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Occurrence of antibodies against *Neospora caninum* and/or *Toxoplasma gondii* in dogs with neurological signs

Ocorrência de anticorpos contra *Neospora caninum* e/ou *Toxoplasma gondii* em cães com sinais neurológicos

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Resumo

Neste estudo objetivou-se verificar a ocorrência de anticorpos contra *Neospora caninum* e/ou *Toxoplasma gondii* em cães com sinais neurológicos. Foram coletadas 147 amostras de sangue, sendo 127 de animais domiciliados (atendidos no Hospital Veterinário da Universidade Federal do Paraná (HV-UFPR) e em clínicas veterinárias da cidade de Curitiba) e 20 de cães errantes da região metropolitana de Curitiba. Os cães apresentavam um ou mais dos seguintes sinais neurológicos: convulsão, paresia ou paralisia, ataxia, alterações de comportamento, alterações sensoriais somáticas e coriorretinite. As amostras foram analisadas pela reação de imunofluorescência indireta (RIFI), na diluição de corte 1:50. Das 147 amostras obtidas, 17 (11,56%) foram positivas para *N. caninum*, 31 (21,08%) foram positivas para *T. gondii* e quatro (2,72%) foram reagentes para ambos os protozoários. Na titulação dos animais positivos, 54,83% (17/31) e 41,18% (07/17) apresentaram títulos ≥1:200 contra *T. gondii* e *N. caninum,* respectivamente. Diferença significativa (P = 0,021, OR = 2,87, IC = 1,1 > 2,8 > 7,4) foi observada para soropositividade ao *T. gondii* entre cães domiciliados (18,11%) e errantes (40%). Sugere-se a inclusão dos exames sorológicos de neosporose e toxoplasmose no diagnóstico de doença neurológica em cães.

Palavras-chave: *Neospora caninum, Toxoplasma gondii*, sorologia, cães, sinais neurológicos.

Abstract

This study aimed to evaluate occurrences of antibodies against *Neospora caninum* and *Toxoplasma gondii* in dogs with neurological signs. Blood samples from 147 dogs were collected: 127 from owned dogs (attended at the Veterinary Teaching Hospital of the Federal University of Paraná (HV-UFPR) and at private veterinary clinics in the city of Curitiba), and 20 from stray dogs found in Curitiba's metropolitan region. The dogs presented one or more of the following neurological signs: seizures, paresis or paralysis, ataxia, behavioral abnormalities, sensory and somatic disorders and chorioretinitis. The samples were analyzed by means of the indirect fluorescent antibody test (IFAT), at a cutoff dilution of 1:50. Out of the 147 samples obtained, 17 (11.56%) were seropositive for *N. caninum*, 31 (21.08%) for *T. gondii* and four (2.72%) for both protozoa. Serum titration on the positive animals showed that 54.83% (17/31) and 41.18% (7/17) had titers ≥ 1:200 against *T. gondii* and *N. caninum*, respectively. A significant difference in seropositivity for *T. gondii* (P = 0.021; OR = 2.87; CI = 1.1 > 2.8 > 7.4) was observed between owned dogs (18.11%) and stray dogs (40%). Inclusion of serological tests for neosporosis and toxoplasmosis is recommended in diagnosing neurological diseases in dogs.

Keywords: *Neospora caninum, Toxoplasma gondii,* serology, dogs, neurological signs.

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Introduction

Neospora caninum and *Toxoplasma gondii* are protozoan parasites with worldwide distribution that cause neuromuscular disease in many animals, including dogs (PARADIES et al., 2007). *Neospora caninum* is capable of infecting various species of domestic and wild animals, and it is considered to be the main cause of abortions among cattle (RIVERA, 2001; DUBEY et al., 2007). Dogs are the definitive hosts of *N. caninum* and are important in the transmission cycle to other animals. In dogs, neosporosis causes neuromuscular, heart, lung and skin diseases (DUBEY, 2003; McINNES et al., 2006).

Few serological-epidemiological studies on neosporosis in dogs presenting neurological signs have been conducted (YILDIZ et al., 2009). In dogs without neurological signs, several studies have demonstrated that the seroprevalence of antibodies against *N. caninum* ranges from 1 to 20%, and it may be up to 100% in endemic areas (ANTONY; WILLIAMSON, 2003; DUBEY et al., 2007). In Brazil, the seroprevalence ranges from 4 to 54.2%, also in dogs without neurological signs (ROMANELLI et al., 2007; FIGUEREDO et al., 2008).

Neurological diseases caused by protozoa are little diagnosed in dogs, and most such cases are attributed to *T. gondii*. Toxoplasmosis in dogs is difficult to diagnose because of its chronic nature, which results from the high infectivity and low pathogenicity of the parasite. Clinical toxoplasmosis may be associated with the canine distemper virus or other immunosuppressive conditions that predispose towards multiplication of the protozoon (DUBEY et al., 2003; MORETTI et al., 2006).

The seroprevalence of *T. gondii* in dogs without neurological signs is high in Brazil, with rates ranging from 21 to 91% (SOUZA et al., 2003; ORTOLANI et al., 2005; FIGUEREDO et al., 2008). Most studies on the prevalence of *N. caninum* and *T. gondii* have been conducted on healthy dogs, and the information on disease occurrence in animals with neurological signs remains limited. Within this context, the aims of this study were to determine the prevalence of antibodies against *N. caninum* and *T. gondii* in dogs with neurological signs.

Material and Methods

Between March 2006 and March 2007, 147 blood samples were collected from dogs with neurological signs, in order to obtain serum. This project was approved by the Ethics Committee of the Federal University of Paraná (UFPR) (Protocol N° 033/2006). The samples were obtained from owned animals that were attended routinely at the Veterinary Hospital of UFPR (HV-UFPR) and at veterinary clinics located in Curitiba (127 dogs); and from stray animals found within the city's metropolitan region, which had been brought into the Zoonosis Control Centers of the municipalities of Pinhais (seven dogs) and São José dos Pinhais (eight dogs) and the non-governmental organization Association of Animal Friends in Campo Magro (five dogs). After neurological and ophthalmic examinations, the clinical files from the neurological examinations on animals with one or more neurological signs were identified and divided according to category of clinical sign. The classical definitions were used to categorize the neurological signs encountered: seizures, paresis or paralysis, ataxia, behavioral abnormalities, sensory and somatic disorders and chorioretinitis.

The indirect fluorescent antibody test (IFAT), was used to detect antibodies against *N. caninum* and *T. gondii* in the animals' serum. Slides were prepared, containing tachyzoites of *N. caninum* (strain NC-1) and *T. gondii* (strain RH), obtained through cell culturing as described by Locatelli-Dittrich et al. (2006). The serum samples were tested at an initial dilution of 1:50, in PBS solution (pH 7.2) and were analyzed in pairs until reaching to the final titer. Conjugated anti-dog IgG was used (SigmaÒ).

The test used for the statistical analysis on proportions between the number of positive samples and the total number of samples studied in the diferent groups was Fisher's exact test ($P \leq 0.05$).

Results

Out of the 147 samples, 52 (35.37%) were positive for *N. caninum* and/or *T. gondii*: 17 (11.56%) for *N. caninum*, 31 (21.08%) for *T. gondii* and four (2.72%) for both. Out of the 127 samples collected from owned animals, 14 (11.02%) were reactive to *N. caninum*, 23 (18.11%) to *T. gondii* and two (1.57%) to *N. caninum* and *T. gondii.* Out of the eight samples collected from stray dogs at the Zoonosis Control Center in São José dos Pinhais, one dog (12.50%) was seropositive for *T. gondii* and one (12.50%) for both protozoa. The seven samples obtained from stray dogs at the Zoonosis Control Center in Pinhais were all positive: three (42.86%) for *N. caninum*, three (42.86%) for *T. gondii* and one (14.28%) for *N. caninum* and *T. gondii.* Out of the five samples obtained from the Association of Animal Friends, four (80%) were positive for *T. gondii* and none for *N. caninum.* A significant difference ($P = 0.0014$; OR = 5.75; CI = 1.7 > 5.7 > 18.5) was observed between the samples collected from dogs at HV-UFPR and from dogs at the non-governmental organization Association of Animal Friends, in relation to the presence of antibodies against *T. gondii.* There was no significant difference ($P > 0.05$) among the other groups analyzed (Table 1).

Through comparing the animals according to their origin, the owned dogs presented occurrence rates of 11.02% (14/127) and 18.11% (23/127) for antibodies against *N. caninum* and *T. gondii*, respectively. Among the stray dogs, the occurrence rates were 15.00% (3/20) for *N. caninum* and 40% (8/20) for *T. gondii*. The seropositivity found for the two protozoa together was 1.57% among the owned dogs and 10.00% among the stray dogs (Table 2). There was a significant difference between the owned and stray dogs regarding the presence of antibodies against *T. gondii* ($P = 0.021$; OR = 2.87; CI = 1.1 > 2.8 > 7.4). There was no significant difference in seropositivity for *N. caninum* or for both protozoa, comparing dogs from different origins ($P > 0.05$).

The titers of the 17 dogs that were seropositive for *N. caninum* were: 1:50 (47.06%; 8/17); 1:100 (11.76%; 2/17); 1:200 (23.53%; 4/17); and 1:800 (17.65%; 3/17). The titers of the 31 dogs that were seropositive for *T. gondii* were: 1:50 (25.80%; 8/31); 1:100 (19.35%; 6/31); 1:200 (32.26%; 10/31); 1:400 (6.45%; 2/31); and 1:800 (16.13%; 5/31). Out of the four dogs that were seropositive

Table 1. Occurrences of antibodies against *Neospora caninum* and/or *Toxoplasma gondii* (IFAT ≥ 50) among dogs with neurological signs in Curitiba and its metropolitan region, Paraná, Brazil.

Region	Dogs	Seropositivity					
		N. caninum		*T. gondii*		*N. caninum* and *T. gondii*	
	n	n	%	n	%	n	%
HV-UFPR/Clinics	127	14	11.02	23[a]	18.11	02	1.57
ZCC São José dos Pinhais	08	00	00	01[ab]	12.50	01	12.50
ZCC Pinhais	07	03	42.86	03[ab]	42.86	01	14.28
NGO Animal Friends	05	00	00	04[b]	80.00	00	00
Total	147	17	11.56	31	21.08	04	2.72

Different letters in the column = P ≤ 0.05; ZCC = Zoonosis Control Center; HV/UFPR = Veterinary Hospital of the Federal University of Paraná; Ngo = non-governmental organization.

Table 2. Occurrences of antibodies against *Neospora caninum* and *Toxoplasma gondii* (IFAT ≥ 50) among owned and stray dogs with neurological signs in Curitiba and its metropolitan region, Paraná, Brazil.

Origin	Dogs	Seropositivity					
		N. caninum		*T. gondii*		*N. caninum* and *T. gondii*	
	n	n	%	n	%	n	%
Owned	127	14	11.02	23	18.11[a]	02	1.57
Stray	20	03	15.00	08	40.00[b]	02	10.00
Total	147	17	11.56	31	21.08	04	2.72

Different letters in the column = P ≤ 0.05.

for both parasites, three presented titers of 1:50 (75%) and one presented a titer of 1:200 (25%).

The neurological abnormalities observed were seizures, paresis or paralysis of the forelimbs and/or hind limbs, ataxia, behavioral changes, sensory abnormalities (such as loss of nociception or proprioception) and chorioretinitis. The dogs were of various ages, both sexes and different breeds, and no trends were revealed in relation to any of these traits.

Discussion

Most of the reports on the seroprevalence of *N. caninum* and *T. gondii*, both in Brazil and in several other countries, have been obtained from asymptomatic dogs. There have been variations in the rates between diferent Brazilian States (GARCIA et al., 1999; AZEVEDO et al., 2005).

There are only a few reports on the seroprevalence of neosporosis and toxoplasmosis among dogs with neurological signs (VARANDAS et al., 2001; GIRALDI et al., 2002; YILDIZ et al., 2009). In the present study, occurrences of antibodies in dogs with neurological signs were greater for *T. gondii* (21.08%) than for *N. caninum* (11.56%), or for both parasites together (2.72%). Results like these were previously described by Mineo et al. (2001), Giraldi et al. (2002), Varandas et al. (2001) and Klein and Müller (2001), among others. In Minas Gerais, the seroprevalences were 3.3, 3.7 and 3.1%, respectively, for *T. gondii*, *N. caninum* and both parasites together, in dogs with neurological, respiratory and/or gastrointestinal diseases (MINEO et al., 2001). In the Northeastern region of the State of São Paulo, the seroprevalences were, respectively, 8.14, 1.36 and 5.76%, for *T. gondii*, *N. caninum* and both of them together, without any correlation between positive serological tests and the presence of neuropathies in the animals (VARANDAS et al., 2001). In Londrina, the seroprevalence of toxoplasmosis was high (35.4 to 77.6%) and neosporosis was not diagnosed (GIRALDI et al., 2002). In Germany, the seroprevalence among dogs with signs was also greater for *T. gondii* (29% and titer ≥ 1:32) than for *N. caninum* (13% and titer ≥ 1:50) (KLEIN; MÜLLER, 2001).

The occurrences of antibodies against *T. gondii* and *N. caninum* among the stray dogs in the present study were 40 and 15%, respectively. In the owned dogs, antibodies against *T. gondii* and *N. caninum* were observed in 18.11 and 11.02% of the dogs, respectively. Comparing the owned and stray dogs, there was a significant difference in relation to the presence of antibodies against *T. gondii* (P = 0.021; OR = 2.87; CI = 1.1 > 2.8 > 7.4), thus demonstrating that access to the streets may be a risk factor for infection and may contribute towards greater seropositivity of *T. gondii*. This result has also been observed among stray dogs in other regions (BARBOSA et al., 2003; MOURA et al., 2009).

For *N. caninum* and for both protozoa together, there was no significant difference between the owned and stray dogs regarding antibody occurrences. This result was similar to the findings from other studies conducted in Bahia and Paraná, which also did not find differences between owned and stray dogs (JESUS et al., 2006; FRIDLUND-PLUGGE et al., 2008). However, there have been reports of lower seroprevalence of *N. caninum* among owned dogs (AZEVEDO et al., 2005) and greater chances of infection among street dogs (GENNARI et al., 2002).

The main neurological signs observed in the dogs that were reactive to *N. caninum* were paresis/paralysis, seizures and ataxia. In the dogs that were seropositive for *T. gondii*, the main signs were also seizures, ataxia and paralysis. However, in Londrina, Paraná, myoclonus was the sign most frequently presented by the dogs that were seropositive for *T. gondii* (GIRALDI et al., 2002). In the present study, the clinical signs were similar for the two diseases, but there were greater occurrences of *T. gondii,* thus corroborating some other results observed previously (MINEO et al., 2001; VARANDAS et al., 2001; GIRALDI et al., 2002).

In the present study, the titers of the dogs that were positive for *N. caninum* ranged from 1:50 to 1:800, with predominance

of the titers 1:50 and 1:200. The titers reported for dogs with clinical neosporosis, measured using IFAT, have been 1:50 and 1:3200 (DUBEY et al., 1998; KLEIN; MÜLLER, 2001), although IgG titers are generally greater than 1:400 (DUBEY et al., 1998; BASSO et al., 2005).

Antibodies against *N. caninum* have also been observed in dogs without neurological signs. Hence, simply the presence of antibodies does not indicate clinical neosporosis (AZEVEDO et al., 2005; VÁCLAVEK et al., 2007; FRIDLUND-PLUGGE et al., 2008). On the other hand, in cases of seronegative dogs or dogs with small titers, neosporosis should not be ruled out. Serological tests should be used to make the clinical diagnosis, but the titers remain undefined and vary according to the serological test. Progressive increases in the antibody titers of class IgG over a period of time are generally indicative of acute infection (DUBEY et al., 1998).

The titers for *T. gondii* presented by the dogs ranged from 1:50 to 1:800, with predominance of 1:50 and 1:200. Other authors have taken into account titers of 1:16 (GIRALDI et al., 2002) or 1:32 (KLEIN; MÜLLER, 2001), but the occurrences observed in the present study may have been greater because of the cutoff point used (1:50). Given that 32.26% of the dogs that were seropositive for *T. gondii* presented high titers (1:200), toxoplasmosis may have been the cause of the neuropathy cases observed.

Serological tests to identify infection by *N. caninum* and *T. gondii* should be done in cases of dogs with neuromuscular signs, even if canine distemper is suspected, because of the similarity of the clinical signs (TARLOW et al., 2005; MORETTI et al., 2006). Most of the stray dogs in the municipality of Pinhais were sent to the Zoonosis Control Center with suspected canine distemper because of the presence of neurological signs, but then the serological tests showed high titers for *T. gondii* (42.86%) and *N. caninum* (42.86%).

Detection of serum antibody titers against *N. caninum* or *T. gondii* in animals with neurological signs obviously does not necessarily establish a cause-effect relationship. Nevertheless, the prevalence of animals with neurological signs and positive findings for one or both of the protozoa was high in the present study. Knowledge of this prevalence, along with descriptions of the neurological signs most commonly observed in these animals, is extremely important for understanding both of these diseases. Neosporosis should certainly be included in the differential diagnosis of neurological diseases like chorioretinitis, canine distemper and rabies, but in Brazil, toxoplasmosis is still the main parasitic disease investigated in the differential diagnosis of neurological diseases. In the present study, just as in Minas Gerais (MINEO et al., 2001), it was observed that the serological test to identify infection by *N. caninum* in dogs that show neuromuscular signs should be included in the differential diagnosis for infection by *T. gondii.*

Conclusion

It was found that a significant percentage of the dogs with neurological signs were infected with the protozoa *N. caninum* (11.56%), *T. gondii* (21.08%) or both (2.72%). Seropositivity for the two diseases was widely distributed among the population studied. This result suggests that clinical veterinarians should be more attentive in drawing up differential diagnoses. Serological tests for toxoplasmosis and/or neosporosis should be requested in the cases of dogs with neurological abnormalities, even if canine distemper or another disease of the central nervous system is suspected, because of the similarity of the clinical signs observed and the possibility of coinfection in the animals.

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