (Juliarena et al. 2016). Longitudinal data from Argentina, the United States, and Japan further document that compared with serostatus alone, proviral load classification is more predictive of transmission risk and clinical

progression (Norby et al. 2016; Porta et al. 2023). In endemic Latin American settings where strict whole-herd seropositive culling is rarely economically feasible, a tiered control approach combining qPCR-based proviral-load classification, segregation or selective culling of HPL animals, replacement from BLV-free sources, and rigorous biosecurity around blood-contaminating procedures appears more realistic and aligns with the WOA-recommended progressive control framework.

Our findings indicate that BLV diagnostic outcomes vary according to animal- and herd-level characteristics and the diagnostic method applied. Holstein cattle tended to have lower ELISA seropositivity than other breeds did, but this difference was not significant after accounting for herd clustering and was absent from the multivariable model. Although Holstein cattle are often reported to be more susceptible to BLV (Nikbakht-Brufeni et al. 2023), the pattern observed here may reflect unmeasured herd-level factors, such as equipment sharing or a longer productive life of crossbred and minority-bred cows. Conversely, Holstein cattle had higher BLV PCR positivity (OR = 2.18; 95% CI: 1.18–4.03), which is more consistent with the international literature on Holstein susceptibility. High PCR detection in older animals likely reflects cumulative exposure and progression toward proviral persistence, a pattern previously described in endemic settings (Juliarena et al. 2016; Porta et al. 2023; Righi et al. 2025).

Milk yield was recorded as a single value per herd; thus, its associations with BLV outcomes are herd-level rather than individual effects. The Q2 category comprised only four herds, which were also the largest sampled (17–63 animals each) and had a higher proportion of Holstein cattle. The lower seroprevalence (32.9% vs. 77.1%) and higher PCR positivity (19.9% vs. 10.0%) observed in these herds therefore most likely reflect differences in herd size, management, replacement policies, and infection history rather than a direct relationship between BLV infection and individual milk production. Accordingly, these results should not be interpreted as evidence that BLV-seropositive cows produce less milk, an effect documented in longitudinal individual-level studies (Norby et al. 2016) that cannot be assessed with the present data. The lack of fit of the ELISA model further suggests between-herd heterogeneity, as the within-herd seroprevalence reached 100% in 18 of the 26 Q1 herds.

After accounting for herd clustering, age ≥ 48 months remained associated with BLV PCR positivity (aOR = 1.87; $p = 0.012$), whereas the association with the Holstein breed was borderline (aOR = 1.88; $p = 0.056$). Both findings are biologically plausible: cumulative exposure throughout productive life increases the probability of stable proviral integration, and Holstein cattle are often managed more intensively, with more frequent blood-contaminating procedures (rectal palpation, injections, and shared equipment), although herd-level management data were not analyzed in the present study. The limited number of PCR-positive animals ($n = 53$) restricted the statistical power for these comparisons.

Given the cross-sectional design and the herd-level nature of milk yield, the diverging patterns observed across herd yield categories—higher ELISA seropositivity in Q1 herds and higher PCR positivity in Q2 herds—cannot be interpreted causally. They may reflect differences in the stage of within-herd infection, with long-established infections and controlled proviral replication in smaller herds versus more recent

transmission and active proviral expansion in larger herds, as well as differences in management. Disentangling these explanations will require longitudinal follow-up with periodic qPCR and individual milk recording, as performed in North American cohorts (Bartlett et al. 2013; Norby et al. 2016).

The combination of high seroprevalence and low PCR detection suggests a large reservoir of latent or immunologically contained BLV infections, characterized by intermittent or absent viremia. This diagnostic pattern complicates eradication efforts, as seropositive but PCR-negative animals may still contribute to transmission through blood-contaminating management practices such as dehorning, rectal palpation, ear tagging, and needle reuse (Kuczewski et al. 2021). Experiences from herd-level control programs in the United States demonstrate that systematic testing and targeted culling strategies based on diagnostic profiles can substantially reduce EBL incidence—although the pace of reduction depends heavily on baseline infection levels and herd management (Ruggiero and Bartlett 2019; Zhao et al. 2025). In Colombia, the absence of mandatory EBL control policies and the common use of shared equipment among farms represent key barriers to progress.

The detection of EBL- and PTB-seropositive animals and of doubtful tuberculin reactors in the evaluated herds reflects a chronic diagnostic and epidemiological landscape with prolonged asymptomatic stages and complex interactions that may affect immunity, productivity, and transmission dynamics. Although BLV is not formally recognized as zoonotic, *M. bovis* and MAP pose clear public health risks, particularly in settings where raw milk consumption persists. Although no tuberculin-positive animals were detected, the four doubtful reactors should be retested according to national regulations, and sustained surveillance remains necessary given the established transmission routes through direct contact and contaminated dairy products (Olea-Popelka et al. 2017). Likewise, the detection of MAP antibodies (4.6%) warrants attention given their long incubation period, diagnostic challenges, and the ongoing debate regarding associations between MAP and Crohn's disease in humans (Chiodini et al. 2012). Together, these findings emphasize the importance of integrated One Health strategies that align animal health, food safety, and public health goals.

The uniformly negative PCR results for both *M. bovis* and MAP in this study warrant interpretation considering well-known biological and analytical constraints. For bTB, whole-blood PCR is intrinsically limited because mycobacteremia in *M. bovis*-infected cattle is intermittent and transient, with bacterial loads typically below the analytical sensitivity of conventional molecular assays; tissue samples from suspected granulomatous lesions or tracheobronchial lymph nodes obtained at slaughter remain the recommended substrates for confirmatory molecular detection. With respect to MAP, the sensitivity of fecal PCR is markedly reduced in subclinically infected animals owing to intermittent and preshedding stages, and most positive results are concentrated in clinically affected or heavy-shedder animals. Beyond these sample-related considerations, the negative PCR findings are also consistent with the low apparent prevalence detected by the reference field tests (no tuberculin-positive and four doubtful reactors for bTB; 4.6% ELISA seropositivity for PTB) and with the typically low pathogen loads expected in subclinical herds, suggesting that true infection prevalence in the studied population is genuinely low rather than the result of analytical insensitivity alone.

This study has notable strengths. Sampling across 30 dairy herds covers a range of specialized dairy systems in the Ubaté Valley, and the combined use of serological and molecular tools allows a more nuanced interpretation of infection status. Access to individual-level demographic data also enabled a detailed evaluation of animal-level epidemiological patterns according to diagnostic outcomes. However, some limitations should be considered when the findings are interpreted. Small sample sizes in certain subgroups—particularly minority breeds—may have reduced statistical power and limited estimation precision. The cross-sectional design prevents causal inference, which is a common constraint in field-based epidemiological studies (LaDronka et al. 2018; Bartlett et al. 2020). In addition, the convenience sampling strategy adopted at both the herd and animal level may introduce selection bias and limit the generalizability of the prevalence estimates and risk associations to the broader Colombian dairy population. Herds were enrolled based on accessibility and willingness to participate, and within-herd sampling depended on the animals available during routine farm visits. As a result, herds with more proactive sanitary practices may be overrepresented, while individual animals selected for sampling may not fully reflect the age, breed, or productive structure of the source population. The estimates reported here should therefore be interpreted as descriptive of the studied population rather than as inferential to the regional or national herd. Because clinical examinations were not conducted and data on other health conditions (e.g., mastitis and lameness) were not collected, their potential role as confounders could not be assessed, and some subclinical or concurrent disorders may have remained unmeasured. More importantly, herd-level management variables that are well established as drivers of EBL, bTB, and PTB transmission—such as biosecurity practices, equipment disinfection protocols, single-use needle policies, calving and calf-rearing management, replacement heifer sourcing, and equipment sharing among neighboring farms—were not analyzed. The absence of these variables from the analysis means that the associations reported here may be subject to substantial residual confounding, particularly for breed, which may covary with management intensity at the herd level. Moreover, milk yield was available only as a herd-level value, so its associations reflect comparisons among herds, driven by only four Q2 herds, rather than individual-level effects. The observed individual-level associations should therefore be regarded as exploratory descriptions of diagnostic patterns rather than as adjusted estimates of independent biological effects, and future multilevel studies that explicitly modeled herd-level covariates are warranted. Predictive values for low-prevalence diseases such as bTB and PTB should also be interpreted with caution, as these metrics lose reliability as the prevalence decreases (Chen et al. 2021). The absence of quantitative PCR for determining the proviral load is a further limitation, since discrimination between low-proviral-load (LPL) and high-proviral-load (HPL) animals — which carry documented implications for transmission risk and culling decisions — could not be performed. Future studies in Colombian herds should incorporate qPCR-based proviral load quantification to better characterize the transmission relevance of seropositive but PCR-negative animals.

Future studies should include longitudinal designs to clarify the temporal relationships and diagnostic dynamics of BLV under field conditions (Gutiérrez et al. 2011). Evaluating combined or sequential diagnostic strategies that integrate serological and molecular methods is essential for improving diagnostic accuracy in endemic herds (Hsieh et al. 2019; Porta et al. 2023; Mamanova et al. 2025).

Expanding similar studies to additional Colombian regions will enable the construction of a more comprehensive national epidemiological profile of EBL, bTB, and PTB, thereby supporting risk-based and regionally adapted control programs.

From a practical standpoint, BLV serological screening could be integrated into the brucellosis and tuberculosis testing rounds mandated by the ICA, proviral load quantification could be prioritized in seropositive replacement heifers and breeding bulls, and low-cost biosecurity measures against iatrogenic transmission (single-use needles, individual palpation sleeves, and disinfection of dehorning and ear-tagging equipment) should be promoted among farm workers and rural veterinarians. Regional pilot programs supported by ICA and AGROSAVIA would provide the evidence needed for a national, context-adapted control framework.

In conclusion, this study provides a field-based diagnostic epidemiological assessment of three major chronic infections affecting Colombian dairy cattle. The high seroprevalence of BLV combined with minimal agreement between serological and molecular tests reflects distinct stages of infection and highlights the limitations of relying on single diagnostic approaches in endemic settings. Differences in epidemiological patterns according to diagnostic method—lower seropositivity in the larger, higher-yielding herds and higher PCR detection in older animals and in those herds—illustrate how diagnostic outcomes capture different biological processes rather than a uniform infection status. Although herd-level management data were not analyzed, these findings emphasize the importance of integrated diagnostic strategies and cautious interpretation of animal-level associations in cross-sectional studies conducted under routine field conditions. Future research incorporating longitudinal designs and herd-level information will be essential to clarify transmission dynamics and support targeted control strategies within a One Health framework.

Declarations

Competing interests

The authors have no relevant financial or nonfinancial interests to disclose.

Ethics approval

Ethical approval was not needed for this study because no experimental procedures were performed. All samples were collected during routine sanitary and diagnostic activities by personnel authorized by the Colombian Agricultural Institute (ICA), in accordance with Colombian animal health and welfare regulations.

Consent to participate

Informed consent was obtained from all the herd owners for the inclusion of their animals in the study.

Consent for publication

Not applicable.

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Author contributions

Jorge Guillermo Noriega Márquez: Conceptualization, Investigation (field sampling), Data curation, Writing – review and editing. **Yolanda Gómez Vargas:** Conceptualization, Methodology, Investigation (laboratory analyses), Writing – review and editing. **Jahir Oswaldo Vargas Domínguez:** Conceptualization, Methodology, Investigation (laboratory analyses), Writing – review and editing. **Diego Ortiz Ortega:** Conceptualization, Writing – review and editing. **Nathalia M. Correa-Valencia:** Formal analysis, Writing – original draft, Writing – review and editing. All authors read and approved the final manuscript.

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Data availability

The dataset supporting the findings of this study is provided in the Supplementary Material (Online Resource 1).

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