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## Occurrence of anti-*Neospora caninum* and anti-*Toxoplasma gondii* antibodies in horses in the Pantanal of Mato Grosso, Brazil

## Ocorrência de anticorpos anti-*Neospora caninum* e anti-*Toxoplasma gondii* em equinos do Pantanal Mato-Grossense, Brasil

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### Abstract

This study aimed to evaluate the occurrence of anti-*Neospora caninum* and anti-*Toxoplasma gondii* antibodies in horses from Pantanal, in Mato Grosso state. Two hundred blood samples were collected from horses in Pantanal of Mato Grosso, Brazil. The samples were analyzed by IFAT for the detection of anti-*Neospora caninum* and anti-*Toxoplasma gondii* antibodies. Antibodies to *N. caninum* were found in 30 (15%) of 200 horses in titers of 50 (25 horses), 100 (two horses), 200 (two horses), and 400 (one horse). Antibodies to *T. gondii* were found in five (2.5%) of 200 horses in titers of 50 (three horses), 200 (one horse), and 400 (one horse). One animal showed antibody titers for both coccidian (titers of 200 for *N. caninum* e 400 for *T. gondii*). The pantaneiros horses were exposed to *Neospora* spp. and *T. gondii*.

**Key words:** Horse, pantanal, toxoplasmosis, neosporosis, immunofluorescence

### Resumo

Este estudo teve como objetivo avaliar a ocorrência de anticorpos anti-*Neospora caninum*. e anti-*Toxoplasma gondii* em cavalos do Pantanal, no estado de Mato Grosso. Duzentas amostras de sangue foram coletadas de equinos do Pantanal Mato-grossense, Brasil. As amostras foram analisadas pela reação de Imunofluorescência Indireta (RIFI) para pesquisa de anticorpos anti-*Neospora caninum* e anti-*Toxoplasma gondii*. Os anticorpos anti *N. caninum* foram detectados em 30 (15%) dos 200 cavalos, nos títulos de 50 (25 cavalos), 100 (dois cavalos), 200 (dois cavalos) e 400 (um cavalo). Os anticorpos anti *T. gondii* foram detectados em cinco (2,5%) dos 200 cavalos, nos títulos de 50 (três cavalos), 200 (um cavalo) e 400 (um cavalo). Em um cavalo verificou-se presença de anticorpos para ambos coccídeos (título de 200 para *N. caninum* e 400 para *T. gondii*). Os equinos pantaneiros foram expostos ao *N. caninum* e *T. gondii*.

**Palavras-chave:** Cavalos, pantanal, toxoplasmose, neosporose, imunofluorescência

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Neosporosis is a disease caused by the *Neospora caninum* and *Neospora hughesi* parasites, which are Apicomplexa protozoa closely related to *T. gondii*. Additionally, *N. caninum* is one of the most cause of abortion in cattle worldwide (DUBEY; SCHARES, 2011). Abortion and neonatal diseases are associated with *N. caninum* infection in horses (VILLALOBOS et al., 2012; KLIGLER et al., 2007) whereas *N. hughesi* infection is associated with neurological diseases, principally equine protozoal myeloencephalitis (EPM) (FINNO et al., 2007; KLIGLER et al., 2007). Domestic dogs and coyotes are known to be the definitive hosts of *N. caninum*, and the definitive host for *N. hughesi* has not yet been identified (DUBEY; SCHARES 2011).

There is no definitive evidence that *T. gondii* causes clinical disease in horses, although antibodies to this protozoa parasite have been reported in horses from many countries, including Brazil (DUBEY et al., 1999). Previous studies in Brazil have indicated that sero-positivity for *T. gondii* in horses ranges from 1.29 to 5.9% (LOCATELLI-DITTRICH et al., 2006; ABREU et al., 2014).

The Pantanal, considered to be the world's biggest wetland area, covers an area of 150,000 km<sup>2</sup> situated in the upper Paraguay River Basin. The greater part lies in Brasil, divided between the states of Mato Grosso and Mato Grosso do Sul, extending into Bolivia and Paraguay. The Pantaneiro horse is a breed that has developed in this region and is widely used for many different activities related to livestock (EMBRAPA, 2005). These animals live in flooded areas for more than four months per year, and the risk of infection with contaminated pasture by fecal material can be high. Disorders with neurological clinical signs in Pantanal horses are commonly diagnosed as trypanosomiasis caused by *Trypanosoma evansi* (SEIDL et al., 2001). *N. caninum* and *T. gondii* has never been reported in Pantaneiros horses in the Pantanal region. The objective of the present study was to determine the occurrence of anti-*N. caninum* and anti-*T. gondii*

antibodies in horses from Pantanal, in Mato Grosso State.

Blood samples were collected from 200 clinically healthy horses from Poconé and Cáceres, cities of Pantanal of Mato Grosso, Brazil, in five different rural properties which have 200 km or more distance from each other in Southern region of the State. The horses were 120 males and 80 females. Ages ranged from 3 to 15 years. The breeds were Pantaneiros (150 horses) and crossbreeds (50 horses). The farm properties size range from 20 km<sup>2</sup> to 8,000km<sup>2</sup>, and all of them had history of deaths of animals with neurological signs and cases of abortion. There other animals in properties, like dogs, cats, cattle and wild animals. The blood samples were collected from the jugular vein. Sera were separated and stored at -20°C until used. The indirect fluorescent antibody test (IFAT) was used to detect antibodies against *N. caninum* and *T. gondii* in the horses' serum. Slides were prepared with tachyzoites of *N. caninum* (NC-1 strain) and *T. gondii* (RH strain), as described by Locatelli Dittrich et al. (2006). Serum samples were tested at an initial dilution of 1:50, in PBS solution (pH 7.2) and the positives were diluted to an endpoint titer. Conjugated anti-horse IgG (Sigma) was used with the dilution of 1:100 the cutoff utilized was 1:50.

Antibodies against *N. caninum* were found in 30 (15%) out of 200 horses. The titers these horses were: 50 (25); 100 (two); 200 (two), and 400 (one). Thirteen females (16%) and 17 males (14%) were seropositive, and antibodies were found in 25 Pantaneiros and five crossbreeds horses. Antibodies to *T. gondii* were found in five males, Pantaneiros horses (2.5%). The titers of the five horses were: 50 (three), 200 (one), and 400 (one). One animal showed antibody titers for both coccidian (titers of 200 for *N. caninum* e 400 for *T. gondii*). All the properties (5/5-100%) had *N. caninum* positive horses, and three properties (3/5-60%) had *T. gondii* positive horses.

The serological tests suggest that horses from Pantanal were exposed to *Neospora* spp., and further studies are necessary to discern which species, *N. caninum* or *N. hughesi*, infected these animals. If the horses are *N. hughesi*-infected it would be necessary to perform the IFAT using *N. hughesi* as the antigen source (PACKHAM et al., 2002).

Antibodies to *N. caninum* were related in horses from some states in Brazil, and the seroprevalence has been reported from 2.5 to 47% (VILLALOBOS et al., 2012; LOCATELLI-DITTRICH et al., 2006). Two studies carried out in Rio de Janeiro, São Paulo, Rio Grande do Sul, and Mato Grosso, detected no antibodies to *N. caninum* in horses (HOANE et al., 2006; DUBEY et al., 1999). Antibodies were not found in 15 horses from Rio de Janeiro, in 70 horses from São Paulo, and in 16 horses from Rio Grande do Sul State (DUBEY et al., 1999). Additionally, antibodies were not found in 28 sera samples from horses from Mato Grosso, without information about gender and breed (HOANE et al., 2006).

In this study, the serum occurrence of antibodies against *N. caninum* was 15%, similar to the another studies carried out in Rio Grande do Sul, in breeding mares (13.8%, IFAT, title 50); in horses of different genders (15.4%), and in carter and Crioulo horses (15.4% and 16.3%, respectively) (SANGIONI et al., 2011; TOSCAN et al., 2010). In Paraná, a similar prevalence was found in carter horses (14.4%; IFAT, title 50) (VILLALOBOS et al., 2012). Reasons for the different seroprevalence reported in horses may be due different management conditions and/or geographic locations. The present study is the first report of the presence of antibodies against *N. caninum* in horses from Pantanal, Mato Grosso.

In this study, antibodies against *T. gondii* were detected in only five horses (2.5%). Antibodies to *T. gondii* have been reported in horses from many Brazilian states; however, seroprevalence studies also suggest that there is a low prevalence of *T. gondii*-positive serology in equine populations (DUBEY et al., 1999; EVERS et al., 2013; ABREU

et al., 2014). In Rio de Janeiro and Paraná, the prevalences of antibodies against *T. gondii* were 16% and 2.7%, respectively (DUBEY et al., 1999; LOCATELLI-DITTRICH et al., 2006). In São Paulo state, the prevalence of *T. gondii* in horses with ataxia was 34.7%, in a modified agglutination test (STELMANN et al., 2011). In Mato Grosso do Sul and Bahia states, the seroprevalences (IFAT, title 64) were 32.8% and 1.5%, respectively (MENDONÇA et al., 2001; LARANGEIRA et al., 1985). Using IFAT (title 16), Evers et al. (2013) found the prevalence of 11.6% in slaughtered horses from several states (Paraná, Minas Gerais, Rio de Janeiro, Goiás, Mato Grosso and Mato Grosso do Sul). The highest prevalence for *T. gondii* was 41.5%, in slaughtered horses of Paraná, São Paulo, Mato Grosso do Sul and Mato Grosso (IFAT, title of 16) (VIDOTTO et al., 1997). The different seroprevalences detected in horses were probably because of differences in the methodology and titles, different management conditions and/or environments. There is the necessity for further investigation of the epidemiology of these parasites in horses of Pantanal region, mainly for the presence of dogs, cats or wild animals. sorologia

The control and prophylaxis measures necessary for bovine neosporosis, already complex, will be further complicated if wildlife plays a role in the domestic and sylvatic cycle of the parasite, as seems likely. Canids are important in the epidemiology of *N. caninum* infection because they are the only hosts reported to date that can excrete the environmentally resistant oocysts. Reports on seroprevalence are of interest because, as occurs with *T. gondii*, seropositive animals might have already shed *N. caninum* oocysts in the environment. In addition, as indicated previously, surveys of *N. caninum* infection in free-ranging canids can provide an estimation of environmental contamination and circulation of *N. caninum* in domestic and wild ecosystems.

All properties had dogs and cats as pets. Domestic dogs and Australian dingos and coyotes are the

only known definitive host of *N. caninum* able to excrete oocysts (KING et al., 2010; GONDIM et al., 2004), however, in others wild canids little is known of *N. caninum* oocyst excretion. Recently, Dubey et al. (2011) observed that gray wolf (*Canis lupus*) may also be an important link in the sylvatic cycle of *N. caninu*, because this species of wolf eliminates oocysts in the feces. Thus, perhaps other wild canids could be definitive hosts, as crab-eating fox (*Cardocyon thous*) and maned wolf (*Chrysocyon brachyurus*), which are both animals present in the Brazilian Pantanal (EMBRAPA, 2002) and identified soropositive animals for *N. caninum* (CANON-FRANCO et al., 2004), however, without studies of presence of oocysts in the faeces. Thus, this would be a possible explanation for the high percentage of seropositive horses for *N. caninum* observed in this study. With respect to *T. gondii*, there are studies demonstrating seropositivity in wild felids in Brazil, especially the jaguar (*Panthera onca*), widely distributed in the Brazilian Pantanal (EMBRAPA, 2002). However, these studies show only seropositivity in captivity animals (SILVA et al., 2001; RAMOS SILVA et al., 2007), and there are no comparative studies on free-living wild cats. Additionally, there are no evaluation of shedding *T. gondii* oocysts in wild cats faeces. Thus, further studies should be conducted to assess the soropresence of *T. gondii* and *N. caninum* in felids and canines of Pantanal, respectively, as well as their role as definitive hosts, to establish its importance in infection of other species that cohabit the region, like the Pantanal horses.

The present study showed that horses in Pantanal region, Mato Grosso, are exposed to *T. gondii* and *N. caninum*. This study reports for the first time the presence of antibodies against these parasites in healthy pantaneiros horses.

### Comission of ethics and animal welfare

Protocol number 2012-082 (CEP/Cuiabá University).

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