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Seroprevalence and risk factors associated with Bovine Leukemia Virus infection in Argentine beef cattle

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Abstract

Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukosis, an endemic disease in dairy cattle of Argentina. However, little is known about the seroprevalence of BLV in beef cattle. In this study, we conducted a cross-sectional study including farms from thirteen provinces of Argentina. A total of 5827 bovine serum samples were collected from 76 farms and analyzed using an in-house developed enzyme-linked immunosorbent assay. Information about herd management was collected through a questionnaire, and univariate and multivariate analyses were performed to detect risk factors associated with BLV infection. Herd-level seroprevalence was 71.05%, while the mean animal-level seroprevalence was 7.23% (median=2.69%; min=0, max=75). Only two provinces had no positive BLV samples. The other eleven provinces showed more than 50% of their farms infected with BLV. The multivariate model revealed that BLV prevalence was significantly associated with the use of animals raised in the same farm for cattle replacement (P=0.005), breeding cows by natural mating with a bull (P<0.001), and weaning calves after 6 months of age (P=0.011). This extensive study revealed that BLV seroprevalence in Argentine beef farms has increased during the last years and allowed identifying some management practices associated with BLV prevalence. These data deserve special attention because BLV infection in beef cattle seems to lead to a dissemination pattern similar to that observed during the last decades in dairy cattle, especially considering that Argentina is the sixth beef producer in the world, with about 5% of global beef production.

Keywords

Leucosis; Beef cattle; Elisa; Prevalence; Herd management; Retrovirus

Introduction

Bovine leukemia virus (BLV), a retrovirus of the genus *Deltaretrovirus*, is the causative agent of enzootic bovine leucosis (Aida et al., 2013). Although the predominant natural hosts for BLV are cattle, other species such as water buffalo, zebu, yak and alpaca can also become naturally infected (Chaves et al., 2012; Lee et al., 2012; Ma et al., 2016; Meas et al., 2000; Molnár et al., 2000; Olaya-Galán et al., 2022; Selim et al., 2020). The disease is widely disseminated throughout the world, with widely different values of herd and animal-level prevalence. However, in New Zealand and many Western European countries, the disease has been eradicated (Acaite et al., 2007; Maresca et al., 2015; Nuotio et al., 2003; Voges, 2009). In North, Central and South America, dairy cattle present high levels of BLV prevalence. In Argentina, between 80 and 99% of dairy farms are infected with BLV (Gutiérrez et al., 2020; Polat et al., 2016; Trono et al., 2001), and animal-level prevalence is as high as 77-90% (Gutiérrez et al., 2012; Monti et al., 2005; Polat et al., 2016).

BLV can infect different cells of the immune system but has preferential tropism for B-lymphocytes (Aida et al., 2013; Rodríguez et al., 2011). Infected animals are mostly asymptomatic carriers of the virus, but approximately 20-30% develop persistent lymphocytosis (a non-malignant proliferation of untransformed B-lymphocytes) (Alvarez et al., 2013; Barez et al., 2015). Up to 10% of BLV-infected animals develop fatal B-cell lymphoma, mostly affecting adult cattle (1-8 years old) (Burny et al., 1988). BLV transmission depends on the transfer of an infected cell carrying a replication-competent provirus. Generally, the main means of BLV spread is horizontal transmission through direct and indirect (iatrogenic) contact with biological fluids. Iatrogenic procedures such as blood extraction, vaccination, castration, dehorning, rectal palpation, tattooing and insemination have been identified as major routes of transmission (Hopkins and DiGiacomo, 1997; Lassauzet et al., 1990), and animals with high levels of proviral

load represent a higher risk for this transmission (Buxton and Schultz, 1984; Mammerickx et al., 1987). BLV can also be transmitted vertically, through perinatal infection (*in utero* or during delivery) and postnatal infection (through consumption of infected colostrum or milk) (Gutiérrez et al., 2015; Jaworski et al., 2016).

Argentina has around 54 million head of cattle and is the sixth beef producer in the world, accounting for almost 5% of global beef production (USDA, 2023). According to the latest national agricultural census, Argentina has around 130,800 beef farms. More than half of beef cattle herds are concentrated in the provinces of Buenos Aires, Cordoba, Santa Fe, Corrientes, Entre Ríos and La Pampa (Insitituo Nacional de Estadística y Censos (Indec), 2021).

Although there are several reports on the high prevalence of BLV in dairy farms of Argentina (Gutiérrez et al., 2011, 2020; Trono et al., 2001), information on the prevalence of BLV infection in beef herds is limited (Alvarez Rubianes, 2004; Disalvo et al., 2016; Panei et al., 2017; Trabattoni and Moriondo, 2016). Local reports on this regard are outdated and reduced to a few areas of Argentina. A study analyzing 1798 animals in the province of La Pampa showed that the animal-level prevalence was 0.17% and that 3 out of 30 (10%) of the beef farms analyzed had at least one animal infected with BLV (Alvarez Rubianes, 2004). Another study evaluating 1957 animals in the province of Buenos Aires showed that the animal-level prevalence was 0.36% and that the herd prevalence in the 90 beef herds included in the study was 6.6% (Panei et al., 2017). A study performed in a breeding farm in the province of Santa Fe showed that the animal-level prevalence in 2013 was 11.9%. This study also showed that, two years later, after removing positive animals from the herd, the overall prevalence was reduced to 3.9% (Trabattoni and

Moriondo, 2016). In another study performed in the southern province of Tierra del Fuego, none of the 516 animals analyzed (from 19 beef herds) was infected with BLV (Disalvo et al., 2016).

In this work, a cross-sectional observational study aimed to estimate the seroprevalence of BLV in beef herds and to identify the risk factors associated with it was conducted for the first time in Argentina. This type of study is essential to define science-based disease management strategies.

Materials and methods

Study design and study population

A cross-sectional study was carried out in beef cattle, including herds from thirteen provinces of Argentina, from 2019 to 2020. The minimal sampling number of animals and farms for BLV seroprevalence estimation was determined with the assumptions of 95% confidence level, 10% error rate, and BLV animal-level seroprevalence of 50%. The number of farms analyzed in each province was proportional to the distribution of farms in each of them. The number of animals to be analyzed in each farm was calculated according to the herd size: for a herd size smaller than 50 animals, 30 animals were needed; for a herd size of 51-100 animals, 40 animals were needed; for a herd size of 101-200 animals, 60 animals were needed; for a herd size of 201-300 animals, 70 animals were needed; for a herd-size of 301-500 animals, 80 animals were needed; and for a herd size greater than 501 animals, 90 animals were needed. Potential explanatory variables were obtained from a checklist questionnaire designed to collect basic data of the farm (size of the herd, productive activities, geographic location and proximity to a dairy farm) as well as management practices (pregnancy diagnosis method, breeding method, use of disposable gloves, needle and syringe manipulation between animals, methods of castration and dehorning, presence of insects in summer, etc.). The questionnaire is available on request.

Sample collection

Serum samples were kindly donated by different Agricultural Experiment Stations (EEA) of the National Institute of Agricultural Technology (INTA) and by laboratories belonging to the National Network of Laboratories from SENASA. These laboratories periodically receive, test and store bovine samples from dairy and beef farms that must have an annual serological test to comply with the Compulsory Determination of Sanitary Status to Brucellosis. In all cases, the owners of the farms gave their consent to use the samples for this study. A total of 5827 bovine serum samples were collected from 76 farms. The samples were assigned to the following categories: cows (n=4715), heifers (n=668), and bulls (n=189); the remaining 255 samples could not be assigned to a category because the information was not available.

Sample analysis: BLV serology

For the identification of BLV-positive animals, anti-BLV p24 antibodies were detected using an in-house developed enzyme-linked immunosorbent assay (ELISA) (Gutiérrez et al., 2009). Briefly, Nunc Polysorp plates were coated with recombinant BLV p24 capsid protein (rp24) produced in *Escherichia coli* at a concentration of 0.5 µg/ml in 50 mM carbonate/bicarbonate buffer, pH 9.6, at 4°C overnight. After washing, plates were blocked with 100 µl of PBS with 10% of equine serum and 0.2% Tween 20 (PBSTE) at 37°C for 2 h. Serum samples were added in a 1:25 dilution in PBSTE and incubated overnight at 4°C. The samples to be tested were added to the plate in duplicate. After incubation and washing, a peroxidase-labeled anti-bovine IgG (KPL) goat antibody was added and incubated at room temperature for 30 min. After washing, the presence of secondary antibody was revealed by incubation with 3,3′,5,5′-tetramethylbenzidine (Sigma) and H_2O_2. The reaction was stopped after 15 min using H_2SO_4 and

the absorbance was read in a microplate reader at 450 nm. Positive (strong and weak) and negative control sera were used. The assay was considered valid if the difference of absorbance between the weak positive and the negative control was higher than or equal to 0.35. Normalized results were obtained as a sample-to-positive ratio, based on the averaged values of replicate wells. The weak positive control serum was used to calculate the ratio; its reactivity was set to 100% and all the samples tested were referred to it. A cut-off level of 25% was established (Gutiérrez et al., 2009).

Statistical analysis

Some of the variables retrieved from the questionnaire were categorized to facilitate the statistical analysis. For example, the breeding method was categorized as AI or natural mating (only or combined with AI). Weaning age was categorized as either older or younger than 6 months of age (using the mean value as a cutoff point). The method of hygiene used for the castration instrument (knife/scalpel), blood needles and blood syringes was classified as physical (when they were rinsed or wiped), chemical (when they were disinfected with a chemical product) or none (when they were reused without performing any hygiene method). On the other hand, information about herd size and pasture area was combined to create a new variable named animal density (animals/ha).

A total of 64 of the 76 farms sampled answered the questionnaire, and only these farms were considered to assess the risk factors associated with animal-level seroprevalence of BLV. All farms with seroprevalence data (n=76) were used in the spatial analysis.

Animal-level seroprevalence was defined as the proportion of positive animals among all the animals tested, whereas herd-level seroprevalence was defined as the proportion of herds with one or more positive animals among all the herds sampled. To assess whether the herds from the farms that had answered the questionnaire were biased, a Mann Whitney test was performed to compare their animal level seroprevalence with that of herds from farms that had not answered the questionnaire.

Animal-level seroprevalence of BLV had a skewed distribution with zero values. To fit this variable to a gamma distribution, a constant of 0.001 was added to all animal-level seroprevalence data. Initially, a univariate analysis with generalized linear mixed model (GLM) was conducted to select explanatory variables potentially associated with animal-level seroprevalence of BLV. All variables with a *P* value <0.15 were selected to be included in the final multivariable model. Multivariable GLM was performed to evaluate the effect of the selected explanatory variables on BLV seroprevalence. A manually conducted backward elimination strategy was followed by removing one variable at a time with the highest *P* value. With each variable removed from the model, the Akaike information criteria (AIC) was checked and, if it resulted higher, the variable was retained in the model. Potential collinearity was evaluated before the multivariable analysis. The correlation between all explanatory variables was tested before including them in the multivariable model. All statistical analyses were carried out using RStudio Team. The models were adjusted using the "glm" function.

Spatial analysis

To identify and test the significance of specific geographic clusters with different BLV seroprevalence, the spatial scan statistic cluster-detection method (Kulldorff and Nagarwalla,

1995) was used. A spatial analysis was performed with the complete data of animal-level seroprevalence of BLV (number of herds=76; number of animals=5827). The dataset was scanned to detect areas where the animal-level seroprevalence of BLV was either significantly lower or higher than that expected by chance. A likelihood-ratio test was calculated for each possible window and the scanning upper limit was set at 50% of the population at risk. Animal-level seroprevalence of BLV was assumed to follow a normal distribution, and the most likely cluster along with secondary clusters based on the Gini index criteria were reported (Kulldorff, 2022). The statistical inference (*P* value) is valid for any continuous distribution. All the analysis was performed using the SaTScan™ software version 9.6.

Results

Seroprevalence and spatial analysis

Of the 76 farms analyzed, 54 had at least one BLV-seropositive animal, resulting in a herd-level seroprevalence of 71.05%. At animal-level, 413 of the 5827 animals tested were positive to BLV, representing a mean seroprevalence of 7.23%. In 72.22% of the positive farms, the animal-level seroprevalence was between 1 and 10%, while in 9.26% of them the animal-level seroprevalence was greater than 30% (Figure 1). The median animal-level seroprevalence was 2.69% (min=0, max=75%). When considering only the farms that had answered the questionnaire (n=64), median animal-level seroprevalence was 2.5% (min=0, max=75%), whereas when considering only those that had not answered the questionnaire (n=12), the median animal-level seroprevalence was 3.72% (min=0, max=50%). No differences in animal-level seroprevalence were found between farms that had answered the questionnaire and those that had not (*P*=0.686). When analyzing the different animal categories, the median BLV seroprevalence

for cows, heifers and bulls in beef farms was 7.6% (min=0, max=72.5%), 0% (min=0, max=87.5%) and 0% (min=0, max=50%) respectively.

Table 1 shows the herd-level and animal-level seroprevalence values obtained for each of the provinces analyzed. Santa Cruz and Chubut were the only two provinces with no BLV-positive samples. The other eleven provinces had more than 50% of their farms infected with BLV, with median animal-level seroprevalence values ranging from 0.6% to 28.09%. The highest median animal-level seroprevalence values were observed in Chaco and Santa Fe (28.09% and 7.24%, respectively). However, no significant geographic cluster of high animal-level BVL seroprevalence was detected. Figure 2 shows the geographical distribution of the 76 farms analyzed in this study and the animal-level seropositivity in beef herds of Argentina.

Descriptive data

The median herd size was 765.5 animals (min=33, max=28742). The median pasture area was 855.5 ha (min=25, max=50000). The median animal density was 0.90 animals/ha (min=0, max=9.33). Most of the farms were breeding farms (48.44%), followed by farms that perform the full productive cycle (breeding, rearing and finishing) (28.12%), breeding and rearing farms (15.62%), and farms raising breeding bulls (bull studs) exclusively or combined with one of the above-mentioned productive activities (7.81%). Most of the farms (75%) did not have any dairy farm nearby (5 km around).

The mean annual replacement rate was 17.09% (SD=6.43%). Most of the farms (79.03%) replaced the cattle with animals raised on the same farm, whereas only 3.23% of the farms purchased animals from other farms and 17.74% of the farms used a combination of both

strategies. Most of the farms (90.60%) used a seasonal breeding strategy with cows and heifers, whereas only 6 out of 64 farms (9.37%) performed breeding throughout the year. In 96.87% of the farms (in the case of cows) and in 85.71% of the farms (in the case of heifers), the breeding system used was natural mating (use of bulls exclusively or combined with artificial insemination, AI), while the rest of the farms used only AI. Pregnancy diagnosis in cows and heifers was a routine practice in 93.75% of the farms. Regarding rectal palpation, in most cases (87.09%) this practice was performed with gloves, but usually without changing gloves between animals; only 1.69% of the veterinarians used one glove per cow, while most of them changed gloves every 10 or more animals (42.37%) or used them until they break (42.37%). Calves were generally weaned between 2 and 9 months of age (mean=6 months). Bull calves were castrated in 89.06% of the farms, generally between 1 week and 3 months of age. The instrument used for castration was mostly a knife or scalpel (87.72%), and to a lesser extent, a rubber ring (7.02%). Regarding the hygiene methods applied at the time of castration, 28.07% of the veterinarians that used a knife/scalpel for castration used a chemical method, 24.56% used a physical method and 40.35% did not clean or disinfect the instrument. Only 22.58% of the farms dehorned their calves and, in all cases, the dehorning method was cut and cauterization. Most farms (67.24%) reported not changing needles between animals during vaccination and not disinfecting them either. During blood extraction, in order to sanitize the needle, only 15.62% of the veterinarians disinfected them between animals and 51.56% used physical methods. The frequency of these practices was similar with syringes. The presence of blood-sucking insects and ticks in summer was common in almost all the farms surveyed and routine control against them was generally practiced. Only 18.52% of the farms vaccinated their animals against bovine anaplasmosis and babesiosis. Finally, the great majority of the farms (96.82%) did not perform BLV diagnosis.

Univariate and multivariate analyses

Eight of the 21 potential explanatory variables evaluated in the univariate analysis were associated with BLV infection (P<0.15). These were the animal replacement strategy (P=0.016), the method for breeding cows (P<0.001), the use of gloves during rectal palpation (P=0.128), the weaning age (P=0.067), the method of hygiene of the knife/scalpel used for castration (P=0.018), performing dehorning (P=0.048), and the presence of *Haematobia irritans* (P=0.008) and ticks (P=0.061) in summer (Table 2). These variables were included in the multivariable model as potential risk factors associated with higher animal-level seroprevalence of BLV. The multivariable model revealed that BLV animal-level seroprevalence was significantly associated with the animal replacement strategy (P=0.005), the method for breeding cows (P<0.001), and the weaning age (P=0.011) (Table 3). Farms that replaced cattle by using animals raised on the same farm had 4.7 times more risk of presenting higher animal-level seroprevalence than farms that replaced cattle by purchasing animals exclusively from external farms (OR=4.7, CI95%=0.72-30.57). When cattle replacement was performed with both animals raised on the same farm and purchased in other farms, the risk was 16 times higher than when replacing animals from other farms exclusively (OR=16.22, IC95%=2.16-121.69). Farms performing natural mating (exclusively or combined with AI) had 98 times more risk of presenting higher animal-level seroprevalence than farms that used exclusively AI to breed their cows (OR=98.55, CI95%=15.22-637.13). Farms that weaned their calves after six months of age had 3 times more risk of presenting higher animal-level seroprevalence than farms that weaned their calves earlier (OR=3.10, CI95%=1.30-7.43).

Discussion

This is the first epidemiological study to assess herd management factors related to the seroprevalence of BLV on beef farms in Argentina. Studies that have addressed BLV prevalence in beef cattle have been performed mostly in countries from Asia. In Japan, the latest reported survey performed in 2013 estimated that the animal-level BLV seroprevalence was 40.9% in dairy cattle and 28.7% in beef cattle (Murakami et al., 2013), and other study showed that herd-level prevalence was 69% (Kobayashi et al., 2014). In Korea and China, the mean BLV seroprevalence in beef cattle was 48% and 1.6%, respectively (Cho et al., 1999; Yang et al., 2016), while in Taiwan, the prevalence of BLV proviral DNA was 11.8% (Chen et al., 2021). In North America, 10.3% of Canadian beef cattle are infected with BLV with a herd-level prevalence of 47.9% (VanLeeuwen et al., 2006), while in the United States, both animal-level and herd-level prevalence rates were 29.2% and 7.7% (Benitez et al., 2020).

The BLV prevalence in dairy cattle varies depending on the country and is generally higher than that in beef cattle. In Argentina, the Pampean region (which includes the provinces of Buenos Aires, Santa Fe, Córdoba, La Pampa and Entre Ríos) is where most of the dairy and beef cattle is distributed (71% of total cattle inventory). The national dairy production is also concentrated in this area, with a high density of dairy farms and dairy industries. In this region, the herd-level prevalence in dairy farms is as high as 99%, and more than 80% of the animals in dairy production are infected (Gutiérrez et al., 2020). Regarding beef cattle, four studies have been conducted in specific areas of the country. These studies have reported that, in Buenos Aires, La Pampa and Tierra del Fuego, herd-level BLV prevalence values range between 0 and 10%, whereas animal-level prevalence values range between 0 and 0.4%. In Santa Fe, the only farm analyzed had 11.9% of its animals infected (Alvarez Rubianes, 2004; Disalvo et al., 2016; Panei et al., 2017; Trabattoni and Moriondo, 2016). The present study is the first serological survey

encompassing several provinces of Argentina, and was aimed to assess the current situation of the BLV seroprevalence in beef cattle as well as the risk factors associated with this prevalence. It should be noted that, in the previously mentioned studies, the samples were analyzed with the agar gel immunodiffusion (AGID) technique. Both AGID and ELISA tests are appropriate to perform prevalence analysis and are recommended by the National Network of the National Service for Animal Health and Food Quality of Argentina (SENASA) and the World Organisation for Animal Health (World Organisation of Animal Health, 2018). The use of the AGID technique is adequate to carry out screening tests in established infections due to its high specificity, whereas ELISA has higher sensitivity and can detect recently infected individuals. Although this study did not include all the Argentine provinces, it covered the Pampean region, which is the main livestock area of the country, where 80% of the country's meat is produced. Our results revealed that the herd-level prevalence was 71.05%, representing at least a 7-fold increase with respect to that previously reported for beef cattle in Argentina (Alvarez Rubianes, 2004; Disalvo et al., 2016; Panei et al., 2017). With the exception of the southern provinces of Santa Cruz and Chubut, where the farms sampled showed no evidence of BLV infection, herd-level prevalence was higher than 50% in all the provinces analyzed, with values higher than 90% in 6 of the 11 provinces with evidence of BLV infection. The animal-level seroprevalence rate in Argentine beef cattle seems to be increasing, reaching a mean value of 7.23% (median: 2.69%). Santa Fe was one of the provinces with a high BVL prevalence rate (mean=17.89%), slightly higher than that reported in 2006 (mean=11.9%) (Trabattoni and Moriondo, 2016). Since this province has an animal-level seroprevalence in dairy farms as high as 80% (Gutiérrez et al., 2012), it is not surprising that beef herds were also highly infected. This could be favored by the fact that dairy cows that are discarded due to reproductive or productive reasons are frequently

sold to beef farms for finishing stage. The province of Chaco showed the highest BLV prevalence rate. This province has very few dairy farms according to the last national agricultural census (Insitituo Nacional de Estadística y Censos (Indec) 2021). However, the owner of the two beef farms analyzed in Chaco used to send the heifers (from 5 to 18 months of age) to be reared in another farm in the north of Santa Fe, where there are several dairy farms nearby (personal communication with the veterinarian). This management practice would probably increase the risk of exposure to BLV in these animals. Therefore, a greater number of beef farms should be analyzed in order to corroborate BVL prevalence in Chaco.

Together, and in concordance with that observed in other countries, our results indicate that BLV seroprevalence has been spreading in Argentine beef herds. The absence of BLV in the southern provinces could be favored not only by the extensive cattle production and very low contact rates, but also by the Patagonic Phytozoosanitary barrier against Foot-and-Mouth disease (FMD). The Colorado River (which marks the boundary between the provinces of Neuquén and Mendoza, and between Rio Negro and La Pampa) serves as this sanitary barrier between zones with different disease status. In this context, the movement of cattle from the northern zone of the river (the FMD-free zone with vaccination) to the southern zone (the FMD-free zone without vaccination) is banned, preventing the introduction of animals from the infected areas of the country.

Our present results also showed that BLV seroprevalence in cows was higher than that in heifers. Higher BLV prevalence in older animals, which may result from the chronic nature of the disease and longer period of exposure to risk factors associated with the transmission of the

infection, has been previously reported in both dairy and beef herds (Erskine et al., 2012; Gutiérrez et al., 2011; Mousavi et al., 2014; Murakami et al., 2013, 2011).

The preliminary univariate analysis showed that some of the factors possibly associated with animal-level seroprevalence were the use of gloves during rectal palpation, the method of hygiene used for the knife used in castration, dehorning, and the presence of *Haematobia irritans* and ticks in summer. These involve transmission through infected blood or the use of devices contaminated with infected blood, and some of them have been identified as risk factors for BLV infection in previous reports (DiGiacomo et al., 1987, 1985; Divers et al., 1995; Kobayashi et al., 2014, 2010; Lassauzet et al., 1990). Transmission by these routes is more likely from animals with high levels of infection, which present high proviral loads (Mammerickx et al., 1987). In this study, these management practices were not found as risk factors for BLV seropositivity in the final multivariate model, but as proviral load was not evaluated, we cannot draw conclusions about possible reasons for these results.

The multivariate analysis identified three risk factors associated with animal-level seroprevalence of BLV: the animal replacement strategy, the method for breeding cows and the weaning age. With regard to the animal replacement strategy, we found that using animals raised in the same farm was associated with an increased risk of BLV infection compared with purchasing animals from other farms exclusively. This finding was contrary to that reported by other groups, who found that purchasing animals from external farms was a risk factor for BLV infection (Benavides et al., 2013; Ramalho et al., 2021). Our observation could be explained by the fact that most farms evaluated (71%) proved to be infected with BLV and that 96.8% of them declared not to perform BLV diagnosis. This favors the coexistence of carrier animals with

healthy animals in the same herd, a fact that undoubtedly increases the risk of within-herd BLV transmission. The finding that farms that replace cattle exclusively from external farms had a lower risk of infection could be explained if the producers purchased the animals from farms dedicated exclusively to raising heifers for sale, and do not have adult cows commingle in the same herd. Therefore, it is likely that these heifers introduced in the farm are not yet infected with BLV. On the other hand, producers from farms that replace cattle with both strategies (purchasing from external farms and using animals from the same herd) are probably less selective, and purchase animals from farms were heifers cohabit with adult cows, increasing the chances that these young animals have already been in contact with BLV-infected animals.

It would be interesting to know how BLV infection initially appeared in beef herds. As mentioned above, one hypothesis may be that this happened through the entry of infected animals from dairy cows discarded for different reasons. Another possibility is that during extreme weather events (such as floods or fires), or because of other factors affecting the regional context, animals are moved to more favorable regions, where they can eventually be cohabiting with dairy cattle in the same area. Considering that BLV seroprevalence in Argentine dairy cattle is higher than 80%, this situation clearly represents a risk of transmission by direct contact from dairy to beef animals.

Regarding the method for breeding cows, performing natural service (as exclusive method or combined with AI) was statistically associated with BLV prevalence compared to performing AI exclusively. Although the natural transmission of BLV through semen could not be experimentally demonstrated, proviral DNA has been detected in semen and smegma of infected bulls (Asadpour and Jafari, 2012; Benitez et al., 2019a, 2019b; Dus Santos et al., 2007), probably related to the presence of infected lymphocytes in the genital tract (Givens, 2018). Therefore,

bull servicing cannot be dismissed as a potential transmission route. Additionally, it has been shown that raw semen samples from seropositive bulls have a pattern of intermittent DNA detection (Rossich, 2008). Indeed, international movement of germ plasm from BLV-infected animals is a matter of sanitary control, since the OIE requires that exporting countries present an international veterinary certificate to ensure that the semen is BLV-free (World Organisation of Animal Health, 2023). Moreover, each country establishes its own protocol for international trade and generally includes the absence of proviral DNA in semen. Nevertheless, no internal control measure exists in our country, and possibly in foreign ones, for genetic material provision. In this context, and in agreement with our results, natural service in Michigan dairy herds has been associated with increased BLV prevalence (Erskine et al., 2012). This practice might facilitate BLV transmission by the transfer of blood due to trauma during copulation. Another study performed in Michigan beef bulls showed that most beef bulls do not become infected with BLV until after they start breeding cows, which suggests that natural service might play a more important role in BLV transmission than previously thought (Zalucha et al., 2013). In our study, although the median BLV seroprevalence in bulls was 0%, 3 of the 17 farms that had bulls had between 23.7 and 50% of their bulls infected. Some authors have proposed that AI could reduce the prevalence of BLV, compared with using natural service (Erskine et al., 2012). Additionally, the use of AI would contribute to avoiding having bulls in the farm, which, as they usually remain in the herd longer than cows, represent a constant source of transmission in the case they were infected with BLV. In this regard, Choi et al. proposed that the combined use of proper AI collection techniques, microscopic evaluation of semen for contaminating leukocytes, and application of a BLV-specific polymerase chain reaction to representative straws of semen

from each ejaculate should provide sufficient confirmation of a BLV-free status, even when using seropositive bulls as donors (Choi et al., 2002).

Finally, weaning calves after 6 months of age proved to be a risk factor for BLV prevalence. Previous studies have described the presence of provirus in milk and colostrum from most BLV-infected cows (Gutiérrez et al., 2015, 2011; Jaworski et al., 2016; Lomonaco et al., 2014). Moreover, infectivity of these secretions has been naturally and experimentally demonstrated (Dimmock et al., 1991; Ferrer et al., 1981; Ferrer and Piper, 1981; Miller and Van Der Maaten, 1979; Romero et al., 1983), especially from cows with high proviral loads (Gutiérrez et al., 2015, 2011), confirming that colostrum and milk could be primary sources of infection to calves. Therefore, allowing calves to stay feeding colostrum and milk from their dams for longer periods would increase the risk of infection, both by consumption of infected secretions and by closer and constant physical contact (Ferrer and Piper, 1981; Johnson et al., 1985; Kono et al., 1983; Lassauzet et al., 1991; Sargeant et al., 1997). In agreement with our results, the association between colostrum feeding and BLV infection rate has been previously reported in dairy and beef farms (Ohno et al., 2015). On the other hand, a study performed by Mekata et al. revealed that BLV infection rates in naturally suckled and artificially reared calves born from dams with high and middle proviral loads were not different, suggesting that BLV transmission through natural suckling is infrequent (Mekata et al., 2021). Furthermore, milk and colostrum can also contain BLV-specific antibodies (Gutiérrez et al., 2015; Jaworski et al., 2016). Therefore, the potential protective role of colostrum and milk in the natural transmission of BLV has also been suggested (Van Der Maaten et al., 1981). Different studies carried out by our group in dairy herds of Argentina have revealed that the colostrum of individual cows shows different provirus/antibody profiles and that milk antibody titers negatively correlate with milk proviral

loads (Jaworski et al., 2016; Lomonaco et al., 2014). Therefore, the consumption of colostrum or raw milk with infected cells or free virus particles and low levels of antibodies may play a critical role in early BLV infections (Ruiz et al., 2018). Although transmission of BLV to calves via these secretions has been demonstrated, the magnitude of the risk of transmission under natural conditions remains a knowledge gap.

Conclusions

This extensive study performed with data collected from beef herds covering more than half of the Argentine provinces revealed that BLV seroprevalence in beef farms has increased during the last years. This is a real problem because when a herd becomes BLV-positive, the animal-level prevalence will likely increase at a fast pace. As BLV-infected animals are mainly asymptomatic and clinical symptoms are potentially developed in a long period of time, when the disease is first detected in a farm, the prevalence is already high. This study has identified some management practices associated with BLV prevalence. In this regard, considering that BLV-infected animals with high proviral loads represent a higher risk of both vertical and horizontal transmission, it would be very useful to evaluate this parameter to add knowledge to viral epidemiology in beef herds. The present results deserve special attention since BLV infection in beef cattle seems to lead to a dissemination pattern similar to that observed during the last decades in dairy cattle, especially considering that Argentina is the sixth beef producer in the world, with about 5% of global beef production.

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Declaration of competing interests

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

The authors declare that they have adhered to the journal's ethical policy. Since the serum samples were donated, no ethical approval was needed for the study. Written informed consent was obtained from the farm owners to participate in the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

GS, IA and VR conceived the study. GS, CM, AIM, MS, IA and VR participated in the design of the study. NP and VR contacted the laboratories for sample collection and performed the experiments. NP, GS, CM, AIM, KT, MS and VR analyzed the data and discussed the results. CM, AIM and MS performed the statistical analysis. NP, AIM and VR, wrote the manuscript. All authors read and approved the final manuscript.

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Figure legends

Figure 1: Frequency distribution of animal-level seroprevalence of BLV among the farms analyzed (n = 76).

Figure 2: Seroprevalence of BVL in Argentine beef cattle. (A) Map of Argentina showing the geographical distribution of the 76 farms analyzed. Black and white dots represent farms that had and had not answered the questionnaire, respectively. Some dots are overlapping because they correspond to farms in the same Municipality. (B) Animal-level seroprevalence of BLV in several provinces of Argentina. The color of each province represents the seroprevalence rate (higher seroprevalence=darker color).

Table 1. Prevalence of Bovine Leukemia Virus (BLV) in beef cattle from Argentina.

Province	Herd-level			Animal-level					
	n	BLV +	Serop (%)	n	BLV +	Serop (%)	Median serop (%)	Min-Max	(%)
Buenos Aires	14	9	64.28	976	61	6.25	1.92	0-50	
Chaco	2	2	100	340	94	27.64	28.09	24.96-31.22	
Chubut	3	0	0	141	0	0	0	0-0	
Córdoba	6	6	100	431	28	6.49	6.88	3.50-11.39	
Corrientes	6	3	50	564	7	1.24	0.60	0.10-3.54	
Entre Ríos	15	8	53.33	987	21	2.12	1.18	0.10-17.60	
La Pampa	1	1	100	100	3	3	*	*	
Mendoza	3	2	66.66	381	9	2.36	0.98	0.10-13.30	
Misiones	3	3	100	221	9	4.07	4.54	2.56-6.10	
Río Negro	2	2	100	175	4	2.28	2.40	2.20-2.60	
Salta	7	6	85.71	577	30	5.19	4.10	0.10-23.78	
Santa Cruz	1	0	0	80	0	0	*	*	
Santa Fe	13	12	92.30	854	147	17.21	7.24	0.10-75.10	
Total	76	54		5807	413				

n: number of herds/animals included; BLV+: positive to BLV; Serop: seroprevalence; * This could not be calculated because there was only one animal-level seroprevalence value

Table 2. Univariate analysis for herd management factors associated with BLV animal-level seroprevalence in Argentine beef farms.

Variable level	n	BLV prevalence (95% CI)	*P* value
Basic farm information			
Animal density (animals/ha)			
-	58		0.620
Productive activity			
Full productive cycle	18	0.056 (0.028;0.109)	
Breeding	3	0.087 (0.053;0.148)	0.378
Breeding and rearing	10	0.037 (0.015;0.092)	
Bull stud (only or combined with other)	5	0.078 (0.021;0.281)	
Presence of a dairy farm nearby			
No	48	0.063 (0.042;0.096)	0.383
Yes	16	0.092 (0.044;0.190)	
Herd management			
Animal replacement strategy			
Own	49	0.053 (0.036;0.79)	
External	2	0.014 (0.002;0.096)	**0.016**
Both	11	0.167 (0.073;0.385)	
Method for breeding cows			
Natural mating (only or combined with AI)	62	0.073 (0.051;0.104)	**<0.001**
AI only	2	0.001 (0.000;0.007)	
Method for breeding heifers			
Natural mating (only or combined with AI)	54	0.063 (0.043;0.093)	0.203
AI only	9	0.123 (0.049;0.319)	
Pregnancy diagnosis			
Yes	60	0.077 (0.049;0.121)	0.611
No	4	0.626 (0.000;1.013)	
Use of gloves during rectal palpation			
Always	54	0.064 (0.044;0.093)	
Sometimes	5	0.038 (0.011;0.131)	**0.128**
Never	3	0.292 (0.059;1.443)	
Weaning age			
≥6 months	51	0.079 (0.053;0.118)	**0.067**
<6 months	11	0.032 (0.014;0.077)	
Method of hygiene for knife/scalpel used in castration			
Physical	14	0.152 (0.072;0.321)	**0.018**
Chemical	16	0.071 (0.035;0.142)	

None	23	0.034 (0.019;0.061)	
Not applicable (use rubber-ring)	4	0.035 (0.009;0.144)	
Perform dehorning			
Yes	14	0.132 (0.061;0.283)	**0.048**
No	48	0.054 (0.036;0.083)	
Method of hygiene for vaccination needle			
Physical	3	0.025 (0.005;0.129)	
Chemical	16	0.064 (0.031;0.130)	0.597
Change (every 10-20 animals)	3	0.060 (0.011;0.310)	
None	39	0.079 (0.049;0.125)	
Method of hygiene for blood syringe			
Physical	36	0.053 (0.034;0.085)	
Chemical	13	0.049 (0.022;0.108)	
Change (every 10-20 animals)	3	0.121 (0.045;0.328)	0.199
Other	1	0.015 (0.000;0.256)	
None	6	0.160 (0.051;0.506)	
Method of hygiene for blood needle			
Physical	33	0.057 (0.035;0.093)	
Chemical	10	0.052 (0.021;0.127)	0.307
Single use	13	0.085 (0.039;0.187)	
Change (every 10-20 animals)	3	0.026 (0.005;0.131)	
None	5	0.185 (0.052;0.657)	
Presence of insects and ticks in summer			
Haematobia irritans			
Always	47	0.084 (0.056;0.123)	**0.008**
Sometimes	13	0.025 (0.011;0.056)	
Stomoxys calcitrans			
Always	26	0.065 (0.038;0.113)	
Sometimes	20	0.102 (0.054;0.191)	0.221
Never	7	0.035 (0.012;0.102)	
Tabanus spp.			
Always	31	0.080 (0.048;0.134)	
Sometimes	23	0.075 (0.041;0.136)	0.250
Never	2	0.013 (0.002;0.103)	
Ticks			
Always	14	0.130 (0.061;0.278)	
Sometimes	2	0.016 (0.002;0.120)	**0.061**
Never	48	0.055 (0.037;0.083)	
Perform insect control			
Yes	53	0.076 (0.051;0.113)	0.459
No	9	0.051 (0.019;0.134)	

Perform vaccination against *Anaplasma marginale* or/and *Babesia bovis and B. bigemina*			
Yes	10	0.040 (0.016;0.098)	0.206
No	45	0.076 (0.049;0.115)	
Perform BLV diagnosis			
Yes	2	0.037 (0.005;0.287)	0.528
No	61	0.073 (0.050;0.105)	

BLV prevalence: animal-level seroprevalence of BLV; AI: Artificial insemination; physical= rinse or wipe, chemical= disinfection with iodine solution or some other disinfectant; -: not applicable; bold numbers represent statistical significance (P<0.15).

Table 3. Final multivariate generalized model for herd management practices associated with BLV seroprevalence.

Variable	Level	OR	95% CI	*P* value
Animal replacement strategy	External (Ref)	1	-	0.005
	Own	4.710	(0.726;30.572)	
	Both	16.223	(2.163;121.690)	
Method for breeding cows	AI only (Ref)	1	-	<0.001
	Natural mating (only or combined with AI)	98.553	(15.224;637.134)	
Weaning age	<6 months (Ref)	1	-	0.011
	≥6 months	3.109	(1.300;7.431)	

Ref: reference category; OR: odds ratio; 95% CI: 95% confidence interval; AI: Artificial insemination

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

- BLV seroprevalence in Argentine beef farms has increased during the last years
- The herd-level seroprevalence of BLV was 71.05% in beef farms in Argentina.
- The mean animal-level seroprevalence of BLV was 7.23% (median=2.69%; min=0, max=75).
- Cattle replacement, breeding method and weaning age were associated with BLV prevalence.

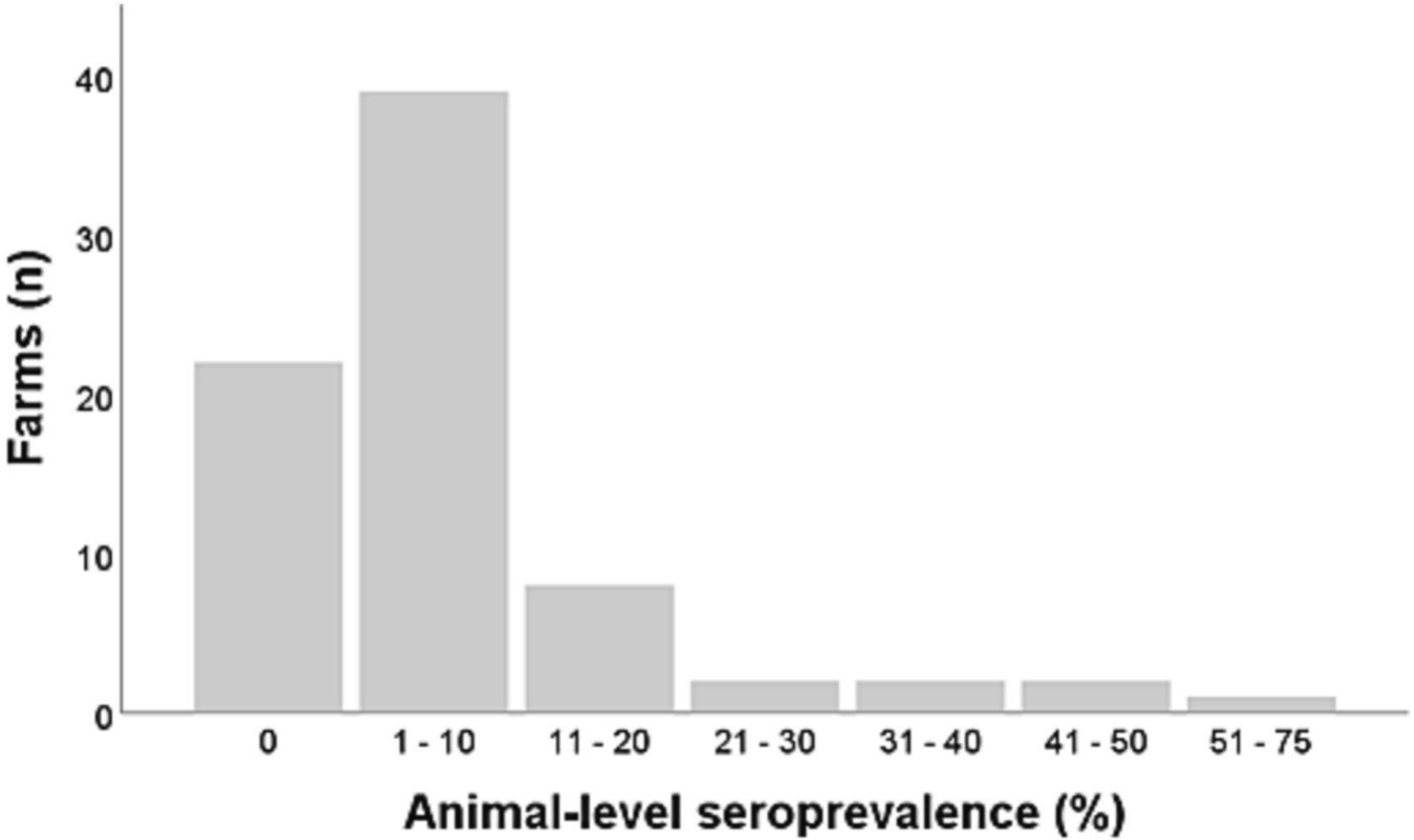
Farms (n)
40
30
20
10
0
0
1 - 10
11 - 20
21 - 30
31 - 40
41 - 50
51 - 75
Animal-level seroprevalence (%)

Figure 1

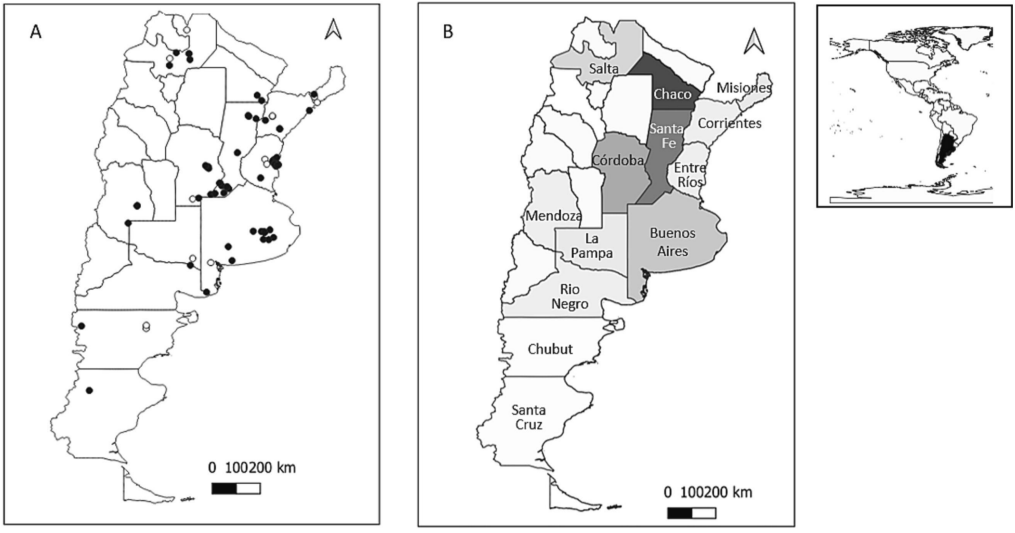
A
0 100200 km
B
Salta
Chaco
Misiones
Corrientes
Santa Fe
Córdoba
Entre Ríos
Mendoza
La Pampa
Buenos Aires
Rio Negro
Chubut
Santa Cruz
0 100200 km

Figure 2