

Occurrence of seropositive sheep (*Ovis aries*) to Bovine Leukemia Virus in Brazil

Ocorrência de ovinos (Ovis aries) soropositivos ao vírus da Leucemia Bovina no Brasil

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Abstract

Occurrence of seropositive sheep (*Ovis aries*) to Bovine Leukemia Virus (BLV) by agar-gel immunodiffusion test (AGID) using the antigen gp51 was surveyed for the period 2005-2007. Samples were collected from sheep in the Brazilian states of Rio Grande do Sul, Paraná, São Paulo, Pernambuco, Maranhão, Pará, Bahia, Mato Grosso, Rondônia, and Acre. Two of 35 (5.7%) flocks were seropositive to BLV, and the rate of seropositive animals was 0.077% (two of 2,592). The two seropositive sheep were female, one 13-month old Santa Inês breed and other of unknown age and breed, both from the state of São Paulo. Distribution of BLV in the ovine population studied proved to be a rare event in Brazil.

Keywords: Bovine Leukemia Virus. Seropositivity. Ovine. AGID test. Brazil.

Resumo

A ocorrência de ovinos sororreagentes ao vírus da Leucemia Bovina (VLB) pelo teste de imunodifusão em gel de ágar (IDGA) utilizando o antígeno gp51 foi avaliada no período de 2005-2007, em diferentes regiões do Brasil. Amostras foram colhidas de ovinos dos Estados do Rio Grande do Sul, Paraná, São Paulo, Pernambuco, Maranhão, Pará, Bahia, Mato Grosso, Rondônia e Acre. Duas em 35 cabanhas (5,7%) e dois em 2592 ovinos (0,077%) foram soropositivos. Os únicos animais com anticorpos contra o VLB eram fêmeas, uma com 13 meses de idade e da raça Santa Inês e a outra não se conhecia a idade e raça, ambas provenientes do Estado de São Paulo. A distribuição da soropositividade na população estudada demonstrou ser rara a infecção pelo VLB em ovinos no Brasil.

Palavras-chave: Vírus da Leucemia Bovina. Soropositividade. Ovinos. IDGA. Brasil.

Introduction

Bovines are the main source of infection of Bovine Leukemia Virus (BLV), a retrovirus in subfamily *Orthoretrovirinae*, genus *Deltaretrovirus*¹. The main route of BLV transmission is iatrogenic, by the use of common syringes, needles, gloves, surgical material, and other types of utensils. Infected cattle may develop Bovine Leukemia (BL), a chronic disease that causes seroconversion, but not all infected animals will develop persistent lymphocytosis and lymphosarcoma along their entire life. Although cattle are the primary natural host for BLV, other species can be infected².

Several studies of experimental infection in sheep, using varying infective materials, doses, and routes of inoculation showed that this species may also be susceptible to BLV. It was observed seroconversion in

infected animals^{3,4,5,6,7,8,9,10} besides the development of lymphocytosis and lymphosarcoma in several organs^{8,9}.

In Brazil, there have been no studies on BL seroprevalence of naturally infected sheep flocks, in contrast to some countries where such investigation has been carried out. In Venezuela, 179 sheep of different ages and breeds were examined for anti-BLV antibodies by AGID every six months, in a total of three collections. These animals were kept on separate pas-

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Received: 03/12/2008

Approved: 09/12/2010

tures, in a farm that had infected bovines with high rates of BL and lymphosarcoma¹¹. Seropositive animals were observed in all of three harvests: in the first one, eight animals from five to six years of age; in the second one, seven new cases from five to six years old; in the third one, a new case, five years of age. These data demonstrated the chronicity of the infection, with greater rates involving older animals.

In Stavropol territory, Russia, it was reported the endemicity of BL in ovine wool breeds¹². Serological tests carried out in three districts in 1989 showed 82 seropositive animals to BLV. *Post mortem* examinations revealed lymphosarcoma. The authors confirmed the occurrence of bovine and ovine seropositive in the same farm. However, risk factors that could spread the agent between the two species were not investigated.

Sheep farming is an expanding industry throughout Brazil, particularly in regions where there was no tradition of economic exploitation of sheep. The trade of living animals is an important activity, and animals are submitted to interstate transportation in Brazil. The Northeast region has the biggest percentage of the total number of sheep produced, in 1975 it was 31.0%, reaching 57.0% in 2003. In the North there has been an increase from 0.47% to 2.8%, in the Southeast an increase from 1.47 to 3.39% and in the Center-west from 0.81% to 5.5. The Southern Region was the only area where there has been a reduction in the number of sheep, from 66.0% to 32.0%¹³.

Antibodies against the BLV in ovine have been reported in Venezuela¹¹ and Russia¹², but in other parts of the world its occurrence was hitherto unknown. The objective of the present study was to evaluate the rates of seropositivity for BLV in sheep from different regions of Brazil.

Material and Method

Serum samples were collected from sheep in breeding units from different Brazilian States and regions,

for the period 2005-2007 (Table 1). The serological tests were done in the *Laboratório de Virose de Bovídeos*, at *Centro de Pesquisa e Desenvolvimento de Sanidade Animal, Instituto Biológico/APTA*, São Paulo state, Brazil.

Samples were submitted to AGID test, which is simple and easy to perform and has been recommended as a standard method by the World Organization for Animal Health (OIE)¹⁴. The BLV-AGID diagnostic kit from Instituto de Tecnologia do Paraná (TECPAR® - Curitiba/Paraná – Brazil) was produced according to OIE standards¹⁴. This kit provided lyophilized positive control serum, lyophilized BLV antigen (glycoprotein gp51), and the diluents. To perform the test according to the manufacturers' protocols, a BLV-AGID punch was obtained. This punch consisted of a rosette arrangement with a central well 4 mm in diameter and six surrounding wells 4 mm in diameter, all 3 mm apart. The wells were filled according to the specifications of the kit insert. The central well received 25 µL of the dissolved antigen. The surrounding wells 1, 3 and 5 received 25 µL of the reference serum and wells 2, 4, and 6 received the same amount of the serum sample to be tested. The tested plates were kept at room temperature (20 - 27 °C) in a closed humid chamber, and read at 24, 48 and 72 hours.

All procedures conformed to Animal Experimentation Ethical Principles adopted by the Brazilian College of Animal Experimentation (COBEA), and approved by the Commission of Ethics in Animal Experimentation of the Biological Institute (CETEA-IB n. 046/08).

Results

Some serum samples that were considered to be suspect in the first analysis were retested in triplicate in the same rosette, and unspecific reactions were considered negative.

A total of 2,592 serum samples collected from sheep in 35 breeding units in Brazil were analyzed, for the

Table 1 - Distribution according to region and state, of seropositive flocks and sheep to BLV by AGID, for the period 2005-2007 - São Paulo - 2007

Region State	Number of flocks analyzed (%)	Number of seropositive flocks (%)	Number of serum samples analyzed (%)	Number of seropositive animals (%)
South				
Rio Grande do Sul	10 (28.6)	0	1008 (38.9)	0
Paraná	03 (8.5)	0	43 (1.7)	0
Subtotal	13 (37.1)	0	1,051 (40.6)	0
Southeast				
São Paulo	05 (14.3)	2 (5.7)	199 (7.7)	2 (0.077)
Northeast				
Bahia	04 (11.4)	0	308 (11.9)	0
Pernambuco	04 (11.4)	0	366 (14.1)	0
Maranhão	03 (8.6)	0	234 (9.0)	0
Subtotal	11 (31.4)		908 (35.0)	
Center-west				
Mato Grosso	01 (2.9)	0	26 (1.0)	0
North				
Pará	02 (5.7)	0	343 (13.2)	0
Rodônia	02 (5.7)	0	55 (2.1)	0
Acre	01 (2.9)	0	10 (0.4)	0
Subtotal	05 (14.3)	0	408 (15.7)	0
Total	35 (100.0)	2 (5.7)	2592 (100.0)	2 (0.077)

period 2005-2007. Only two ovine sera were considered true reactor for BLV, they were retested by AGID test in the same rosette, tree time, and the precipitin line of identity was confirmed in all the repetitions. The rate of flocks seropositive was 5.7% and to animals was just 0.077% (Table 1). The two seropositive sheep were a 13 months old female Santa Inês, and the other was of unknown age and breed, from different flocks of São Paulo state, Southeast region. The seropositive animals didn't present lymphosarcoma.

Sampling represented all the Brazilian regions, and the greatest number of serum samples came from the Southern region (40.6%), and the Northeast region (35.0%) (Table 1), both considered the most important areas of sheep farming in the country¹². The Southern region was represented by Rio Grande do Sul (38.9%) and Paraná States (1.7%), followed by the Northeast region represented by the States of Bahia (11.9%), Per-

nambuco (14.1%) and Maranhão (9.0%). The Southeast region was represented only by the State of São Paulo (7.7%) and the Northern region (15.7%) represented by the States of Pará (13.2%), Rodônia (2.1%) and Acre (0.4%). A smaller number of samples came from the Center-west region, the State of Mato Grosso (1.0%).

Predominant breeds were Santa Inês (meat production), 37.8%, followed by Texel (wool production), 24.0%; in 26.7% of the cases, breed was unknown. Most sheep, 82.5%, were females, 12.5% were males and in 4.9% of the samples, gender was unknown. Most, 44.8%, were adults (more than one year old) and 20.8% were less than one year old; age was unknown for 34.4% (Table 2).

Discussion

The susceptibility of ovine to experimental BLV infection was proved by several authors^{3,4,5,6,7,8,9,10}. The

Table 2 - Distribution according to breed, gender and age, of sheep screened by AGID for infection with BL in Brazil, for the period 2005-2007 - São Paulo - 2007

Variables	Number of serum samples analyzed (%)	Number of positive sera (%)
Breed		
Santa Inês	980 (37.8)	1 (0.038)
Texel	621 (24.0)	0
Crossbred	133 (5.1)	0
Lacaune	102 (3.9)	0
Dorper	63 (2.4)	0
Unknown	693 (26.7)	1 (0.038)
Gender		
Male	325 (12.5)	0
Female	2,139 (82.5)	2 (0.077)
Unknown	128 (4.9)	0
Age		
Young	538 (20.8)	0
Adult	1,162 (44.8)	1 (0.038)
Unknown	892 (34.4)	1 (0.038)

BLV causes in ovine seroconversion, but not all infected animals will develop persistent lymphocytosis and lymphosarcoma along their entire life.

Despite sampling in the present epidemiological study evaluated sheep from all breeding regions in Brazil¹³, the low rate of seropositivity in the population studied shows that the natural occurrence of sheep infected by BLV in Brazil is rare. Studies carried out in Venezuela¹¹ and Russia¹² proved that sheep is naturally infected by BLV, and although risk factors for the spread of the virus were not investigated, these two reports have epidemiological value and show the occurrence of seropositive animals in field conditions, and they justify the need for the investigation of BL occurrence in sheep in other regions or different breeding conditions, in order to assess the importance of the disease. Since BL is a chronic disease, all age ranges should be examined in incidence studies involving sheep as is done for other species.

In the present study, most samples came from the South and Northeast regions, where there is

the highest proportion of sheep in Brazil. The seropositive sheep were found only in the state of São Paulo (SP), in the Southeast region of the country where sheep breeding is developing and the trade is very intensive. Seroepidemiological surveys on BL in bovine herds in the state of São Paulo indicate high seroreactor rates¹⁵, however, since the main route for BLV transmission is iatrogenic², this may be considered as a potential source of infection for sheep.

In bovines naturally infected by BLV, intrauterine and post-natal transmission by colostrum and milk are well-known ways for the maintenance of the agent in the herd². However, there are no reports in the literature on the forms of BLV transmission in sheep flocks, perhaps due to the low occurrence of seropositive animals in natural conditions. An experimental study showed that one ewe inoculated with BLV produced two lambs that later developed BL¹⁶. One showed lymphosarcoma, and the other showed high lymphocyte counts and seropositive results with AGID. However, the author did not deter-

mine if the lambs were infected before or after birth. The present epidemiological survey diagnosed two seropositive females to BLV, one adult ewe (of reproductive age) and another of unknown age, supporting future studies on the natural and experimental transmission of BLV between ewe and lamb.

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Conclusion

The extremely low incidence of seropositive animals shows that this disease has no economic impact on the sheep populations studied in Brazil. Nevertheless, sanitary measures are important to keep sheep flocks free from BLV.