

# Occurrence of antibodies against *Neospora caninum* in wild pigs (*Sus scrofa*) in the Pantanal, Mato Grosso do Sul, Brazil

## Ocorrência de anticorpos contra *Neospora caninum* em porcos-monteiro (*Sus scrofa*) do Pantanal, Mato Grosso do Sul, Brasil

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### Abstract

Serum samples from 83 free living wild pigs (*Sus scrofa*) from the Nhecolândia region, Pantanal do Mato Grosso, Brazil, were examined for the presence of antibodies against *Neospora caninum* by means of the indirect fluorescence antibody test (IFAT) with a cut off of 50. Antibodies were present in 10.8%, with titers of: 1:200 in one animal, 1:400 in four and 1:800 in four. Analysis using the  $\chi^2$  test showed an association between sex and presence of antibodies, with females showing occurrence of 20.5% and males 2.3% ( $p = 0.017$ ). No association was found between age and occurrences of antibodies against this parasite. This was the first observation of *N. caninum* antibodies in Brazilian wild pigs from Pantanal.

**Keywords:** *Sus scrofa*. *Neospora caninum*. Pantanal. Antibodies. IFAT. Brazil.

### Resumo

Amostras de soro de 83 porcos-monteiro (*Sus scrofa*) selvagens, oriundos da região de Nhecolândia, Pantanal do Mato Grosso, Brasil, foram examinadas para a presença de anticorpos contra *Neospora caninum* pela reação de imunofluorescência indireta (RIFI) com ponto de corte de 50. A ocorrência de anticorpos foi de 10,8%, com um animal apresentando título de 1:200, quatro com 1:400 e quatro com 1:800. Quando analisados pelo teste do  $\chi^2$ , foi observada a existência de associação entre sexo e presença de anticorpos, com as fêmeas apresentando ocorrência de 20,5% e os machos 2,3% ( $p = 0,017$ ). Não houve associação entre idade e ocorrência de anticorpos contra este parasito. Esta é a primeira observação de porcos-monteiro da região do Pantanal, apresentando anticorpos contra *N. caninum*.

**Palavras-chave:** *Sus scrofa*. *Neospora caninum*. Pantanal. Anticorpos. RIFI. Brasil.

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*Neospora caninum* is an important cause of abortion among cattle worldwide. Dogs (*Canis lupus familiaris*), coyotes (*Canis latrans*), dingoes (*Canis lupus dingo*) and gray wolves (*Canis lupus*) (MCALLISTER et al., 1998; GONDIM et al., 2004; KING et al. 2010; DUBEY et al., 2011) have an important role in the epidemiology of this parasite because they are the definitive hosts, shedding the environmentally resistant oocysts in their feces.

*Neospora caninum* has already been found infecting wild animals like herbivores, carnivores, rodents and marine mammals; however this parasite has only been successfully isolated from a few species (reviewed by GONDIM, 2006). The role of birds in *N. caninum* epidemiology is yet to be determined. Mineo et al. (2011) analyzed serum samples from 294 birds of 17 species in Brazil and none of them were seropositive for *N. caninum*, using the indirect fluorescent antibody test (IFAT  $\geq$  20). However, using immunohistochemical techniques, *N. caninum* cysts were found in the muscle tissue of two Psittaciformes birds.

Pantanal is a huge floodplain and comprises areas of Brazil, Paraguay and Bolivia and it is one of the richest wild life areas of Brazil (ABREU et al., 2010). Wild pigs, *Sus scrofa* L. (Artiodactyla, Suidae), are one of the most invasive animal species worldwide (LOWE et al., 2000), and in Pantanal, wild pigs are the main hunting target, whereas the endemic species are protected by federal law and hunting is forbidden (DESBIEZ et al., 2011). Brazilian Pantanal has the country's largest wild pig population, which has become established over the last 200 years (DESBIEZ et al., 2011).

*Neospora caninum* antibodies in wild pigs (*Sus scrofa*) have been detected in the Czech Republic (BÁRTOVÁ et al., 2006), in Spain (ALMERÍA et al., 2007) and in the USA (BEVINS et al., 2013). In Pantanal region, *N. caninum* antibodies were detected in other species of wild animals (IFAT  $\geq$  1:50) as pampas-deer (*Ozotoceros bezoarticus*), with 75% occurrence, 12 of 16 samples examined were positive (TIEMAN et al., 2005), and jaguars (*Panthera onca*), with 72.7% occurrence and 8 of 11 samples positive (ONUMA et al., 2014).

To date, no reports on seropositivity for *N. caninum* among *Sus scrofa* in Brazil have been published. Therefore, the purpose of this study was to survey the occurrences of antibodies against *N. caninum* in wild pigs in the Pantanal region, Brazil,

where this invasive species has contact with other wild and domestic animals.

Wild pig samples were collected in Nhecolândia (18°59'15"S; 56°37'03"W), a southeastern subregion of the Pantanal, state of Mato Grosso do Sul, Brazil. A detailed description of the area can be found elsewhere (RAMOS et al., 2014). The wild pigs were captured in forested areas or by water bodies using traditional local method with the aid of dogs and a lasso. After the pigs had been caught, they were sedated as described by Fontana (2011) for blood sample collection. Samples from 83 individuals were collected; serum was obtained after centrifugation; and this was identified and stored at -20°C until assayed for antibodies against *N. caninum*.

The capture and handling had previously been authorized by the federal environmental authorities (SISBIO license 21416-1).

Individual serum samples were tested for the presence of IgG antibodies against *N. caninum* by means of IFAT, as previously described by Paré et al. (1995), using 1:50 dilution as the cut-off value (ALMERÍA et al., 2007; AZEVEDO et al., 2010).

The association of each qualitative variable (age and sex) with seropositivity for *N. caninum* was evaluated by means of the  $\chi^2$  test with a significance level of 5%. The SPSS 17 software was used to perform the analyses.

The overall occurrence rate of antibodies against *N. caninum* was 10.8%, i.e. nine of the 83 animals examined were positive. The antibody titers were 200 in one animal, 400 in four, and 800 in four. In the Brazilian Pantanal area, *Sus scrofa* was introduced by European settlers about 200 years ago. Mourão et al. (2002) considered feral pig one important species in terms of the animal biomass it represents. Despite the low inference about the relation of this species with the native populations of fauna and other animals in the region (DESBIEZ et al., 2011), the role of this species in the epidemiological chain of diseases for humans and animals is an aspect that deserves attention (RAMOS et al., 2014).

Table 1 presents the occurrence rate of antibodies against *N. caninum* according to age (adult versus juvenile) and sex (male versus female). Sex was a qualitative variable that presented a significant association ( $p = 0.017$ ), such that 2.3% of males and 20.5% of females were positive.

Table 1 – Occurrences of antibodies against *N. caninum* among wild pigs (*Sus scrofa*) according to age and sex, in the Pantanal region, Brazil – 2015

	Number of animals			P
	Examined	Positive	Occurrence (%)	
<b>Age</b>				
Adult	60	7	11.7	0.729
Juvenile	23	2	8.7	<b>0.017</b>
<b>Gender</b>				
Male	44	1	2.3	
Female	39	8	20.5	
<b>Total</b>	<b>83</b>	<b>9</b>	<b>10.8</b>	

The prevalence found in the present study was similar to other studies with the same animal species. Using the same technique and also the same cut-off, Almería et al. (2007) found a prevalence of 0.3% in Spain (one out of the 298 animals examined was positive). Also using IFAT and a cut-off of 40, Bártová et al. (2006) found that 18.1% of the animals were positive in the Czech Republic (102 out of the 565 animals examined). In the USA, Bevins et al. (2013) found a prevalence of 15.8%, i.e. 74 out of the 467 wild pigs examined were positive for *T. gondii* antibodies, using a commercial ELISA kit. However, when compared with other wild animal studies in the same region (TIEMANN et al., 2005; ONUMA et al., 2014) the prevalence was lower, indicating that the wild pigs probably are less important in the epidemiology of *N. caninum*. Among domestic pigs, little is known about *N. caninum* infection with regard to abortion or transplacental infection. Jensen et al. (1998) experimentally infected pregnant pigs with *N. caninum*, and they observed disease caused by the parasite in the mothers and also transplacental infection. However, naturally-acquired porcine

infection and disease has not been described. Helmick et al. (2002) examined serum samples from 454 breeding sows that had aborted or were considered infertile, and all of them were negative according to IFAT.

Domestic pigs in northeastern Brazil were examined for antibodies against *N. caninum* (AZEVEDO et al., 2010) and occurrence rate of 3.1% (4/130) was found, i.e. lower than what was observed in the present study among wild pigs using the same technique and cutoff.

*N. caninum* is a very important and prevalent parasite among cattle in Pantanal area (ANDREOTTI et al., 2004). The wild pigs of the Pantanal, including the animals of the present study, live on cattle ranches, much closer to cattle than the majority of domestic pigs. Indeed, wild pigs in the Pantanal were observed consuming bovine carcasses found in the ground (Piovezan, personal observation). This close contact probably increases the chances of infection, through wild pigs' ingestion of bovine placentae and fetal material, given that these animals are omnivorous.

In the USA, coyotes and white tailed deer (*Odocoileus virginianus*) are involved in the sylvatic cycle of the parasite (GONDIM, 2006). However, in South America, very little is known about the epidemiology of *N. caninum* in sylvatic environments and the role of the wild carnivores and the animals that are part of their alimentary chain. Due to the economic importance and wide biodiversity of Pantanal, more studies should be done in this region about the relationship between wild and domestic animals and *N. caninum* infection.

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