

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

Anticorpos contra *Neospora* spp. em equinos de duas regiões geográficas do Estado de Santa Catarina, 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 $\geq 1:50$). 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 $\geq 1:50$). 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 (oito), 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 significativa ($P=0,06704$) 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.

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In a study carried out in Brazil, Dubey et al. (1999) did not detect 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, 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 ($P \leq 0.05$) (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 (Protocol 73/09 of Aug 10, 2009).

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 seroconversion (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, $\geq 1:50$) for *Neospora* spp. from the Mountain and Coastal regions, Santa Catarina State, Brazil, for analyzed variable and total 2012.

Variables		Animals		Positive ¹		Positive ²		P
		n	%	n	%	n	%	
Region	Mountain	311	50.6	8	2.6	8	32.0	0.0670
	Coastal	304	49.4	17	5.6	17	68.0	
Sex	Male	270	43.9	10	3.7	10	40.0	0.8376
	Female	345	56.1	15	4.3	15	60.0	
Breed	Mixed	166	27.0	2	1.2	2	8.0	0.0356
	Pure	449	73.0	23	5.1	23	92.0	
Age	< 6 months	04	0.7	0	0.0	0	0.0	0.4861
	6–12 months	11	1.8	1	9.1	1	4.0	
	1–5 years	197	32.0	10	5.1	10	40.0	
	6–10 years	227	36.9	9	4.0	9	36.0	
	> 10 years	176	28.6	5	2.8	5	20.0	
Contact with animals	Dog	130	21.2	03	2.3	03	12.0	0.0075
	Cat	07	1.1	00	-	00	-	
	Dog and Cat	90	14.6	01	1.1	01	4.0	
	Dog and Bovine	184	29.9	12	6.5	12	48.0	
	Dog, Cat and Bovine	167	27.2	04	2.4	04	16.0	
	Dog and Pig	02	0.4	00	-	00	-	
	Bovine	13	2.1	04	30.8	04	16.0	
	Equine	07	1.1	00	-	00	-	
	Dog and Chicken	05	0.8	00	-	00	-	
	No Contact	10	1.6	01	10.0	01	4.0	
Reproductive Disorders	Abortion	21	3.4	0	-	0	-	0.9372
	Stillborn	01	0.2	0	-	0	-	
	Mummified	0	-	0	-	0	-	
	Return Season	39	6.3	01	2.6	01	4.0	
	No Disorders	543	88.2	24	4.4	24	96.0	
	Abortion+Return Season	09	1.5	0	-	0	-	
	Endometritis	01	0.2	0	-	0	-	
	Abortion + Return Season + Stillborn	01	0.2	0	-	0	-	
Neurologic Disorders	Yes	03	0.5	0	-	0	0	1.0
	No	612	99.5	25	4.1	25	100	
Total		615	100	25	-	25	100	

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-Shehadeh 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.

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