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Serological survey of Bovine leukemia virus infection in dairy cattle herds in the Central-Eastern mesoregion of Paraná state, southern Brazil

Avaliação sorológica da infecção pelo vírus da leucemia bovina em rebanhos leiteiros da mesorregião Centro-Oriental do estado do Paraná, Brasil

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Highlights

EBL is endemic in dairy cattle herds in the Central-Eastern mesoregion of Paraná State.
Herd size (100–200 lactating cows) is a risk factor for BLV infection.
Intensive management practices increased risk of BLV infection in dairy cattle herds.

Abstract

Bovine leukemia virus (BLV) is an oncogenic retrovirus belonging to the subfamily *Orthoretrovirinae* and genus *Deltaretrovirus.* It is the etiological agent of enzootic bovine leukosis, an infectious disease that affects cow longevity and milk production, potentially leading to economic losses. Serological surveys provide valuable information on BLV circulation in dairy cattle herds. This study evaluated the

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serological profile of BLV infection in high-yield dairy cattle herds in the Central-Eastern mesoregion of Paraná State, Southern Brazil. Whole-blood samples were collected from 628 lactating cows in 43 dairy cattle herds between October 2019 and January 2020. Serum samples were stored at –80 °C until processing. Serological tests were performed using a commercially available ELISA kit to detect antibodies against glycoprotein 51 (gp51) in the BLV envelope. Herds were considered positive when at least one cow tested positive for anti-gp51 antibodies. The data obtained were categorized by type of management (intensive and semi-intensive) and the number of lactating cows per herd (<100, 100–200, and >200) and subjected to statistical analyses using chi-square and odds ratio tests at a 95% confidence level. Notably, all 43 (100.0%) evaluated herds were seropositive for BLV, with 10 herds (23.3%) showing 100% positivity in the evaluated cows. Of the sampled cows, 82% (515/628) were seropositive for BLV. The results of the statistical analyses for both factors were compared with the frequency of seropositive cows. Herds with 100–200 lactating cows exhibited a higher chance of being positive than those with <100 or >200 lactating cows. Moreover, herds under intensive management practices showed a 1.87-times greater chance of becoming infected with BLV than those under semi-intensive management. The serological survey results indicated that BLV infection is endemic in the Central-Eastern mesoregion of Paraná State. Enzootic bovine leukosis is a clinically silent retroviral disease, with overt clinical manifestations occurring only sporadically. Nevertheless, BLV infection is associated with reduced animal productivity and significant economic losses. Therefore, only coordinated actions to raise awareness among all stakeholders in the dairy production chain regarding the epidemiological importance of BLV and its routes of transmission will help to reduce the prevalence of seropositive animals and herds in endemic regions.
Key words: Bovine retrovirus. Dairy cow. Enzootic bovine leukosis. ELISA gp 51 test. Health risk factor.

Resumo

O vírus da leucemia bovina (*Bovine leukemia virus* - BLV) é um retrovírus oncogênico que pertence à subfamília *Orthoretrovirinae* e ao gênero *Deltaretrovirus.* BLV é o agente etiológico da Leucose Enzoótica Bovina (LEB), uma doença infecciosa que impacta a longevidade das vacas e a produção de leite ocasionando perdas econômicas para esta importante cadeia produtiva. Estudos sorológicos fornecem informações valiosas sobre a circulação de BLV em rebanhos leiteiros. Este estudo avaliou o perfil sorológico para a infecção por BLV em rebanhos leiteiros de alta produção na mesorregião Centro-Oriental do estado do Paraná, região sul do Brasil. Amostras de sangue foram coletadas de 628 vacas lactantes em 43 rebanhos no período de outubro de 2019 a janeiro de 2020. As amostras de soro foram armazenadas a -80 °C até o processamento. A avaliação sorológica foi realizada por meio de um sistema de ELISA comercial (IDEXX Leukosis Blocking Antibody Test) que detecta anticorpos para a glicoproteína 51 (gp51) presente no envelope do BLV. Rebanhos foram considerados positivos quando pelo menos uma vaca testou positivo para anticorpos anti-gp51. Os dados obtidos foram distribuídos pelo tipo de manejo (intensivo e semi-intensivo) e número (<100, 100-200 e >200) de vacas lactantes em cada rebanho e submetidos à análise estatística utilizando os testes de qui-quadrado e razão de chances, com intervalo de confiança de 95%. Todos os 43 (100,0%) rebanhos avaliados foram soropositivos para BLV e 10 (23,3%) rebanhos apresentaram 100% de positividade nas vacas avaliadas. Das vacas amostradas, 82% (515/628) foram soropositivas para BLV. Os resultados

da análise estatística em ambos os fatores analisados foram comparados com a soroprevalência encontrada. Rebanhos com 100 a 200 vacas lactantes tiveram maior chance de positividade do que os rebanhos com <100 e >200 vacas lactantes. Rebanhos que utilizavam manejo intensivo das vacas tiveram 1,87 vezes mais chances de infecção por BLV. Os resultados deste estudo sorológico mostram que na mesorregião Centro Oriental do Paraná a infecção por BLV é endêmica. A LEB é uma retrovirose silenciosa na qual a manifestação de sinais clínicos é um evento esporádico. Redução na produtividade animal, com consequente prejuízo econômico, é uma das mais importantes consequências da infecção. Somente com ações que objetivem conscientizar todos os atores da cadeia produtiva do leite sobre a importância da infecção e sobre as formas de transmissão do BLV será possível reduzir as taxas de animais e rebanhos soropositivos em regiões endêmicas.

Palavras-chave: Retrovírus bovino. Vaca leiteira. Leucose enzoótica bovina. Teste ELISA gp 51. Fator de risco à saúde.

Introduction

Bovine leukemia virus (BLV; officially named *Deltaretrovirus bovleu*) is a retrovirus belonging to the family *Retroviridae,* subfamily *Orthoretrovirinae,* and genus *Deltaretrovirus* (ICTV, 2024). It is the etiological agent of enzootic bovine leukosis (EBL), an infectious disease that naturally affects cattle (Bos taurus and B. indicus), yaks, water buffaloes, sheep, and capybaras (Kuczewski et al., 2021; Nishikaku et al., 2022; Olaya-Galán et al., 2022; Selim et al., 2020; WOAH, 2024). BLV infection imposes a significant economic burden on global cattle populations, particularly dairy cattle herds (Rola-Łuszczak et al., 2013; WOAH, 2024). However, several countries, particularly in Asia, Oceania, and Western Europe, are officially BLV-free due to sustained surveillance programs (WOAH, 2024).

To date, no country in the Americas is considered free from BLV, and, in Brazil, EBL is considered endemic, particularly in dairy cattle herds, where the seroconversion rate ranges from 8.9% (59/661) to 81.1% (30/37) (Carneiro et al., 2003; Meirelles et al., 2009). In Brazil, EBL is a notifiable disease that must be reported monthly based exclusively on clinical manifestations, without the need for laboratory confirmation (MAPA, 2013).

BLV infection is often subclinical; approximately 70% of BLV-infected cattle exhibit subclinical infection, which is asymptomatic and easily overlooked (Zhao et al., 2025). Persistent lymphocytosis occurs in 30% of seropositive animals older than three years, with a small proportion (1–5%) possibly developing lymphomas (Mirsky et al., 1996). Clinical manifestations vary according to the location and number of lymphomas and include persistent lymphocytosis, weight loss, decreased milk production, enlarged lymph nodes, weakness or general debility, and neurological manifestations (WOAH, 2024).

BLV may cause immune dysfunction and decreased immune response to vaccination in infected animals (Frie et al., 2016; Puentes et al., 2016). Infected animals may also exhibit increased susceptibility to mastitis, diarrhea, and pneumonia (Emanuelson et al., 1992; Sandev et al., 2004). Additionally, increased culling rates (Benitez et al., 2022) and reduced milk production

(López-Sánchez et al., 2025; Nekouei et al., 2016; Norby et al., 2016; Ott et al., 2003; Yang et al., 2016) and conception (Emanuelson et al., 1992) rates have been reported in BLV-seropositive cattle herds.

The acquisition of seropositive animals is the most common route of BLV introduction into herds. Management practices such as vaccination and medication without changing needles, collection of blood and other biological samples, dehorning, ear-tagging, and pregnancy diagnosis are the most frequent forms of horizontal or intraherd spread of infection (Monti et al., 2005). Vertical transmission occurs via transplacental transmission through contact with maternal blood or secretions during the peripartum period (Moraes et al., 1996) and through the ingestion of colostrum or milk containing the virus during the postpartum period (Ferrer & Piper, 1981; Moraes et al., 1996; Úsuga-Monroy et al., 2021).

The Central-Eastern mesoregion of Paraná State is home to prominent dairy cattle herds, characterized by large volumes, high productivity, and high-quality milk. However, to date, no comprehensive serological surveys have assessed the prevalence of BLV infection in the herds and cows of this region. Therefore, this study aimed to evaluate the serological profile of BLV infection in high-producing dairy cattle of this mesoregion in Paraná, Southern Brazil.

Materials and Methods

Herds and cows

Serum samples were collected in the municipalities of Arapoti, Castro, Carambeí, Palmeira, and Ponta Grossa in Paraná State, southern Brazil. The required number of dairy cattle herds and blood serum samples was determined using the EpiInfo 7.4.2.0 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). The total number of lactating cows in these five municipalities was approximately 63,500, according to data from the 2017 Census of Agriculture (IBGE, 2017). Because no data were available on the prevalence of EBL-seropositive cows in the Central-Eastern mesoregion of Paraná State, the prevalence was assumed to be 50%, and the design effect was set at 1.5, with a 95% confidence interval (CI) (Thrusfield, 2018). The sample size in each municipality, which accounted for approximately 1% of the total number of milked cows, was determined using the proportional allocation method (Thrusfield, 2018). The numbers of herds and cows sampled varied among municipalities. The serum samples analyzed in this study were derived from those previously evaluated by Camilo et al. (2022) (Table S1).

This study evaluated 43 high-yield dairy herds, predominantly of the Holstein breed, with an average milk production of 32.3 L/d per cow, ranging from 25.4 to 42.1 L. The herds represented approximately 3% of dairy herds in the five municipalities and varied significantly in size, ranging from 33 to 3,773 cows and 19 to 1,771 lactating cows.

Between October 2019 and January 2020, blood samples were collected from 628 lactating cows, none of which exhibited clinical signs of EBL. The serum samples were stored at −80 °C until analysis. This study was approved by the Ethics Committee on Animal Use of the Universidade Estadual de Londrina (CEUA; protocol no. 1835.2019.45).

Serological analyses

Serum samples were analyzed using the IDEXX Leukosis Blocking Antibody Test (IDEXX Laboratories, Inc., Montpellier, France). This commercial kit detects antibodies against the gp51 antigen of the BLV envelope. Results are expressed as the percentage of the sample relative to the negative control (S/N%), according to the manufacturer's instructions. Serum samples with S/N% ≤ 40% were classified as positive for BLV antibodies. Herds were considered BLV-positive when at least one animal tested positive.

Statistical analyses

The collected data were analyzed using the chi-square test of independence and odds ratio (OR; 95% CI) to assess the associations between the type of management (intensive and semi-intensive) and number of lactating cows per herd (<100, 100–200, and >200) and the rate of seropositivity for BLV infection. Significant risk association was defined as a CI with a lower limit greater than one. Statistical significance was set at $p = 0.05$, and the results are presented as total numbers and percentages. OR analysis was conducted using an online calculator available at https://www.medcalc.org/calc/odds_ratio.php, according to the manufacturer's instructions.

Results

The total frequencies of BLV-seropositive herds and lactating cows were 100% (43/43) and 82% (515/628), respectively. The seropositivity rate in herds was 30–100%, with all cows testing seropositive in 10 (23.2%) of the 43 herds. The number and percentage of positive cows in each herd are presented in Table S1.

Notably, the highest frequency of BLV seropositivity (84.6%; 391/462) was observed under intensive management, which was significantly higher than the 74.7% (124/166) observed under semi-intensive management (X^2 [1,628] = 8.1659; p = 0.004268). Moreover, OR analysis of the chi-square test results revealed that herds under intensive management were 1.87-times more likely to have BLV-infected cows than those under semi-intensive management (Table 1).

Table 1
Distribution of Bovine leukemia virus seropositive lactating cows in dairy cattle herds from the Central-Eastern mesoregion of Paraná State, Brazil, according to the herd management

Management	Herds (*n*=)	Cows evaluated		OR	p-value
		Total	Positive (%)		
Intensive	30	462	391 (84.6)a	1.87	0.004268
Semi-intensive	13	166	124 (74.7)b		
Total	43	628	515 (82.0)		

[a, b] in the same column, indicate a statistical difference among management types.

Analyses of lactating cows and their seropositivity in relation to the number of BLV-positive animals in each herd revealed that herds with 100–200 lactating cows exhibited a higher chance of having more cows with evidence of contact with BLV than those with <100 (X^2 [1,386] = 18.3377; p = 0.000019) or >200 (X^2 [1,473] = 4.0239; p = 0.044860) lactating cows. Chi-square and OR analyses demonstrated that herds in this category were 3.12 and 1.7-times more likely to have seropositive cows than those with <100 or >200 lactating cows, respectively (Table 2).

Table 2
Distribution of Bovine leukemia virus seropositive lactating cows in dairy cattle herds from the Central-Eastern mesoregion of Paraná State, Brazil, according to the herd size

Number lactating cows	Herds (n=)	Cows evaluated		OR	p-value
		Total	Positive (%)		
<100	12	155	111 (71.6)[a]	3.1254	0,000019
100-200	15	231	205 (88.7)[b]		
>200	16	242	199 (82.2)[a]	1.7037	0,04486
Total	43	628	515 (82.0)		

[a, b] in the same column, indicate a statistical difference in dairy herd size.

Discussion

EBL is a chronic and silent viral infection affecting adult cows (Yang et al., 2016). Owing to its lack of obvious symptoms in most animals, BLV infection is neglected in many regions worldwide (Suárez Archilla et al., 2022). Despite advances in experimental vaccine research (WOAH, 2024), no commercial vaccine is currently available for EBL control. BLV infection is also neglected in Brazil because of the absence of an official control and eradication program.

Paraná is the second-largest milk producer in Brazil; of its 10 mesoregions, the Central-Eastern mesoregion exhibits the highest milk productivity in the country (IBGE, 2024). Dairy cattle herds in the area are characterized by cows with superior genetics that benefit from nutritional and zootechnical management superior to the national average. Most dairy operations in the region use intensive breeding systems, primarily compost barns and free-stall systems. Despite these measures, veterinary assistance can cause iatrogenic transmission via fomites, such as palpation gloves.

From the first serological study conducted in Paraná State in 1983 (Kantek et al., 1983) to this study, the number of herds with seropositive animals increased 2.45 times, and the number of infected cows increased 3.81-fold. Over 37 years, the number of herds with seropositive cows increased by 245%, and the number of infected cows increased by 396% (Table 3). In 1983, 38.5% (94/244)

of cows were seropositive in the Central-Eastern mesoregion (Kantek et al., 1983), which is 2.13-times lower than that observed in this study.

Table 3
Studies evaluating the detection of antibodies against bovine leukemia virus in dairy cattle herds in Paraná State, southern Brazil, including serum samples from cows older than two years

Collection year	Paraná Mesoregion	Dairy herds (n)		Dairy cows (n)		
		Sampled	Positives (%)	Sampled	Positives (%)	Reference
1983	Central-Eastern Metropolitan Northwest North Central West Southeast	184	75 (40.8)	637	137 (21.5)	Kantek et al. (1983)
1994	North Central	6	5 (83.3)	175	45 (25.7)	Carvalho et al. (1996)
2000	North Central	23	23 (100.0)	519	230 (44.3)	Leuzzi Júnior et al. (2003)
2007	Metropolitan	5	not informed	169	118 (69.8)	Barros Filho et al. (2010)
2020	Central-Eastern	43	43 (100.0)	628	515 (82.0)	**This study**

Vertical transmission of BLV occurs when a calf becomes infected during pregnancy in a BLV-positive cow, resulting in the calf being born positive for the virus or becoming positive shortly after birth if it consumes colostrum or milk containing the virus (Moraes et al., 1996; Úsuga-Monroy et al., 2021).

Two risk factors for BLV infection in dairy cattle herds were identified in this study: Management type (intensive) and herd size (100–200 lactating cows). The main modes of BLV transmission in dairy cattle herds may explain the identification of these risk factors.

BLV transmission occurs primarily through the transfer of infected lymphocytes in blood and other biological fluids. Therefore, management conditions that increase the probability of blood transfer between animals play a critical role in viral dissemination. In intensive dairy systems, high animal density and close contact among animals possibly facilitate horizontal viral transmission. Routine management and veterinary procedures, including vaccination, blood sampling, rectal palpation, dehorning, and other interventions, are performed more frequently in these systems. When needles, syringes, or surgical instruments are reused without adequate disinfection, even small

quantities of blood containing infected lymphocytes may be sufficient to transmit the virus. The shared use of equipment capable of causing microlesions further contributes to iatrogenic transmission. Additionally, hematophagous insects may act as mechanical vectors by transferring infected blood between animals (Yang et al., 2016).

Small herds (<100 lactating cows) frequently adopt semi-intensive management systems, which possibly reduces the risk of BLV infection. Although intensive management predominates in large herds (>200 lactating cows), characteristics typical of these production systems may partially contribute to maintaining a relatively stable prevalence of EBL within the herd. Large dairy herds generally have higher culling rates than small and medium-sized herds. Culling is commonly associated with factors such as low milk yield, reproductive disorders, hoof problems, and mastitis, conditions that become more frequent as cows age. Consequently, the replacement rate is higher in large herds than in small or medium-sized herds, where animals tend to remain in production for longer periods. This dynamic possibly contributes to maintaining a relatively stable prevalence of EBL, as older multiparous cows, which are more likely to be BLV-seropositive, are removed more frequently and replaced by younger heifers, which exhibit a higher probability of being BLV-seronegative. These herd dynamics may partly explain why, in the present study, large herds were not identified as a risk factor for BLV infection, in contrast to medium-sized herds.

The implementation of biosecurity measures aimed at interrupting the transmission chain to prevent iatrogenic infections and reduce the risk of BLV transmission (Leuzzi Junior et al., 2004; Monti et al., 2005).

Studies quantifying the direct and indirect economic losses caused by EBL remain scarce. In the USA, BLV infects approximately 40% of the cattle population, causing an approximately $525 million annual economic loss to the dairy industry (Nikbakht Brujeni et al., 2023; Ott et al., 2003). Therefore, awareness should be raised among producers and professionals regarding direct (treatment costs and occasional deaths) and indirect (interference with indicators of production and reproductive efficiency) economic losses in the production chain caused by BLV infection in dairy cattle herds.

EBL prevention measures should be integrated into comprehensive biosecurity programs to progressively reduce the within-herd prevalence of BLV infection. Because of sustained surveillance efforts, several Western European countries are now recognized as free of BLV infection (Polat et al., 2017), supported by official animal health programs dedicated to EBL eradication (EFSA, 2015).

The milk production chain plays a significant socioeconomic role across all regions of Paraná. Although the frequency of BLV infection in dairy cattle herds has been documented at local and regional levels, the prevalence of EBL in dairy cattle herds in Paraná remains unclear. Considering its economic and animal health implications, more comprehensive epidemiological studies are necessary to facilitate the development of effective EBL control and prevention strategies.

The municipalities of Castro and Carambei in the Central-Eastern region of Paraná, where this study was conducted, are the first and second largest milk producers in Brazil, yielding 484 and 293 million liters, respectively, in 2024. In addition to their production quantity, these municipalities, along with Arapoti, have herds of cows exhibiting the highest milk productivity (kg milk/d/cow) in Brazil. To the best of our knowledge, the present study was the first conducted in this dairy region to determine the occurrence and frequency of BLV infection in lactating cows. The results provide important insights into the current state of knowledge regarding this viral infection in dairy cattle herds of the Central-Eastern mesoregion of Paraná State.

Conclusion

EBL is caused by a retrovirus endemic to dairy cattle herds in Paraná State, southern Brazil. This study identified the number of lactating cows in the herd (100–200 lactating cows) and type of management (intensive) as significant risk factors for BLV infection in dairy cattle herds of the Central-Eastern mesoregion of Paraná. The lack of knowledge regarding the direct and indirect economic impacts of BLV infection on the production system has led to its neglect by animal health managers in the milk production chain.

The absence of official control and eradication programs, along with the lack of incentives for producers to voluntarily implement measures to reduce infection risk in dairy cattle herds, has resulted in a high frequency of herds and adult cows being infected with BLV in Paraná State. By gradually adopting both external and internal biosecurity practices to mitigate infection risk, producers can reduce the prevalence of EBL.

Supplementary table

Table S1. Distribution of serum samples from lactating cows (>2 years old) per dairy herd and results of commercial ELISA tests for the detection of anti-gp51 antibodies from Bovine leukemia virus in the Central-Eastern mesoregion of Paraná state, southern Brazil, from 2019 to 2020.

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