

Communication

[Comunicação]

Occurrence of antibodies anti-*Neospora* spp. in cart horses and Crioula breed horses from Rio Grande do Sul State

[Ocorrência de anticorpos anti-*Neospora* spp. em cavalos de carroça e equinos da raça Crioula no estado do Rio Grande do Sul]

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The protozoa of the genus *Neospora* spp. (Apicomplexa, Sarcocystidae) are capable of infecting equines causing different clinical manifestations. However, the pathogenesis of these protozoa in horses has not been fully explained yet. Two species of the genus *Neospora* spp. have already been described parasitizing equines - *Neospora caninum* and *Neospora hughesi*. The infection by *N. caninum* is mainly characterized by reproductive problems and neonatal illness, and *N. hughesi* is associated with myeloencephalitis (Dubey and Porterfield, 1990; Lindsay, 2001).

N. caninum was firstly described in dogs in Norway (Bjerkas et al., 1984), from there on, it has been described in many warm-blooded species (Dubey and Lindsay, 1996). *N. hughesi* has been reported for the first time by Marsh et al. (1998) who isolated a protozoan very similar to *N. caninum* (however, with structural and molecular differences) from brain and spinal marrow of a horse that was showing signs compatible with equine protozoal myeloencephalitis (EPM).

The biological cycle of *N. caninum* was described by McAllister et al. (1998) and involves definitive (DH) and intermediate (IH) hosts. The definitive ones are dogs and coyotes (McAllister et al., 1998; Gondim et al., 2004) and, besides sheltering the agent, they are

characterized by excreting oocysts in their feces. These oocysts sporulate in the environment becoming infective for the IH, group in which fit several mammalian species (Dubey and Lindsay, 1996).

N. caninum can disseminate itself in susceptible populations by the horizontal route (sporulated oocysts), and by the vertical transmission via placenta (by tachyzoites). In the stage of tachyzoite, the protozoan is able to accomplish parasitaemia and new cells invasion. The infected IH develop cysts containing bradyzoites (slow multiplication form), and the DH are infected by eating tissues containing these cysts (Dubey et al., 2007).

The biological cycle of *N. hughesi* was not entirely elucidated until the moment; its definitive host is unknown, as well as other possible intermediate hosts besides equines. Consequently, the infection routes for *N. hughesi* in horses are not determined (Hoane et al., 2006). The discovery of *N. hughesi* as a possible etiological agent of EPM turned out to be of great importance in its diagnosis, since *Neospora* spp. has been reported in cases of this pathology (Marsh et al., 1998). However, it is still undefined if both protozoan species are able to cause this kind of myeloencephalitis.

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In Brazil, studies concerning prevalence of *Neospora* spp. in equines are rare, with detection indexes ranging from 2.5% (Hoane et al., 2006) to 47% (Locatelli-Dittrich et al., 2006). Besides, Dubey et al. (1999a) tested serum samples of 101 Brazilian horses and found no antibodies anti-*Neospora* spp. The aim of the present study was to evaluate the frequency of antibodies anti-*Neospora* spp. and to compare the occurrence of these antibodies in cart horses and Crioula breed horses, all from central region of Rio Grande do Sul State.

Serum samples were obtained from 241 horses aging from four to 14 years old, being 91 from cart horses and the other 123 from Crioula breed horses. The blood samples were collected from jugular vein in vacutainer tubes. After the retraction of the clot, the serum was collected and kept under low temperature or frozen until processing. The cart horses were used to carry loads and most of them had no defined breed and Crioula breed horses were from stud farms. Only animals that were in a strictly characteristic epidemiological situation were included in each group.

The search for antibodies anti-*Neospora* spp. was made using indirect fluorescent antibody test (IFAT). The IFAT was performed on microscope slides containing tachyzoites of NC-1 strain, fixed with methanol. Serum samples were diluted into PBS (1:50) and used as primary antibody (incubated at 37°C for 30min in a humid chamber). As secondary antibody, equine anti-IgG^{®1} conjugate to fluorescein (diluted to 1:75 and incubated as previously described) was utilized. The slides were analyzed using an epifluorescent microscope and samples that showed peripheral or diffuse fluorescence were considered positive.

The statistical analysis of the data was done by the Tukey test with significance level of 5%. This test was performed using the SAS/1996 software.

The frequency of antibodies against *Neospora* spp., including both populations, was 15.9% (34/214). The frequency of positive serology in the cart horses was 15.4% (14/91) and in Crioula breed was 16.3% (20/123). There was no significant difference in antibodies frequency between the analyzed groups of horses.

The frequency of antibodies anti-*Neospora* spp. was high. Dubey et al. (1999a,b) did not detect antibodies anti-*Neospora* spp. in horses neither from Brazil (0/101) nor from Argentina (0/76). Hoane et al. (2006) found a prevalence of 2.5% (24/961) in equine serum samples from different regions of Brazil, although the prevalence was 3.5% (5/172) in the States of Rio Grande do Sul, Santa Catarina, and Paraná. Villalobos et al. (2006) detected a frequency of 10.3% (114/1106) for antibodies anti-*Neospora* spp. in horses in the State of São Paulo. Also, in mares with history of reproductive problems, as abortion on the third gestational trimester or neonatal mortality, the percentage of positive animals increased to 15.1% (73/483).

In the United States, Cheadle et al. (1999) and McDole and Gay (2002) found, respectively, 11.5% and 10% of seropositive horses for *Neospora* spp.. However, in other studies, the indexes of antibodies detection were higher. Dubey et al. (1999c) noticed 23.3% of seropositivity in equines in an abattoir in the USA. In Italy, Ciaramella et al. (2004) found a prevalence of 28% (42/150).

In the present study, it was not taken into account neither the report of possible reproductive problems nor the occurrence of EPM. Probably, the highest indexes of anti-*Neospora* spp. antibodies should be found in serum samples from animals and/or from farms with a history of reproductive or neurological diseases. Anyway, the results found here show that these protozoa are present in a relevant form in both populations of horses.

The similar frequency of seropositivity found in cart horses and Crioula breed horses could be due the two routes of infection by *Neospora* spp.: vertical (transplacental) and horizontal (ingestion of sporulated oocysts) transmissions. However, it is well known that the epidemiological aspects concerning both studied populations are totally different. Due to the open character of the population of cart horses (because of large mobility) a major infection source to these animals, probably, is the intake of sporulated oocysts present in the environment after the excretion by DH. Additionally, it should be considered that some of these horses may have been infected by vertical route. In Crioula breed horses, which belong to a controlled population

(with restricted mobility), it is presumable that both infection forms may have similar importance.

Other studies concerning the infection routes of *N. caninum* and *N. hughesi* in horses are necessary. Furthermore, it should be noted that, due to the pattern of the pathogenesis of these protozoa in equines, neosporosis should be included in the diagnosis of reproductive

problems in mares and in cases of EPM. The protozoa of the genus *Neospora* spp. are present in the studied equine populations. Further studies need to be performed with the aim of defining the matter related to transplacental passage of these protozoa in horses.

Keywords: horse, *Neospora caninum*, *Neospora hughesi*, IFAT

RESUMO

O objetivo deste estudo foi avaliar a frequência de detecção de anticorpos contra *Neospora* spp. em cavalos de carroça e em cavalos da raça Crioula. Para tal, 214 amostras de soro foram coletadas e analisadas pela técnica de imunofluorescência indireta, das quais 91 eram de cavalos de carroça e 123 de cavalos da raça Crioula, todas provenientes da região central do Rio Grande do Sul. As frequências de anticorpos detectadas foram: 15,9% (34/214) na população total estudada, 15,4% (14/91) nos cavalos de carroça e 16,3% (20/123) nos cavalos da raça Crioula. Estes resultados sugerem que a infecção por *Neospora* spp. está presente igualmente nas duas populações. Assim, devido à importância e ao padrão da patogênese da neosporose em equinos, *Neospora* spp. deve ser incluído no diagnóstico de problemas reprodutivos em éguas e em casos de problemas neurológicos em equinos.

Palavras-chave: equino, *Neospora caninum*, *Neospora hughesi*, RIFI

ACKNOWLEDGEMENTS

We are thanked to Professors Rudi Weiblen and Eduardo Furtado Flores to make available the Laboratory of Virology at the UFSM for the accomplishment of this study.

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