Neospora spp. antibodies in horses from two geographical regions of the state of Santa Catarina, Brazil

Anderson Barbosa de Moura1*; Márcio Orides da Silva1; Juliana Antunes Farias1; Achilles Vieira-Neto1; Antonio Pereira de Souza1; Amélia Aparecida Sartor1; Joandes Henrique Fonteque1; Silvério Bunn1

1Centro de Ciências Agroveterinárias – CAV, Universidade do Estado de Santa Catarina – UDESC, Lages, SC, Brasil

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Abstract

The aims of this study were to determine occurrences of Neospora spp. IgG antibodies in horses from two geographical regions of the state of Santa Catarina, southern Brazil, and identify risk factors for infection. Analyses were performed on 615 samples using the immunofluorescent antibody test (IFAT ≥ 1:50). Out of the 615 samples, 25 (4.1%) were positive for Neospora spp. The titers for Neospora spp. were distributed as follows: 1:50 (13), 1:100 (eight), 1:200 (three) and 1:400 (one). Out of the 311 samples taken in the mountain region, eight were positive (2.6%). Among the samples from the coastal region (304), 17 had Neospora spp. antibodies, thus indicating occurrence of 5.6%. Although no statistically significant difference was observed (P = 0.06704), the prevalence among animals of the coast was 2.2 times higher than that of the mountain region. Contact with dogs and/or cattle (P = 0.007596) were identified as risk factor for Neospora spp. infection.

Keywords: Neospora spp., equines, IFAT, epidemiology.

Resumo

O presente estudo objetivou determinar a ocorrência de anticorpos anti-Neospora spp. em equinos das regiões serrana e litorânea de Santa Catarina e identificar possíveis fatores de risco para a infecção. Foram analisadas 615 amostras por meio da reação de imunofluorescência indireta (RIFI ≥1:50). Das 615 amostras, 25 (4,1%) foram positivas para Neospora spp. A recíproca dos títulos de anticorpos anti-Neospora spp. nos equinos foi assim distribuída: 1:50 (13), 1:100 (oitoo), 1:200 (três) e 1:400 (um). Das 311 amostras da Serra Catarinense, oito foram positivas (2,6%). Das amostras da Região Litorânea (304), 17 apresentaram anticorpos anti-Neospora spp., indicando uma prevalência de 5,6%. Não foi observada diferença estatística significante (P=0,06704) entre as soroprevalências para Neospora spp. em equinos das diferentes regiões, porém os animais do litoral de Santa Catarina apresentaram prevalência 2,2 vezes maior que aqueles da região serrana. O contato dos equinos com cães e/ou bovinos (P=0,007596) foi identificado como fator de risco para infecção por Neospora spp.

Palavras-chaves: Neospora spp., equinos, RIFI, epidemiologia.

Introduction

Neosporosis is a disease that affects cattle (BAAR et al., 1991), dogs (DUBEY et al., 1988a), sheep (DUBEY et al., 1990), goats (DUBEY et al., 1992), deer (WOODS et al., 1994), buffalos (RODRIGUES et al., 2004) and horses (LINDSAY et al., 1996; DAFT et al., 1997). Among horses, the disease can be caused by N. caninum and N. hughesi. The definitive host for N. hughesi has not yet been determined, so it remains unknown how horses are exposed to this parasite. It is also unclear whether horses can become infected with the related species N. caninum (HOANE et al., 2006). There are still uncertainties regarding the consequences of infection either by N. caninum or by N. hughesi in horses (LOCATELLI-DITTRICH et al., 2006a).

Neosporosis causes abortion, neonatal diseases and visceral and neurological dysfunction among horses. Cases of neosporosis among horses have been discovered in the United States (DUBEY; PORTERFIELD, 1990; GRAY et al., 1996; LINDSAY et al., 1996; CHEADLE et al., 2000; DUBEY et al., 2001; FINNO et al., 2007) and in France (PRONOST et al., 1999; PITEL et al., 2003; LEON et al., 2009). In Brazil, Locatelli-Dittrich et al. (2006b) and Toscan et al. (2010) suggested that vertical transmission of Neospora spp. was occurring and, in the United States, Pusterla et al. (2011) identified endogenous transplacental infection in horses.
In a study carried out in Brazil, Dubey et al. (1999) did not detected anti-
Neospora spp. antibodies among horses (n=101) in three municipalities. In another survey carried out among animals 
in different states and regions of Brazil, seroprevalence of 2.5% 
was observed (HOANE et al., 2006). In the state of Paraná, 
seroprevalence for Neospora spp. of 30% to 47% was observed 
among mares and 22.2% among precolostral foals (LOCATELLI-
DITTRICH et al., 2006b).

Horse neosporosis still has not been sufficiently studied in 
Brazil. Although some seroepidemiological studies relating to 
horse neosporosis have already been conducted here in Brazil, 
the lack of reference data throughout the state of Santa Catarina 
was the motive for carrying out this study.

Materials and Methods

Between August 2009 and July 2011, blood samples (n = 615) 
were taken from horses in the mountain region (n = 311) and 
coastal region (n = 304) of the state of Santa Catarina, southern 
Brazil, in 59 herds. The blood samples were obtained as random 
convenience samples from healthy animals. The exceptions were 
72 and three samples from horses for which there were records 
of problems of a reproductive or neurological nature, respectively, 
within the preceding 12 months, although these animals were 
seen to be healthy at the time of sample collection. Information 
on the animals was obtained by means of an epidemiological 
questionnaire (management, frequency and type of reproductive 
disorders in the last 12 months, although these animals were 
seen to be healthy at the time of sample collection. Information 
on the animals was obtained by means of an epidemiological 
questionnaire (management, frequency and type of reproductive 
disorders in the last 12 months, type of diet and contact with 
dogs and other animals). The data were entered into tables and 
were analyzed by means of the Fisher’s exact and chi-square tests 
(P ≤ 0.05) (R Development Core Team, 2009), in order to correlate 
the results with the risk factors analyzed.

Tachyzoites of the NC1 strain of N. caninum were used as 
antigens (DUBEY et al., 1988b) in order to perform the 
immunofluorescent antibody test (IFAT) as described by 
Conrad et al. (1993). Serum samples were screened at 1:50 
(DUBEY et al., 1999; BJÖRKMAN; UGGLA, 1999) and positive 
samples were tested at dilutions ranging from 1:100 to 1:400. 
Positive and negative serum controls were used in comparisons 
with all reactions.

This research project was approved by the Ethics Committee 
on Animal Experiments of CAV/UDESC (Protocol 1.05.09) and 
by the Ethics Committee on Research Involving Human Beings 

Results and Discussion

Out of 615 samples, 25 (4.1%) were positive for Neospora 
spp. The reciprocity of antibody titers among the horses was 
1:50 (thirteen), 1:100 (eight), 1:200 (three) and 1:400 (one). 
Out of the 311 samples from the mountain region, eight samples 
were found to be positive (2.6%). Among the animals from the 
coastal region (304), 17 had antibodies against Neospora spp., 
thus indicating an occurrence of 5.6%. Villalobos et al. (2006) 
used IFAT (1:50) to investigate antibodies against Neospora spp. 
among horses in the state of São Paulo. Both healthy animals and 
mares with reproductive problems were evaluated, and the results 
showed that 5.8% of the healthy horses were positive, similarly 
to what was observed in the present study.

The antigens that are common to both species of Neospora 
have demonstrated cross-reactions (MARSH et al., 1996, 1998; 
DUBEY et al., 2001; PACKHAM et al., 2002; GONDIM et al., 
2009). Animals with N. hughesi infection also demonstrated 
antibodies when the antigen was N. caninum, and therefore 
the titers were fewer (PACKHAM et al., 2002). A distinction 
between N. caninum and N. hughesi can only be made by means 
of molecular characteristics (HOANE et al., 2005). In this respect, 
the current results cannot be focused specifically on either of the 
species of Neospora spp. that affect horses.

In the present study, the titer 1:50 represented 52% (13/25) 
of the seropositive samples, thus suggesting that there was chronic 
infection, compatible with healthy animals. Ratios of 1:50 and 1:100, 
in the immunofluorescent antibody test (IFAT), are considered to 
be positive, and use of the 1:50 ratio can increase the sensitivity of 
the diagnosis (McDOLE; GAY, 2002; VARDELEON et al., 2001).

Studies on the seroprevalence of Neospora spp. in horses in Brazil 
have presented diverse results, ranging from 0% (DUBEY et al., 
1999, using the agglutination test, 1:40) to 47% (LOCATELLI-
DITTRICH et al., 2006b, using IFAT, 1:50). The latter, higher 
seroprevalence, which was found in the state of Paraná, could be 
attributable to different management conditions and/or geographical 
position, or exposure to parasites.

The results from this study are the first to detect Neospora spp. 
antibodies in horses in the state of Santa Catarina. Hoane et al. 
(2006), evaluated 961 horse serum samples from 10 Brazilian states 
(São Paulo, Minas Gerais, Paraná, Santa Catarina, Rio Grande 
do Sul, Bahia, Rondônia, Mato Grosso, Mato Grosso de Sul and 
Goiás) in order to detect NhSAG1 antigen (ELISA), and 2.5% 
(24/961) were found to be seropositive for Neospora spp. Out of 
the 24 horses in that study that were from the state of Santa 
Catarina, none of them presented antibodies against Neospora spp.

Most studies do not reflect a real prevalence, since the samples 
is not always statistically determined. In addition, the fluctuation 
of Neospora spp. antibody levels observed in pregnant mares 
(HOFFMANN-KORMANN et al., 2008) may occur due to 
individual or hormonal variations. This also resembles cases of 
recent seraconversion (immune window), which gives rise to 
difficulty in comparing results. Furthermore, maternal antibodies 
can cause false-positive results for up to three to four months 
(DUARTE et al., 2004) or up to 10 months of age among 
the foals (TOSCAN et al., 2010), since transfer can occur 
(PUSTERLA et al., 2011). In the present study, 15 animals were 
younger than 12 months and, of these, only one had antibodies 
against Neospora spp. (Table 1).

A difference in seroprevalence for Neospora spp. was observed in 
the present study between horses from different regions. Although 
not statistically significant (P = 0.06704), the animals from Santa 
Catarina’s coastal region presented occurrence that was 2.2 times 
higher than was seen among the horses from the mountain 
region (Table 1). The odds ratio observed (0.45), less than one, 
indicates that the animals of the Santa Catarina mountains had 
lower occurrence of antibodies against Neospora spp. compared 
with animals from the coastal region, but without statistical
Table 1. Frequency of positive equine (IFAT, ≥1:50) for *Neospora* spp. from the Mountain and Coastal regions, Santa Catarina State, Brazil, for analyzed variable and total 2012.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Animals</th>
<th></th>
<th>Positive¹</th>
<th>Positive²</th>
<th>P</th>
</tr>
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<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
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<td><strong>Region</strong></td>
<td></td>
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<td>311</td>
<td>50.6</td>
<td>8</td>
<td>2.6</td>
<td>8</td>
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<tr>
<td>Coastal</td>
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<td>49.4</td>
<td>17</td>
<td>5.6</td>
<td>17</td>
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<td>270</td>
<td>43.9</td>
<td>10</td>
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<td>10</td>
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<tr>
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<td>56.1</td>
<td>15</td>
<td>4.3</td>
<td>15</td>
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<td><strong>Breed</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>166</td>
<td>27.0</td>
<td>2</td>
<td>1.2</td>
<td>2</td>
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<tr>
<td>Pure</td>
<td>449</td>
<td>73.0</td>
<td>23</td>
<td>5.1</td>
<td>23</td>
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<td><strong>Age</strong></td>
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<td>&lt; 6 months</td>
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<tr>
<td>6–12 months</td>
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<td>1.8</td>
<td>1</td>
<td>9.1</td>
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<td>1-5 years</td>
<td>197</td>
<td>32.0</td>
<td>10</td>
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<td>10</td>
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<tr>
<td>6-10 years</td>
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<td>36.9</td>
<td>9</td>
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<tr>
<td>&gt; 10 years</td>
<td>176</td>
<td>28.6</td>
<td>5</td>
<td>2.8</td>
<td>5</td>
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<tr>
<td>Dog</td>
<td>130</td>
<td>21.2</td>
<td>03</td>
<td>2.3</td>
<td>03</td>
</tr>
<tr>
<td>Cat</td>
<td>07</td>
<td>1.1</td>
<td>00</td>
<td>-</td>
<td>00</td>
</tr>
<tr>
<td>Dog and Cat</td>
<td>90</td>
<td>14.6</td>
<td>01</td>
<td>1.1</td>
<td>01</td>
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<tr>
<td>Dog and Bovine</td>
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<td>29.9</td>
<td>12</td>
<td>6.5</td>
<td>12</td>
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<tr>
<td>Dog, Cat and Bovine</td>
<td>167</td>
<td>27.2</td>
<td>04</td>
<td>2.4</td>
<td>04</td>
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<tr>
<td>Dog and Pig</td>
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<td>00</td>
<td>-</td>
<td>00</td>
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<td>30.8</td>
<td>04</td>
</tr>
<tr>
<td>Equine</td>
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<td>00</td>
<td>-</td>
<td>00</td>
</tr>
<tr>
<td>Dog and Chicken</td>
<td>05</td>
<td>0.8</td>
<td>00</td>
<td>-</td>
<td>00</td>
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<tr>
<td>No Contact</td>
<td>10</td>
<td>1.6</td>
<td>01</td>
<td>10.0</td>
<td>01</td>
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<td>-</td>
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<tr>
<td>Stillborn</td>
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<td>0.2</td>
<td>0</td>
<td>-</td>
<td>0</td>
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<tr>
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<td>-</td>
<td>0</td>
<td>-</td>
<td>0</td>
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<tr>
<td>Return Season</td>
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<td>6.3</td>
<td>01</td>
<td>2.6</td>
<td>01</td>
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<tr>
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<td>24</td>
<td>4.4</td>
<td>24</td>
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<td>-</td>
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<td>0</td>
<td>-</td>
<td>0</td>
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<tr>
<td>Abortion + Return Season + Stillborn</td>
<td>01</td>
<td>0.2</td>
<td>0</td>
<td>-</td>
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<td><strong>Neurologic Disorders</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>03</td>
<td>0.5</td>
<td>0</td>
<td>-</td>
<td>0</td>
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<tr>
<td>No</td>
<td>612</td>
<td>99.5</td>
<td>25</td>
<td>4.1</td>
<td>25</td>
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<tr>
<td><strong>Total</strong></td>
<td>615</td>
<td>100</td>
<td>25</td>
<td>4.1</td>
<td>25</td>
</tr>
</tbody>
</table>

Positive¹ = Relation among the positive animals within a category and the total of animals of its category. Positive² = Relation among the positive animals within a category and the total of positive animals. p = descriptive level of the Fischer exact's test.

difference, since the confidence interval (95% CI = 0.16-1.11) contains the value 1.0. It is possible that the animals that live in the coastal region have a higher chance of contact with infectious forms (sporulated oocysts of *Neospora* spp.), which are common in warmer and humid environments. High temperatures can cause rapid sporulation of oocysts (Dubey et al., 2007). Wouda et al. (1999) observed that abortion epidemics among dairy cows usually occurred in the hot and humid summer. Furthermore, Abo-Shehada and Abu-Halaweh (2010) identified that cold climates can reduce the seropositivity for *Neospora* spp. Another explanation for this could be that high average temperatures and high humidity stimulate the growth of fungi. Mycotoxins are suspected of being the cause of immunosuppression among cattle, which can cause an upsurge of latent infection due to *N. caninum* (Dubey et al., 2007). Although these studies were carried out on cattle, their conclusions can be used to explain the results relating to the environment that were obtained in the present study.

An analysis on the risk factors found that horses belonging to a defined breed (P = 0.03568) demonstrated higher seropositivity. Among the horses that were positive for neosporosis, 92% (23/25) demonstrated a defined breed pattern. However, 73% (449/615) of the total number of animals evaluated were identified as animals belonging a breed (Table 1), thus suggesting that the higher occurrence among these animals might have resulted from the sampling, as indicated by the observed OR (0.23), less than 1. In a study on 91 cart horses (no defined breed) and 123 Crioula breed horses in Rio Grande do Sul, Toscan et al. (2011) found that there was high seropositivity in both groups (15.4% and 16.3%, respectively), and no difference between the distinct populations was observed. However, in Santa Maria, Rio Grande do Sul, in
traction horses (no pure breed), Sangioni et al. (2011) also found high seropositivity (15.4%) by means of IFAT (1:50).

Close contact of horses with dogs and/or cattle presented a association (P = 0.007596) with Neospora spp. antibodies. The observed odds ratio (2.24), greater than 1.0, indicates that the group of animals that have contact with cattle and/or dogs showed higher occurrence of antibodies against Neospora spp. Among all the positive horses, 96% (24/25) had been in contact with dogs and/or cattle, which are the most important species in the biological cycle of N. caninum (DUBEY; SCHARES, 2011). This demonstrates the importance of these species in introducing and/or maintaining infection on the farm, and perhaps in relation to its spread to horses.

The additional features that were analyzed (gender, age and reproductive and neurological disorders) did not revealed any association with occurrences of Neospora spp. antibodies among the horses in the current experiment.

Conclusion

The occurrence of Neospora spp. antibodies among analyzed horses was low and the contact with dog and/or cattle is a risk factor for infection these animals.

References


Hoffmann-Kormann DCS, Locatelli-Dittrich R, Richartz RRTB, Hoffmann-Kormann DCS, Locatelli-Dittrich R, Richartz RRTB, Richartz RRTB.
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