

# A 13-year retrospective study on the serologic diagnosis of bovine leukemia virus in samples from Southern Brazil at the Virology Section, UFSM

## Retrospectiva de 13 anos de diagnóstico sorológico do vírus da leucose bovina em amostras da região Sul do Brasil no Setor de Virologia - UFSM

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### Highlights

This study presents retrospective data on bovine leukemia virus infection in Southern Brazil from 2011 to 2023.

The findings reveal an increasing prevalence of the disease across both individual animals and herds in the region.

The results underscore the significance of continuous diagnostic surveillance and the implementation of control and prevention measures for bovine leukemia virus infection.

### Abstract

Bovine leukemia virus (BLV) is a widespread oncogenic *Deltaretrovirus* responsible for significant economic losses in the global dairy industry and remains endemic in Brazilian cattle. This 13-year retrospective study (2011–2023) reports serologic BLV diagnoses conducted at the Virology Section, Universidade Federal de Santa Maria, Rio Grande do Sul, Brazil. The dataset comprised 11,727 cattle from 1,086 herds (mostly dairy) across 189 counties in three states forming Brazil's second-largest dairy basin: Rio Grande do Sul (RS), Santa Catarina (SC), and Paraná (PR). Samples were tested using

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agar gel immunodiffusion (AGID) or enzyme-linked immunosorbent assay (ELISA). Overall, animal seroprevalence was 38.1% (4,467/11,727) and herd prevalence 64.5% (700/1,086). The three states showed similar animal prevalence rates 39.1% in RS, 37.6% in SC, and 35.9% in PR with herd prevalence of 63.5%, 65.2%, and 67.2%, respectively. Both animal and herd seroprevalence exhibited an upward trend from 2011 (19.6% and 57.5%) to 2023 (60.4% and 73.8%). These findings confirm the continued widespread circulation of BLV in dairy herds across Southern Brazil and highlight the need for official and/or voluntary control measures to mitigate infection prevalence and associated economic losses.

**Key words:** Bovine leukosis. Persistent infection. Serologic diagnostic. ELISA. AGID.

## Resumo

O vírus da leucose bovina (BLV) é um *Deltaretrovirus* oncogênico amplamente difundido, associado a importantes perdas econômicas na pecuária leiteira em todo o mundo e é endêmico nos rebanhos bovinos brasileiros. Apresenta-se aqui um relato retrospectivo de 13 anos (2011-2023) de diagnóstico sorológico de BLV realizado no Setor de Virologia - Universidade Federal de Santa Maria. O estudo incluiu amostras de 11727 bovinos de 1086 rebanhos (principalmente leiteiros) localizados em 189 municípios de três estados que compõem a segunda bacia leiteira do Brasil, Rio Grande do Sul (RS), Santa Catarina (SC) e Paraná (PR). As amostras foram testadas por imunodifusão em ágar-gel (IDGA) ou ensaio imunoenzimático (ELISA). No geral, a soroprevalência animal foi de 38,1% (4467/11727) e a prevalência de rebanho 64,5% (700/1086). Os três estados (RS, SC e PR) apresentaram prevalências de animais semelhantes (39,1%, 37,6% e 35,9%, respectivamente). As prevalências do rebanho foram 63,5% (RS), 65,2% (SC) e 67,2% (PR). No conjunto, o rebanho e a soroprevalência dos animais apresentaram tendência crescente de 2011 (57,5% e 19,6%, respectivamente) a 2023 (73,8% e 60,4%). Esses resultados demonstram que o BLV continua circulando amplamente nos rebanhos bovinos dos três estados do Sul e indicam a necessidade de medidas oficiais e/ou voluntárias para controlar e reduzir a prevalência e as perdas associadas à infecção e doença pelo BLV.

**Palavras-chave:** Leucose bovina. Infecção persistente. Diagnóstico sorológico. ELISA. IDGA.

Bovine leukemia virus (BLV) is a widespread *Deltaretrovirus* of the family *Retroviridae*, species *Deltaretrovirus bovine* (ICTV, 2023), associated with enzootic bovine leukosis, a condition commonly characterized by persistent lymphocytosis and, in some cases, by fatal lymphosarcoma (Polat et al., 2017). Virtually all BLV-infected cattle remain lifelong carriers, and most infections are subclinical (Tsutsui et al., 2016). Evidence indicates that apparently healthy

carriers may exhibit immune dysregulation, decreased milk production (Nekouei et al., 2016), and reproductive failure (Bartlett et al., 2013). Collectively, these effects lead to significant economic losses in the cattle industry (Polat et al., 2017; Nakada et al., 2023). Beyond its importance to animal health, growing evidence suggests possible zoonotic potential, including a putative association with human breast cancer (Schwingel et al., 2019).

Transmission among cattle occurs primarily through iatrogenic procedures, although transplacental, insect-borne, artificial insemination, and ingestion of contaminated colostrum or milk may also contribute to viral dissemination (Choudhury et al., 2015). Diagnosis of BLV infection is typically based on serologic detection using tests such as agar gel immunodiffusion (AGID) and enzyme-linked immunosorbent assay (ELISA) (World Organization for Animal Health, [WOAH], 2018). Control measures involve identifying and isolating or culling seropositive animals, along with implementing biosecurity practices to limit intra- and inter-herd transmission (Ruggiero et al., 2019).

Between the 1990s and 2010s, several studies reported that BLV infection was widely distributed among Brazilian herds, with higher prevalence in dairy cattle (Del Fava & Pituco, 2004; Rodakiewicz et al., 2018). This study presents 13 years of retrospective data on serologic BLV diagnosis performed at the Virology Section, Universidade Federal de

Santa Maria (RS). Serum samples from 11,727 cattle (mostly dairy) across 1,086 herds in 189 counties in three states Rio Grande do Sul (RS), Santa Catarina (SC), and Paraná (PR) were received for diagnostic testing between 2011 and 2023. Samples were analyzed by AGID (Instituto de Tecnologia do Paraná – Tecpar; n = 7,821) or ELISA (IDEXX®; n = 3,906) according to the manufacturers' instructions.

Table 1 presents the results of the tests. Out of 11,727 serum samples, 4,467 (38.1%) tested positive for BLV antibodies. RS accounted for 63.2% of the samples, followed by PR (25.5%) and SC (11.3%). The three states exhibited similar seropositivity rates 39.1% in RS, 37.6% in SC, and 35.9% in PR. Among 1,086 herds, 700 (64.5%) contained at least one seropositive animal. Herd prevalence showed a similar pattern, with slightly higher values in PR (67.2%), followed by SC (65.2%) and RS (63.5%). These findings confirm the wide distribution of BLV infection mainly in dairy herds throughout Southern Brazil.

**Table 1**  
**Detection of antibodies against bovine leukemia virus (BLV) in bovine serum samples by state, 2011–2023 (Virology Section - SV/UFSM)**

State	Sample		Herd	
	Total	Positive: n (%)	Total	Positive: n (%)
RS	7,408	2,895 (39.1%)	693	440 (63.5%)
SC	1,323	497 (37.6%)	201	131 (65.2%)
PR	2,996	1,075 (35.9%)	192	129 (67.2%)
Total	11,727	4,467 (38.1%)	1,086	700 (64.5%)

Nearly two-thirds of the examined herds had at least one seropositive animal, and approximately one-third of all cattle tested were BLV-positive. Because infected cattle remain lifelong carriers and continuous sources of infection, seropositive animals pose a constant threat to susceptible individuals, promoting viral persistence and spread within herds. Under these conditions, BLV becomes endemic across most cattle populations.

Previous serologic surveys from the 1990s to 2010s, based on non-probabilistic sampling, documented BLV prevalence ranging from 9.2% to 32.6% in RS, 35% to 42.1% in SC, and 18.4% to 56.3% in PR (Cordeiro et al., 1994; Del Fava & Pituco,

2004; Barros et al., 2010; Rodakiewicz et al., 2018). In contrast, the present dataset, comprising thousands of samples collected over 13 years from hundreds of herds across numerous municipalities, provides an updated and comprehensive overview of the current epidemiological scenario in Southern Brazil. Moreover, the results demonstrate an increasing prevalence trend over time, suggesting ongoing viral dissemination in the region (Table 2). While many Western European countries have eradicated BLV, the infection remains endemic in Eastern Europe and throughout the Americas (Choudhury et al., 2015; Polat et al., 2017; Porta et al., 2023; Rúa Giraldo et al., 2023).

**Table 2**

**Detection of antibodies against bovine leukemia virus (BLV) in bovine serum samples by year, 2011–2023 (Virology Section - SV/UFSM)**

Year	Animal (n)	Positive n (%)	Herd (n)	Positive n (%)
2011	1,395	274 (19.6%)	87	50 (57.5%)
2012	315	68 (21.6%)	46	25 (54.3%)
2013	1,376	306 (22.2%)	142	69 (48.6%)
2014	1,357	444 (32.7%)	131	86 (65.6%)
2015	865	367 (42.4%)	85	50 (58.8%)
2016	964	182 (18.9%)	76	37 (48.7%)
2017	1,004	388 (38.6%)	94	64 (68.1%)
2018	800	237 (29.6%)	85	63 (74.1%)
2019	1,302	815 (62.6%)	121	91 (75.2%)
2020	631	367 (58.2%)	55	47 (85.5%)
2021	620	405 (65.3%)	48	39 (81.3%)
2022	527	269 (51%)	51	31 (60.8%)
2023	571	345 (60.4%)	65	48 (73.8%)
Total	11,727	4,467 (38.1%)	1,086	700 (64.5%)

A few submissions lacked information about the production type, but it is estimated that more than 90 percent were from dairy operations. Therefore, the analyzed samples largely represent dairy cattle distributed across western Paraná, Santa Catarina, and Rio Grande do Sul. This continuous area constitutes Brazil's second-largest dairy macro-basin. Historically, BLV infection has been more prevalent in dairy herds, where it causes the greatest economic losses, including decreased milk yield and prolonged intervals between calvings and lactations (Polat et al., 2017).

In addition to factors that favor the high frequency of infection in dairy herds such as greater animal density, intensive management, multiple opportunities for viral transmission, and the long productive lifespan of dairy cows BLV dissemination in Southern Brazil has been strongly influenced by large-scale importation of dairy heifers and cows from Uruguay and Argentina during the 1980s and 1990s. At that time, few or no sanitary restrictions concerning BLV were imposed on imported animals. Furthermore, limited awareness of the disease, its mostly subclinical presentation, and the absence of official or voluntary control programs contributed to the progressive spread of infection in the region (Del Fava & Pituco, 2004).

Our findings reveal a broader distribution and higher prevalence of BLV compared with most previous reports. Herds with elevated prevalence may reflect high technological intensification, since management practices such as rectal palpation, vaccination, and other iatrogenic procedures facilitate viral transmission. These factors, combined with historical

and structural aspects of regional dairy production, likely account for the extensive spread and high prevalence observed in this study. Meanwhile, in 2017, 5,950 samples from beef calves destined for export from Rio Grande do Sul were tested, revealing a seroprevalence of only 0.12 percent (7/5,950).

Because no official control or eradication programs currently exist, voluntary individual or collective initiatives could help prevent further dissemination and gradually reduce BLV prevalence in the region. Actions such as certification of BLV-free herds, animals, and dairy products, along with mandatory negative testing for animal trade and participation in official fairs, may support progress toward this goal. Strengthening diagnostic capacity would also be beneficial. In this regard, sanitary requirements for live-cattle export markets have already placed some pressure on herd health status in certain Brazilian states. However, these regulations currently apply only to beef operations and may, in the future, encourage producers and authorities to implement control strategies for dairy herds as well.

This report provides an updated overview of BLV infection in a representative portion of the Southern Brazilian dairy basin. A limitation of this study is that samples were obtained from routine diagnostic submissions and therefore represent only part of the cattle population, which may not fully reflect regional prevalence. Moreover, some farms may have submitted samples at multiple time points, potentially inflating the number of positive results per herd. Compared with earlier long-term studies, these results indicate no improvement in

the sanitary status regarding BLV. Without systematic and coordinated control efforts, BLV infection is expected to continue spreading and increasing in prevalence among cattle in the region.

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## Declaration of Conflicts of Interest

The authors declare no conflicts of interest. The funding agencies had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## References

- Barros, I. R., F<sup>o</sup>., Guimarães, A. K., Biondo, A. W., Krüger, E. R., Wammes, E. V., Ollhoff, R. D., Piekarz, C. H., & Sponchiado, D. (2010). Soroprevalência da Leucose enzoótica em bovinos leiteiros criados na região metropolitana de Curitiba - Paraná. *Arquivos do Instituto Biológico*, 77(3), 512-520. doi: 10.1590/1808-1657v77p5112010
- Bartlett, P. C., Norby, B., Byrem, T. M., Parmelee, A., Ledergerber, J. T., & Erskine, R. J. (2013). Bovine leukemia virus and cow longevity in Michigan dairy herds. *Journal of Dairy Science*, 96(3), 1591-1597. doi: 10.3168/JDS.2012-5930
- Choudhury, B., Finnegan, C., Phillips, A., Horigan, M., Pollard, T., & Steinbach, F. (2015). Detection of bovine leukaemia virus antibodies and proviral DNA in colostrum replacers. *Transboundary and Emerging Diseases*, 62(5), 60-61. doi: 10.1111/TBED.12182
- Cordeiro, J. L. F., Deschamps, F. C., Martins, E., & Martins, V. M. V. (1994). Identificação e controle da leucose enzoótica bovina (LEB) em um rebanho leiteiro. *Pesquisa Agropecuária Brasileira*, 29(8), 1292-1296. doi: 10.1590/S0100-204X1994000800009
- Del Fava, C., & Pituco, E. M. (2004). Infecção pelo vírus da leucemia bovina (BLV) no Brasil. *Instituto Biológico*, 66(1/2), 1-8. [http://www.biologico.sp.gov.br/uploads/docs/bio/v66\\_1\\_2/fava.pdf](http://www.biologico.sp.gov.br/uploads/docs/bio/v66_1_2/fava.pdf)
- International Committee on Taxonomy of Viruses (2023). *Virus taxonomy: 2023 release*. ICTV. <https://talk.ictvonline.org/taxonomy>
- Nakada, S., Fujimoto, K., Kohara, J., & Makita, K. (2023). Economic losses associated with mastitis due to bovine leukemia virus infection. *Journal of Dairy Science*, 106(1), 576-588. doi: 10.3168/jds.2021-21722
- Nekouei, O., VanLeeuwen, J., Stryhn, H., Kelton, D., & Keefe, G. (2016). Lifetime effects of infection with bovine leukemia virus on longevity and milk production of dairy cows. *Preventive Veterinary Medicine*, 133(C), 1-9. doi: 10.1016/j.prevetmed.2016.09.011



- Polat, M., Takeshima, S. N., & Aida, Y. (2017). Epidemiology and genetic diversity of bovine leukemia virus. *Virology Journal*, 14(1), Article 209. doi: 10.1186/s12985-017-0876-4
- Porta, N. G., Suarez-Archilla, G., Miotti, C., Molineri, A. I., Alvarez, I., Trono, K., Signorini, M., & Ruiz, V. (2023). Seroprevalence and risk factors associated with bovine Leukemia virus infection in argentine beef cattle. *Research in Veterinary Science*, 164(5), 104999. doi: 10.1016/j.rvsc.2023.104999
- Rodakiewicz, S. M., Fernandez, M. L., Munhoz, M. L., Yamakawa, F. H. S., Urío, M., Forell, F., Ferraz, S., Portes, V. M. P., & Costa, U. M. D. (2018). Heterogeneity determination of bovine leukemia virus genome in Santa Catarina state, Brazil. *Instituto Biológico*, 85(1), 1-7. doi: 10.1590/1808-1657000742016
- Rúa Giraldo, C. C., López Herrera, A., & Ruiz-Cortés, T. (2023). Bovine leukosis virus, bovine viral diarrhea, and bovine neosporosis seroprevalence in specialized dairy herds in Antioquia-Colombia. *Tropical Animal Health and Production*, 55(5), 294. doi: 10.1007/s11250-023-03685-2
- Ruggiero, V. J., Norby, B., Benitez, O. J., Hutchinson, H., Sporer, K. R. B., Droscha, C., Swenson, C. L., & Bartlett, P. C. (2019). Controlling bovine leukemia virus in dairy herds by identifying and removing cows with the highest proviral load and lymphocyte counts. *Journal of Dairy Science*, 102(10), 9165-9175. doi: 10.3168/jds.2018-16186
- Schwingel, D., Andreolla, A. P., Erpen, L. M. S., Frandoloso, R. & Kreutz, L. C. (2019). Bovine leukemia virus DNA associated with breast cancer in women from South Brazil. *Scientific Reports*, 9(1), 1-7. doi: 10.1038/s41598-019-39834-7
- Tsutsui, T., Kobayashi, S., Hayama, Y., & Yamamoto, T. (2016). Fraction of bovine leukemia virus-infected dairy cattle developing enzootic bovine leukosis. *Preventive Veterinary Medicine*, 124, 96-101. doi: 10.1016/j.prevetmed.2015.11.019
- World Organization for Animal Health (2018). *Manual of diagnostic tests and vaccines for terrestrial Animals*. [https://www.woah.org/fileadmin/Home/eng/Health\\_standards/tahm/A\\_summry.htm](https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/A_summry.htm).

