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Article

Serological determination of enzootic bovine leukosis virus (EBLV) in the municipality of Paipa, Boyacá (Colombia)

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Abstract:

Enzootic Bovine Leukosis (EBL) is an economically important infection of dairy cattle, caused by the Enzootic Bovine Leukemia Virus (EBLV). The usual method of spread of EBLV infection is horizontal transmission, through direct and indirect exposure of susceptible animals to infected lymphocytes from blood or milk. After infection, animals appear to be clinically healthy during the first years after infection, but between 30 and 70 % of animals may develop persistent lymphocytosis and 0.1 to 10 % of cattle suffer from lymphosarcoma. This infection is detected by serological tests, usually by the enzyme-linked immunosorbent assay (ELISA). The objective of this research was to determine the seroprevalence of EBLV in bovine females from the municipality of Paipa (Boyacá). The epidemiological study was Descriptive Observational (Cross-sectional) with simple random sampling, where 1000 serum samples were collected, which were processed using the indirect ELISA technique implementing the commercial kit SERELISA® BLV Ab Mono Blocking. A seroprevalence of 31.1 % (311/1000) was determined, finding a statistically significant association between breed, age and seropositivity for the virus.

Key words: Bovine diseases, Leukosis, Seroprevalence, ELISA.

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Introduction

EBL or also known as Bovine Viral Leukosis (BVL) is a persistent infectious disease caused by a retrovirus that belongs to the genus *Deltaretrovirus* and attacks cattle, mainly milk-producing animals[1,2]. Cattle are the only species that is naturally infected with EBLV, although it is possible to experimentally infect sheep, goats, horses, deer, rabbits, rats, guinea pigs, cats, among others[3].

The virus mainly affects B lymphocytes, it is transmitted horizontally and vertically, the first being the most important source of contagion[4], which is mainly caused by arthropods such as horse flies and by the ingestion of colostrum from an infected cow. In addition, it can occur due to iatrogenic infection, which occurs through surgical instruments or sleeves contaminated with infected blood that remains as a residue of a rectal palpation[5].

The disease does not spread rapidly between herds, however, within the affected herds, the seropositivity can be up to 80 %. The usual incubation period is 4 to 5 yr. Infection is rare in animals younger than 2 years and its maximum frequency occurs between 4 to 8 years of age[3]. Animals infected with the virus usually have no visible signs, however, exophthalmia is the most specific sign of the disease, in which degeneration of the retroocular tissue and internal structures of the eye occurs[6,7]. Other characteristic symptoms of the disease are loss of appetite, weight loss, general weakness and, sometimes, neurological manifestations. Superficial lymph nodes may become swollen and may be felt under the skin or by rectal examination[8].

It is a disease that affects milk production. Animals infected with EBLV have been shown to have a decrease in milk production ranging from 2.5 to 5 % compared to the herd[9-12]. In addition to this, there is greater susceptibility to the appearance of other diseases such as mastitis, diarrhea and pneumonia[7]. While more than 20 countries have successfully eliminated EBLV through control programs, the prevalence of the virus can be up to 90 % in endemic areas such as Eastern Europe, South America, and several Asian countries[13].

In Colombia the disease is considered notifiable (ICA Resolution 3714 of 2015)[14]. However, there is no program to prevent the spread of EBLV in cattle, which would help prevent the appearance of the disease, contributing to the mitigation of its economic impact[15]. In the case of EBL, animals positive for the virus and those with persistent lymphocytosis can only be diagnosed through laboratory techniques, while animals with lymphosarcoma can be easily diagnosed by the veterinarian in the field[16].

In Boyacá, little research has been done on the disease[8], which shows the lack of information about it in the area. In turn, in the municipality of Paipa, the seroprevalence of the virus and the relationship that exists with its manifestation in dairy production are unknown, a condition that is considered of great relevance, since this is a municipality with an agro-industrial economic profile with a strong livestock sector, and a marked trend towards growth. Considering the above, the objective of this research was to determine the seroprevalence of EBLV in the municipality of Paipa, Boyacá.

Material and methods

Geographical location

The study was conducted in Paipa (Boyacá), a Colombian municipality located in the center-east of Colombia. It is located in the Tundama province of the department. According to the data from the 2005 census, it has a population of 27,274 inhabitants. In the regional economic structure, the municipality participates extensively with various products in each of the economic sectors. In the primary sector, agriculture, livestock and mining are carried out. Within agriculture, oats, barley, corn, wheat, potatoes and legumes are grown and in livestock, products such as milk and meat are obtained[17].

Sample size

According to the National Livestock Census conducted by the Colombian Agricultural Institute (2019)[18], in the municipality of Paipa, a bovine population of 22,975 heads of cattle was registered, where 16,968 of these individuals were females. Taking into account this information, a sample of 1,000 bovine females was determined from the following formula, obtained through the statistical program OpenEpi, version 3: $[DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1) + p*(1-p)]$; where: d= confidence limits as a % of 100 (absolute +/-%) = 3%; n= population size (22,975); p= hypothetical frequency % of the result factor in the population = 50%+/-3; $Z_{1-\alpha/2}$= two-sided Z value, 1.96 for a 95 %

confidence interval; α= tail probability, e.g., 0.05 for a 95 % confidence interval; design effect (for group surveys-DEFF).

Variables evaluated

The variables mounting and artificial insemination, type of milking implemented in each farm, breed and age of the sampled animals were evaluated.

Sampling and processing

Blood samples were obtained from females older than 2 yr of age and belonging to the Ayrshire, Holstein, Jersey and Normande breeds; prior to taking the blood sample, the area was disinfected with alcohol to facilitate the collection and avoid its contamination. By puncture in the coccygeal vein using needle caliber 16 and 18 of 3 inches, 7 ml of blood were extracted, implementing the vacuum tube system (Vacutainer type, red or yellow top). These tubes were labeled, refrigerated in polystyrene coolers and transported to the Veterinary Parasitology laboratory of the Pedagogical and Technological University of Colombia (UPTC, for its acronym in Spanish), where they were centrifuged at 2,500 rpm for 10 min to separate the cells from the serum. Then with a Pasteur pipette, the serum was transferred to an Eppendorf tube for storage at -20 °C[19]. The samples were processed with the indirect ELISA technique using the commercial kit SERELISA® BLV Ab Mono Blocking (Zoetis, United States) with a sensitivity of 97 % and specificity of 98 %, following the manufacturer's instructions.

Statistical analysis

The epidemiological study was descriptive observational (cross-sectional) with simple random sampling, where the target population was made up of dairy cattle from the municipality of Paipa, while the study population were bovine females of the Holstein, Ayrshire, Jersey and Normande breeds that were two years or older. The data obtained were processed in the statistical program IBM SPSS Statistics 19. The chi-square test was performed to determine if there was a relationship between the presence of antibodies against EBLV and the variables evaluated ($P \leq 0.05$), where the reference categories for age was the age group of 2-3 yr and for the breed it was the Ayrshire. The variables that presented statistical significance, where the *P* value was ≤0.05, were analyzed by logistic regression.

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Ethical considerations

The study was conducted under the conditions of Law 576 of 2000 and Law 84 of 1989 of the Republic of Colombia. Informed consent was obtained from the owners of the cattle prior to the collection of the samples.

Results

A seroprevalence of 31.1 % was determined for EBLV (311/1000) in females from the municipality of Paipa. The Jersey breed was the one with the highest seroprevalence (39.2 %), followed by the Holstein, Ayrshire and Normande breeds, with 38.1, 36.7 and 11.3 % respectively. Regarding the age groups evaluated, it was determined that females between 3 and 4 years old have the highest seroprevalence (36.7 %), followed by individuals older than 4 years and the group of cattle between 2 and 3 yr old, with 23.4 and 21 % respectively.

Likewise, the breed and age of the sampled individuals presented significant differences ($P \leq 0.05$). Regarding age, the age group of 3 to 4 yr presented a statistically significant association ($P=0.000$; $P \leq 0.05$). With respect to the breed, the females of the Normande breed presented a significant association ($P=0.000$; $P \leq 0.05$), in addition to having a value of OR<1, it can be considered that these cattle have less susceptibility to present antibodies against EBLV (Table 1).

Table 1: Analysis of age and breed as risk factors associated with EBLV infections

Variable	Parameter	OR	CI	*P*-value
Age	2-3 years			
	3-4 years	2.106	1.466 – 3.025	0.000
	> 4 years	1.111	0.688 – 1.794	0.667
Breed	Ayrshire			
	Holstein	1.065	0.748 – 1.517	0.726
	Jersey	1.111	0.759 – 1.626	0.587
	Normande	0.220	0.138 – 0.351	0.000

Results are presented as unadjusted Odds Ratio (OR) and 95 % confidence intervals (CI).

Of 651 pregnant females by natural mounting, 32.6 % (212) were seropositive for EBLV, while of 525 cows pregnant by artificial insemination, 30.8 % (162) had antibodies against the virus. However, none of these variables presented a statistically significant association with the appearance of the disease ($P>0.05$).

Regarding the milking characteristics implemented in the farms, of the females subjected to manual milking, 30.5 % (210/688) were seropositive for the disease, while, of the mechanically milked cattle, 31.7 % (91/446) presented antibodies against the virus. Finally, it should be noted that there was no statistical association between the seropositivity for the disease and the type of milking implemented ($P \geq 0.05$).

Discussion

There are several studies carried out on EBL at the national level. Prevalences of 24.9 % have been reported in the Andean region, 14.4 % for the Caribbean, 15.3 % in the Piedemonte Llanero and 1.5 % in Córdoba using the immunodiffusion technique[20]. On the other hand, implementing the ELISA technique in Pasto, the prevalence found was 19.8 %[21], 15 % in Yopal[22], 13.5 % in Toca (Boyacá)[8], 16.32 % and 16.07 % in Patía and Mercaderes (Cauca)[23]. Finally, by molecular tests, the departmental distribution of the disease by animal and farm evaluated was established in Cundinamarca (69 and 90 %), Boyacá (71 and 94 %), Antioquia (73 and 100 %), Meta (85 and 100 %), Nariño (14 and 75 %) and Cesar (17 and 75 %)[24].

At the international level the prevalences are variable, after the implementation of the ELISA technique, values of 14.6 % have been established in Chile[25], 92.7 % and 46.38 % in Peru[1,26], 5.6 % in Ecuador[27], from 11 to 100 % in Thailand[28]. It should be noted that the variation in the results can occur due to the number of animals sampled and the techniques implemented for the diagnosis of the disease. In addition to this, the form of transmission of the virus must be taken into account, which can occur through milk and objects that are contaminated with infected lymphocytes, so the sanitary and management practices implemented in each sampled herd could influence the transfer of the virus from one animal to another[6].

With respect to breeds, in the present study, a greater seroprevalence was found in the Jersey breed. This differs from the results obtained by Romero *et al* in 2015[29], where this breed presented a prevalence of 11.9 %. Likewise, during this study, statistical significant differences were found between this variable and the appearance of antibodies against the virus, results that agree with those reported by Hernandez *et al*[30], who affirm that there is a strong dependence between the breed group and the seropositivity measured by ELISA ($P<0.01$). However, this does not coincide with what was reported by other researchers[31], who determine that there is no association between the breed variable and the presence of EBL.

On the other hand, the Normande breed is less likely to have the disease compared to individuals of the other breeds, which indicates that it acts as a protective factor against EBLV. This could occur in the first place because individuals of this breed have breed

characteristics that make them less susceptible to different pathologies and also, not being considered as a specific breed biotype for milk production, it is less likely to have the virus, since it should be taken into account that dairy breeds are more susceptible to the presence of the disease as reported by other studies[7,10,32].

In addition to the above, it is important to note that the study developed by Hernandez *et al*[30] indicates a strong breed effect on the dynamics of infection with EBLV, where Creole cattle such as Harton del Valle had a lower rate of infection with the virus, animals that became infected developed less lymphocytosis, had a higher immune response and maintained a lower proviral load compared to the Holstein breed.

Regarding age, there were statistical significant differences, which is consistent with what was stated by Betancur and Rodas[31], who identified a higher frequency of infection depending on the age range. Likewise, Hernandez *et al*[30] established that the presence of EBLV depends on the age of the animal; females between 3 and 4 years of age had the highest seroprevalence, after the implementation of molecular techniques and ELISA, they determined that animals older than 4 years had higher percentages of infection, observing a marked reduction in prevalences in younger animals. In this way, it can be established that there is greater susceptibility to the virus as the age of the animal increases, which could be explained by the existence of accumulated exposure to the virus by maintaining contact with infected animals[31,33]. In addition, Gutiérrez *et al*[34] indicate that passive immunity in young animals can alter the percentages of infection measured by ELISA in a herd with high prevalence of EBLV.

On the other hand, the risk factors that predispose to the presence of the disease may be given mainly by the management and practices that are carried out in each of the farms, this being an aspect to take into account to prevent the appearance of the disease and control its spread[8,22]. Although in the present study no statistically significant association was found with the reproductive variables evaluated, it was established that there is a high seroprevalence of the virus in females who are pregnant by natural mounting (32.6 %). This is because horizontal transmission occurs mainly due to the presence of infected lymphocytes in biological fluids such as semen[35]. In addition, Bonifas and Ulcuango[27] determined that direct mounting contributes to the spread of infection due to the use of EBL-positive bulls. In addition to this, rectal examination is a potential route of transmission of the virus, but transmission is related to other factors, such as the number of palpations with a common glove, the level of contamination of the glove with infected lymphocytes and the age of the animals[36].

When relating the disease to the type of milking of the farms, no statistically significant differences were found between this variable and the disease. However, 30.5 and 31.7 % of cattle subjected to manual and mechanical milking, respectively, were seropositive for EBLV. Previous studies have reported that in dairy farms, when having different types of milking, greater intervention is required during this process, facilitating the iatrogenic

dissemination of viral particles through milk, equipment or hands of the operators, generating that the females are more prone to the appearance of the disease[5,37,38,39].

Conclusions and implications

High seroprevalence of EBLV was determined in bovine females from the municipality of Paipa (Boyacá), finding statistical association with the breed and age of the individuals evaluated. It is considered that the early diagnosis of the disease will allow the establishment of effective programs to control it, preventing the spread of the virus in the region. In addition to this, future research is required to compare the influences of each risk factor (type of dehorning, implementation of hypodermic needles, mode of implementation of palpation sleeves, size of the herds, among others) responsible for transmission within the herd, thus contributing to the probability that the disease will reach low epidemic proportions in the future.

Conflict of interest

The authors of this article declare that there is no type of conflict of interest, nor any economic, personal, political relationship, financial or academic interest that may influence their judgment.

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