# PREVALENCE OF ANTI-*Neospora caninum* ANTIBODIES IN DOGS IN THE URBAN AREA OF CAMPO GRANDE, MS, BRAZIL[1]

JACQUELINE M. DE OLIVEIRA[2]; MARIA DE FATIMA C. MATOS[3]; LEANDRA M. OSHIRO[4]; RENATO ANDREOTTI[5]

**ABSTRACT:**- OLIVEIRA, J.M. DE; MATOS, M. DE F.C.; OSHIRO, L.M.; ANDREOTTI, R. **Prevalence of anti-*Neospora caninum* antibodies in dogs in the urban area of Campo Grande, MS, Brazil.** [Prevalência de anticorpos anti-*Neospora caninum* em cães da área urbana do município de Campo Grande, MS, Brasil]. *Revista Brasileira de Parasitologia Veterinária*, v. 13, n. 4, p. 155-158, 2004. Embrapa Gado de Corte, BR 262, km 4, Caixa postal 154, Campo Grande, MS 79002-970, Brazil. E-mail: andreott@cnpgc.embrapa.br

*Neospora caninum* is an obligate intracellular protozoan that can infect domestic and wild canids, as well as ruminants and equines. It was described as causing neuromuscular alterations and death in dogs. In order to determine the prevalence of this parasite in the population of dogs in Campo Grande, MS, Brazil, 245 blood samples were collected from animals originating from the four health districts of the city. Sera were submitted to indirect fluorescence antibody test (IFAT) for detection of anti-*N. caninum* antibodies, using the NC-1 strain. Sixty-five reactive samples were detected, accounting for a prevalence of 26.53%. Prevalences were similar across all health districts: North, 30.61%; South, 27.27%; East, 24.32%; and West, 23.94%. Titers ranged from 1:50 to 1:400, as follows: 1:50 (29.23%), 1:100 (47.69%), 1:200 (18.46 %), and 1:400 (4.62%). The study revealed a statistically significant association with age ($p < 0.05$), with values of 31.38% for adult dogs and 10.52% for young ones. No statistical difference was found between male and female animals. The results provided evidence for the presence of *N. caninum* in all health districts of Campo Grande, MS, showing that dogs in these areas are exposed to infection with this parasite.

KEY WORDS: *Neospora caninum*, dogs, indirect fluorescence antibody test**,** prevalence, Campo Grande.

**RESUMO**

*Neospora caninum* é um protozoário intracelular obrigatório que pode infectar canídeos domésticos e selvagens, ruminantes e eqüinos, que tem sido descrito como causa de alterações neuromusculares e morte em cães. Para determinar a prevalência desse parasita na população de cães da cidade de Campo Grande, MS, foram coletadas 245 amostras de sangue de animais provenientes dos quatro distritos sanitários da cidade. Os soros foram submetidos à Reação de Imunofluorescência Indireta (RIFI) para detecção de anticorpos anti-*N. caninum*, utilizando-se a cepa NC-1. Foram detectadas 65 amostras reagentes, representando uma prevalência de 26,53%. As prevalências entre os diferentes distritos sanitários foram similares: Norte, 30,61%; Sul, 27,27%; Leste, 24,32%; Oeste, 23,94%. Os títulos encontrados variaram de 1:50 a 1:400, a saber: 1:50 (29,23%), 1:100 (47,69%), 1:200 (18,46%) e 1:400 (4,62%). Verificou-se associação estatisticamente significativa em relação à variável idade ($p < 0,05$), com 31,38% para cães adultos e 10,52% para cães jovens positivos a RIFI. Não houve diferença estatística significativa entre machos e fêmeas. Os resultados encontrados evidenciaram a presença de *N. caninum* nos diferentes distritos sanitários de Campo Grande, indicando que os cães dessa região estão expostos à infecção por esse parasita.

PALAVRAS-CHAVE: *Neospora caninum*, cães, imunofluorescência indireta, prevalência, Campo Grande.

## INTRODUCTION

*Neospora caninum* is a protozoan of the phylum Apicomplexa, class Sporozoasida, family Sarcocystidae, subfamily Toxoplasmatinae, that can infect domestic and wild canids, as well as ruminants and equines. It was first described as causing neuromuscular alterations and death in dogs (DUBEY et al., 1988). Domestic dogs, which have been considered the only definitive hosts of this parasite, shed oocysts in their feces after ingesting tissue cysts of *N. caninum*

[1] Supported by Fundect/MS and CNPq.

[2] Agência de Defesa Sanitária Animal e Vegetal – IAGRO.

[3] Universidade Federal de Mato Grosso do Sul – UFMS

[4] Bolsista do CNPq.

[5] Embrapa Gado de Corte, BR 262, km 4, Caixa postal 154, Campo Grande, MS 79002-970. E-mail: andreott@cnpgc.embrapa.br

(LINDSAY et al., 1999). Recently, however, the presence of oocysts of *N. caninum* has been demonstrated in the feces of coyotes (*Canis latrans*), thus identified as definitive hosts of this protozoan (GONDIM et al., 2004).

Studies on the prevalence of anti-*N. caninum* antibodies have revealed that neosporosis has a wide geographical distribution, as it has been reported from many parts of the world, including Australia, New Zealand, Europe, Korea, Japan, Thailand, and the Americas. Dogs and bovines are the chief species exposed to this parasite; both dairy and beef cattle can be affected (ANDERSON et al., 2000).

Clinical and subclinical infections with *N. caninum* in dogs are epidemiologically relevant because domestic dogs (*Canis familiaris*), the principal definitive hosts of the parasite, are capable of shedding oocysts in the environment, thus constituting a risk factor for the occurrence of miscarriage associated with *N. caninum* in bovines (PARE et al., 1998). Studies conducted in Canada, Japan, and the Netherlands have reported a positive correlation between canine and bovine neosporosis (WOUDA et al., 1999). Dijkstra et al. (2002) found that dogs living on farms with evidence of postnatal infection with *N. caninum* were fed placenta from cows and defecated on feeding alleys and silage storage, whereas on farms without evidence of infection such habits were not frequent. The study supported the hypothesis that farm dogs might become infected by fetal fluids or placental material from infected cattle, and might subsequently cause postnatal infection in noninfected bovines by shedding oocysts.

Recently, *N. caninum* has received considerable attention for its large impact on the dairy industry, given the economic losses related to breeding failures and to a decrease in productivity (DUBEY, 2003).

*Neospora caninum* can cause necrotic lesions that become visible within a few days, leading to cellular death by active multiplication of tachyzoites, and potentially producing severe neuromuscular diseases in dogs, bovines, and possibly other species as well. Large numbers of neural cells are destroyed, including cranial and spinal nerves, affecting the conductivity of these cells (CANTILE; ARISPICI, 2002).

Dogs of any age can develop clinical neosporosis, which may be generalized - when several organs are involved, including the skin - or localized. The most severe cases of neosporosis occur in young, congenitally infected cubs. Juvenile dogs develop hind limb paresis that evolves to paralysis. Neurological signs are dependent on the site parasitized. Hind limbs are more severely affected than front limbs and are often in rigid hyperextension. Other possible dysfunctions include difficulty in swallowing, paralysis of the jaw, muscle flaccidity and atrophy, and heart failure (DUBEY, 2003). Congenital and postnatal neosporosis have been experimentally induced in dogs, although the typical signs of congenital neosporosis, observed in naturally occurring cases, have not been reproduced (BUXTON et al., 2002).

In addition to cells of the nervous system, other cells can be infected, among them those of the vascular endothelium, myocytes, and dermal cells. Reported cases of cutaneous neosporosis are related to immunosuppressed or aged dogs (DUBEY et al., 1988).

Precise diagnostic techniques for detecting animals infected with *N. caninum* are central for understanding the epidemiological aspects of neosporosis. IFAT is regarded as the reference test, against which the other methods are compared.

Cutoff values for IFAT differs among laboratories, but the most frequently used ones are 1:50 for dogs and 1:160-1:640 for bovines. This divergence among laboratories hampers direct comparison of results. Tachyzoites of *N. caninum* isolated either from bovines or dogs have also been used as antigens for IFAT at several laboratories, without indication that antigenic differences among isolates may affect test accuracy. IFAT for *N. caninum* has been applied to a large number of animal species (BJORKMAN; UGGLA, 1999).

The purpose of this study was to determine the prevalence of anti-*N. caninum* antibodies in sera from dogs living in the urban area of Campo Grande, MS, Brazil, and to identify the association of the factors sex, age, and geographical health district with the frequencies of reactive titers.

## MATERIAL AND METHODS

**Animals.** Sample size was calculated with the formula proposed by Thrusfield (1994), $N = Z^2\ P(1 - P)/e^2$, with a 95% confidence interval and a maximum error of 5%. For the expected prevalence, a 20% level was adopted, which corresponds to the mean value found in epidemiological studies conducted in other Brazilian localities (BELO et al., 1999, MEIRA SANTOS et al., 1999, SILVA et al., 2001, SOUZA et al., 2002, CAÑÓN-FRANCO et al., 2003), resulting in a total of 245 samples. The entire sample was stratified so that the number of samples from each health district was proportional to the entire estimated dog population, according to data provided by Center for Zoonosis Control of the municipality.

**Collection of samples**. The blood samples were collected by the Center for Zoonosis Control of Campo Grande during the antirabies vaccination campaign, from September to October, 2003, and graciously supplied for analysis of anti-*N. caninum* antibodies. All the samples were drawn from dogs that lived in the urban area of the municipality and did not have known clinical history of any disease. The samples were drawn from the brachial or jugular veins into sterile Vacutainer tubes without anticoagulant. The tubes were centrifuged at 2500 rpm for 10 minutes and the sera thus obtained were stored at –20 ºC until analysis.

**Indirect fluorescence antibody test (IFAT).** The samples were examined for the detection of anti-*N. caninum* antibodies using the indirect immunofluorescence antibody test (IFAT). The antigen was produced in our laboratory using tachyzoites of *N. caninum*, strain NC-1 (DUBEY et al., 1988), graciously supplied by Dr. Rosangela Locatelli-Dittrich, of Universidade Federal do Paraná at Curitiba. The rabbit anti-canine IgG conjugate was purchased from VMRD (USA). The samples were tested at an initial dilution of 1:50 and the positive samples were then tested at 1:100, 1:200, 1:400, and 1:800 (LOCATELLI-DITTRICH, 2002).

**Statistical analysis.** For analysis of the results, the data were grouped by health district, sex, and age, and submitted to a chi-square test with one degree of freedom for the variables sex and age and three degrees of freedom for the variable health district, using Epi-Info 6.04b statistical analysis software and adopting a significance level of 0.05.

## RESULTS AND DISCUSSION

Sixty-five reactive samples were identified, accounting for a prevalence of 26.53% (65/245). The serology results were analyzed by health district, sex, and age.

As shown in Table 1, anti-*N. caninum* antibodies were detected in sera from every health district of the city. The West district had the lowest frequency (23.94%; 17/71) and the North district the highest one (30.61%; 15/49), though no statistically significant differences were found across districts.

Table 2 shows the results for the variables sex and age. Although the percentage of seroreactive males (30.71%; 43/140) was higher than that of females (20.95%; 22/105), no statistically significant difference was found between them. When the frequency for juvenile animals (10.52%; 6/57) was compared with that for adults (31.38%; 59/188), a statistically significant difference was found ($p < 0.05$).

Titers ranged from 1:50 to 1:400, as follows: 1:50 (29.23%), 1:100 (47.69%), 1:200 (18.46%), and 1:400 (4.62%). The highest titer observed was 1:400. Basso et al. (2001) reported that the antibody titers in dogs with clinical signs were not different from those in dogs without reported clinical signs. Fernandes et al. (2004) observed the occurrence of titers higher than 1:800 in 7 of 450 dogs. In their study, only one dog showed neurological alterations, seen as convulsions and locomotion difficulties. None of the seropositive dogs had any previous clinical symptoms - evidence that a large portion of the infection occurs in subclinical form.

Table 1. Prevalence of reactive dog sera to anti-*Neospora caninum* antibodies by indirect fluorescence antibody test in the health districts of Campo Grande, MS, Brazil.

| Health district | Reactive | Nonreactive | Total | Seroreactive samples in each health district (%) |
|---|---|---|---|---|
| North | 15 | 34 | 49 | 30.61 |
| South | 24 | 64 | 88 | 27.27 |
| East | 9 | 28 | 37 | 24.32 |
| West | 17 | 54 | 71 | 23.94 |
| Total | 65 | 180 | 245 | |

$\chi^2$ calculated = 0.78 $\chi^2$ tabulated = 7.81

Table 2. Prevalence of anti-*Neospora caninum* antibodies in dogs from Campo Grande, MS, Brazil, by age and sex, using indirect fluorescence antibody test.

| Variables | Total examined | Reactive | Prevalence | |
|---|---|---|---|---|
| Sex | | | | |
| Males | 140 | 43 | 30.71 | $\chi^2$ = 2.93 |
| Females | 105 | 22 | 20.95 | p = 0.0868 |
| Total | 245 | 65 | 26.53 | |
| Age | | | | |
| Juveniles (<1 yr) | 57 | 6 | 10.52 | $\chi^2$ = 9.76 |
| Adults (=1 yr) | 188 | 59 | 31.38 | p = 0.0018 |
| Total | 245 | 65 | 26.53 | |

The prevalence found in the present investigation (26.53%; 65/245) was similar to that obtained from a preliminary study in the urban area of the same municipality (SILVA et al., 2001) comprising 40 dogs. Cañón-Franco et al. (2003) found anti-*N. caninum* antibodies in 13 (8.3%) out of 136 dogs older than six months in the state of Rondônia. Souza et al. (2002) detected anti-*N. caninum* antibodies in 29 (21.6%) out of 134 dogs living on dairy-cattle farms in the state of Paraná. Basso et al. (2001), in Argentina, found a prevalence of 26.2% in urban dogs and of 48% in dogs coming from dairy farms. All of these surveys were based on IFAT titers of 1:50 or higher.

Gennari et al. (2002) found frequencies of 10% for domiciled dogs and 25% for stray ones in the state of São Paulo. Meira Santos et al. (1999) obtained a prevalence of 18% for dogs in the state of Bahia, whereas Belo et al. (1999) found 35% for stray dogs in the state of São Paulo.

Regarding the variable sex, no statistically significant difference was found in this study, in agreement with the results obtained by Souza et al. (2002) and by Varandas et al. (2001)—a feature explained by the fact that both male and female dogs are exposed to the same risk conditions. According to Dubey (2003), the existence of breed predisposition or diferential sex susceptibility to neosporosis in dogs is still unknown.

Cañón-Franco et al. (2003), observed that seroprevalence values were similar among dogs of different ages, breeds, and diets, despite the higher values found for stray dogs and those fed homemade diets.

Fernandes et al. (2004), observed the occurrence of anti-*N. caninum* antibody in 14% of the 450 serum samples analyzed, with 10.7% in dogs coming from the urban area, 18.9% in those from the peri-urban area, and 21.7% in those from the rural area, showing that the prevalence in rural area is higher. According to the authors, this may be explained by the fact that rural dogs are at greater risk of exposure to the parasite because they have contact with placentas and aborted fetuses of infected bovines. The present study, on the other hand, was based on dogs living in the urban area; however, because cattle-raising is a major economic activity in the surroundings areas, the diet of urban dogs often includes beef - and such habit might justify the prevalence observed.

Age did play a role in the results obtained in the present study, as the prevalence found for adult dogs was higher than that for juvenile ones, which is in agreement with data reported by Souza et al. (2002), who also detected an increase in seroprevalence with age. These findings are suggestive of a postnatal exposure to the parasite. Barber and Trees (1998) reported that the rate of vertical transmission of neosporosis in dogs was low, since 80% of the offspring of seropositive mothers had not been infected until birth time, indicating that postnatal infection is significant in this species. Varandas et al. (2001), however, did not find any significant differences regarding the parameter age, suggesting that dogs of any age are subject to the same risk of infection.

To our knowledge, the present study reports for the first time in Campo Grande, MS, the prevalence of anti-*Neospora caninum* antibodies in a stratified random sample of the dog population in the city.

**Acknowledgments:**- The authors are thankful to Centro de Controle de Zoonoses de Campo Grande (CZZ), Agência Estadual de Defesa Sanitária Animal e Vegetal (IAGRO), and Embrapa Gado de Corte for the support provided for this study, and to Fundect-MS and CNPq-Brazil for the funding for this project and the grant awarded to L.M.O.

## BIBLIOGRAPHIC REFERENCES

ANDERSON, M. L.; ADRIANARIVO, A. G.; CONRAD, P. A. Neosporosis in cattle. *Animal Reproduction Science***,** v. 60-61, n. 1, p. 417-431, 2000.

BARBER, J. S.; TREES, A. J. Naturally occurring vetical transmission of Neospora caninum in dogs. *International Journal for Parasitology*, v. 28, n. 1, p. 57-64, 1998.

BASSO, W.; VENTURINI, L.; VENTURINI, M. C.; MOORE, P.; RAMBEAU, M.; UNZAGA, J. M.; CAMPERO, C.; BACIGALUPE, D.; DUBEY, J. P. Prevalence of *Neospora caninum* infection in dogs from beef-cattle farms, dairy farms, and from urban áreas of Argentina. *Journal of Parasitology*, v. 87, n. 4, p. 906-907, 2001.

BELO, M. A. A.; REZENDE, P. C. B.; CASTAGNOLLI, K. C.; BRESCIANI, K. D. S.; COSTA, A. J. Pesquisa de anticorpos anti-*Neospora caninum* em cães criados sob diferentes condições sanitárias. In: SEMINÁRIO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 11, 1999, Salvador. *Anais...* Salvador: CBPV, 1999. p. 228.

BJORKMAN, C.; UGGLA, A. Serological diagnosis of *Neospora caninum* infection. *International Journal for Parasitology*, v. 29, n.10, p. 1497-1507, 1999.

BUXTON, D.; MCALLISTER, M. M; DUBEY, J. P. The comparative pathogenesis of neosporosis. *Trends Parasitology*, v. 18, n. 12, p. 546-552, 2002.

CAÑÓN-FRANCO, W. A.; BERGAMASCHI, D. P.; LABRUNA, M. B.; CAMARGO, L. M.; SOUZA, S. L.; SILVA, J. C.; PINTER, A.; DUBEY, J. P.; GENNARI, S. M. Prevalence of antibodies to Neospora caninum in dogs from Amazon, Brazil. *Veterinary Parasitology*, v. 115, n. 1, p. 71-74, 2003.

CANTILE, C.; ARISPICI, M. Necrotizing cerebellits due to *Neospora caninum* infection in a old dog. *Journal of Veterinary Medicine*, v. 49, n. 1, p. 47-50, 2002.

DIJKSTRA, T.; EYSKER, M.; SCHARES, G.; CONRATHS, F. J.; WOUDA, W.; BARKEMA, H. W. Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with *Neospora caninum* tachyzoites. *International Journal for Parasitology*, v. 8, n. 8, p. 747-752, 2001.

DUBEY, J. P.; HATEL, A. L.; LINDSAY, D. L.; TOPPER, M. J. Neonatal *Neospora caninum* infection in dogs: Isolation of the causative agent and experimental transmission: *Journal of the American Veterinary Medical Association***,** v. 193, n. 10, p.1259-1263, 1988.

DUBEY, J. P. *Neospora caninum* and neosporosis in animals. *Korean Journal Parasitology*, v. 41, n. 1, p. 1-16, 2003.

FERNANDES, B. C; GENNARI, S. M; SOUZA, S. L; CARVALHO, J. M; OLIVEIRA, W. G; CURY, M. C**.** Prevalence of anti-*Neospora caninum* antibodies in dogs from urban, periurban and rural areas of the city of Uberlandia, Minas Gerais-Brazil. *Veterinary Parasitology*, v. 123, n. 1-2, p. 33-40, 2004.

GENNARI S. M.; YAI, L. E.; D'AURIA, S. N.; CARDOSO, S. M.; KWOK, O. C.; JENKINS, M. C.; DUBEY, J. P. Occurrence of *Neospora caninum* antibodies in sera from dogs of the city of Sao Paulo, Brazil. *Veterinary Parasitology*, v. 106, n. 2, p. 177–179, 2002.

GONDIM, L. F.; MCALLISTER, M. M.; PITT, W. C.; ZEMLICKA, D. E. Coyotes (Canis latrans) are definitive hosts of *Neospora caninum*. *International Journal for Parasitology*, v. 34, n. 2, p. 159-161, 2004.

LINDSAY, D. S.; DUBEY, J. P.; DUNCAN, R. B. Confirmation that dogs is a definitive host for *Neospora caninum*. *Veterinary Parasitology*, v. 82, n. 4, p. 327-333, 1999.

LOCATELLI-DITTRICH, R. *Diagnóstico sorológico, isolamento, cultivo e caracterização molecular de neospora caninum em bovinos leiteiros e em eqüinos no Estado do Paraná, Brasil.* 2002. 184 p. Tese (Doutorado) - Universidade Federal do Paraná, Curitiba, 2002.

MEIRA SANTOS, P. O.; VIANA DE JESUS, E. E; PINHEIRO, A. M.; ALMEIDA, M. A. O.; GUIMARÃES, J. E.; SOUZA, R. M.; GONDIM, L. F. P. Freqüência de anticorpos contra *Neospora caninum* em cães criados no Estado da Bahia. In: SEMINÁRIO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 11., Salvador. *Anais*...Salvador: Colégio Brasileiro de Parasitologia Veterinária, 1999. p. 228.

PARE, J.; FECTEAU, G.; FORTIN, M.; MARSOLAIS, G. Seroepidemiologic study of *Neospora caninum* in dairy herds. *Journal of the American Veterinary Medical Association*, v. 213, n. 11, p. 1595-1598, 1998.

SILVA, E. A.; BARROS, J. C.; SANTOS, F.; ANDREOTTI, R. Sorologia de *Neospora caninum* em gado de corte e em cães na região de Campo Grande – MS. In: CONGRESSO BRASILEIRO DE BUIATRIA, 5, 2001, Campo Grande. *Anais...* Campo Grande: CBB, 2001. 1 CD ROM.

SOUZA, S. L. P.; GUIMARÃES, J. S.; FERREIRA, F.; DUBEY, J. P.; GENNARI, S.M. Prevalence of *Neospora caninum* antibodies in dogs from dairy cattle farms in Paraná. *The Journal of Parasitology*, v. 88, n. 2, p. 408-409, 2002.

THRUSFIELD, M. *Veterinary epidemiology*. 2. ed. Edinburgh: Blackwell Science, 1994. 412 p.

VARANDAS, N. P.; RACHED, P. A.; COSTA, G. H. N.; SOUZA, L. M.; CASTAGNOLLI, K. C.; COSTA, A. J. Freqüência de anticorpos anti-*Neospora caninum* e anti-*Toxoplasma gondii* em cães da região nordeste do Estado de São Paulo. Correlação com neuropatias. *Semina: Ciencias Agrarias*, v. 22, n. 1, p. 105-111, 2001.

WOUDA, W.; DJEKSTRA, T.; KRAMER, A. M. H.; MAANEN, C. V.; BRINKHOF, J. M. A. Seroepi-demiological evidence for a relationship between *Neospora caninum* infections in dogs and cattle. *International Journal for Parasitology*, v. 29, n.10, p. 1677-1682, 1999.

Received on September 27, 2004.
Accepted for publication on December 28, 2004.