

Seroreactivity to bovine leukemia virus in goat (*Capra hircus*) in Brazil

Sorreatividade ao vírus da leucemia bovina em caprinos (Capra hircus) no Brasil

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Abstract

Goats are susceptible to Bovine Leukemia Virus (BLV) infection and may develop after experimental inoculation, the lymphosarcoma. Occurrence of goats (*Capra hircus*) seroreactors to BLV by agar-gel immunodiffusion test (AGID) in breeding animal's submitted to interstate transportation in Brazil was surveyed for the period 2005-2007. Samples were collected from 298 animals in 19 municipalities from the states of Rio Grande do Sul, Paraná, São Paulo, Maranhão, Bahia, Pernambuco, and Pará prior transportation. No seroreactors were found, no matter the region of origin, breed, gender and age. The greatest number of samples came from the Northeast region 133 (44.63%), represented by the States of Pernambuco (35.9%) and Maranhão (4.7%), followed by the Southeast region represented by the State of São Paulo 103 (34.56%), followed by the Southern region 59 (19.8%) represented by Rio Grande do Sul (19.46%) and Paraná States (0.34%). A smaller number of samples came from the Northern region 3 (1.01%) represented by the State of Pará. Predominant breeds were Boer (meat production), 71.14%, followed by Anglo Nubian (meat and milk production), 2.68%, Saanen (milk production) 2.35%, and Toggenburg (milk production) 1.34; in 22.48% of the cases, breed was unknown. Most goat, 61.41%, were females, and 35.59% were males. Most, 51.01%, were adults (more than one year old) and 43.96% were less than one year old; age was unknown for 5.03%. Nevertheless, breeders and technicians should enforce strict hygiene/sanitary measures to commercialization and transportation, because goat breeding is expanding to become an important economic activity in Brazil, and the BLV virus represents a potential risk for goat health.

Keywords: AGID. Bovine Leukemia Virus. Goats. Seroreactors.



Resumo

Caprinos são susceptíveis à infecção pelo Vírus da Leucemia Bovina (VLB) e podem desenvolver, após inoculação experimental, o linfossarcoma. Estudou-se a ocorrência de caprinos sororeagentes ao VLB pelo teste de imunodifusão em gel de ágar (IDGA), em animais reprodutores submetidos ao trânsito interestadual no Brasil, no período de 2005-2007. Um total de 298 soros oriundos de 19 municípios dos Estados do Rio Grande do Sul, São Paulo, Paraná, Maranhão, Bahia, Pernambuco e Pará foram colhidos antes do transporte dos animais. Nenhum dos caprinos foi sororeagente, independente da região de origem, raça, sexo e idade. O maior número de amostras veio da Região Nordeste - 133 (44,63%), representada pelos Estados de Pernambuco (35,9%) e Maranhão (4,7%), seguidos pela Região Sudeste, representada pelo Estado de São Paulo - 103 (34,56%), seguida pela Região Sul - 59 (19,8%) representada pelos Estados do Rio Grande do Sul (19,46%) e Paraná (0,34%). O menor número de amostras veio da Região Norte - 3 (1,01%), representada pelo Estado do Pará. As raças predominantes foram Boer (produção de carne) - 71,14%, seguidas pela Anglonubiana (produção de carne e leite) - 2,68%, Saanen (produção de leite) - 2,35%, e Toggenburg (produção de leite) - 1,34; em 22,48% dos casos, a raça era desconhecida. A maior parte dos caprinos era fêmea - 61,41%, e 35,59% eram machos. Com relação à idade, a maioria eram adultos - 51,01% (idade acima de um ano), e 43,96% tinham menos de um ano de idade; a idade era desconhecida em 5,03% dos animais. Técnicos e criadores devem ser orientados para evitar disseminação de enfermidades pelo comércio e transporte de animais reprodutores entre diferentes regiões, tendo em vista que a caprinocultura está se expandindo e se fortalecendo economicamente no Brasil, e que o VLB representa um risco potencial à saúde dos caprinos.

Palavras-chave: Caprinos. IDGA. Sororeatividade. Vírus leucemia bovina.

Introduction

Bovine Leukemia Virus (BLV) is a *Deltaretrovirus* (ICTV, 2016), whose main route of transmission is iatrogenic, by the use of common syringes, needles, gloves, surgical material, and other types of utensils. Bovines are the main source of infection and animals may develop a chronic disease that causes seroconversion, persistent lymphocytosis and lymphosarcoma (Johnson and Kaneene, 1992).

BLV natural infection has been reported in cattle (*Bos taurus* and *Bos indicus*), water buffaloes, and capybaras (OIE, 2016).

In goats, a serological survey in Rio de Janeiro State, Brazil (Gouveia et al., 1984) detected 7.3% (28/382) seroreactors by agar gel immunodiffusion (AGID). There was an increase of seroreactivity in older categories: up to 4 months old 3.1% (4/120), from 4.1 up to 12 months old 3.3% (4/98) and more than 12 months old 13.3% (20/130). Brito (1985) tested by AGID 347 goats in the South of Goiás State, Brazil, and Silva et al. (1988) examined by AGID 340 serum samples from 44 properties and 14 municipalities in Minas Gerais State, Brazil, and no seroreactors were found. Olson

and Baumgartener (1975) in Germany examined 479 goats and did not find animal's seroreactors. Lin et al. (1990) did a serological survey in dairy goats in 14 geographical regions of Taiwan, and 1.5% (120/7,855) serum samples were reactors to AGID; in five regions, there were only negative reactions to AGID, and in the others, the rate of seropositive animals ranged from 0.1% to 11.5%.

Several species can be infected by inoculation of the virus; sheep are very susceptible to experimental inoculation and develop tumours more often and at a younger age than cattle. A persistent antibody response can also be detected after experimental infection in deer, rabbits, rats, guinea pigs, cats, dogs, sheep, rhesus monkeys, chimpanzees, antelopes, pigs, goats and buffaloes (OIE, 2016).

Goats experimentally inoculated with BLV seroconverted (Hoss and Olson, 1974; Paulsen et al., 1974; Ressang et al., 1976; Mammerickx et al., 1981; Mammerickx et al., 1987) and some developed lymphocytosis and lymphosarcoma in several organs (Olson et al., 1981; Kettmann et al., 1984). It was demonstrated the presence of BLV proviral DNA in peripheral lymphocytes and in lymph nodes of experimentally infected goats

by molecular hybridization studies (Olson et al., 1981), southern blot analysis (Kettmann et al., 1984) and by PCR (Jun et al., 1997).

Mammerickx et al. (1981) reported results of several out cross-transmission trials, starting from cows, sheep and goats as donors to infect the same series of animal species. The experiments showed that BLV remains infectious for the cow, sheep and goat, whatever the donor species (cow, sheep and goat). Some years later, Popescu et al. (1995) described the localization of the receptor gene encoding a polypeptide that represents part of the putative BLV cell receptor and which selectively binds to the viral envelope glycoprotein gp 51 on cattle, goat and sheep. It was used nonradioactive *in situ* hybridization and simultaneous fluorescent R-banding, thus confirming the homology, based on banding patterns, among the chromosomes of these three species.

Goat farming is an expanding industry throughout Brazil, and the trade of living animals is an important activity for genetic improvement, when goats are sold and transported to different regions in the country. However, goats are susceptible to BLV infection and may develop after experimental inoculation, the lymphosarcoma. The objective of the present study was to evaluate the rates of goat seroreactors to BLV, in breeding animals submitted to interstate transportation, because this virus represents a potential risk factor for goat health.

Materials and methods

Serum samples were collected from breeding goats prior to interstate transportation in breeding units from different Brazilian States and regions, for the period 2005-2007. The serological tests were done in the *Laboratório de Vírus de Bovídeos*, at *Centro de Pesquisa e Desenvolvimento de Sanidade Animal, Instituto Biológico/APTA*, São Paulo State, Brazil.

Screening for anti-BLV antibodies was performed using AGID (a prescribed test for international trade) in accordance with the Manual of diagnostic tests and vaccines for terrestrial animals of the World Organization for Animal Health (OIE, 2016). The BLV diagnostic kit produced by the "Paraná

Technology Institute" (TECPAR® Curitiba/Paraná - Brazil) was used according to the manufacturer's instructions. This kit provided lyophilized bovine positive control serum and bovine leukosis antigen for the AGID test, and the diluents. To perform the test according to the manufacturers' protocols, an AGID punch was obtained. This punch consisted of a rosette arrangement with a central well 4 mm in diameter and 6 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 agar was prepared according to the kit protocol.

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 nº. 050/08).

Results and discussion

A total of 298 serum samples collected from goat prior to interstate transportation in 19 municipalities in Brazil were analyzed, for the period 2005-2007. The rate of seroreactors to flocks was 0.0% and to animals was 0.0%, no matter the region of origin, breed, gender and age.

The greatest number of samples came from the Northeast region 133 (44.63%), represented by the States of Pernambuco (35.9%) and Maranhão (4.7%), followed by the Southeast region represented by the State of São Paulo 103 (34.56%), followed by the Southern region 59 (19.8%) represented by Rio Grande do Sul (19.46%) and Paraná States (0.34%). A smaller number of samples came from the Northern region 3 (1.01%) represented by the State of Pará (Table 1).

Predominant breeds were Boer (meat production), 71.14%, followed by Anglo Nubian (meat and milk production), 2.68%, Saanen (milk production) 2.35%, and Toggenburg (milk production) 1.34; in 22.48% of the cases, breed

was unknown. Most goats, 61.41%, were females, and 35.59% were males. Most, 51.01%, were adults (more than one year old) and 43.96% were less than one year old; age was unknown for 5.03%.

Despite the samples for interstate transportation had come from different Brazilian States, no samples came from the State of Rio de Janeiro, where Gouveia et al. (1984) found 8.9% seropositivity, with higher rates in older animals. The evidence of natural infection in goats to BLV in Rio de Janeiro State, who belongs to the Southeast region, where goat breeding is developing and the trade is very intensive, justifies the need for the investigation of BLV occurrence in other regions of Brazil, with different breeding and epidemiological conditions, in order to assess the importance of the disease. Most samples having come from the Northeast, where there is the highest proportion of goats in the country – 91.4% (IBGE, 2013), and from the Southeast, where only the State of São Paulo (SP) was examined. Seroepidemiological survey in the state of Rio de Janeiro by Gouveia et al. (1984) indicated seropositivity in goats, but it was not explained the type of animal management and other risk factors. However, there are no reports in the literature on the forms of BLV transmission in goat flocks, perhaps due to the low occurrence of seroreactor animals in natural

conditions. A study carried out by Lin et al. (1990) in Taiwan showed the occurrence of seropositive goats in field conditions, too, but few studies have been done in the world involving this specie. The low rate of goat's seroreactors found in the international literature and no seroreactors found in the present study may be explained by the type of animal management used in the flocks. Goats are usually bred without direct or indirect contact with bovines, making the risk of interspecies transmission low. Nonetheless, since the main route for BLV transmission is iatrogenic, surveys should not be discontinued in areas where there are high infection rates of BLV in cattle, because this is a potential risk factor for goats, because in Brazil several surveys showed high rates of seropositivity in cattle and dissemination in all the States examined (Del Fava and Pituco, 2004).

In Brazil, a seroepidemiological survey performed in sheep (*Ovis aries*) (Del Fava et al., 2010) reported a low occurrence of seroreactors to BLV by AGID in animals submitted to interstate transportation: 5.7% (2/35) flocks were seroreactors, and 0.07% (2/2.592) sheep. The seroreactor sheep were female, one 13-month old Santa Inês breed and other of unknown age and breed, both from the state of São Paulo. Similarly, to goats at the present research, these sheep were

Table 1 - Distribution of flocks screened by AGID for infection with BLV in Brazil, by region and state, for the period 2005-2007

Region	State	Number of examined flocks	Number of examined animals	Number of positive samples
		Total (%)	Total (%)	Total
Southern	Rio Grande do Sul	3 (15.79)	58 (19.46)	0
	Paraná	1 (5.26)	1 (0.34)	0
	Subtotal	4 (21.05)	59 (19.8)	0
Northeast	Bahia	2 (10.53)	12 (4.03)	0
	Pernambuco	4 (21.05)	107 (35.91)	0
	Maranhão	2 (10.53)	14 (4.7)	0
	Subtotal	8 (42.11)	133 (44.63)	0
Southeast	São Paulo	6 (31.58)	103 (34.56)	0
	Subtotal	6 (31.58)	103 (34.56)	0
Northern	Pará	1 (5.26)	3 (1.01)	0
	Subtotal	1 (5.26)	3 (1.01)	0
Total		19 (100.00)	298 (100.00)	0

usually bred without direct or indirect contact with bovines too, making the risk of interspecies transmission low. 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 herd (Johnson and Kaneene, 1992). However, there are no reports in the literature on the forms of BLV transmission in goat and sheep, perhaps due to the low occurrence of seroreactor animals in natural conditions. Occurrence of BLV in small ruminant flocks in Brazil are a rare event, but it may be a warning in order to identify risk factors to prevent new infections and to keep these one in a free status of infection.

The susceptibility of goat to experimental BLV infection was proved by several authors, who demonstrated that goats seroconverted, despite different protocols varying viral doses, types of material (total blood, colostrum, milk, lymphocyte culture), routes of administration (oral, subcutaneous, intraperitoneal, intradermal) and different dosages (Hoss and Olson, 1974; Paulsen et al., 1974; Ressang et al., 1976; Mammerickx et al., 1981; Olson et al., 1981; Mammerickx et al., 1987; Kettmann et al., 1984; Jun et al., 1997). Some animals developed lymphocytosis and lymphosarcoma (Olson et al., 1981; Kettmann et al., 1984). It was demonstrated the BLV infection by the presence of proviral DNA in peripheral lymphocytes and in lymph nodes of experimentally infected goats by molecular hybridization studies (Olson et al., 1981), southern blot analysis (Kettmann et al., 1984) and by PCR (Jun et al., 1997). Mammerickx et al. (1981) demonstrated by experimental inoculation, that it is possible cross-transmission of BLV among cows, goats and sheep, proving that the BLV remains infectious for these species, whatever the donor (cow, goat and sheep), thus, there is a risk of these species act as reservoir for each one of them. Some years latter, Popescu et al. (1995) described the location of the gp51 receptor gene of BLV in cattle, goats and sheep, thus confirming the homology, based on banding patterns, among the chromosomes of these three species.

However, since the main route for BLV transmission is iatrogenic, surveys should not be discontinued in Brazilians areas where there are

high infection rates of BLV in cattle, because it was demonstrated, according to Mammerickx et al. (1981), the possibility of experimental cross-transmission of BLV between goats and cattle, and it should be considered that the iatrogenic transmission is a potential risk factor for goats. And more else, it is important to know the occurrence of vectors transmitters in the flocks, because it was showed that the horse fly *Tabanus fuscicostatus* transmitted the BLV from cattle to goats (Foil et al., 1989).

AGID test for BLV is one of the prescribed serological tests for international trade of cattle (OIE, 2016). The AGID test is a specific, but not very sensitive test for detecting antibody in serum samples from individual animals, it is simple and easy to perform and has proven to be highly useful and efficient as a basis for eradication schemes. The TECPAR® commercial AGID test kit follows the recommendations of OIE (2016): it contains reference sera (known positive and negative control serum), and the antigen specific glycoprotein gp51 of BLV. AGID test has been used in seroepidemiological surveys in goats in Brazil (Gouveia et al., 1984; Brito, 1985; Silva et al., 1988), in Germany (Olson and Baumgartener, 1975) and in Taiwan (Lin et al., 1990). The different seropositivity rates among these studies may be related to epidemiological characteristics of the herds, and to different sensitivity and specificity of the AGID tests used. Despite the Enzyme-linked immunosorbent assay (ELISA) be a very specific and sensitive test for detecting anti-BLV antibodies in serum samples, and be recommended by OIE (2016) as serological test, there are only available ELISA kits for cattle. The present study used AGID test for detecting antibodies against the BLV, but in future studies it may be used the PCR for antigen detection, because PCR may be used as complementary diagnosis to serology for confirmatory testing (OIE, 2016).

Conclusion

No seroreactors submitted to interstate transportation in Brazil were found, no matter the region of origin, breed, gender and age. These results suggest that BLV has no economic

impact on the breeding goat population studied, despite goat be susceptible to BLV infection and may develop after experimental inoculation, the lymphosarcoma. Nevertheless, strict hygiene/sanitary measures should be enforced by breeders and technicians to commercialization and transportation in order to minimize transmission, because goat breeding is expanding to become an important economic activity in Brazil.

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