

## PREVALENCE AND ASSOCIATE FACTORS TO THE INFECTION FOR LENTIVIRUS OF SMALL RUMINANTS IN CAPRINE OF TOCANTINS STATE, BRAZIL

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### ABSTRACT

The aim of this study was to estimate the prevalence of caprine blood reagents to small-ruminant lentiviruses (SRLV), through a non probabilistic sampling of herds in Tocantins State. In order to detect the anti-SRLV antibodies, 843 blood samples were analyzed using the microimmunodiffusion test in agarose gel – MICRO-IDGA. The frequency of blood reagent goats was 2.7% (23/843). Five focus, being two in the city of Araguaína, and one in each of the cities of Babaçulândia, Filadélfia and Miracema do Tocantins had been identified. The results were distributed according to micro-region in the following manner: 10.0 (20/200) and 1.4%

(3/207) for the North and Miracema micro-regions, respectively. The Saanen was the breed with the highest percentage of blood reagent animals (11.7%; 7/60), the undefined breed animals 0.6% (2/310) and Anglo-Nubian (3.0%; 14/466). Regarding age, 1.9% (6/314) and 3.2% (17/529) of animals under and over 24 months of age, respectively, tested positive. 2.4% (4/166) of males and 2.8% (19/677) of females tested positive. The SRLV infection occurs at a low prevalence among goats in Tocantins State, affecting mainly animal of the Saanen breed and from the north region.

KEY WORDS: Brazil, CAEV, epidemiology, microimmunodiffusion.

### RESUMO

#### PREVALÊNCIA E FATORES ASSOCIADOS À INFECÇÃO POR LENTIVÍRUS DE PEQUENOS RUMINANTES EM CAPRINOS NO ESTADO DO TOCANTINS

Objetivando estimar a prevalência de caprinos sororreagentes aos Lentivírus de Pequenos Ruminantes (LVPR), através de uma amostragem não probabilística em rebanhos no Estado do Tocantins, foram analisadas 843 amostras de soros, utilizando o teste de imunodifusão em gel de agarose – MICRO-IDGA para a detecção de anticorpos anti-LVPR. A frequência de animais sororreagentes foi de 2,7% (23/843). Foram identificados 5 focos, sendo dois no município de Araguaína e um em Babaçulândia, Filadélfia e Miracema do Tocantins. De acordo com a região, os resultados foram assim distribuídos: 10,0% (20/200) e 1,4% (3/207) para as

microrregiões Norte e Miracema, respectivamente. Entre as raças, a Saanen foi a que apresentou o maior percentual de animais sororreagentes, 11,7% (7/60), os sem raça definida 0,6% (2/310) e a anglo-nubiana 3,0% (14/466). De acordo com a idade, os animais com idade inferior e superior a 24 meses apresentaram 1,9% (6/314) 3,2% (17/529), respectivamente. Os machos apresentaram 2,4% (4/166) de positivos e as fêmeas 2,8% (19/677). Os resultados indicam que os LVPR ocorrem com baixa prevalência no Estado do Tocantins, afetando principalmente animais da raça saanen e da região norte.

Palavras-Chaves: CAEV, microimunodifusão, epidemiologia, Brasil.

## INTRODUCTION

The productive chain analysis of national caprine raising has shown a great expansion potential. In this sense, it is essential the concern about the herds' sanitary state, once the sanitary demands for animal commerce have been intensified. This way, an improvement of the installations hygiene conditions and the assurance of herds free of certain diseases may result in added value to the animals and their products (CASTRO & MELO, 2001).

The absence of a national program of essential genetic improvement for the expansion of milk caprine raising has shown the necessity of importing specialized breeds animals. With the import of these animals that come from European countries, many emergent sanitary problems have been reported, specially small ruminants lentivirosis (SRLV) (CASTRO, et al., 1994; ASSIS & GOUVEIA, 1994).

The SRLV are infecto-contagious diseases of slow evolution, caused by a virus from the Retroviridae family and Lentivirinae subfamily, which is presented in four clinical forms: nervous, respiratory, mammary, and articular. The most frequent clinical form is the articular in adult caprines, whereas the leucoencephalomyelitis in broods within one and four months of age is the least frequent (DAWSON, 1980; EAST et al., 1993; CALLADO et al., 2001).

The principal transmission way of the SRLV is through the intake of colostrum or of infected goats' milk. However, the horizontal transmission may occur through secretions and excretions, contaminated tools and equipments, and long contact between healthy and infected animals (ADAMS et al., 1983; ALVES, 1999; PETERHANS et al., 2004). This infection is insidious presenting the clinical signs within months or years after the infection, whilst most of the animals do not present symptomatology. Nevertheless, the production patterns are affected, specially in milk goats SMITH & CUTLIP, 1988; GREENWOOD, 1995).

In Brazil, the first description of SRLV in caprine was made by MOOJEN et al. (1986) in Rio Grande do Sul State. Serological studies with probabilistic

sampling have shown the presence of positive animals in many States, principally in commercial exploration milk herds. The observed prevalences varied from 0.73% to 43.0% in researches in the following States: Pernambuco (SARAIVA NETO et al., 1995), Ceará (PINHEIRO et al., 2001), São Paulo (LEITE et al., 2004), Paraíba (BANDEIRA, 2009), Rio Grande do Norte (SILVA et al., 2005), Bahia (OLIVEIRA et al., 2006), Piauí (SAMPAIO JÚNIOR, 2007), and Rio de Janeiro (MOREIRA et al., 2007).

Considering the growth of caprine raising in Tocantins and the lack of information about SRLV in caprine in the State, the objective of this work was to estimate the prevalence of caprine blood reagents to SRLV in Tocantins State, and to verify whether there is association among microregion, breed, age, sex, raising system and herds with associated species, and the SRLV occurrence.

## MATERIAL AND METHODS

Tocantins State, with an area of 286,706 Km<sup>2</sup>, is located in the Northern region of the country, in Legal Amazonia, and has the geographical coordinates: longitude - 46° 00' and 51° 00' of Greenwich and latitude - 05° 00' and 13° 00' S. The vegetation is very varied, from cerrado (savanna) meadow, cerradão, rupestrian grassland, to transition equatorial Forest, under gallery forest form. The climate is semi-humid, with average annual temperature of 26°C. Rainfall varies from 1,500mm to 1,800mm/year, and the characterize a distribution which defines two periods: dry from May to August, and rainy from September to May (NASCIMENTO, 2007).

The SRLV prevalence in caprine was estimated based on a non-probabilistic sample study, involving the municipalities of Araguatins, Araguaína, Xambioá, Babaçulândia, Filadélfia, Barra do Ouro, Araguacema, Dois Irmãos do Tocantins, Miracema do Tocantins, Pium, Paráíso, Palmas, Natividade, Dianópolis and Ponte Alta do Bom Jesus (Figure 1). The number of animals used in this research was calculated by the Epi-Info software, version 3.3.2 (DEAN et al., 1992), through the following formula, described by KISH (1965):

$$N = Z \cdot Z [ P (1 - P) ] / D \cdot D,$$

Where:

N – number of samples to estimate the prevalence in infinite population;

P – expected prevalence (5%);

Z – determining factor of the 90% reliability level ( $Z=1.64$ ); and

D – sampling error (25% P)

This way, "N" equal to 818 samples was obtained. The number of collected samples per property varied from 16 to 30, in a total of 843 samples in 29 properties. The animals' age was estimated based on the number of teeth the animals presented: first change (less than 24 months), and more than two changes (more than 24 months) (JARDIM, 1985).

The blood was collected through vein puncture of the jugular, by the use of vacuum system. After the blood clot retraction, the samples were centrifuged at 800g for ten minutes and transferred to freezing tubes (1.5mL), which remained at -20°C until the processing moment.

For the detection of antibodies against SRLV the microtechnique of agar gel immunodiffusion (AGID) was used, by the means of a commercial kit<sup>1</sup>, composed of antigen, positive pattern serum, and agarose solution at 1% in borate buffer, according to the manufacturer's recommendations.

For the data analysis, the Pearson's Chi-Square test or Fisher's exact test were used, when the conditions for the Chi-Square test utilization were not verified. A 5% significance level was used in the decisions about the statistical tests. The software used for obtaining the statistical calculations was Epi-Info, version 3.2.2 (DEAN et al., 1992).

## RESULTS AND DISCUSSION

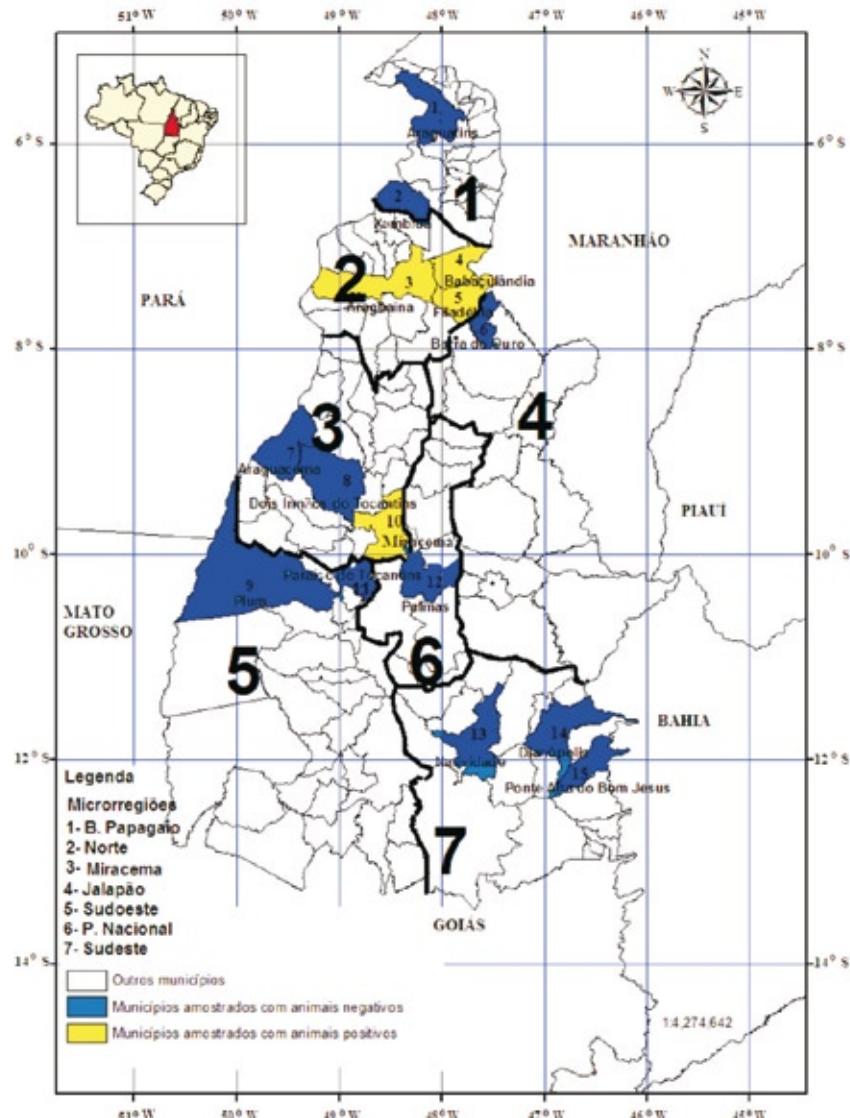
From the 843 serum samples were examined by the AGID test for the research of antibodies against SRLV, 23 (2.7%) were positive. Five flocks were identified, being two in the municipalities of Araguaina, and one in each of the municipalities of Babaçulândia, Filadélfia and Miracema do Tocantins (Figure 1). From the 29 herds, Five (17.2%) presented at least one blood reagent animal.

The low prevalence found by this research may be possibly explained by the recent herd formation in the State, composed basically by anglo-nubian and undefined breed (UB) animals (UB) that, according to SARAIVA NETO (1995), have presented prevalence level inferior to the one of the European milk breeds. Besides, practices which tend to increase the risk of horizontal transmission, such as feedlot and the use of collective bottle, are not adopted by the researched properties (ADAMS et al., 1983; ALVES, 1999). However, these results are considered important due to possibility of SRLV dissemination to other caprine herds. Moreover, the breeding systems intensification intending to increase productivity might offer conditions for the virus dissemination COSTA et al., 2007).

Blood reagent animals to SRLV were found in two microregions, being 10% among the animals of northern microregion and 1.4% among the animals from Miracema (Table 1). Such results indicate significant association between the SRLV prevalence and the microregions ( $p<0.05$ ). The high positivity found in the northern microregion is possibly due to fact that the animals belong to exploration herds for Milk production.

In the studied properties only five breeds were reported (Anglo-nubian, Saanen, undefined breed – UB –, Boer and Jamnapari), demonstrating recent adhesion to caprine raising by the State breeders. It was also verified that the positivity for SRLV was higher among the Saanen animals, with 11.7% (7/60), followed by the Anglo-nubian, 3.0% (14/452), and last by the undefined breed animals (UB), 0.6% (2/310). According to the result analysis, significant association between breed and SRLV occurrence was confirmed ( $P < 0.05$ ) (Table 2).

One reason for the smaller prevalence in undefined breed animals (UB) with regards to pure breed animals is possibly related to the management system, because some practices that may increase horizontal transmission, such as feedlot and the use of collective bottles, occasionally adopted at some properties, are usually employed for the management of pure milk herds (SARAIVA NETO et al., 1995). The presence of SRLV among almost all milk animals may represent a dissemination risk of the agent to the undefined breed animals (UB) (PINHEIRO et al., 2001; BANDEIRA, 2005).



**FIGURE 1:** Geographical representation of the municipalities in Tocantins State representative of the sample SRLV in caprine.

**TABLE 1** - Distribution of caprine positive to agar gel microimmunodiffusion (MICRO-AGID) for small ruminants lentiviruses, per microregion in caprine herds from Tocantins State, 2006.

Microregion	MICRO – AGID					
	Positive				Negative	
	N	n	%	n	%	
Bico do Papagaio	46	0	0,0	46		100,0
North	200	20	10,0	180		90,0
Jalapão	60	0	0,0	60		100,0
Miracema	207	3	1,4	204		98,6
Southwest	90	0	0,0	90		100,0
Porto Nacional	150	0	0,0	150		100,0
Southeast	90	0	0,0	90		100,0
Total	843	23	2,7	820		97,3

P = 0,0001, CHI-Square test

**TABLE 2** - Distribution of caprine positive to agar gel microimmunodiffusion (MICRO-AGID) for small ruminants lentiviruses, per breed from Tocantins State, 2006.

Breed	MICRO – AGID					
	Positive				Negative	
	N	n	%	n	%	
UB	310	2	0,6	308	99,4	
Anglo-nubian	466	14	3,0	452	97,0	
Saanen	60	7	11,7	53	88,3	
Total	843	23	2,7	813	97,3	

P = 0.0001, by Chi-Square test

As regards the animals' age, the prevalence of positive blood reagents to SRLV was 3.2% among animals within 2nd and 4th change, and 1.9% among the animals in the age group until 1st change. The results analysis did not prove significant association between age group and SRLV occurrence ( $p>0.05$ ) (Table 3). The superiority of positivity between older animals may be explained by a longer exposure time to the virus, due to the slow evolution of the infirmity (MCGUIRE, 1987). A long time is necessary for the animal to produce levels of antibody which are detectable at AGID test, what may occur 18 months after detection by PCR (polymerase chain reaction) or even it may never occur (WAGTER et al., 1998).

Table 4 shows the results of the serological tests relative to the animal's sex. The prevalence of positive animals to SRLV was only 0.4% higher among the females than among the males (2.8% x 2.4%) and a significant association was not proved between sex and SRLV occurrence ( $p>0.05$ ). These results confirm

the ones by CRAWFORD & ADAMS (1981) and SARAIVA NETO (1995), Who stated that there were no factors related to the sex that would predispose the animal to SRLV infection. However, FERNANDES et al. (2003) reported that due to the longer time the females remain together in the herd and in the same stall, while reproductive males remain in separated stalls, there can be a higher dissemination of the virus among the females.

According to the management system, positive animals were observed in 6.6% (1/15) and 28.4% (4/14) of the properties that adopt extensive and semi extensive breeding, respectively (Table 5). The frequency of positive animals was higher in the semi-extensive system, but there was no significant difference between breeding system and the occurrence of positive animals per herd ( $p > 0.05$ ). The breeding system is an important factor to be considered, once the agglomeration favours the SRLV dissemination (CASTRO & MELO, 2001; FERNANDES et al., 2003).

**TABLE 3** - Distribution of caprine positive to agar gel microimmunodiffusion (MICRO-AGID) for small ruminants lentiviruses, per age (change) from Tocantins State, 2006.

Age (change)	MICRO – AGID					
	Positive				Negative	
	N	n	%	n	%	
Until 1 <sup>st</sup>	314	6	1,9	308	98,1	
From 2 <sup>nd</sup> to 4 <sup>th</sup>	529	17	3,2	512	96,8	
Total	843	23	2,7	820	97,3	

P = 0.2616, by Chi-square test

**TABLE 4** – Distribution of caprine positive to agar gel microimmunodiffusion (MICRO-AGID) for small ruminants lentiviruses, per sex from Tocantins State, 2006.

Sex	MICRO – AGID				
	Positive		Negative		%
	N	n	n	%	
Males	166	4	162	2,4	4
Females	677	19	658	2,8	19
Total	843	23	820	2,7	23

P= 1.000, by Fisher exact's test.

**TABLE 5** -Distribution of caprine positive to agar gel microimmunodiffusion (MICRO-AGID) for small ruminants lentiviruses, per breeding system from Tocantins State, 2006.

Breeding system	MICRO – AGID				
	Positive		Negative		%
	N herd	n	n	%	
Extensive	15	1	14	6,6	93,4
Semi-Extensive	14	4	10	28,6	71,4
Total	29	5	24	17,2	82,8

P = 0.1432, by Fisher exact's test.

In Table 6 the results relative to herds with or without species association are found. In the herds where caprine are reared the percentage of blood reagent animals was of 18.7% (3/16), and in the herds with association of caprine and ovine it was of 15% (2/15). There was no significant association ( $p > 0.05$ )

between the caprine + ovine associated management or the single management of caprine and the occurrence of SRLV in the studied herds (Table 6). However, filogenetic studies indicate the existance of transmission between caprine and ovine (OLIVER et al., 1985; CASTRO et al., 1999; SHAH et al., 2004).

**TABLE 6** -Distribution of caprine positive to agar gel microimmunodiffusion (MICRO-AGID) for small ruminants lentiviruses, per herd with species association from Tocantins State, 2006.

Species	N	AGID		
		Herd	Positive n (%)	Negative n (%)
Caprine	16	3	(18,7)	13 (81,3)
Caprine + Ovine	13	2	(15,4)	11 (84,6)
Total	29	5	(17,2)	24 (82,8)

P = 0.6038, by Fisher exact's test.

## CONCLUSION

The SRLV is present, with low prevalence, in caprine herds form Tocantins State, mainly in the north

microregion and in animals of the Saanen breed. These data confirm the importance of implementing measures to control small ruminants lentiviruses in the State.

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