ARTÍCULO ORIGINAL

Frequency of seropositive equines for Theileria equi in the Southern Rio Grande do Sul State, Brazil

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ABSTRACT

A study on equine theileriosis was carried out in the southern region of the Brazilian state of Rio Grande do Sul (RS). Blood samples were collected from 113 mares from an equine breeding farm located in the city of Bagé, latitude 31°30'S and a longitude of 54°10'W. The serological testing was carried out with the use of indirect fluorescence test (IFAT) and compared with Nested Polymerase Chain Reaction (nPCR). Among the sera collected from 118 horses, 25 were found positive to Theileria equi by the IFAT, while by nPCR 17 positive for T. equi was observed, corresponding to a frequency of 22.1% and 15.0%, respectively. The racial analysis showed 15.05% (14) thoroughbred and 55% (11) Crioulo breed horses to be positive by IFAT.

Key words: *Theileria equi*, IFAT, prevalence.

INTRODUCTION

Equine piroplasmosis, caused by *Theileria equi* and *Babesia caballi*, is considered to be the most important tick-borne disease of horse in tropical and subtropical areas¹. Clinical manifestation of the disease is variable including fever, anemia, icterus, lethargy, and in some cases death².

In endemic countries like Brazil, the control of equine piroplasmosis is important to keep international markets open to the horse industry³. For this reason many countries prohibit the importation of horses because of the high prevalence of asymptomatic carrier animals in the region. The southern area of the Rio Grande do Sul State thoroughbred racing industry is particularly badly affected by equine

piroplasmosis, acute infections resulting in missed training sessions and races and hence serious loss of income to owners and trainers. Income is also lost to owners through abortion in stud mares which are *T. equi* carriers.

It has also been shown that strenuous exercise, such as that experienced with horse-racing, can cause sub-clinical infections to become acute^{4,5}.

Carrier animals are responsible for the maintenance of the infection⁶. Parasites are present, in these animals, in very low numbers in the blood and they may not be detected in Giemsa-stained blood smears⁷.

Equine piroplasmosis can be made by microscope examination of Giemsa-stained blood smears or by specific polymerase chain reaction⁷, but complement fixation test is

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recommended for the detection of carrier animals⁸. Using the conventional thin blood smear examination, indirect fluorescence antibody test, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) babesiosis has been documented in Brazil⁹⁻¹³.

Dermacentor (Anocentor) nitens ticks are the vectors for *B. caballi*¹⁴ and, in Brazil, it is known that *Rhipicephalus (Boophilus) microplus* can transmit *T. equi*¹⁵⁻¹⁷.

The state of Rio Grande do Sul has an area of 281.749 Km² and the population of horses is estimated to be 468.447 head, equivalent to 8.15% of the national herd. The region has a subtropical weather, precipitation between 400 and 1200 mm per year. The climate is characterized by a distinct dry season (winter) from June to September and a wet season (summer) from December to March¹⁸.

In the present study, we performed an epidemiological study on equine piroplasmiasis in one of the most important area for the international horse industry in Rio Grande do Sul State.

MATERIAL AND METHODS

For the purpose of this study, blood samples from 113 mares were collected from an equine breeding farm located in the city of Bagé, Rio Grande do Sul State, Brazil, latitude 31°30' S and a longitude of 54°10' W.

From all mares 93 were Thoroughbred racehorses raised in stables and without limited access to the pasture and without contact with ticks and 20 of the Crioulo horse (male and female) breed with access to the pasture and raised with cattle infected with *R. microplus*.

The blood samples were collected from the jugular vein and placed into two tubes using a vacutainer blood collection set. One tube contained ethylenediaminetetraacetic acid (EDTA, at a concentration of 1 mg.ml⁻¹) was centrifuged (500 g for 5 min) and the supernatant and the buffy coat were removed. Blood samples were immediately used for DNA isolation. The second tube contained no anticoagulant, and the blood was left at room temperature to coagulate. Then the sera was collected and kept at -20°C until used.

For the nest PCR, DNA from 250µl of each blood sample was prepared using the Purogene kit (Gentra Systems, Minneapolis, MN, USA)

according to the manufactures protocol. The final pellet was dissolved in 30µl of water and reaction was performed as described by Nicolaiewsky¹⁹.

Blood from un-infected horse DNA was obtained from Veterinary College of Federal University of Pelotas, Rio Grande do Sul State, Brazil and used as a negative control. For examination of blood parasites, blood films were prepared at the time of sampling and stained with Giemsa stain. Both thin and thick blood smears were stained with Giemsa Stain²¹ and observed with Bright-field light microscopy at 1000x for the presence of the *T. equi* and *B. caballi*. No apparent clinical signs were observed on all the sampled horses by macroscopic examination.

The slides were prepared with infected horses erythrocytes in which are visible as compact inclusion by IFAT staining. IFAT was performed according to the Cunha¹⁰, using Anti-Horse IgG conjugate with FITC produced in rabbit. The positive control was used a reference sample previously identified¹⁰. Serum samples were diluted at 1:80 dilution in phosphate buffered saline (PBS), pH 7.4 and applied at 10 µl/well and were graded either as negative (-) or positive (+ to ++++). The size, appearance and density of staining were compared with the positive control where a positive reaction was seen as apple green fluorescent inclusion within the infected erythrocytes, while a negative reaction gave a uniformly red counter stain or uniform greenish staining (Nikon UV microscope, 490 nm excitation/520 nm emission). The differences between different parameters were evaluated by using the chi-square test (χ^2).

RESULTS AND DISCUSSION

Among the sera collected from 118 horses, 25 were found positive to *T. equi* by the IFAT, while by nPCR 17 positive for *T. equi* was observed, corresponding to a prevalence of 22.1% and 15.0%, respectively (Table 1). The distribution of the positive results for groups and the average prevalence of the infection for *T. equi* by IFAT are showed in Table 2. None of the horses showed signs of clinical piroplasmiasis and *B. caballi* were not detected by examination of Giemsa-stained blood smears neither by nPCR. The agreement between the tests was of 87.6%

and observed itself high association between the techniques ($\chi^2 = 42.13$; $p < 0.001$). Using the nPCR we observed that the sensibility and the specificity of the EMA-1 gene of *T. equi* were the same found by Nicolaiewsky¹⁹.

In 3 samples the results of the nPCR were positive and the results of IFAT negative. This suggests that being a recent infection might not have the time necessary for an antibody response to the infection. Weiland²² observed that horses artificially infected with *T. equi* and *T. caballi* delayed of 3 to 20 days to produce antibodies, which might corroborate with our observation. In other hand, 11 animals were observed positive results for IFAT and negative for nPCR, probably these horse are not anymore in the acute phase of the infection or the sample are not well manipulated.

Transmission of piroplasmosis is usually influenced by the dynamics of vector populations, and these are directly influenced by climatic conditions²⁴. In Rio Grande do Sul State the tick *R. microplus* is widespread and probably serve as agents of transmission for this hemoparasite¹⁵.

The percentage of seropositive animals in the studied population was relatively high but below than the seroprevalences to *T. equi* found in other studies in different states of Brazil. Authors have demonstrated occurrences varying from 49.2% to 100% in the southern and south-eastern states with different epidemiologic conditions but with high tick infections^{9-13,25}.

The effects of the variation of the prevalence has been observed with different categories and breeding systems in different regions of Brazil^{25,26}. It is evident in this study, whereas Crioulo breed horses were grazed with cattle in the same pasture had higher titers than the thoroughbred horses.

The management of the horses appears to be an important factor for the prevalence of *T. equi* infections. Among horses raised with access to pasture there was a significant difference in the seropositive percentage of reactors (15.05%) compared with horses without access to pasture (55%).

Our data are corresponding with another study that found 17.5% of the horses infected in professional stud farms, whereas the horses were grazed without contact with cattle¹⁶.

Among the positive animals the antibodies titers ranged from 1:80 to 1:1280, with 64% of the titers between 1:320 and 1:640, being these

Table 1. Distribution of IFAT and nPCR results

Assays/Results		IFAT		Total
		Positive	Negative	
nPCR	Positive	14	3	17
	Negative	11	85	96
	Total	25	88	113

Table 2. Prevalence of antibodies against *T. equi* in horses' thoroughbred and Crioulo, by IFAT

Categories	N ¹	P ²	(%)
Thoroughbred	93	14	15.05
Crioulo	20	11	55.00
Total	113	25	22.12

1- N: Number of Animals; 2- P: Positive for *T. equi*.

two titers more frequent. A similar serologic distribution was observed with horses breeding with access to pasture²⁶.

The prevalence and the titers average of the Crioulo breed were higher than for animals without access to the pasture (thoroughbred) (Table 2 and Table 3). These results are explained for the breeding with herds of other species, in native pasture, where have a higher contact with bovine ticks.

These data are in agreement with described by other studies, whereas the authors found high prevalence in horses utilized for to work with cattle and breeding in pasture considered infected with ticks^{27,16}.

Previous studies have shown that exist correlation between the occurrence of *Rhipicephalus microplus* and the prevalence of theileriosis^{27,14,28}.

The prevalence of reagents' horses to *T. equi* in this study demonstrated that the region is an endemic area of equine theileriosis. In addition, the animals with contact to bovine herds are more exposed and prone to *T. equi* infection than stable animals.

Our results confirm the importance of the control of *T. equi* and demonstrate that the horse population of Southern Rio Grande do Sul State is exposed to *T. equi* infection and suggest that equine piroplasmosis is widespread in the area. Therefore, we anticipate that future research will maximize the effectiveness of the present

Table 3. Absolute and relative frequencies of titers against *T. equi*, by IFAT

Titers	Categories				Total	
	Thoroughbred N ¹	(%)	Crioulo N ¹	(%)		
80	2	14.3	1	9.1	3	12.0
120	2	14.3	1	9.1	3	12.0
320	5	35.7	3	27.3	8	32.0
640	4	28.6	4	36.4	8	32.0
1280	1	7.1	2	18.1	3	12.0
Total	14	100.0	11	100.0	25	100.0

study for clarifying the epidemiology of the equine piroplasmosis.

RESUMO

Foi realizado um levantamento sobre theileriose eqüina em uma propriedade da região sul do estado do Rio Grande do Sul (RS). Amostras de sangue foram coletadas de 108 éguas de um haras localizado no município de Bagé, latitude 31°30' Sul e longitude 54°10' Oeste. A sorologia foi realizada com o uso da imunofluorescência indireta (IFAT) e utilizou-se a técnica de Nested Reação em Cadeia Polimerase (nPCR). Do total de amostras examinadas, 22% (24) foram positivas por IFAT e 15% (16) por nPCR). Na análise por raça, 15.05% (14) dos animais Puro Sangue Inglês (PSI) foram soro-positivos, e dos animais da raça Crioula (RC), 55% (11) foram positivos por IFAT.

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