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# Serodetection of antibodies against bovine enzootic leukosis virus in confined Holstein cattle herds[1]

Diorges H. Setim[2], Carlos Bondan[3], Márcio M. Costa[4], Bruna L. Braun[5], Brenda L.R. Marcuzzo[5], Ana S. Betto[6], Adriana C. Motta[2,5]*

Diorges Henrique Setim – https://orcid.org/0000-0001-9247-5238
Carlos Bondan – https://orcid.org/0000-0002-4827-2609
Márcio Machado Costa – https://orcid.org/0000-0002-8630-1781
Bruna Letícia Braun – https://orcid.org/0009-0000-6280-6104
Brenda Liz Rozanski Marcuzzo – https://orcid.org/0009-0005-1149-0003
Ana Stela Betto – https://orcid.org/0009-0005-1825-7431
Adriana Costa da Motta – https://orcid.org/0000-0002-3947-0419

**ABSTRACT.-** Setim DH, Bondan C, Costa MM, Braun BL, Marcuzzo BLR, Betto AS, Motta AC. **Serodetection of antibodies against bovine enzootic leukosis virus in confined Holstein cattle herds**. *Pesuisa Veterinária Brasileira 46:e07835, 2026.* Laboratório de Patologia Animal, Hospital Veterinário, Escola de Ciências Agrárias, Inovação e Negócios, Universidade de Passo Fundo, BR-285 Km 292,7, São José, Passo Fundo, RS 99052-900, Brazil. E-mail: acmotta@upf.br

The enzootic bovine leukosis (EBL) virus is the etiological agent of a permanent and incurable infection in cattle. The virus integrates into the host's DNA and replicates continuously, primarily infecting B lymphocytes and negatively affecting the immune response of infected cattle. With oncogenic potential, the virus is responsible for the development of lymphomas in adult cattle; however, neoplasia is uncommon, occurring in approximately 2–5% of infected animals. Most seropositive cattle remain subclinical, which may lead to decreased immunity, particularly when associated with other diseases. This study aimed to estimate herd-level and within-herd seroprevalence of antibodies against EBL in confined dairy cow herds in the northern region of Rio Grande do Sul, Brazil. Four commercial farms using Free Stall or Compost Barn systems were selected by convenience, and all cows older than six months of age were sampled, totaling 750 animals. Serum samples were tested using a commercial blocking enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against EBL virus. Descriptive statistics were used to calculate herd-level and within-herd seroprevalences and their 95% confidence intervals (95% CI). All herds had at least one seropositive animal (herd-level prevalence: 100%). Within-herd seroprevalence varied across the four herds, ranging from 51% in the São Luiz Gonzaga herd to 65.2% in the Salvador das Missões herd, and the overall seroprevalence among all sampled cows was 55.1% (95% CI: 51.2–58.9). These results demonstrate a high level of EBL seropositivity among dairy cows in the four confined herds evaluated in this study in the northern region of Rio Grande do Sul and indicate similarities in management practices and seroprevalence across these farms.



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[2] Graduate Program in Bioexperimentation, Escola de Ciências Agrárias, Inovação e Negócios (ESAN), Universidade de Passo Fundo (UPF), BR-285 Km 292,7, São José, Passo Fundo, RS 99052-900, Brazil. *Corresponding author: acmotta@upf.br

[3] Setor de Grandes Animais, Hospital Veterinário (HV), Escola de Ciências Agrárias, Inovação e Negócios (ESAN), Universidade de Passo Fundo (UPF), BR-285 Km 292,7, São José, Passo Fundo, RS 99052-900, Brazil.

[4] Faculdade de Medicina Veterinária (FAMEV), Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil.

[5] Laboratório de Patologia Animal, Hospital Veterinário (HV), Escola de Ciências Agrárias, Inovação e Negócios (ESAN), Universidade de Passo Fundo (UPF), BR-285 Km 292,7, São José, Passo Fundo, RS 99052-900, Brazil.

[6] Student, Veterinary Medicine Course, Escola de Ciências Agrárias, Inovação e Negócios (ESAN), Universidade de Passo Fundo (UPF), BR-285 Km 292,7, São José, Passo Fundo, RS 99052-900, Brazil.

INDEX TERMS: Epidemiology, enzootic bovine leukosis, dairy cows, antibodies.

**RESUMO.-** [**Sorodetecção de anticorpos contra o vírus da leucose enzoótica bovina em rebanhos de bovinos confinados da raça Holandesa.**] O vírus da leucose enzoótica bovina (LEB) é o agente etiológico de uma infeção permanente e incurável nos bovinos. O vírus integra-se no DNA do hospedeiro e replica-se continuamente, infectando principalmente os linfócitos B e afetando negativamente a resposta imunitária dos animais infectados. Com potencial oncogênico, o vírus é responsável pelo desenvolvimento de linfomas em bovinos adultos; no entanto, a neoplasia é pouco frequente, ocorrendo em aproximadamente 2–5% dos animais infectados. A maioria dos bovinos soropositivos mantém-se assintomática, o que pode levar a uma diminuição da imunidade, sobretudo quando associada a outras doenças. Este estudo teve como objetivo estimar a soroprevalência de anticorpos contra o vírus da LEB em rebanhos leiteiros confinados na região norte do Rio Grande do Sul, Brasil, tanto a nível de rebanho como dentro de cada rebanho. Foram selecionadas quatro fazendas comerciais que utilizam sistemas de confinamento em "Free Stall" ou em "Compost Barn", por conveniência, e foram amostrados todos os bovinos com mais de seis meses de idade, totalizando 750 animais. As amostras de soro foram testadas através de um ensaio de imunoabsorção enzimática (ELISA) de bloqueio comercial para detecção de anticorpos contra o vírus da LEB. Foram utilizadas estatísticas descritivas para calcular a soroprevalência ao nível do rebanho e dentro de cada rebanho, bem como os seus respetivos intervalos de confiança a 95% (IC 95%). Todos os rebanhos apresentaram pelo menos um animal soropositivo (prevalência ao nível do rebanho: 100%). A soroprevalência dentro de cada rebanho variou entre 51% no rebanho de São Luiz Gonzaga e 65,2% no rebanho de Salvador das Missões, e a soroprevalência global entre todas as vacas amostradas foi de 55,1% (IC 95%: 51,2–58,9). Estes resultados demonstram um elevado nível de soropositividade para o vírus da LEB entre vacas leiteiras nos quatro rebanhos confinados avaliados na região norte do Rio Grande do Sul e indicam similaridade de manejo e de soroprevalência entre as fazendas.

TERMOS DE INDEXAÇÃO: Epidemiologia, leucose enzoótica bovina, vacas leiteiras, anticorpos.

## INTRODUCTION

The enzootic bovine leukosis (EBL) virus is a retrovirus that causes a permanent infection in the host. Currently, there is no commercially available vaccine for EBL in Brazil. Therefore, a positive serology indicates that the animal is a carrier (Blagitz et al. 2017). The virus infects a new host through circulating leukocytes, so a small amount of blood is sufficient to transfer the virus from one bovine to another (Sugimoto et al. 1994).

The main forms of transmission are iatrogenic, through the sharing of needles, gloves, and blood transfusions (Alvarez et al. 2013); biological, via hematophagous insects acting as carriers of the virus; and vertical transmission. Once infected, the virus primarily affects B lymphocytes and negatively influences antibody production (Frie et al. 2016). This characteristic of EBL can have an adverse effect on the herd's immunization process, causing seropositive cattle to fail to produce antibodies satisfactorily in response to administered vaccines (Meas et al. 2002, Blagitz et al. 2017).

In a herd with a cow that tests positive for EBL, the incidence of tumor formation, specifically the tumoral form (lymphomas) of EBL, is low, resulting in only 2 to 5% of deaths. In this context, positive cows may not exhibit lymphomas caused by EBL and account for most cattle in the herd. These positive cows might or might not show persistent leukocytosis, which is a nonspecific parameter for diagnosing the disease (Sugimoto et al. 1994, Lo et al. 2021, Pluta et al. 2021).

Due to the increasing technological advancements in dairy cattle farming in Rio Grande do Sul (RS), particularly in the northern region, changes have likely occurred in the epidemiological presentation of EBL, possibly resulting in a rise in the number of positive cattle within the herds, given the scarcity of recent information on the epidemiology of EBL in RS. In this context, the objective of the present study was to estimate the herd-level and within-herd seroprevalence of antibodies against EBL in commercial herds of confined dairy cows in the northern region of Rio Grande do Sul and to describe management practices of these farms.

## MATERIALS AND METHODS

**Ethical approval.** The protocol was approved by the Ethics Committee on the Use of Animals (CEUA) of the "Universidade de Passo Fundo" (UPF), under number 020/2022.

**Population and sampling.** A cross-sectional observational study was conducted to estimate herd-level and within-herd seroprevalence of antibodies against enzootic bovine leukosis virus (BLV) in confined Holstein dairy herds in northern Rio Grande do Sul (RS), Brazil. Four commercial Holstein dairy farms using Free Stall or Compost Barn confinement systems were included. Farms were selected by convenience from the municipalities of Vila Maria, Ibiraiaras, São Luiz Gonzaga, and Salvador das Missões, in the northern region of RS. Inclusion criteria were location in northern RS, use of a confinement system, absence of a previous clinical or laboratory

diagnosis of enzootic bovine leukosis (EBL), only one farm per municipality, and owner's consent to sample animals and collect management data. The study was conducted from April to June 2023.

The four farms were located in distinct municipalities (Vila Maria, Ibiraiaras, São Luiz Gonzaga, and Salvador das Missões), separated by distances ranging from approximately 60 to 380 km (information obtained from road distances between municipalities). These municipalities are part of the northern region of Rio Grande do Sul, where previous serological surveys have reported EBL circulation (Moraes et al. 1996, Frandoloso et al. 2008). However, none of the included herds had a prior clinical or laboratory diagnosis of EBL, and the farms were not selected based on known high-risk status, but solely on convenience and the use of confinement systems.

All selected herds operated under intensive confinement systems (Free Stall or Compost Barn), and no extensive or semi-intensive grazing herds were included. Therefore, the objective of the present study was not to compare different production systems, but rather to describe the detection of EBL specifically in confined Holstein dairy herds under intensive management conditions.

**Sample collection and epidemiological data.** Venous blood samples were collected from all cattle over six months of age on the studied farms. The blood was centrifuged to obtain serum, which was stored in sterile vials and frozen at -18 °C until the day of the enzyme-linked immunosorbent assay (ELISA) test. The ELISA test for diagnosing EBL relies on an ultrapurified viral lysate standardized for detection according to the European standard for serum (E5), with a dilution of 1:100 in negative bovine serum. The test procedures were carried out following the manufacturer's instructions (IDEXX® – Leukosis Blocking Ab). According to the manufacturer's validation data (IDEXX Laboratories, IDEXX Leukosis Blocking Ab Test, technical data sheet), the test sensitivity is reported as 100% and the specificity as 95%. The assay demonstrates excellent sensitivity in well-characterized samples; however, specificity may be slightly lower. As with any antibody-based test, samples collected during the early phase of infection, before effective seroconversion, are expected to yield negative results. This reflects the biological dynamics of antibody production rather than a limitation of the test itself, and should be considered when interpreting serological results.

Information was collected on farm management, including sharing of needles during vaccinations and medication administration, use of artificial insemination or natural mating, glove changes between rectal palpation procedures, provision of colostrum negative for EBL, control of vectors, and testing of animals for EBL. Herds with at least one positive animal were considered positive. Herd-level seroprevalence is treated as a binary (herd-level) outcome, indicating whether at least one seropositive animal is present in the herd or whether no seropositive animals are detected. In contrast, within-herd seroprevalence describes the proportion of seropositive and seronegative animals in the herd, based on the diagnostic tests performed and described in this study.

**Statistical analysis.** Descriptive statistics were used to summarize herd characteristics and management practices. Herd-level seroprevalence was defined as the proportion of farms with at least one seropositive animal among all tested farms. Within-herd seroprevalence was defined as the proportion of seropositive animals among all tested animals within each herd. Overall and herd-specific seroprevalences were calculated along with their respective 95% confidence intervals (95% CI) using the binomial approximation. In addition, the 95% confidence intervals for seroprevalence were calculated under a simple binomial assumption that treats all sampled animals as independent observations. Because animals were clustered within only four herds, these intervals do not account for intra-herd correlation and may underestimate the true uncertainty around the prevalence estimates. Data were organized, and calculations were performed in Microsoft Excel (Microsoft Corporation, Redmond/WA, USA).

## RESULTS

Four farms from different municipalities in the northern region of Rio Grande do Sul were tested, totaling 750 samples from confined Holstein dairy cows. Only one farm was sampled in each municipality. All four farms had at least one seropositive animal for EBL, resulting in a herd-level seroprevalence of 100%. Within-herd seroprevalence varied across the four herds, ranging from 51.0% in the São Luiz Gonzaga herd to 65.2% in the Salvador das Missões herd. The overall seroprevalence among all sampled cows was 55.0% (95% CI: 51.2–58.9). Table 1 shows the municipalities where the farms were located, the confinement system, ELISA test results, and within-herd seroprevalence.

All evaluated farms operated under confinement systems and, therefore, had similar reproductive and sanitary management practices. All used artificial insemination, and none used natural mating for reproduction. Vaccinations and medication administration were performed with shared needles in all herds. The gloves used for rectal palpation were not changed between animal assessments. The farms did not have structured colostrum banks with systematic selection of colostrum from EBL-negative cows. Vector control was implemented, but it did not result in complete elimination of hematophagous insects. None of the farms had previously tested their animals for EBL before this study.

## DISCUSSION

Our results showed that four evaluated farms were positive for EBL, resulting in a herd-level prevalence of 100%. Within-herd seroprevalence ranged from 51% to 65.2%, and the overall prevalence among all sampled cows was 55%. These values are higher than those reported in previous studies conducted in the same state (Moraes et al. 1996, Frandoloso et al. 2008), in which the herd-level prevalence ranged from 33.5% to 38.4% and the within-herd prevalence ranged from 6.9% to 30.75%. Although differences in study design, sampling strategy, and production systems limit direct comparisons, our estimates are higher than those previously reported in dairy herds from Rio Grande do Sul (Moraes et al. 1996, Frandoloso et al. 2008). However, these findings do not allow us to infer a true temporal trend in EBL prevalence in the region.

In this study, we chose to test all animals older than six months of age in each herd to minimize the risk of misclassifying herd infection status that can occur when only a small sample of the population is tested, particularly when disease prevalence is expected to be around 50%. When only a few animals are randomly tested, there is a risk of selecting only negative animals and erroneously inferring that the herd is free of infection. By performing a census within each herd, we reduced this risk and obtained more accurate within-herd prevalence estimates.

The serological survey conducted by Moraes et al. (1996) remains one of the largest studies on EBL in RS. However, it is not clearly reported whether all animals in each herd were sampled or whether only a subset was tested. In addition, the herds probably represented a variety of breeds and management systems, as indicated by the diversity of breeds described in that study. Since then, approximately three decades have passed, and dairy herds in the region have undergone major changes, including increasing adoption of confinement systems and reproductive biotechnologies, which raise animal density and handling frequency, and may consequently increase infection pressure.

In the present study, all herds consisted of confined Holstein cows, which likely represent a higher-risk epidemiological scenario compared with extensive or mixed production systems. The herds shared several management characteristics that are compatible with enhanced EBL transmission: all used artificial insemination and did not use bulls for natural mating, vaccinations and drug administrations were performed with shared needles, rectal palpation gloves were not changed between animals, and there were no structured colostrum banks with systematic selection of colostrum from EBL-negative cows. Although vector control was implemented on the farms, it did not achieve complete elimination of hematophagous insects. These conditions are consistent with previous reports indicating that EBL transmission is strongly associated with iatrogenic procedures (sharing needles, gloves, and instruments), mechanical transmission by biting insects, and vertical transmission (Sugimoto et al. 1994, Meas et al. 2002, Alvarez et al. 2013).

Beyond their epidemiological implications, the management practices observed in the evaluated herds may also affect animal welfare and milk production. High EBL seroprevalence has been associated with impaired immune function, increased susceptibility to other infectious diseases, and potential changes in hematological parameters (Frie et al. 2016, Blagitz et al. 2017), which can contribute indirectly to reduced productive performance and increased culling. In intensive confinement systems, where animals are handled frequently for reproductive and sanitary procedures, inadequate biosecurity (e.g., sharing needles and gloves) may lead to more clinical interventions and a higher burden of chronic infections, thereby compromising welfare. Furthermore, subclinical EBL infection can interact with other production diseases (such as mastitis), potentially affecting milk yield and quality, although these outcomes were not measured in the present study. Future studies should incorporate indicators of animal welfare and milk quality to quantify the broader impacts of EBL and related management practices in intensive dairy herds.

Our sampling frame did not include herds managed under extensive or semi-intensive conditions. Consequently, we were unable to compare EBL seroprevalence across different production systems within this study. The focus was restricted to confined Holstein herds, which likely represent a higher-risk epidemiological scenario due to increased animal density and frequent handling. Future studies including both confined and pasture-based herds are needed to formally assess the impact of production systems on EBL transmission dynamics.

Our analysis was descriptive and did not include inferential statistical tests to quantify the association between specific management practices and serological status for EBL. However, the uniformly high prevalence across the four herds is consistent with the hypothesis that intensive confinement, frequent animal handling, and the use of shared equipment can considerably increase the transmission pressure of EBL.

Animal movement is another important factor in the spread of EBL. In the region studied, buying and selling Holstein heifers and cows is common practice, which facilitates the introduction of infected animals into herds with no prior exposure or low prevalence. The introduction of EBL-positive cattle into herds that do not implement specific biosecurity measures will likely result in rapid virus spread, and a high percentage of the herd may become seropositive in a relatively short period. This scenario is particularly likely in herds that do not

test animals prior to introduction, use shared needles, have limited vector control, and do not manage colostrum based on the donor cow's EBL status (Bartlett et al. 2014, Kuczewski et al. 2021).

All four farms evaluated in this study had a history of using shared needles. After communicating serological results and providing technical guidance on EBL and its control, two farms switched to using individual disposable needles. In addition, one farm that already maintained a colostrum bank began selecting colostrum exclusively from cows that tested negative for EBL. Although the impact of these management changes could not be measured in this cross-sectional study, these interventions are consistent with current recommendations for EBL control and may contribute to reducing herd prevalence over time.

When comparing our results with those of previous research, we observed higher seroprevalence estimates in the confined Holstein herds evaluated here than those reported in earlier surveys of dairy herds in the state. Nevertheless, due to methodological differences between studies (e.g., herd selection, sampling strategy, breeds, and production systems), these comparisons should be interpreted cautiously and cannot be used to demonstrate a significant temporal increase in EBL prevalence.

This study has some limitations that should be considered in the interpretation of the results. Firstly, the rural properties were selected for convenience, and only four confined Holstein herds were evaluated. Thus, the results cannot be generalized to all dairy herds in northern Rio Grande do Sul, particularly those managed under extensive or semi-intensive conditions. Secondly, the cross-sectional design and descriptive nature of the analysis do not allow for causal inference or quantification of the effects of individual risk factors on the transmission of EBL. Thirdly, only serological status was evaluated, without complementary molecular diagnoses or assessment of clinical manifestations and productive impacts, such as milk production, reproductive performance, and culling rates. Finally, we relied on the high sensitivity and specificity reported by the ELISA manufacturer and did not adjust the apparent prevalence for test accuracy.

Despite these limitations, the present study provides updated information on the seroprevalence of EBL in confined Holstein dairy herds in the northern region of RS. It suggests that intensive production systems combined with iatrogenic procedures may play an important role in maintaining high levels of infection. The high prevalence rates observed within and at the herd level highlight the need for systematic control programs for EBL in intensive dairy herds, including routine testing, rigorous biosecurity measures, and targeted management of procedures involving blood and colostrum. Future studies with a larger number and greater diversity of herds, combined with longitudinal follow-up and multivariate analyses, are needed to better characterize the risk factors for EBL transmission and evaluate the effectiveness of specific control strategies under field conditions.

## CONCLUSION

Our study shows a high proportion of enzootic bovine leukosis (EBL)-seropositive cows in the four confined Holstein herds evaluated in the northern region of Rio Grande do Sul. Despite originating from different farms, the management practices used in the herds and the serological results are very similar. The positivity rate for EBL among cows indicates that preventive measures must be applied effectively and continuously in high-producing commercial dairy herds. Future longitudinal studies are needed for a detailed assessment of the disease spread.

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**Data availability statement.-** The data used in this study are available and can be accessed upon request from the corresponding author.

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**Table 1. Description of data obtained from different properties in northern Rio Grande do Sul**

| City | Confinement system | No. positive cows | No. negative cows | No. of cows tested | Total number of cows in the herd | Prevalence (%) | Lower 95% CI (%) | Upper 95% CI (%) |
|---|---|---|---|---|---|---|---|---|
| Vila Maria | Free stall | 21 | 17 | 38 | 44 | 55.2 | 39.4 | 71 |
| Ibiraiaras | Free stall | 84 | 76 | 160 | 220 | 52.5 | 44.7 | 60.2 |
| São Luiz Gonzaga | Compost barn | 188 | 180 | 368 | 384 | 51 | 45.9 | 56.1 |
| Salvador das Missões | Compost barn | 120 | 82 | 184 | 212 | 65.2 | 58.3 | 72.1 |
| TOTAL | | 413 | 355 | 750 | 860 | 55 | 51.5 | 58.6 |

Samples collected from confined dairy cows and tested for enzootic bovine leukosis; CI = confidence interval.