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Association of antibodies against *Neospora caninum* in mares with reproductive problems and presence of seropositive dogs as a risk factor

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ABSTRACT

Sera from 112 mares from 5 horse-breeding farms was examined for the presence of antibodies to *Neospora caninum* and *Toxoplasma gondii* by an indirect fluorescent antibody test (IFAT), as well as from dogs and cattle present on these properties for the presence of antibodies to *N. caninum*. Among the 112 mares, 35 had a history of reproductive problems in the last breeding season and 77 had no reproductive problems. The rates of seroprevalence of *N. caninum* in mares with and without a history of reproductive problems were 25.71% and 6.49% and from *T. gondii* 2.85% and 1.29%, respectively. In dogs and cattle, the rates of seroprevalence of *N. caninum* were 10.52% and 15.55%, respectively. A positive correlation was found between the presence of antibodies against *N. caninum* ($p = 0.010$) in mares and the occurrence of reproductive problems using the Fisher's exact test. Significantly higher seroprevalence for *N. caninum* in mares was observed on the farm that had seropositive dogs ($p = 0.018$). Cattle on this farm were also seropositive. No significant differences in seropositivity were found on farms where dogs were seronegative, or absent. This result suggests, for the first time, the presence of seropositive dogs as a risk factor for *N. caninum* in mares and the necessity for further investigation of the epidemiology of this parasite in horse-breeding farms with reproductive problems and the presence of cattle and dogs. This is the first report on the occurrence of antibodies against *N. caninum* in horses from the state of Santa Catarina, Brazil.

1. Introduction

Equine neosporosis is caused by the parasites *Neospora caninum* and *Neospora hughesi*, which are obligate intracellular protozoans. The definitive hosts of *N. caninum* are dogs, coyotes and wolves and intermediate hosts include diverse animal species such as: cattle, sheep, dogs and horses (Dubey and Schares, 2011). Infection by *N. caninum* can occur by horizontal and vertical transmission (Dubey et al., 2007). The definitive host of *N. hughesi* is still unknown (Hoane et al., 2006).

Abortion and neonatal diseases are associated with *N. caninum* infection in horses (Villalobos et al., 2006; Kligler et al., 2007) whereas *N. hughesi* infection is associated

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with neurological diseases, principally equine protozoal myeloencephalitis (EPM) (Finno et al., 2007; Kligler et al., 2007). In Brazil, the seroprevalence of *Neospora* spp. ranges from 2.5 to 47% in healthy horses (Hoane et al., 2006; Locatelli-Dittrich et al., 2006).

The presence of dogs on farms plays an important epidemiological role in the infection of cattle and other intermediate hosts, and as such, are a potential risk factor for the occurrence of *N. caninum* in these species (Basso et al., 2001). Canine infection can occur by the ingestion of carcasses, fetuses and the placental remains of contaminated bovine and other intermediate hosts (Gondim et al., 2002).

Toxoplasma gondii is another protozoan that is being investigated in equines, which is one of the domestic species most resistant to infection by this parasite. Infection in horses is generally inapparent, being characterized by the maintenance of titer and the presence of tissue cysts (Langoni et al., 2007). In general, the seroprevalence is lower than in other livestock species (Dubey and Jones, 2008). Previous studies in Brazil have indicated that seropositivity for *T. gondii* in horses ranges from 2.7 to 5.9% (Locatelli-Dittrich et al., 2006; Coiro et al., 2012).

This study aimed to correlate the seroprevalence of *N. caninum* and *T. gondii* in mares with the occurrence of reproductive problems and to investigate the presence of dogs and cattle with the seroprevalence of *N. caninum*, identifying if these animals represent a risk factor for equine neosporosis in the states of Paraná and Santa Catarina in southern Brazil.

2. Materials and methods

2.1. Animals

Five horse-breeding farms in the states of Paraná and Santa Catarina in southern Brazil were chosen to estimate the occurrence of *N. caninum* and *T. gondii* antibodies. Blood was obtained from the jugular vein of 112 horses in August 2011. They were all adult females from the following breeds: Crioula, Mangalarga Marchador and Thoroughbred. All mares were in good physical condition and were subjected to routine veterinary examinations. The mares analyzed from each farm were for the last 3 years on the same farm. Among the 112 mares, 35 had a history of reproductive problems in the last breeding season, of which 29 had one or more abortions registered, principally between the 7th and 9th month of gestation, 2 mares presented embryonic absorption and 4 mares presented histories of stillbirths.

Blood samples from 19 dogs of different breeds and ages and of both sexes from 4 properties were collected for serological analysis. Of the 5 farms, only 1 had horses, dogs, and cattle. On this farm, 22 blood samples were collected from mares, of which 10 had a history of reproductive problems and 12 were without a history of reproductive problems; there were 7 blood samples from dogs and 45 blood samples from cattle (only samples from mature female bovines were collected). The cattle and dogs did not present clinical signs of neosporosis.

2.2. Serology

Sera were separated and stored at −20° C until used. The indirect fluorescent antibody test (IFAT) was utilized to test for circulating IgG antibodies specific for antigens from *N. caninum* and *T. gondii*, NC-1 and RH strains respectively. Specific conjugates were used for each species: anti-horse IgG (Sigma–Aldrich®, St Louis, MO, USA), anti-dog IgG (Sigma–Aldrich®, St Louis, MO, USA), and anti-bovine IgG (Sigma–Aldrich®, St Louis, MO, USA) antibodies.

For dog and horse serum samples the initial screening dilution was 1:50 in accordance with Locatelli-Dittrich et al. (2006, 2008), respectively, in phosphate buffered saline (PBS), and the positives were diluted to an endpoint titer. In cattle, the cut-off dilution was 1:100 (Locatelli-Dittrich et al., 2001). Only samples that presented complete peripheral tachyzoite fluorescence were considered positive. Previously known positive and negative sera for *N. caninum* in horses, dogs and cattle were included on each slide according to the species analyzed as well as horses for *T. gondii*.

2.3. Statistical analysis

The statistical analysis of the association between the seroprevalence for *N. caninum* and *T. gondii* and the occurrence of reproductive problems, as well as, the association between the seroprevalence for *N. caninum* in mares and the presence of dogs and cattle was performed by Fisher's exact test, considering $p < 0.05$.

3. Results

The overall frequencies of occurrence of antibodies against *N. caninum* in mares, dogs, and cattle were 12.5% (14/112), 10.52% (2/19), and 15.55% (7/45), respectively

Table 1
Seroprevalence (%) of *Neospora caninum* in mares with and without history of reproductive problems, and in dogs and cattle analyzed by farm.

Farms	Mares (IFAT ≥ 1:50)		Dogs (IFAT ≥ 1:50)	Cattle (IFAT ≥ 1:100)
	With reproductive problems	Without reproductive problems		
1	50.0 (5/10)	16.66 (2/12)	28.57 (2/7)	15.55 (7/45)
2	16.66 (1/6)	1.85 (1/54)	0.0 (0/8)	–
3	0.0 (0/2)	28.57 (2/7)	–	–
4	22.22 (2/9)	0.0 (0/1)	0.0 (0/2)	–
5	12.0 (1/8)	0.0 (0/3)	0.0 (0/2)	–
Total	25.71 (9/35)	6.49 (5/77)	10.52 (2/19)	15.55 (7/45)