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Seroprevalence and risk factors associated with leukosis in cattle from Villlavicencio, Colombia

Soroprevalência e fatores de risco associados à leucose em bovinos de Villlavicencio, Colômbia

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Abstract
Bovine leukosis is caused by an oncogenic virus of the genus *Deltaretrovirus*, causing losses associated with decreased production indicators and restrictions on exports of cattle and cattle products. The disease has a prolonged incubation period of between 1–5 years and the antibodies can be detected 2–3 weeks post infection. The disease can present asymptomatically, and develop persistent lymphocytosis or lymphosarcoma. The objective of this study was to estimate the prevalence and risk factors associated with bovine leukosis in Villavicencio, Colombia. Blood samples were taken from 636 animals, and obtained randomly from 24 herds. The samples were analysed using a Competition ELISA kit for the detection of anti-gp51 antibodies. Information on possible risk factors was collected, then OR and X^2 were calculated, and statistically significant with $p < 0.05$ variables were included in a linear regression multivariate analysis. The general seroprevalence was 24.6% and the herd seroprevalence was 83.3%. The seroprevalence was 21.3% in males and 25.0% in females. The risk factors identified were abortion, non-bearing cows, artificial insemination, and use of common needles, Creole breed and participation in cattle exhibitions. The study confirmed the presence of bovine leukosis associated with reproductive and management factors.
Keywords: bovine persistent lymphocytosis; bovine lymphoma; bovine chronic infectious viruses; bovine leukosis; ELISA.

Resumo
A leucose bovina é causada por um vírus oncogênico do gênero Deltaretrovirus, causando prejuízos associados à queda dos indicadores produtivos e restrições à exportação de bovinos e derivados. A doença tem um período de incubação prolongado entre 1 e 5 anos e os anticorpos podem ser detectados 2 a 3 semanas após a infecção. A doença pode se apresentar de forma assintomática, e evoluir para linfocitose persistente ou linfossarcoma. O objetivo do estudo foi estimar a prevalência e os fatores de risco associados à leucose bovina em Villavicencio, Colômbia. Amostras de sangue foram coletadas de 636 animais, obtidos aleatoriamente de 24 rebanhos. As amostras foram analisadas com o kit Competition ELISA para detecção de anticorpos anti-gp51. Foram coletadas informações sobre possíveis fatores de risco, se realizo um analise univariado entre as variáveis e a presença da seropositividad a leukosis bovina mediante o cálculo do OR e X^2, as variáveis estatisticamente significativas com $p<0,05$ foram incluídas em uma análise multivariada de regressão linear. A soroprevalência geral foi de 24,6% e a soroprevalência do rebanho foi de 83,3%. A soroprevalência foi de 21,3% em machos e 25,0% em fêmeas. Os fatores de risco identificados foram: aborto, vacas não reprodutivas, inseminação artificial e uso de agulha comum, raça crioula e exposições de gado. O estudo confirmou a presença de leucose bovina associada a fatores reprodutivos e de manejo.
Palavras-chave: linfocitose persistente bovina; linfoma bovino; vírus infeccioso crônico bovino; leucose bovina; ELISA.

1. Introduction

Bovine leukosis is one of the most common neoplastic diseases in cattle, having a high prevalence and causing great economic losses for the livestock sector ([1]). The disease is caused by an oncogenic RNA virus of the Retroviriade family, genus *Deltaretrovirus*, with tropism for B lymphocytes causing persistent lymphocytosis. More than ten different genotypes of bovine leukosis virus strains have been identified circulating in various geographical locations throughout the world, the most predominant being genotypes 1, 4 and 6 ([2]). The disease has a slow clinical course with an incubation period of between 1–5 years, affecting cattle older than two years to a greater extent. However, some animals infected with the virus do not show visible signs and may be asymptomatic for their entire lives, 30% of infected animals may develop persistent lymphocytosis (LP), while 2–5% may

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develop lymphosarcoma ([3]).

Transmission of bovine leukosis occurs through provirus-infected lymphocytes, which are transmitted iatrogenically through contact with blood and through surgical or handling procedures, such as blood extraction, castration, vaccination, dehorning and palpation ([4]). Additionally, by vertical transmission, the foetus can be contaminated by the transplacental and intrauterine routes or through the birth canal, and even after birth through the consumption of colostrum ([5]). Transmission by flies and arthropods has recently been reported ([4]). The disease has been associated with breast cancer in women from milk consumption, where molecular PCR studies have identified bovine leukosis genes in breast cancer tissue ([6]). In addition, a review study showed that the disease is associated with an increased risk of breast cancer ([7]).

Livestock suffer economic losses associated with confiscations in animal processing plants because of the presence of clinical lymphosarcoma in the carcass. However, animals with the disease can present significant alterations in immune function, which can cause reduced milk production, infectious diseases and reproductive inefficiency ([1]). In many European countries, bovine leukosis is under official control: 20 countries of the European Union have obtained disease-free status. However, in Latin America, official programmes to control the disease are still lacking. In Colombia, diseases not subjected to official control, including bovine leukosis, cause up to 30% of infertility and abortions, low pregnancy rates and high neonatal mortality, with losses of more than 108 million dollars annually ([8]). Villavicencio is one of the main livestock centres in Colombia; the presence of bovine leukosis in the region is unknown. This study aims to estimate the prevalence and risk factors associated with bovine leukosis in the municipality of Villavicencio.

2. Materials and Methods

2.1. Study area and population

A cross-sectional epidemiological study was carried out in the municipality of Villavicencio, which has a population of 108,109 cattle ([9]). The sample size was determined following the postulates of Dohoo (2003) ([10]), determined considering a hypothetical seroprevalence of 31.1% ([11]), a confidence level of 95%, design effect of 1.95 and a population of 108,109 according to the Colombian Agricultural Institute (ICA) census. A total sample of 636 cattle was calculated using SPSS software.

2.2. Selection and collection of samples

The animal samples were selected by stratified random sampling of 24 farms of the dual-purpose system from five villages of Villavicencio, the average herd size was 15 animals, with a minimum of three and a maximum of 69. The study was carried out between March and August of 2017. Blood samples of 0.5 mL were taken from the jugular vein of each animal into sterile tubes without anticoagulant (Vacutainer), considering surveys of all the criteria of asepsis and disinfection, which were conducted in isothermal cells to the Animal Reproduction and Genetics Laboratory of the Universidad de los Llanos for processing. The samples were centrifuged at 2,000g for 10 minutes, and the sera were extracted and transferred to Eppendorf tubes using a Pasteur pipette and stored at −20 °C until analysis.

2.3. ELISA test

The samples were analysed using the ID Screen BLV Competition ELISA kit from Innovative Diagnostics Vet, which allows the detection of anti-gp51 antibodies. The reading was carried out using a spectrophotometer that measures the optical densities of samples and controls with which a relationship of the percentage of competition was made. Consequently, the optical density was determined at 450 nm, the positive samples whose values were greater than or equal to 0.50 optical density with a wavelength of 450 nm, values below were considered negative. The kit has a sensitivity of 96.3% and a specificity of 99.6%.

2.4 Statistics and risk analysis

For the risk factor analysis an epidemiological questionnaire was applied per farm, where information was collected on the populations and species of domestic animals, productive activities, infrastructure, technical assistance, sanitation and biosafety practices. The questionnaire was applied and standardised in coordination with the national agricultural authorities, namely, the Colombian Agricultural Institute (ICA). Univariate analysis was performed using Pearson's chi-square test, 2x2 table and OR calculation to assess the relationship between bovine leukosis and the variables. Variables with $p<0.05$ in the univariate analysis were included in the multivariate logistic regression model. Statistical analyses were performed using SPSS, version 20 software (SPSS Inc., Chicago, Il, USA).

2.5. Ethical statement

In this study, the animals received treatment following the animal experimentation rules described in the International Guiding Principles for Veterinary Research Involving Animals and the owners of the animals signed informed consent before their inclusion in the study. The study was approved by the Ethics Committee of the Faculty of Agricultural Sciences of the Universidad de Ciencias Aplicadas y Ambientales (UDCA) N. 001–2017.

3. Results

The general seroprevalence was 24.6% (95% CI: 21.04–28.7) and the herd seroprevalence was 83.33%. Regarding the sex, females presented a seroprevalence of 25.04% (95% CI: 21.2–29.39) and males 21.31% (95% CI: 11.85–35.53) (Table 1). The age range of the animals was divided into four groups: age 0–1 year with a prevalence of 22.3% (95% CI: 14.2–33.57); age 1–2 years with a prevalence of 20.9% (95% CI: 11.89–34.23); age 2–3 years with a prevalence of 17.8% (95% CI: 8.25–33.76); and older than four years with a prevalence of 26.5% (95% CI: 21.9–31.73).

The villages with the highest prevalence were Amor with 41.0% (95% CI: 31.3–52.7) followed by Apaiay with 30.9% (95% CI: 19.6– 46.4) and Bella Suiza with 26.1% (95% CI: 19.8–33.6) (Figure 1). No positive cases were detected on four farms (16.6%) and a seroprevalence above 50% was presented on six farms (25%).

Table 1. Seroprevalence of bovine leukosis according to population characteristics from Villavicencio, Colombia.

General variables	Total animals	Positive animals	Prevalence (%)	95% CI
Age ranges				
Age 0–1	21	94	22.3%	14.2–33.57%
Age 1–2 years	14	67	20.9%	11.89–34.23%
Age 2–3 years	8	45	17.8%	8.25–33.76%
Older than 4 years	114	430	26.5%	21.9–31.73%
Sex				
Female	144	575	25.04%	21.2–29.39%
Male	13	61	21.31%	11.85–35.53%

Confidence Interval: CI

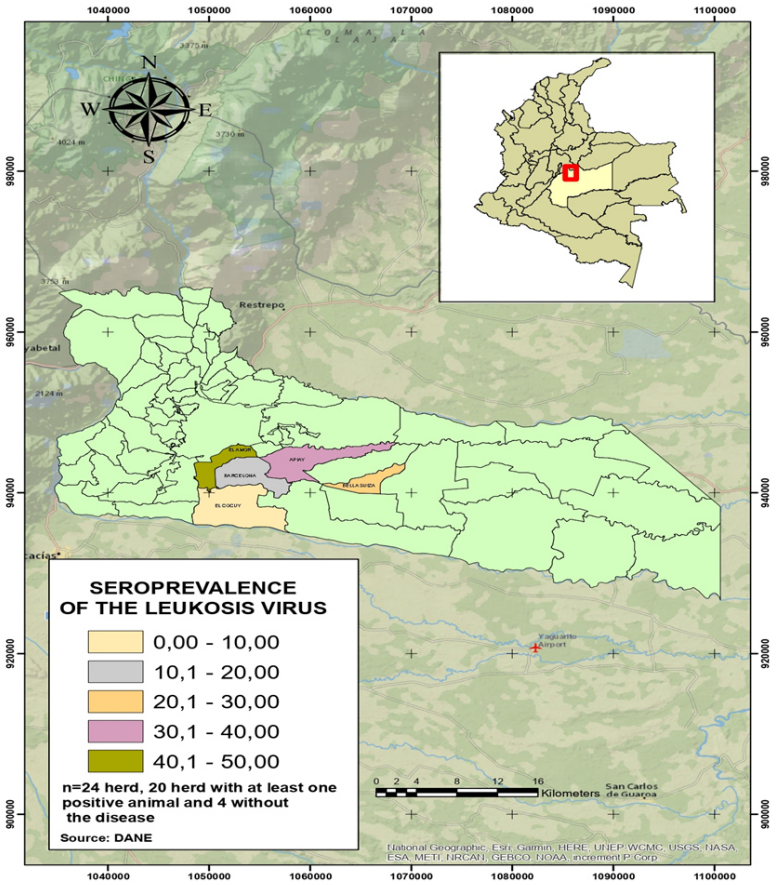

Figure 1. Seroprevalence of antibodies of bovine leukosis in the municipality of Villavicencio, Colombia.

In the analysis of risk factors, abortion (OR = 5.138 95% CI: 3.378–9.595), non-bearing cows (OR = 2.682 95% CI: 1.804–3.985), artificial insemination (OR = 2.036 95% CI: 1.408–2.945), use of a common needle (OR = 2969 95% CI: 1.891–4.659), Creole breed (OR = 2.206 95% CI: 1.321–3.047) and livestock exhibitions (OR = 5276 95% CI: 2.989–9.313) were identified as risk factors (Table 2). The protective factors were the use of a bull for direct mounting (OR = 0.584 95% CI: 0.386–0.884) and concentrate storage (OR = 0.321 95% CI: 0.220–0.468). In the analysis according to breed, the Pardo (OR = 0.324 95% CI: 0.200–0.523), Angus (OR = 0.361 95% CI: 0.176–0.742), Zebu (OR = 0.391 95% CI: 0.249–0.631) and Girolando (OR = 0.523 95% CI: 0.351–0.778) were considered as protective factors. The results of the multivariate logistic regression model are shown in Table 3.

Table 2. Analysis of risk factors associated with the seroprevalence of bovine leukosis in Villavicencio, Colombia.

Variables	X^2	P -value	OR	95% CI
Female	0.413	0.520	1.234	0.650–2.342
Male	0.413	0.520	0.811	0.427–1.539
Under 1 year old	0.326	0.568	0.859	0.509–1.448
1–2 years old	0.579	0.447	0.787	0.424–1.461
2–3 years old	1.243	0.265	0.641	0.292–1.408
Older than 4 years old	2.381	0.123	1.368	0.918–2.037
Abortion	50.226	0.000	5.138	3.378–9.595
Non-bearing cows	24.802	0.000	2.682	1.804–3.985
Reproduction by natural mating	6.570	0.010	0.584	0.386–0.884
Artificial insemination	14.537	0.000	2.036	1.408–2.945
Pardo breed	22.659	0.001	0.324	0.200–0.523
Angus breed	8.265	0.004	0.361	0.176–0.742
Cebu breed	17.553	0.000	0.391	0.249–0.631
Girolando breed	10.418	0.001	0.523	0.351–0.778
Creole breed	10.913	0.001	2.206	1.321–3.047
Common needle	23.757	0.000	2.969	1.891–4.659
Livestock exhibitions	38.729	0.001	5.276	2.989–9.313
Buried dead animals	6.958	0.008	2.314	1.224–4.377
Concentrates on pallets	36.569	0.000	0.321	0.220–0.468

Statistically significant ($P < 0.05$), Confidence Interval: CI

Table 3. Multivariate logistic regression analysis associated with the seroprevalence of leukosis in cattle from Villavicencio, Colombia.

Variable	β	Exp(B)	P -value	95% CI
Abortion	0.244	1.968	0.002	0.092–0.395
Non-bearing cows	0.453	2.682	0.000	0.289–0.618
Pardo breed	−0.306	0.324	0.000	−0.430–0.183
Zebu breed	−0.148	0.391	0.001	−0.231–0.064
Girolando breed	0.186	0.523	0.001	0.072–0.300
Livestock exhibitions	0.224	5.276	0.002	0.081–0.367

Potential risk factors ($P < 0.05$) were selected for inclusion in the multivariate model. Statistically significant $P < 0.05$; CI: confidence interval (95%).

4. Discussion

The present study showed a herd seroprevalence of 83.3% for the municipality of Villavicencio. A high herd seroprevalence of 94.2% has been reported in the US in a study of 103 dairy herds from 11 states ([12]). In Canada, a herd seroprevalence of 79% was reported in a study of seven provinces ([13]). However, in Turkey, a low herd seroprevalence of 11.82% was identified from the analysis of 28,982 animals from 1,116 herds ([14]). The seroprevalence at the animal level in Villavicencio was 24.6%; similar results were found in Iran in the analysis of 429 blood samples from industrial dairy herds with a seroprevalence of 25.4% ([15]). In Japan, a total of 5,420 bovines from 209 farms was analysed, reporting a global seroprevalence of 28.6% ([16]). In the United Arab Emirates, 957 bovine sera were analysed by ELISA, observing a seroprevalence of 25.7% ([17]). In Egypt, dairy cattle were studied in four provinces in the Nile Delta with a seroprevalence between 16.2% and 20.3%, depending on the province ([18]).

In Latin America, similar results were observed in Argentina, in the province of Corrientes, where a seroprevalence of 32.53% in 126 animals was reported through the analysis by agar gel immunodiffusion tests ([19]). However, in some regions, low prevalence rates are still reported. For example, in Paraiba-Brazil a seroprevalence of 10.8% was reported from the analysis

of 2,067 animals ([20]); in the state of Rio Grande do Sul-Brazil, a prevalence of 9.2% was determined ([21]), while in the Brazilian Amazon a seroprevalence rate of 8.9% ([22]) was noted. In Chile, in the Los Ríos and Los Lagos regions, a seroprevalence of 15.6% was reported from the analysis of 4,360 animals by ELISA tests ([23]). In Colombia, high prevalence rates of the disease were determined in the department of Santander, where a seroprevalence of 73% was reported from the analysis of 360 samples from 75 herds ([24]). A study in various regions of Colombia identified a seroprevalence of 42.7% at the animal level and 67.7% at the herd level; in Antioquia, a seroprevalence of 53.9% was identified, in Boyacá seroprevalence was between 31.1% and 78%, in Cesar between 77.1% and 79.9%, in Córdoba between 5.1 and 19.5%, in Cundinamarca between 35% and 36%, and 26.5% in Nariño ([25]). It is important to consider that serological diagnosis can identify antibodies 2–3 weeks post infection, while PCR diagnosis identifies the presence of virus, being a complementary technique, mainly in cases such as young animals ([1]).

The analysis of risk factors did not identify a correlation between the disease and the variables of sex and age; although the range with the highest prevalence were animals over four years of age, and under one year of age, which may be associated with maternal immunity; there are various reports on the increase in bovine leukosis in adults ([14,26]). An association of the disease with reproductive symptoms such as abortion and non-bearing cows was observed. Few studies have associated the disease with abortion; malignant lymphoid neoplasia has been reported in two foetuses and their abortion was assumed to have been caused by the bovine leukosis virus ([27]). In Montería-Colombia, a study was conducted on animals with reproductive problems, identifying the circulation of disease with a high percentage of bovine leukosis ([28]). Diseases can occur concomitantly or evidence of immunosuppression in cattle infected with leukosis has been reported. The disease causes immunological disturbances at the level of cellular immunity, modifying the number and profile of T cells, which could increase the risk of the presentation of other infectious diseases ([29]). In Michigan, United States, co-infections with reproductive diseases, specifically between leukosis and mycobacteria, have been reported ([30]). Artificial insemination is a risk factor; several studies explored the transmission of the disease by this route, however, the findings are diverse. The presence of the virus has been identified in semen ([31, 32]); however, other studies have reported the absence of the virus in the semen of positive animals ([33]) as well as low viral transmission through semen ([34]). In Chile, the presence of the disease was associated with artificial insemination ([35]); the authors state that procedures involving artificial insemination, such as rectal palpation and glove contamination, can transmit high viral loads through the rectal mucosa. Natural mating was a protective factor in this study. In the United States, the transmission of bovine leukosis was evaluated during natural mating between a BLV-infected bull and non-infected heifers, not identifying transmission ([36])

The study of biosafety practices as risk factors identified common needles as a risk factor, Hutchinson et al. ([37]) reported similar results associating the transmission of the disease with the reuse of needles; however, experimental studies conducted previously by Weber et al., ([38]) showed that the number of infectious lymphocytes that pass during injection with common needles is too small to induce infection. Therefore, the different management practices that may intervene with other additional factors must be analysed. The mobility of animals, the entry of new animals and contact with animals have been reported as sources of disease transmission ([39]). In the present study, we identified livestock exhibitions as a risk factor. In Brazil, acquiring new animals in the last year was identified as a risk factor ([20]) and in Canada, farms that did not buy cows in the last five years were more likely to be free of bovine leukosis ([13]). Concentrates on pallets was observed as a protection factor, as well as a risk factor for buried dead animals, showing the importance of animal management to prevent horizontal transmission. However, more studies are required to fully understand the transmission mechanisms and substantiate control programmes.

This study identified the Pardo, Angus, Zebu and Girolando breeds as protective factors, while the Creole breed was a risk factor. Several studies have reported a higher presence of the disease in dairy cattle ([16]). In Turkey, Holstein cattle had a higher risk of being infected by leukosis compared to Brown Swiss cattle ([14]). In Colombia, a higher risk was observed associated with the Holstein and Normande breeds ([11]). Dairy breeds presented a greater exposure to sources of infection, such as routine palpation, milking and vaccination needles, among others. However, it is important to highlight the widespread use of the Creole breed for a dual purpose (milk–meat) in the study region, which could influence the results obtained. Similarly, the best management given to them should be considered for high value breeds. The association of genetics and disease has recently been studied, where the genetic diversity of the innate and adaptive immune system in animals provides antigen-presenting cells with variability in the immune response to particular pathogens for individuals ([1]). Studies have associated the disease with familial lineages; the DRB3 and DQA1 alleles of BoLA Class II were found to be associated with resistance or susceptibility ([40]). Therefore, breed influences the presentation of the disease, so future genetic findings may be used in the control of the disease.

5. Conclusions

This study shows that the seroprevalence of bovine leukosis was 24.6% at the individual and 83.3% at the in the herd, being slightly higher in females and in those individuals aged four years and older. Leukosis is not an officially monitored in Colombia; it is mainly associated with reproductive risk factors and management, such as abortion, non-breeding cows, artificial insemination, use of common needles, Creole breed and participation in livestock exhibitions.

Declaration of conflict of interest

The authors declare that there are no conflicts of interest.

Author contribution

Conceptualization: J. Conde-Muñoz, N. Reyes-Bernal, M. F. Guatibonza-Garzon, J. C. Tobon, D. L. Valero, B. L. G. Barragan. *Data curation*: J. Conde-Muñoz, N. Reyes-Bernal, M. F. Guatibonza-Garzon, J. C. Tobon, D. L. Valero, B. L. G. Barragan. , *Formal Analysis*: J. Conde-Muñoz, N. Reyes-Bernal, M. F. Guatibonza-Garzon, D. L. Valero, B. L. G. Barragan. *Investigation*: J. Conde-Muñoz, N. Reyes-Bernal, M. F. Guatibonza-Garzon, J. C. Tobon, B. L. G. Barragan. *Methodology*: J. Conde-Muñoz, N. Reyes-Bernal, M. F. Guatibonza-Garzon, J. C. Tobon, D. L. Valero, B. L. G. Barragan. *Project administration*: J. C. Tobon e B. L. G. Barragan. *Resources*: J. C. Tobon and B. L. G. Barragan. *Funding acquisition*: J. C. Tobon. *Software*: J. Conde-Muñoz, N. Reyes-Bernal, M. F. Guatibonza-Garzon, D. L. Valero, B. L. G. Barragan. *Supervision*: J. C. Tobon and B. L. G. Barragan. *Writing (original draft)*:J. Conde-Muñoz, N. Reyes-Bernal, M. F. Guatibonza-Garzon, D. L. Valero, B. L. G. Barragan. *Writing (review & editing):* J. C. Tobon and B. L. G. Barragan.

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