Acta Scientiae Veterinariae, 2023. 51: 1922.

RESEARCH ARTICLE
Pub. 1922

ISSN 1679-9216

Natural Association between Bovine Leukemia Virus and Reproductive Infectious Diseases

Laureana De Brun[1], Marcos Mionetto[1], Federico Rodríguez[1], Scott Brandl[1], Silvia Hubner[2], Geferson Fischer[2] & Rodrigo Puentes[1]

ABSTRACT

***Background*:** Enzootic bovine leukosis (EBL) is a widespread infectious disease caused by the bovine leukemia virus (BLV), which results in immune system dysfunction. The resulting immunosuppression may lead to an increased prevalence of other diseases. Dairy cows infected have altered immune function associated with decreased milk production and shortened lifespan and decreased immune response to immunization. BLV infection, however, is often asymptomatic, so any connection between subclinical infection and common reproductive diseases remains unknown. This study aimed to describe the relationship between naturally occurring subclinical BLV and infectious reproductive diseases seroconversion in the field.

***Materials, Methods & Results*:** The diseases investigated included Bovine viral diarrhoea (BVD), Bovine alfaherpesvirus 1 (BoHV-1), Bovine gammaherpesvirus 4 (BoHV-4), Chlamydiosis, Leptospirosis, Brucellosis and Neosporosis in dairy cattle. Six hundred fifty-five sera samples from the northern and south-central regions of Uruguay, from asymptomatic female Holstein and Holstein crosses without a history of vaccination against reproductive diseases were processed using reference diagnostic methods (Seronautalization, ELISA, MAT, Rose Bengal Plate test). The seroprevalence of BLV was 20.0%. Seroprevalence of reproductive diseases BVD, BoHV-1 and BoHV-4 were 99.3%, 41.2% and 27.3% of the populations, respectively, and the total seroprevalence of Leptospirosis, bovine *Neospora caninum* and Chlamydiosis were 19.8%, 29.8% and 33.0% respectively. The results revealed positive associations between naturally contracted BLV and the presence of antibodies against BoHV-1 ($P = 0.002$), as well as between naturally contracted BLV and presence of antibodies against *Leptospira* spp. ($P = 0.028$).

***Discussion*:** BLV infection can impact innate and adaptive immune system cells and alter the proper functioning of uninfected cells. BLV infection may also induce changes in the complex balance of cytokine expression, cell proliferation, and programmed cell death in T- and B-lymphocytes, which is critical for immune competence and effective response to infectious challenges. The progression of BLV infection has a substantial effect on host defense mechanisms. Indeed, low-magnitude serologic responses to a commercial foot-and-mouth disease vaccine and a J5 *Escherichia coli* vaccine have been observed. These results are supported by recent trial studies showing a reduced immune response to vaccination against BoHV-1 and *Leptospira* spp. in asymptomatic animals infected with BLV. These are 2 of the most prevalent infectious reproductive diseases in cattle worldwide, and our results provide evidence that a link between BLV and susceptibility to these diseases may exist. Although there is evidence of the co-occurrence of these diseases, it remains unknown whether there is a direct or indirect effect of BLV on infertility, embryonic loss, or abortion. Another possibility is that natural infection with these reproductive pathogens (BoHV-1, *Leptospira*, or others) promotes BLV expression, negatively affecting the farms where these pathogens are endemic. Considering the high seroprevalence of BLV in dairy herds in North and South America where the infection is endemic, it was explored BLV's role as an immunosuppressant by quantifying its co-occurrence with diseases that affect reproductive performance in breeding herds. Future work should clarify the role of BLV and the co-occurring pathogens in causing infertility or abortions.

Keywords: BLV, herpesvirus, Infectious Bovine Rhinotracheitis (IBR), Leptospirosis, reproductive performance.

DOI: 10.22456/1679-9216.129251

Received: 4 March 2023 Accepted: 15 July 2023 Published: 17 August 2023

[1]Departamento de Patobiología, Facultad de Veterinaria (Fvet), Universidad de la República (UdelaR), Montevideo, Uruguay. [2]Laboratorio de Virología e Inmunología, Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil. CORRESPONDENCE: L. Brun [laureanadebrun@gmail.com]. Departamento de Patobiología, Fvet - UdelaR. Ruta 8 Km 18. Montevideo, Uruguay.

INTRODUCTION

Bovine leukemia virus (BLV) is a δ-retrovirus with a worldwide distribution that commonly infects B cells [7] . Cows with the infection often do not express clinical symptoms in cases referred to as subclinical or aleukemic and persistent lymphocytosis [3] .

These immune-level responses to BLV often cause dysfunction or detectable alterations during the generation of an immune response against another pathogen, or against an immunization with commercial vaccines [4,5,9] . However, there have been few studies conducted in the field that investigate the natural immune response produced against these or other pathogens when cattle have a subclinical BLV infection, and the resulting practical importance to animal health remains unknown.

Standout highlight several common infectious and parasitic diseases that affect reproduction in cattle, including Neosporosis, Bovine viral diarrhoea (BVD), Bovine alfaherpesvirus 1 (BoHV-1), Bovine gammaherpesvirus 4 (BoHV-4), Chlamydiosis, Leptospirosis and Brucellosis. These diseases are responsible for reproductive losses which are a major economic concern worldwide. If antibodies are present for these diseases in unvaccinated animals, it indicates prior exposure to the pathogen. In this way, it could be used to associate BLV infection with reproductive, productive or health parameters [4,5,9,11] .

The objective of this study was to associate subclinical BLV with the presence of antibodies against common bovine diseases to demonstrate the possible effects of BLV on disease susceptibility. If the prevalence of these diseases is higher in cows with subclinical BLV, it will demonstrate the effects of BLV on animal health.

MATERIALS AND METHODS

Animal sampling

A total of 655 serum samples from asymptomatic female Holstein and Holstein crosses without a history of reproductive vaccination were used in this study. Samples representing cows in production with an average parity of 2 to 4 calvings, originating from the northern and south-central regions of Uruguay in the departments of Florida, Durazno and Tacuarembó were selected. All samples were taken from the serum bank at the Faculty of Veterinary - University of the Republic - Uruguay.

ELISA for detection of antibodies against BLV

It was diagnosed BLV by detecting anti--gp51 antibodies with a commercial ELISA test[1] (VMRD). The samples were processed according to the manufacturer's instructions and the readings were made at 620 nm in a visible range spectrophotometer[2].

Virus neutralization (VN) test for BoHV-1 and BVD virus diagnosis

Madin-Darby bovine kidney (MDBK, originally ATCC- CCL22) cells were used to propagate Bovine herpesvirus 1 (Los Angeles strain) and BVD virus (NADL strain) as challenge viruses in the neutralization assays [12]. Neutralizing antibodies to BoHV-1 and virus BVD were assayed in a 24-h virus/serum incubation period, as recommended for international trade. The test results were expressed as the reciprocal of the dilution of serum that neutralized the virus in 50% of the wells [12]. Samples with an antibody titer equal to or greater than 1/16 were considered positive [2].

Indirect ELISA for reproductive pathogens antibody detection

It was used a commercially available indirect ELISA for BoHV-4[3], *Neospora caninum*[4] and *Chlamydophila abortus*[4] (IDEXX). The tests were performed according to the manufacturer's instructions and results were read at an optical density of 450 nm in a spectrophotometer.

Rose Bengal Plate test (*RBPT*) *for Brucellosis*

RBPT was performed according to previous studies [1]. The RBPT antigen[5] was obtained from Virbac laboratories. An equal volume of serum sample and antigen (0.03 mL each) were placed on the slide and mixed thoroughly. The appearance of definite clumping/agglutination within 3 min was considered a positive reaction while no clumping/agglutination was considered negative.

Microscopic agglutination test (MAT) for Leptospira spp. *Diagnostic*

The MAT was conducted with live antigens, as recommended by the World Organisation for Animal Health [13]. Briefly, serum samples were screened at a 1:100 dilution and a collection of 12 serovars was employed as antigens (*Leptospira interrogans* serovar: Ballum, Bratislava, Canicola, Grippotyphosa, Hardjo, Hardjobovis, Hebdomadis, Copenhageni, Pomona, Pyrogenes, Taras-

sovi, Wolfi), to determine the adequate antigen battery for each animal. All samples with agglutinating activity at dilution of 1:200 were considered positive.

Statistical analysis

The sample population was divided into BLV positive and control (BLV negative) animals based on the results of the BLV ELISA test. Disease states were classified as categorical variables. A Chi square test was applied (STATA v14) between each disease and BLV infection individually, to determine the likelihood of obtaining the results by chance. Results were reported as significant at $P = 0.05$.

RESULTS

The seroprevalence of BLV was 20.0% across the 3 sampling locations (131/655). Seroprevalence of reproductive BVD, BoHV-1 and BoHV-4 were 99.3%, 41.2% and 27.3% of the populations, respectively, and the total seroprevalence of Leptospirosis was 19.8%. Many of the animals that tested positive to *Leptospira* spp. had antibodies against more than 1 serovar. Those serovars in decreasing order were Tarassovi 76.9% (100/130), Hardjo 71.5% (93/130), Hardjo bovis 56.9% (74/130), Hebdomadis 30.7% (40/130), Wolfi 16.1% (21/130), Icterohaemorrhagiae 11.5% (15/130), Bratislava 3.85% (5/130), Pyrogens 3.85% (5/130), Pomona 3.08% (4/130), Canicola 3.08% (4/130), Grippotyphosa 0.769% (1/130) and 0% Ballum (0/130).

The seroprevalence of bovine *Neospora caninum* and Chlamydiosis were 29.8% and 33.0%, respectively. Finally, no animals were seropositive for bovine brucellosis. A summary of the results for each disease for all samples can be seen in Table 1.

Antibody detection revealed the co-occurrence of BLV with 2 of the diseases investigated. A positive association was found between BLV and BoHV-1 ($P = 0.002$) and BLV and Leptospirosis ($P = 0.028$). No differences were found when comparing the seropositivity of BLV versus the each serovars of *Leptospira* spp.

The association was not significant for Neosporosis, Chlamydiosis and BoHV-4, and the association could not be determined for BVD virus and brucellosis because 99% of the animals were seropositive for BVD virus and 100% were seronegative for brucellosis by RBPT.

DISCUSSION

In this study, was sought to determine if there is a direct relationship between BLV infection and seropositive tests for the most common infectious reproductive diseases around the world. This is the first study that explores the relationship between BLV in unvaccinated cattle that were exposed to productive and/or reproductive pathogens in the natural environment.

The results showed a positive association between BLV infection and the presence of antibodies against BoHV-1 ($P = 0.002$) and *Leptospira* spp. ($P = 0.026$). These are 2 of the most prevalent infectious reproductive diseases in cattle around the world and the results provide evidence that a link may exist between BLV and susceptibility to these diseases. The direct and indirect effect of asymptomatic BLV infection should be

Table 1. Serological results for each BLV positive and control groups.

	BLV+	Prevalence BLV+	Total analyzed BLV+	Control (BLV-)	Prevalence Control (BLV-)	Total analyzed BLV-	Significance *P*	Total
BVD	129	98.5%	131	522	99.6%	524		655
BoHV-1	75	57.3%	131	221	42.2%	524	*0.002	655
Chlamydiosis	41	38.7%	106	102	36.8%	280		386
Neosporosis	40	32.8%	122	90	28.7%	314		436
BoHV-4	19	22.4%	85	130	28.3%	460		545
BLV	131	100%	131	524	100%	524		655
Leptospirosis	35	26.7%	131	95	18.1%	524	*0.028	655
Brucellosis	0	0%	131	0	0%	524		655

*Statistical difference ≤ 0.05. BLV+: Positive Bovine leukemia virus; BLV-: Negative Bovine leukemia virus; BVD: Bovine viral diarrhea; BoHV-1: Bovine herpesvirus-1; BoHV-4: Bovine herpesvirus-4.

further verified because the co-occurrence demonstrated here may be an indication that this virus itself may be interfering with reproductive performance in breeding herds. Another possibility is that natural infection with these reproductive pathogens (BoHV-1, *Leptospira*, or others) promote BLV expression, representing a negative effect in the farms where these pathogens are endemic.

Although evidence of the co-occurrence of these diseases, it remains unknown whether there is a direct or indirect effect of BLV on infertility, embryonic loss or abortion. Previous studies, have reported that BLV-seropositive cows had a 7% lower rate of conception compared to seronegative cows [11]. Similarly, in a previous experiment was found a 26% higher conception rate in heifers free of antibodies against BLV [8]. It was no possible to demonstrate whether the effect on reproductive performance was a direct result of the virus on pregnancy or whether it favored the infection with some specific reproductive pathogen. This experiment shows a positive association between BLV and reproductive pathogens, which is in agreement with the results obtained previously and reinforces the hypothesis that there may be an indirect effect of BLV on reproduction.

The effect of BLV infection on the immune response of asymptomatic animals has been studied both *in vitro* and *in vivo*, analyzing both the cellular and humoral response. Changes observed in the innate or adaptive immune response of BLV infected cattle is more pronounced in animals with persistent lymphocytosis, where a relative increase in the population of B lymphocytes to the detriment of different T lymphocyte subpopulations has been shown [6]. It was recently demonstrated using a commercial vaccine that BLV-positive cows produce significantly lower IgM and IgG2 titers against BoHV-1, *L*. Hardjo, and *L*. Pomona [5]it is essential to understand the circumstances by which BLV negatively affects the immune system of infected cattle. To address this question, BLV- and BLV+ adult, lactating Holstein dairy cows were vaccinated with Bovi-Shield GOLD® FP® 5 L5 HB and their immune response to vaccination was measured over the course of 28 days. On day 0 prior to vaccination and days 7, 14 and 28 post-vaccination, fresh PBMCs were characterized for T and B cell ratios in the periphery. Plasma was collected to measure titers of IgM, IgG1 and IgG2 produced against bovine herpesvirus 1 (BHV1. They observed no difference in CD4+ or CD8+ T cell activation in BLV-positive cows, however MHC-II expression in B lymphocytes from positive cows was reduced. It has also been shown that the immune response to pathogens in animals carrying BLV produces less IgG2 against *E. coli* [4], as well as IgM and IgG1 against the foot-and-mouth disease virus [9]. Collectively, these references demonstrate that BLV-positive cows respond differently to vaccination for these pathogens.

It may, however, be possible that reproductive pathogens favor BLV infection and proliferation as shown in previous studies. Studies suggested that macrophage-derived COX-2 products, such as PGE2, regulate virus expression and disease progression in BLV infection, indicating that PGE2 may stimulate BLV tax and pol mRNA levels and may also stimulate BLV expression from infected B cells while the COX-2 inhibitor, NS-398, inhibited the quantity of BLV mRNA detected. Furthermore, they showed that BoHV-1 and *Brucella abortus*, 2 common opportunistic infections in cattle, activated COX-2 mRNA expression [10]. These results observed *in vitro* could provide insight into the positive association between BLV and BoHV-1 found by natural exposure to both pathogens in this study.

CONCLUSION

In conclusion, it was found a natural association between BLV and 2 important reproductive diseases in cattle. This complements previous evidence that BLV can interfere with the optimal productive performance of infected cattle or that certain infectious diseases favor BLV expression.

MANUFACTURERS

[1]VMRD Inc. Pullman, WA, USA.
[2]Thermo Fisher Scientific Inc. Waltham, MA, USA.
[3]Bio-X Diagnostics. Rochefort, Belgium.
[4]IDEXX Laboratories Inc. Eau Claire, WI, USA.
[5]Virbac Uruguay. Montevideo, Uruguay.

***Acknowledgments*.** We thank the veterinarians Dr. Agustin Furtado for the data provided with respect to farmers in the study.

***Funding*.** This work was supported by the authors' previous project (FSSA_X_2014_1_105283). Financial support was provided by the Agencia Nacional de Investigación e Innovación (ANII).

***Declaration of interest*.** The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. The authors alone are responsible for the content and writing of paper.

REFERENCES

1 **Alton G., Jones L. & Pietz D.E. 1975.** Serological methods In: *Laboratory Techniques in Brucellosis*. 2nd edn. Geneva: World Health Organization, pp.64-124.

2 **Anziliero D., Martins M. Weiss M., Monteiro F.L., Ataide C.F., Weiblen R. & Flores E.F. 2014.** Resposta sorológica aos herpesvirus bovino tipos 1 e 5 e vírus da diarreia viral bovina induzida por vacinas comerciais. *Ciência Rural*. 45(1): 58-63. DOI: 10.1590/0103-8478cr20130167

3 **Bartlett P.C., Sordillo L.M., Byrem T.M., Norby B., Grooms D.L., Swenson C.L., Zalucha J. & Erskine R.J. 2014.** Options for the control of bovine leukemia virus in dairy cattle. *Journal of the American Veterinary Medical Association*. 244(8): 914-922. DOI: 10.2460/javma.244.8.914

4 **Erskine R.J., Bartlett P.C., Sabo K.M. &Sordillo L.M. 2011.** Bovine leukemia virus infection in dairy cattle: Effect on serological response to immunization against J5 *Escherichia col*i Bacterin. *Veterinary Medicine International*. 2011: 915747. DOI: 10.4061/2011/915747

5 **Frie M.C., Sporer K.R., Wallace J.C., Maes R.K., Sordillo L.M., Bartlett P.C. & Coussens P.M.** 2016. Reduced humoral immunity and atypical cell-mediated immunity in response to vaccination in cows naturally infected with bovine leukemia virus. *Veterinary Immunology and Immunopathology*. 182: 125-135. DOI 10.1016/j.vetimm.2016.10.013

6 **Kabeya H., Ohashi K. & Onuma M. 2001.** Host Immune Responses in the Course of Bovine Leukemia Virus Infection. *Journal of Veterinary Medical Science*. 63(7): 703-708.

7 **Meirom R., Moss S. & Brenner J. 1997.** Bovine leukemia virus-gp51 antigen expression is associated with CD5 and IgM markers on infected lymphocytes. *Veterinary Immunology and Immunopathology*. 59(1-2): 113-119. DOI: 10.1292/jvms.63.703

8 **Puentes R., De Brun L., Algorta A., Álvarez J.P., Sacco G., Oliveira M. & Llambi S. 2016.** Horizontal Transmission Dynamics of Bovine Leukemia Virus (Blv) and Negative Effect on Reproductive Performance in Naturally Infected Holstein Heifers. *Science And Animal Health*. 4(3): 294. DOI:10.15210/SAH.V4I3.8295

9 **Puentes R., De Brun L., Algorta A., Da Silva V., Mansilla F., Sacco G., Llambí S. & Capozzo A.V. 2016.** Evaluation of serological response to foot-and-mouth disease vaccination in BLV infected cows. *BMC Veterinary Research*. 12(1): 196. DOI: 10.1186/s12917-016-0749-x

10 **Pyeon D., Diaz F.J. & Splitter G.A. 2000.** Prostaglandin E2 Increases Bovine Leukemia Virustax and pol mRNA Levels via Cyclooxygenase 2: Regulation by Interleukin-2, Interleukin-10, and Bovine Leukemia Virus. *Journal of Virology*. 74(12): 5740-5745. DOI: 10.1128/jvi.74.12.5740-5745.2000

11 **Vanleeuwen J.A., Haddad J.P., Dohoo I.R., Keefe G.P., Tiwari A. & Tremblay R. 2010.** Associations between reproductive performance and seropositivity for bovine leukemia virus, bovine viral-diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum* in Canadian dairy cows. *Preventive Veterinary Medicine*. 94(1-2): 54-64. DOI: 10.1016/j.prevetmed.2009.11.012

12 **World Organization for Animal Health (OIE). 2018.** Diarrea viral bovina. In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Chap. 2.4.8. Paris: OIE, pp.1-22. https://www.woah.org/fileadmin/Home/esp/Health_standards/tahm/3.04.07_BVD.pdf

13 **World Organization for Animal Health (OIE). 2021.** *Leptospirosis: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Chap. 3.1.12 Paris: OIE, pp.1-14. https://www.woah.org/fileadmin/Home/esp/Health_standards/tahm/3.01.12_Leptospirosis.pdf