EQUINE NEOSPOROSIS: SEARCH FOR ANTIBODIES IN CEREBROSPINAL FLUID AND SERA FROM ANIMALS WITH HISTORY OF ATAXIA*

NEOSPOROSE EQUINA: PESQUISA DE ANTICORPOS NO LIQUIDO CEFALORRAQUIDIANO E SORO SANGUÍNEO DE ANIMAIS COM HISTÓRICO DE ATAXIA

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ABSTRACT. Stelmann U.J.P., Ullmann L.S., Langoni H. & Amorim R.M. [**Equine neosporosis: search for antibodies in cerebrospinal fluid and sera from animals with history of ataxia**]**.** Neosporose equina: pesquisa de anticorpos no liquido cefalorraquidiano e soro sanguíneo de animais com histórico de ataxia. *Revista Brasileira de Medicina Veterinária, 33(2):99-102, 2011*. Programa de Pós-Graduação em Medicina Veterinária. Departamento de Clínica Veterinária. Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista Júlio de Mesquita Filho, Campus de Botucatu, Distrito de Rubião Júnior, s/n, Botucatu, 18.618-970, SP. Brasil. E-mail: stelmann.ppgctia@gmail.com

Equine neosporosis is caused by the protozoans *Neospora caninum* and *N. hughesi*. Its clinical signs include hindlimb paralysis, incoordination, ataxia, and abortion. Serum and cerebrospinal fluid (CSF) samples were collected from 38 equines with history of ataxia for indirect fluorescent antibody test (IFAT) in order to detect antibodies to *N. caninum*. Of the tested serum samples 15/26 (57.6%) were positive, whereas all CFS samples were negative. These seronegative CFS samples suggest that *N. caninum* has no relation to the manifestation of the neurological clinical signs of ataxia.

KEY WORDS. *Neospora caninum*, *Neospora hughesi*, equine, cerebrospinal fluid, serum, Indirect fluorescent antibody test (IFAT).

RESUMO. A neosporose equina é causada pelos protozoários *Neospora caninum* e *N. hughesi*. Os sinais clínicos são, entre outros, paralisia dos membros posteriores, incoordenação, ataxia e aborto. Amostras de soro e de líquido cefalorraquidiano (LCR) foram coletadas de 38 equinos com histórico de ataxia. Para pesquisa de anticorpos anti-*N. caninum* foi utilizada a reação de imunofluorescência indireta (RIFI). Das amostras de soro testadas neste grupo de equinos, 15/26 (57,6%) mostraram-se positivas, enquanto que as amostras de LCR foram negativas. Os resultados negativos, nas amostras de LCR, sugerem que *N. caninum* não teve relação com a manifestação dos sinais clínicos neurológicos de ataxia.

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PALAVRAS-CHAVE. *Neospora caninum*, *Neospora hughesi*, equino, Liquido cefalorraquidiano , soro, Reação de Imunofluorescência indireta.

INTRODUCTION

Neosporosis is a disease that affects different animal species including equines (Lindsay et al. 1996, Daft et al. 1996). In the latter, it is caused by the protozoans *Neospora caninum* and *Neospora hughesi*, obligate intracellular parasites presenting many features still under epidemiological study (Hoane et al. 2006, Locatelli-Dittrich et al. 2006). Clinical signs include blindness, weight loss, hindlimb paralysis, unusual behavior, chewing difficulty, incoordination, ataxia, and abortion (Walsh et al. 2000). *Neospora caninum* is often associated with cases of abortion and stillbirths, while *N. hughesi* is related to the onset of neurological disease such as myeloencephalitis, which can also be caused by *Sarcocystis neurona* (Duarte et al. 2004). However, the presentation forms of *N. hughesi* in equines, as well as its definitive and intermediate hosts, remain unknown (Hoane et al. 2006).

Neospora hughesi has not been isolated in South America and most studies on seroprevalence have used *N. caninum* tachyzoites as antigen, not allowing the differentiation among the *Neospora* species that infect equines (Dubey et al. 1999a,b, Patitucci et al. 2004, Locatelli-Dittrich et al. 2006). Dubey et al. (1999a) investigated 101 English Thoroughbred horses in Brazil for the presence of serum antibodies to *S. neurona*, *Toxoplasma gondii*, and *N. caninum*. Only anti-*S. neurona* and *T. gondii* antibodies were detected in 36 and 16 animals, respectively.

There is scarce information to evaluate the seroprevalence of *Neospora* spp. in equines worldwide; however, antibodies have been detected in populations from different states of the United States with 10.0% (Mcdole & Gay 2002), 11.5% (Cheadle et al. 1999), 17.0% (Vardeleon et al. 2001), and 21.3% seroprevalence (Dubey et al. 1999b). Considering other countries, the reported values are 0% in Brazil (Dubey et al. 1999a) and in Argentina (Dubey et al. 1999c), 23.0% in France, (Pitel et al. 2001), and 2.0% in South Korea (Gupta et al. 2002).

Considering the lack of information about the importance of *Neospora* spp. infection in equines and the non-inclusion of neosporosis in the differential diagnosis of equine protozoal myeloencephalitis (EPM), the main neurological disease affecting equines in the Americas, this study aimed to evaluate the frequency of serum antibodies to *Neospora caninum*, besides a possible association between the presence of anti-*N. caninum* antibodies in CSF and proprioceptive ataxia in the horse group analyzed, since most studies have been using serum instead of CSF samples to detect antibodies to *N. caninum*.

MATERIAL AND METHODS

Twenty-six serum and cerebrospinal fluid (CSF) samples were collected from equines with history of ataxia, independently of breed, gender and age. All animals belonged to properties in the State of São Paulo, Brazil.

Indirect fluorescent antibody test (IFAT) was carried out according to Dubey et al. (1988). Slides were previously sensitized with *Neospora caninum* tachyzoites NC-1 strain kept in cultivation of Vero cells in RPMI 1640 medium supplemented with 10% fetal bovine serum.

First, CSF and serum samples were selected by adopting as cutoff point a titer of 2 in sterile phosphate buffered saline (PBS) pH 7.2 (0.0084M Na2HPPO4, 0.14MNaCl), added of 1% bovine serum albumin. Each slide received serum and CSF positive and negative samples as control, at the same dilution as that of the tested samples (1:2). Reactions in which total fluorescence was surrounding the surface of tachyzoites were considered positive, according to Paré et al. (1995). The sera considered reagent (titer>2) were twofold diluted until 1:64 (1:2, 1:4, 1:8, 1:16, 1:64) in order to detect the highest dilution still presenting fluorescence signal.

Descriptive statistics was used to analyze the results.

RESULTS AND DISCUSSION

IFAT was the serological diagnostic test chosen for this study since it is considered the standard test for the canine species, which is most frequently used in works with the investigated agent (Bjorkman & Uggla 1999). According to Vardeleon et al. (2001), IFAT identifies all reagent samples and is considered highly sensitive. Antigens can be obtained from *N. caninum* strains isolated from dogs and cattle, and slight antigenic variations among the isolates do not affect the test efficacy (Bjerkas et al. 1994).

Of the serum samples tested in this group of equines, 15/26 (57.6%) were seropositive, whereas all CSF samples were negative. Although a positive serum sample was used as control for CSF in IFAT due to the lack of a CSF sample positive to *Neospora caninum*, the reaction was initiated at a very low dilution (1:2) in an attempt to detect samples with lower titers. The test quality can also be assured by using an anti-antibody linked to a fluorescein (conjugate) for serum samples, with 57.6% positivity, confirming the quality of both used material and chosen technique. Considering that there was no reagent sample in the first dilution (1:2), all CSF samples were believed to be negative to *N. caninum* antibodies.

In the diagnosis of equine neosporosis, infections by *N. caninum* and *N. hughesi* must be considered. As the main *Neospora* isolated from equines is *N. hughesi*, it can be inferred that this species is the predominant causal agent of equine neosporosis; however, the relative importance of both species remains unknown (Jakubek et al. 2006). Based on differences in proteins of the internal transcribed spacer regions (ITS1) of DNA and on the morphology of tissue cysts, there is a high degree of antigenic similarity between *N. hughesi* and *N. caninum*, and their number of common antigens is sufficient for anti-*N. hughesi* antibodies to cross-react to *N. caninum* in serological tests (Marsh et al 1998, Walsh et al. 2000, Packham et al. 2002). Differentiation between the serological responses to both agents will only be possible if tests using monoclonal antigens specific for each species are employed.

The choice of IFAT, used in this study, is also justified by the adequate specificity in its interpretation since a positive diagnosis is identified when there is total fluorescence from the whole external surface of the tachyzoite fixed in the slide, whereas partial fluorescence, probably due to cross-reaction to other coccidia, is discarded. In addition, IFAT allows result comparison since most researchers use this same technique to diagnose neosporosis in equines.

The presence of antibodies to *Neospora* spp. in CSF is indicative of clinical infection, but studies in equines infected by Neospora and respective diagnostic tests are scarce; besides, a titer for *N. hughesi* in CSF samples must be established (Packham et al. 2002, Jakubek et al. 2006).

The obtained results (57,6% seroprevalence) suggest these equines were exposed to *N. caninum*, which does not indicate, however, an active infection (Vardeleon et al. 2001).

As a cross-reaction between *N. caninum* and *N. hughesi* may occur, it is impossible to identify which of these species is responsible for the infection (Walsh et al. 2000).

It is clear that there was no infection by *N. caninum* in the central nervous system (CNS) since there was no positive CSF sample, which justifies the present findings. Thus, in this group of equines the protozoan *N. caninum* has no relation to the clinical neurological manifestations of ataxia. Further studies must be carried out to better understand the epidemiological aspects of neosporosis in equine species.

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