Research Note

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Prevalence of antibodies against *Neospora* spp. and *Sarcocystis neurona* in donkeys from northeastern Brazil

Prevalência de anticorpos contra *Neospora* spp e *Sarcocystis neurona* em jumentos do nordeste do Brazil

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Abstract

Sarcocystis neurona and *Neospora hughesi* are coccidian protozoa that can cause neurological illness in horses in America. In this study we report seroprevalence of *Neospora* spp. and *S. neurona* in sera of 333 donkeys from the northeastern region of Brazil. Antibodies to *Neospora* spp. were detected in 2% (7 donkeys) of 333 sera tested by the indirect fluorescent antibody test (IFAT) with a cut-off dilution of 1:40. Antibodies to *S. neurona* were found in 3% (10 donkeys) of the samples tested by IFAT (cut-off ≥50) and 21% (69 donkeys) by the direct agglutination test (SAT ≥50). The SAT and IFAT results for *S. neurona* showed a poor concordance (value of Kappa=0.051). This is the first report of *Neospora* spp. antibodies in Brazilian donkeys and the first detection of antibodies against *S. neurona* in this animal species.

Keywords: *Neospora hughesi*, *Neospora caninum*, *Sarcocystis neurona*, donkey, Brazil.

Resumo

Sarcocystis neurona e *Neospora hughesi* são protozoários coccídios que infectam equídeos e são responsáveis por doenças neurológicas nessas espécies. Neste estudo, a soroprevalência de infecção por *S. neurona* e *Neospora* spp. foi determinada em amostras de 333 soros de jumentos da Região Nordeste do Brasil. Anticorpos contra *Neospora* spp. foram detectados em 2% (7 jumentos) dos 333 animais examinados pela reação de imunofluorescência indireta (RIFI), com ponto de corte de 40. Anticorpos contra *S. neurona* foram detectados em 3% (10 jumentos) das amostras pela RIFI (ponto de corte de 50) e em 21% (69 jumentos) pela técnica de aglutinação direta (SAT - ponto de corte de 50). SAT e RIFI, para diagnóstico de *S. neurona,* apresentaram uma baixa concordância (Kappa = 0,051). Essa é a primeira observação de anticorpos anti-*N. caninum* em jumentos brasileiros e a primeira detecção de anticorpos contra *S. neurona* nessa espécie.

Palavras-chave: *Neospora hughesi*, *Neospora caninum*, *Sarcocystis neurona*, jumentos, Brasil.

Introduction

Equine protozoal myeloencephalitis (EPM) is a neurological disease of horses that is caused primarily by *Sarcocystis neurona* and less frequently by *Neospora hughesi* (DUBEY et al., 2001, 2015). *N. hughesi* infection has also been reported to cause abortion in horses (PUSTERLA et al., 2014).

Horses become infected with *S. neurona* after ingesting sporocysts shed by opossums, *Didelphis* spp. In North America, *D. virginiana* is the definitive host for *S. neurona,* and in South America the opossum *D. albiventris* is a proven definitive host (DUBEY et al., 2001). The definitive host for *N. huguesi* is unknown. Serologically antibodies against *N. hughesi* will cross-react with *N. caninum* antigen and antibodies to *N. caninum* will react to *N. hughesi* antigen (GONDIM et al., 2009). Sera reactive to *N. caninum* antigen in the present study were considered positive for antibodies to *Neospora* spp.

In Brazil, antibodies to *Neospora* spp. and *S. neurona* have been reported in horses (see reviews DUBEY & SCHARES, 2011; DUBEY et al., 2015) but there is no report for these infections in donkeys.

Information regarding *Neospora* spp. and *S. neurona* exposure in donkeys (*Equus asinus*) from other countries is scarce (MACHACOVÁ et al., 2013; BLANCO et al., 2014). Donkeys

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are used traditionally for working roles, however in some parts of the world they are increasingly being used for milk production.

Here we report seroprevalence of *Neospora* spp. and *S. neurona* in donkeys from Brazil.

Materials and Methods

Serum samples were obtained from 333 donkeys (*Equus asinus*) from rural properties, located in the northeastern region of Brazil (Table 1). We used a convenience sampling technique and the animals included in the sample were those available at the time of the survey. All animals were from both genders and different ages, and were mainly bred for working roles. All procedures were conducted in accordance with the Animal Protocols approved by the Ethic Committee of the Faculty of Veterinary Medicine, University of São Paulo, Brazil.

The indirect fluorescent antibody test (IFAT) was used to detected antibodies against *Neospora* spp. and was conducted according to the method described by Dubey et al. (1988) using tachyzoites of NC-1 *N. caninum* isolate as antigen. *Sarcocystis neurona* merozoites of the SN3 isolate (GRANSTROM et al., 1992) were used as the antigen in *S. neurona* IFAT and in the direct agglutination test (SAT) for *S. neurona*. For IFAT, tachyzoites or merozoites were collected from cultures, washed in PBS (NaCl 0.142M; KCl 0.003M; Na_2HPO_4 0.008M; NaH_2PO_4 0.001M, pH 7.4), counted with an hemocytometer to a concentration of $2x10^7$/mL, distributed in 12-well slides, air dried, fixed with methanol and stored at –20°C until used. Sera were distributed on the wells, incubated at room temperature in wet chambers for 30 min, and then the slides were soaked in PBS three times for 5 min; after the slides were air dried, the conjugate was applied, and the slides were incubated and processed as described above. Fluorescein-labeled affinity-purified antibodies against horse IgG were used as conjugate in both IFATs. For *S. neurona* IFAT, the cut-off value was 1:40 and for *N. caninum*, 1:50. Every positive serum was retested using a twofold serial dilution. Positive and negative *S. neurona* and *N. caninum* horse samples were added in each slide. The SAT was performed according Lindsay & Dubey (2001) with a cut-off value of 1:50. Positive and negative control sera were used to validate the results of each SAT.

The proportions of positive samples from the Brazilian states were compared for *Neospora* spp. and *S. neurona* using the Fisher's exact test (ZAR, 2010) with a P value <0.05 being significant.

Results and Discussion

The prevalence of antibodies to *Neospora* spp. in donkeys was 2% (95% CI: 0.8%-4.3%) and only donkeys from the states of Alagoas and Pernambuco were positive (Table 1). Occurrence of *S. neurona* was 21% (95% CI: 17%-26%) by SAT (titers between 50 and 200) and 3% (95% CI: 1.5%-5.5%) by IFAT (titers between 40 and 160) and positive donkeys were found in all five analyzed States by SAT, but not in the state of Paraíba by IFAT (Table 1). Five of the 10 IFAT positive donkeys were also SAT positive. The SAT and IFAT results for *S. neurona* showed a poor agreement (value of Kappa=0.051).

In Italy, occurrence of antibodies to *Neospora* spp. in donkeys was 11.8% using a competitive-ELISA (MACHACOVÁ et al., 2013) and in Colombia, 11 of the 56 examined donkeys presented antibodies against *Neospora* spp. measured by Dot-ELISA (BLANCO et al., 2014). Due the different methodology used in the studies, comparisons between these studies and the present study are difficult to make.

The results for the comparisons between the proportions of positive animals from the Brazilian states are shown in Table 1. For *S. neurona* statistical analyzes were done using both the SAT and IFAT results. For the SAT results, significant differences (P <0.05) were observed for *S. neurona* prevalence between the samples from Alagoas and the samples from Pernambuco, Piauí and Rio Grande do Norte, and between the samples from Pernambuco and Piauí. No differences were found between the prevalence value and location for the IFAT results for *S. neurona* and also when antibodies to *Neospora* spp. were analyzed.

This is the first report of *Neospora* spp. antibodies in Brazilian donkeys and the first detection of antibodies against *S. neurona* in this animal species.

This is the first study related to *S. neurona* occurrence in donkeys and the prevalence of 21% (95% CI: 17%-26%) by SAT found was lower than the prevalence of 36.0% to 69.6% found in Brazilian horses (DUBEY & SCHARES, 2011; PIVOTO et al., 2014). In this study there was a poor agreement between IFAT and SAT. There is no comprehensive study of the sensitivity and

Table 1. Prevalence of antibodies to *Neospora* spp. and *Sarcocystis neurona* in donkeys from northeastern Brazilian States.

State	*Neospora* spp.			*Sarcocystis neurona*				
	IFAT			SAT			IFAT	
	Examined	Positive	%	Examined	Positive	%	Positive	%
Alagoas	74	3	4.0[a]	70	27	38.6[a]	2	2.9[a]
Paraíba	30	0	0.0[a]	30	6	20.0[abc]	0	0.0[a]
Pernambuco	117	4	3.4[a]	117	28	23.9[b]	5	4.3[a]
Piauí	77	0	0.0[a]	77	5	6.5[c]	2	2.6[a]
Rio Grande do Norte	35	0	0.0[a]	35	3	8.6[bc]	1	2.9[a]
TOTAL	**333**	**7**	**2.0**	**329**	**69**	**21.0**	**10**	**3.0**

Different letter in the columns P <0.05 (Fisher's exact test).

specificity of the SAT in equids (DUBEY et al., 2001, 2015). Immunoblot is considered the golden test for seroprevalence studies of *S. neurona* in horses, but it is expensive and laborious. DUARTE et al. (2003) showed that IFAT could be an alternative to immunoblot for *S. neurona* antibody detection in horses, with good specificity and sensitivity. Cross-reactivity of both tests with other protozoa from donkeys is not known.

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