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Detection of *Neospora* sp. antibodies in cart horses from urban areas of Curitiba, Southern Brazil

Detecção de anticorpos anti-*Neospora* sp. em cavalos de carroceiros de áreas urbanas de Curitiba, Sul do Brasil

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Abstract

Neospora caninum is a protozoan parasite which affects dogs as definitive hosts and several mammalian species as intermediate hosts mainly causing abortions and central nervous system disorders. The reemerging population of cart horses for carrying recycling material in urban areas of major cities in Brazil may have an impact on disease spreading, and these animals may be used as sentinels for environmental surveillance. Thus, the present study investigated the frequency of *Neospora* sp. antibodies in cart horses from Curitiba and surrounding areas, Paraná State, Southern Brazil. IgG antibodies against *Neospora* sp. were detected using indirect fluorescence antibody test (IFAT), and titers equal to or higher than 1:50 were considered reactive. Of all samples, 14/97 (14.4%) were positive: 2/29 (6.9%) were younger than 5; 5/26 (19.2%) between 6 and 9; and 6/31 (19.4%) older than 10 years of age. One of the 11 animals with unknown age was positive (9.1%). Cart horses are likely to be more exposed to dog feces and to *Neospora* sp. oocyst contamination in urban settings and a lower frequency of disease in dogs may have a negative impact on horse infection risk in these areas.

Keywords: *Neospora* sp., cart horse, indirect immunofluorescence, Brazil.

Resumo

Neospora caninum é um protozoário parasita que afeta cães como hospedeiros definitivos e diversos mamíferos como hospedeiros intermediários, envolvido em abortos e distúrbios do sistema nervoso central. A população reemergente de cavalos de carroceiros utilizados para transportar material reciclável em áreas urbanas de grandes cidades brasileiras na disseminação de doenças, e estes animais podem ser utilizados como sentinelas para vigilância ambiental. Deste modo, no presente estudo foi investigada a frequência de anticorpos anti-*Neospora* sp. em cavalos de carroceiros da Região Metropolitana de Curitiba, Estado do Paraná, Sul do Brasil. Anticorpos da classe IgG anti-*Neospora* sp. foram detectados utilizando a reação de imunofluorescência indireta (RIFI), e títulos maiores ou iguais a 1:50 foram considerados reagentes. Do total de amostras testadas, 14/97 (14,4%) foram positivas: 2/29 (6,9%) tinham menos do que 5,5/26 (19,2%) entre 6 and 9, e 6/31 (19,4%) mais de 10 anos de idade. Um dos 11 animais positivos não tinha a idade conhecida (9,1%). Como cavalos de carroceiros possivelmente estejam mais expostos a oocistos de *Neospora* sp. em ambientes urbanos, uma baixa freqüência da doença em cães pode ter impacto negativo no risco de infecção de cavalos nestas áreas.

Palavras-chave: *Neospora* sp., cavalos de carroceiros, imunofluorescência indireta, Brasil.

Neospora caninum is a protozoan parasite structurally and biologically related to *Toxoplasma gondii* due to a similar life cycle. The difference is that *Neospora* sp. has dogs and other canids as definitive hosts and affects mainly cattle herds while *Toxoplasma gondii* has felids as definitive hosts and is more pathogenic in humans, sheep and goats. *Neospora caninum* has been associated to abortions and central nervous system disorders in several mammalian species and dogs have been reported as their main definitive hosts (McALLISTER et al., 1998; DUBEY, 2003; DUBEY et al., 2007). Dogs, coyotes, fox and wild dogs are known

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definitive hosts (McALLISTER et al., 1998; LINDSAY et al., 1999; BASSO et al., 2001; WAPENAAR et al., 2006; KING et al., 2010). Horses can serve as an intermediate host. Tachyzoites of *Neospora caninum* were first found in a fetus lung (DUBEY; PORTERFIELD, 1990) and tachyzoites of *Neospora hughesi* were isolated from an adult horse, both in the US (MARSH et al., 1998). In horses, neosporosis causes abortions, and neonatal, visceral and neurological disorders (LOCATELLI-DITTRICH et al., 2006). *Neospora caninum* is related to abortions and *Neospora hughesi* with neurological disease.

Curitiba is currently the eighth most populated city in Brazil and has approximately 3,000,000 inhabitants in its metropolitan area (IBGE, 2010). According to the local center for disease control, around 1,500 owners and their cart horses move around daily in Curitiba and surrounding areas to collect recycling materials. Horses are mainly fed with grass from these urban areas, and most horses belong to poor working people who cannot afford veterinary care. As dogs are definitive hosts of *Neospora caninum* and ingestion of their feces may infect other animals (McALLISTER et al., 1998), contaminated grass intake by horses in urban areas may increase the risk of *Neospora* infection. The aim of the present study was to investigate the frequency of antibodies against *Neospora* sp. in cart horses in Curitiba and São José dos Pinhais (Curitiba surroundings), Paraná State, Southern Brazil.

A total of 97 samples were collected from apparently healthy cart horses from urban areas of Curitiba and surroundings. These horses were mainly used for carrying rudimentary carts of recycling materials and they were voluntarily taken by their owners to the local center for disease control. There were no inclusion criteria in this study and no animals had neurological signs. A total of 97 crossbreed horses, 51 males and 46 females, with a mean age of 15.3 ± 4.2 years (from 4 months to 22 years) were studied. There were 12 mares and 17 horses younger than 5 years; 13 mares and 13 horses aged between 6 and 9 years; and 12 mares and 19 horses older than 10 years old. Age was unknown for 9 mares and 2 horses. Blood samples were collected by vein puncture, sera separated and stored at-20 °C until processing at Instituto Biológico, São Paulo State, Brazil. The present study was approved by Universidade Federal do Paraná Animal Ethics Committee (protocol number 027/10).

Horse samples were screened at dilutions of 1:50 and 1:100 antibodies against *Neospora caninum* using an Indirect Fluorescent Antibody Test (IFAT).

The study results are summarized in Table 1 and 2. Antibodies to *Neospora* sp. were found in 14/97 (14.4%) of horses tested. Among positive horses (14), 6 had titers of 1:50 (42.8%), 6 1:100 (42.8%), and 2 1:200 (14.3%). Of the 14 positive horses, 2/29 (6.9%) were younger than 5; 5/26 (19.2%) between 6 and 9; and 6/31 (19.4%) older than 10 years of age. One of the 11 animals with an unknown age was positive (9.1%).

Because of *N. caninum* and *N. hughesi* serological cross-reactivity we could not confirm which species infected these horses, but the serological results indicated that cart horses have been exposed to *Neospora* sp. (MARSH et al., 1998; WALSH et al., 2000). The frequency of anti-*Neospora* sp. antibodies found in the present study (14.4%) was similar to that previously reported in healthy horses from Israel, with 95/800 (11.9%) positive samples (KLIGLER et al., 2007) and in reproductive-age mares from a horse farm in Brazil, with 16/116 (13.8%) animals found positive (TOSCAN et al., 2010). In a study in Chile, 47/145 horses (32.0%) were positive and 8/21 (38.09%) were positive working horses (PATITUCCI et al., 2004), which is a rate higher than that found in this study that also studied working horses. In Italy, 42/150 (28.0%) healthy horses were positive: 14/63 (22.2 %) younger than 5 years, 13/45 (28.8%) aged between 6 and 9 years; and 15/42 (35.7%) older than 10 years old (CIARAMELLA et al., 2004). In all age groups, the prevalence was higher than that found in this study. In Southern Brazil 17/36 adult mares (47.0%) from farms in Paraná state were positive (LOCATTELI-DITTRICH et al., 2006). Finally, our rates were higher than that reported in adult horses from Sao Paulo State (114/1106; 10.3%) (VILLALOBOS, et al., 2006) and horses older than 10 years old from Paraná state (5/146; 3.4%) (HOANE et al., 2006).

A serological survey of *Neospora* sp. conducted in dogs of Curitiba and surrounding areas showed 50/197 (25.38%) and 23/181 (12.71%) positive samples in rural and urban areas, respectively (FRIDLUND-PLUGGE et al., 2008). Interestingly, these results have shown a pattern: higher prevalence of seropositive dogs from rural when compared to those from urban areas. Likewise, a study found a higher prevalence with 17/36 (47.0%) seropositive horses in rural areas (LOCATTELI-DITTRICH et al., 2006) when compared to 14/97 (14.4 %) seropositive cart horses in the present study. Carnivores are likely to become infected by ingesting tissues containing bradyzoites while herbivores become infected by the ingestion of food or water contaminated by *Neospora* sp. sporulated oocysts (DUBEY et al., 2007). Since horses and dogs may share the same environment in these areas, we believe that the higher frequency of positive horses seen in rural areas may be contaminated pasture with *Neospora* sp. oocysts from feces of infected dogs and the higher prevalence in dogs may be explained by greater access to carcasses, fetuses and placentas from farm

Table 1. Serum antibodies to *Neospora* sp. in cart horses from urban areas of Curitiba and São José dos Pinhais, Southern Brazil, according to gender and city.

Local	Animals	Positive		Total
		Males (%)	Females	
Curitiba	25	0	1 (4.0%)	1 (4.0%)
São José dos Pinhais	72	8 (11.1%)	5 (6.9%)	13 (18.0%)
Total	97	8 (8.2%)	6 (6.1%)	14 (14.4%)

Table 2. Serum antibodies to *Neospora* sp. in cart horses from urban areas of Curitiba and São José dos Pinhais, Southern Brazil, according to age and titer.

Positive	Age (years old)			Total
Titer	≤5	6-9	≥10	
1:50	0	3	2	5
1:100	1	1	4	6
1:200	1	1	0	2
Total	2	5	6	13

infected animals. In conclusion, cart horses are more likely to be exposed to dog feces and to *Neospora* sp. oocyst contamination in urban settings, and a lower frequency of disease in dogs may have a negative impact on horse infection risk in these areas.

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