Epidemiological study of bovine leukemia virus in dairy cows in six herds in the municipality of Pasto, Nariño*

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Abstract

Introduction. Enzootic bovine leukosis is a highly infectious disease caused by a deltaretrovirus of the retroviridae family, which affects bovines of all ages and that generates a high economic impact on the dairy herds. This is caused by the high costs of symptomatic treatments, premature deaths and replacement of ill animals, the reduction of the milk production and the restrictions of importation and exportation imposed by some countries. **Objective.** To determine the prevalence of bovine leukemia virus (BLV) in its two different forms of disease presentations (persistent lymphocytosis and lymphosarcoma) and the factors associated with the seropositivity of the virus in dairy herds from Pasto, (Nariño, Colombia). **Materials and methods.** The study included six specialized dairy herds from Pasto, Colombia. A total of 242 blood samples were taken from 24 months of age or older cows and were analyzed using the indirect ELISA test to determine the seropositivity. The management practices were evaluated in each herd and an analysis binary logistic regression was used to find associations with seropositivity. **Results.** A seroprevalence of 19.8% was determined. Out of 48 positive animals, 13 had a total count over 10000 leukocytes/mm3, and 6 of these (12.5%) developed persistent lymphocytosis (PL) according to their age. No cases of lymphosarcoma or malignant lymphoma were found in the study. Concerning the management practices, the replacement of animals in different herds or in livestock fairs is associated to farms with a higher prevalence. **Conclusions.** Surveillance programs for dairy herds should include diagnostic tests for BLV. Only a small number of animals show consistent changes with lymphocytic or clinical disease. In addition, early diagnosis allows efficient control programs in the replacement of animals and it also prevents the spread of the virus in dairy herds.

Key words: bovine leukemia, dairy herds, persistent lymphocytosis, lymphosarcoma

Estudio epidemiológico del virus de leucemia bovina en vacas de seis hatos del municipio de Pasto, Nariño

Resumen

Introducción. La leucosis enzoótica bovina es una enfermedad altamente infecciosa causada por un deltaretrovirus de la familia retroviridae, que afecta a bovinos de todas las edades y especialmente genera un gran impacto económico en los hatos lecheros. Esto se debe a los grandes costos de los tratamientos de los síntomas, muertes prematuras y reemplazo de los animales enfermos, disminución de la producción de leche y restricciones a la importación y la exportación por parte de algunos países. **Objetivo.** Determinar la prevalencia del virus de leucemia bovina (VLB) en dos formas de presentación de la enfermedad (linfocitosis persistente y linfosarcoma) y los factores asociados con seropositividad del virus en hatos de Pasto, Nariño, Colombia. **Materiales y métodos.** El estudio incluyó a seis hatos lecheros especializados de Pasto, Nariño, Colombia. Un total de 242 muestras de sangre se tomaron de vacas con 24 meses de edad o mayores y se

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analizaron con la prueba ELISA indirecta, para determinar la seropositividad. Las prácticas de manejo fueron evaluadas en cada hato y un análisis de regresión logística binaria se practicó para encontrar asociaciones con la seropositividad. **Resultados.** Se determinó una seroprevalencia de 19.8%. De 48 animales positivos, 14 tuvieron un conteo total mayor a 10000 leucocitos /mm^3 y 6 de ellos (12.5%) desarrollaron linfocitosis persistente (LP) según la edad. No se encontraron casos de linfosarcoma ni de linfoma maligno en el estudio. Entre las prácticas de manejo, el reemplazo de animales en distintos hatos o ferias ganaderas se asocia con las fincas de más prevalencia. **Conclusiones.** Los programas de vigilancia para hatos lecheros deberían incluir exámenes de diagnóstico para VLB. Solamente un pequeño número de animales muestra cambios consistentes con enfermedad linfocítica o clínica. Además, el diagnóstico precoz permite programas de control eficiente en el reemplazo de animales, además de prevenir la propagación del virus en los hatos lecheros.

Palabras clave: leucemia bovina, hatos lecheros, linfocitosis persistente, linfosarcoma.

Estudo epidemiológico do vírus de leucemia bovina em vacas de seis curais do município de Pasto, Nariño

Resumo

Introdução. A leucosis enzoótica bovina é uma doença altamente infecciosa causada por um deltaretrovirus da família retroviridae, que afeta a bovinos de todas as idades e especialmente gera um grande impacto econômico nos curais leiteiros. Isto se deve aos grandes custos dos tratamentos dos sintomas, mortes prematuras e substituição dos animais enfermos, diminuição da produção de leite e restrições à importação e a exportação por parte de alguns países. **Objetivo.** Determinar a prevalência do vírus de leucemia bovina (VLB) em duas formas de apresentação da doença (linfocitosis persistente e linfosarcoma) e os fatores associados com soro positivo do vírus em curais de Pasto, Nariño, Colômbia. Materiais e métodos. O estudo incluiu a seis curais leiteiros especializados de Pasto, Nariño, Colômbia. Um total de 242 mostras de sangue se tomaram de vacas com 24 meses de idade ou maiores e se analisaram com a prova ELISA indirecta, para determinar a seropositividade. As práticas de manejo foram avaliadas em cada cural e uma análise de regressão logística binária se praticou para encontrar associações com a seropositividade. **Resultados.** Determinou-se uma seroprevalencia de 19.8%. De 48 animais positives, 14 tiveram uma contagem total maior a 10000 leucocitos /mm3 e 6 deles (12.5%) desenvolveram linfocitosis persistente (LP) segundo a idade. Não se encontraram casos de linfo sarcoma nem de linfoma maligno no estudo. Entre as práticas de manejo, a substituição de animais em diferentes curais ou feiras de gado se associa com as herdades a mais prevalência. **Conclusões.** Os programas de vigilância para curais leiteiros deveriam incluir exames de diagnóstico para VLB. Somente um pequeno número de animais mostra mudanças consistentes com doença linfocítica ou clínica. Ademais, o diagnóstico precoce permite programas de controle eficiente na substituição de animais, além de prevenir a propagação do vírus nos curais leiteiros.

Palavras importantes: leucemia bovina, curais leiteiros, linfocitosis persistente, linfo sarcoma.

Introduction

Enzootic Bovine Leukosis (EBL) is a neoplastic disease of the reticuloendothelial system caused by bovine leukemia virus (BLV)[1]. All breeds are susceptible, although the incidence is higher in dairy cows. It rarely occurs in animals which are younger than 2 years old and the incidence increases with age[2, 3].

In Colombia, the EBL was identified for the first time in 1957 from clinical and necropsy cases[4]. The prevalence of the disease is variable— in dairy cattle there are reports of prevalence of 24.9% in the Andean region, 14.4% in the Caribbean region and 15.3% in the Orinoquia region[5]. Other studies show a higher prevalence in central and northern Colombia. The prevalence was 32% and 40% respectively[6] and in Antioquia the prevalence was 37.5% in heifers and 79.1% in adult cows[7].

The main form of iatrogenic transmission is attributed to management practices such as injections, vaccinations, dehorning[8], castration, rectal palpation[9] and tattoos, carried out in minimum hygiene, which have been demonstrated experimentally[10] Vertical transmission has

been reported transplacentally or through the ingestion of colostrum and milk[11-13]. It has also been described the role played by blood-sucking insects such as *Tabanus sp*[14].

The BLV is integrated into the DNA of lymphocytes and can produce a polyclonal B cell expansion which is usually manifested as an increase of lymphocytes in blood persistent lymphocytosis (PL)[15] and monoclonal expansion accumulating lymphoid tissue of cattle (lymphosarcoma or lymphoma)[16].

Most animals remain asymptomatic throughout their life. However, about 30% of cattle infected with BLV develop PL between their 3rd and 6th year of age[17]. Lymphosarcoma or lymphoma occur in less than 5% of infected animals and it is present in animals 5 years old or older; which may be preceded by PL but it is not necessary for its appearance[18, 19]. Clinical signs may include enlarged lymph nodes, weight loss, decreased milk production, and other clinical signs related to the location of the tumor[20, 21].

BLV proviral load is correlated with the titer of antibodies against envelope antigens, specifically against the viral proteins gp51 and p24[22]. BLV infection stimulates a strong humoral immune response against these proteins which form the basis for the detection of serological tests[23]. The most common diagnostic tests are the Agar Gel Immunodiffusion (AGID) and Absorbent Immunosorbent Assay (ELISA) which have been developed in order to detect antibodies against gp51[24]. In a study in Chile, the ELISA test had a similar ability to detect positive animals to the PCR with a sensitivity of 97%[25]. That is why this method was used in the present study.

There is evidence that the virus can infect in vitro cells from different animal species, as sheeps. Hence it is important to monitor the BLV diagnostic, which must include constant surveillance of the genome in order to detect genetic variations and emergence of new strains acquiring the ability to affect humans[26].

In most countries nowadays, the EBL is a notifiable disease[27]. In Colombia there are not established health policies to prevent, control and eradicate BL. Additionally to date it is not considered a reportable disease to any entity.

The objective of this study was to determine the prevalence of BLV; its forms of manifestation in dairy herds in the municipality of Pasto-Colombia and to establish the association of population management variables in regards to the seropositivity to BLV.

Materials and methods

A cross sectional study was performed in six specialized dairy farms in the municipality of Pasto, Nariño, ecosystems above 2500 m to 3200 m where dairy herds, randomly selected, with the following inclusion criteria: farms with information system or veterinary assistance who keeps records of population practices.

The sample size was determined by a simple random sampling strategy, based on cow population over 24 months of age (Census of 2010 FMD vaccination) with the purpose of correlating their age with the appearance forms of the disease.

$$n = \frac{N*Z2*P*(1-P)}{N*e2 + Z2*P*(1-P)}$$

Where:
N: number of dairy cows in the region (14145 cows)
P: prevalence expected of EBL (30%)
e: accepted error (in this study 10%)
Z: confidence level (α= 0.05)

A number of 242 blood samples were obtained by venipuncture of the coccygeal vein whit vacutainer and red tubes without anticoagulant for obtaining serum. Samples were processed at the Clinical Laboratory of the Universidad de Nariño, where white blood counts (WBC) was held. Those samples with counts greater than 10000 leukocytes/mm^3 were performed blood smears to obtain the differential count. Animals which had lymphocytosis were re-sampled 6 months after the first sample to determine whether they developed PL or not (table 1). This count was corrected according to the animal age by Bendixen key which confirms that the increase is due to the BLV cells and not due to their age. Under normal conditions the number of lymphocytes decreases with age of the animal[28]. In the same laboratory,

Table 1. Bendixen key[29]

AGE (Years)	LYMPHOCYTES COUNTS/mm^3		
	Normal	Suspect	Positive
0-1	<11.000	11.000-13.000	>13.000
1-2	<10.000	10.000-12.000	>12.000
2-3	<8.500	8.500-10.500	>10.500
3-4	<7.500	7.500-9.500	>9.500
4-5	<6.500	6.500-8.500	>8.500
5-6	<6.000	6.000-8.000	>8.000
>6	<5.500	5.500-7.500	>7.500

serum samples were processed to detect the presence of antibodies against BLV by indirect Enzyme Linked Immunosorbent Assay -ELISA (Commercial Kit SV ANOVA BLV GP51). BLV apparent prevalence was calculated as the proportion of positive reactors animals (48 cows) to ELISA over the total number of animals sampled (242) for the study. Positive animals were subject to a clinical examination to establish the development of neoplasias and corroborate histopathologically with lymphosarcoma.

An epidemiological survey was conducted on each campus of the study, aimed to find variables associated with seroprevalence of BLV. These variables were: 1. Source of replacement animals; 2. Biosecurity practices in the population management (changing palpation sleeves, disinfection of surgical and obstetric instruments, needles and syringes exchange among animals), 3. Management during calving and reuse of medical equipment of individual treatments (needles, syringes).

Data was recorded on an Excel spreadsheet. Each variable was identified as “0” when the response was negative and as “1” when it was positive. Polytomous variables were transformed into dummy variables to be examined dichotomously. These variables were analyzed descriptively and the association with the presence or absence of antibodies to BLV was determined by logistic regression in the statistical program STATA 9®

This project was approved in the record 001 of October 13, 2010, by the Ethics Committee On Animal Research of the Universidad de Nariño, and it was classified as minimal risk.

Results

Out of 242 animals sampled, 48 animals were found seropositive, giving a seroprevalence of 19.8% for the VLB in the municipality of Pasto. The distribution of positive animals in sampled herds was as follows in table 2:

Out of 48 seropositive animals, 13 (27.08%) had counts over 10000 leukocytes/mm^3 and 6 (12.5%) developed persistent lymphocytosis according to their age. Only one animal infected to present an ocular neoplasia, which –at the histopathology study – was diagnosed as squamous cell carcinoma.

A descriptive analysis was performed of the most important variables associated with seropositivity transmission and BLV (table 3)

When performing logistic regression, co linearity was found in most variables. Therefore, the variables could not be associated with seropositivity to BLV. Nonetheless, the replacement variable was found to have an increased risk of infection when the replacement is from other farms. In-farm replacement has an inverse association in accordance with the information of table 4.

Table 2. Distribution of seropositive animals BLV in the municipality of pasto

Farms	Animals Sampled	Positive Animals	Seroprevalence (%)
A	54	13	24%
B	51	14	27%
C	20	13	65%
D	35	0	0%
E	34	2	5%
F	48	6	13%
TOTAL	242	48	19.8%

Table 3. Description of variables associated with the handling and transmission of BLV seropositivity in dairy cows on the municipality of Pasto

Variable Analized	Yes	No
In-farm replacement	86%	14%
Purchase of replacement animals	57%	43%
Sleeves and Needle exchange	100%	0%
Syringes exchange	57%	43%
Disinfection of surgical instruments	100%	0%
Bulls allowed to mate with a cow	29%	71%
Artificial insemination	100%	0%
Embryo transfer	29%	71%
Presence of calving sites	86%	14%
Ectoparasite control	86%	14%

Table 4. Logistic regression analysis for variables associated with seropositivity management of BLV in dairy cows of the municipality of Pasto, Nariño

Variable Analized	Or	P Value	Confidence Interval (95%)
External animal replacement (farms or fairs)	2,59	0,014	1.208916 - 5.576702
Animal replacement from own farm	0,22	0,007	.0775403 – 6629104

Discussion

The seroprevalence found in this study (19.8%) is lower than expected in dairy farms because it assumes a greater risk due to management practices and the time animals live in farms[30]. Nonetheless, the result is similar to the ones found in the following specific studies: animals with reproductive disorders where the prevalence was 21% (Monteria, Cordoba)[31], and dairy herd in high altitude tropical areas with seroprevalence of 15.4%[32]. In this region, geographical conditions and management are similar to the one in the study area. It is evident that the variety of results among farms found in the study (table 1) ranges from 0% -*farm D*- to 65% -*farm C*-, confirming that there is a variety of farm prevalence with a low number of positive reactors[33].

It is important to clarify that only some animals develop all stages of the disease and that the order of their appearance does not suggest a sequence of them[34]. For this reason, the infection is not always visible; therefore, it must be implemented a diagnostic routine test in order to identify the virus at the farm.

The PL condition is an important factor because transmission is more effective when the infecting cattle have it[35]. These cattle have approximately 25-35% of infected cells with pro-virus, whereas animals which do not develop PL have less than 5% of infected lymphocytes[22]. The number of animals that developed PL is low[6]. This coincides with those reported by Van *et al* who found that only one third of infected animals develop this condition[36].

In technified production systems such as those included in this study, it has been demonstrated a relationship among the genetic potential for milk production (genotype BoLA-A), the resistance, the susceptibility to BLV, the development of PL, and proviral load. The latter suggests that the resistance to PL is associated with longevity in the herd when the infection is prevalent[37]. This was observed in *farm A*, which has high production cows with high longevity --14 years of age-- in some animals in production. In this herd, there were no cases of PL, alterations in milk production, or its quality. This is consistent with studies in Canada where it was found that BLV infection did not affect the quantity and quality of milk during the 305 days of lactation[38, 39].

In this study only one animal presented an ocular neoplasia compatible with one of the anatomical locations of lymphosarcoma in Holstein cows[40]. However, this was diagnosed as squamous cell carcinoma. There were no other cases with neoplasias because less than 5% of infected cattle with BLV develop lymphosarcoma or malignant lymphoma[41]. Additionally, the difficulty and the limitation of their diagnosis it is the result of animals dying without evident clinical signs. These dead animals are sent for human consumption and they are never subject to necropsy in order to establish tumor development e.g. lymphosarcoma. These tumors are often found in the rumen, heart, spleen, intestine, liver, kidney, lung and uterus[42].

In the variables analyzed in the survey, horizontal transmission was considered because of infected lymphocytes present in biological fluids such as blood, semen and rarely saliva[43]. This transmission is higher in situations of confinement where the risk increases by the presence of tissues and fluids especially during calving[44].This risk decreases when using birth sites or paddocks, which were found in 86% of the farms under study.

Other variables in the iatrogenic transmission of BLV are management practices with minimum hygiene conditions which have been experimentally demonstrated[45]. In the analyzed farms, the criteria of biosecurity were met—change of needles/ gloves and disinfection of surgical instruments (table 2). In terms of the role of blood-sucking insects, it was taken into account the implementation of control programs for ectoparasites used in 86% of the properties. The entry of replacement animals from other farms and fairs was the only variable associated with seropositivity. This can be explained because the health status of these animals is not evaluated and required by their owners. The only diagnostic tests are the ones from Official Control Diseases (Foot and mouth disease, brucellosis and bovine tuberculosis). The official control measures for bovine leucosis adopted by countries such as Lithuania in the process of its eradication include the following: detection or virus monitoring, border

surveillance, movement of animals control inside the country, and sanitary slaughter[46].

The risk that represents this type of management was evident in *farm C* where, on one hand, the entrance of animals is free and permanent without any sanitary control. This coincides with the highest BLV prevalence found in this study (65%). On the other hand, the lowest infection prevalence (0%) was found in *farm D,* where replacement animals come from the same farm and bought animals are subject to various diagnostic tests in order to ensure their health. The previous biosecurity measures were recommended by a study conducted in Minneapolis about the considerations that must be taken at the time to establish replacement programs[47].

The economic importance of this disease is that BLV infection generates premature replacements, reduced slaughter value, mortality loss, abortion, reproductive alterations, and treatment costs. Additionally, there is an immunodeficiency and increased susceptibility to other diseases[48], resulting not only in the decreased exports competitiveness, but also in the increased concerns about consumer safety[49].

Conclusions

Control of BLV in infected dairy farms in the municipality of Pasto must be improved by focusing particularly on the risk and protective factors. A small number of animals showed consistent changes with lymphocytic or clinical disease, however among the surveillance programs for dairy herds should include diagnostic tests for BLV. In addition, early diagnosis allows efficient control programs in replacement animals preventing the spread of the virus in the region. Future research is required to compare the influences of each factor responsible for within-herd transmission and to facilitate more rational prioritization of control measures. Health policies for prevention, control, and eradication of the BLV should be set

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