

## Occurrence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in dairy cattle from the northern region of the Paraná State, Brazil

[*Ocorrência de anticorpos contra Neospora caninum e Toxoplasma gondii em bovinos leiteiros da região Norte do estado do Paraná*]

L. Ogawa<sup>1</sup>, R.L. Freire<sup>1</sup>, O. Vidotto<sup>1</sup>, L.F.P. Gondim<sup>2</sup>, I.T. Navarro<sup>1\*</sup>

<sup>1</sup>Depto. Medicina Veterinária e Preventiva – Universidade Estadual de Londrina  
Caixa Postal 6001  
86051-970 – Londrina, PR

<sup>2</sup>Universidade Federal da Bahia, Salvador, BA

### ABSTRACT

Three-hundred and eighty-five serum samples were taken from dairy cows on 90 farms in 12 counties from the northern region of the Paraná State, Brazil. The samples were analyzed by IFAT for the detection of anti-*Neospora caninum* and anti-*Toxoplasma gondii* IgG antibodies. Forty-five (12%) samples were seropositive to *N. caninum*, while 102 (26%) samples were seropositive to *T. gondii*. Only four animals were seropositive to both coccidia. No significant difference was observed between the *N. caninum* serology and any of the variables studied, such as dairy cattle management, milk production, reproductive problems, feeding, and presence of dogs, cats and rodents. These data suggest that neosporosis is present among dairy cattle in the studied geographic region and the simultaneous detection of serum positive animals to both types of coccidian parasite demonstrates the independent occurrence of these coccidia in dairy cows.

Keywords: dairy cattle, seroepidemiology, *Neospora caninum*, *Toxoplasma gondii*

### RESUMO

Foram analisadas, por meio da imunofluorescência indireta, 385 amostras de soros de vacas, pertencentes a 90 propriedades leiteiras de 12 municípios da região Norte do estado do Paraná. Foram observados 45 (12%) sororeagentes ao *Neospora caninum* e 102 (26%) ao *Toxoplasma gondii*. Apenas quatro animais apresentaram títulos de anticorpos para ambos os coccídios. Não foi observada diferença significativa na associação entre a sorologia do *N. caninum* e as variáveis relacionadas ao manejo, produção de leite, problemas reprodutivos, alimentação, presença de cães, gatos e roedores. Os resultados sugerem que neosporose e toxoplasmose estão disseminadas nos rebanhos leiteiros da região Norte do estado do Paraná, e a frequência simultânea de anticorpos anti-*N. caninum* e anti-*T. gondii*, demonstra sua ocorrência independente em vacas leiteiras.

Palavras-chave: bovino leiteiro, seroepidemiologia, *Neospora caninum*, *Toxoplasma gondii*

---

Colaboradores: E.R.M. Marana, A.P.M.E. Santos

Recebido para publicação em 5 de maio de 2003

Recebido para publicação, após modificações, em 7 de julho de 2004

\*Autor para correspondência (*corresponding author*)

E-mail: italmar@uel.br

## INTRODUCTION

*Neospora caninum* is a recently recognized protozoan parasite of worldwide distribution in livestock and companion animals. It was misdiagnosed as *Toxoplasma gondii* until 1988 because of morphological similarities. Since the first report as a cause of abortion in cattle (Thilsted and Dubey, 1989), neosporosis has become a growing concern in dairy and beef cattle reproduction due to important economic losses.

Neosporosis in cattle can occur by congenital, faecal-oral and lactogenic routes (Uggla et al., 1998; De Marez et al., 1999). Dogs inoculated orally with mouse tissue containing *N. caninum* cysts shed oocysts in the faeces (McAllister et al., 1998). De Marez et al. (1999) showed *N. caninum* fecal-oral route experimentally in calves.

Serologic studies have reported the association between dogs presence with neosporosis seroprevalence in cows on dairy farms (Paré et al., 1998; Bartels et al., 1999). However, vertical *N. caninum* transmission is considered the main route of infection in dairy herds (Hietala and Thurmond, 1999; Davison et al., 1999).

In Brazil, Brautigam et al. (1996) detected antibodies to *N. caninum* in beef and dairy cattle sera from farms in the states of São Paulo and Mato Grosso do Sul. Gondim et al. (1999a) described the first Brazilian case of cattle abortion caused by *N. caninum*. Moreover, several researchers have detected anti-*N. caninum* antibodies in dairy cattle (Melo and Leite, 1999), beef cattle (Hasegawa et al., 1999), buffalo (Fujii et al., 2001) and dog (Gennari et al., 2002; Souza et al., 2002) sera, indicating that neosporosis is present in some regions of Brazil.

Toxoplasmosis, a cosmopolitan zoonosis, has also been studied because of the taxonomic proximity of *T. gondii* to *N. caninum* and to verify the presence of concomitant infections. However, *T. gondii* has not been indicated as an important cause of reproductive problem in cattle (Dubey, 1986). Domestic cat is the main definitive host of *T. gondii*, responsible for the dissemination of the oocysts in the environment

(Dubey and Beattie, 1988). Preliminary serological studies performed in dairy cattle, involving the same region of the present study, have shown frequencies of *T. gondii* varying from 25.8% to 48.5% (Marana et al., 1995; Garcia et al., 1999).

The objectives of this study were to determine the seroprevalence of *N. caninum* and *T. gondii* in dairy cows and evaluate the association of neosporosis with the environmental variables of dairy farms.

## MATERIAL AND METHODS

Ninety dairy farms (out of 1917) in 12 counties (out of 27) from the northern of Paraná State were selected according to the voluntary participation of the owners. The counties, located between 23° latitude south and 51° longitude West of Greenwich, have a mesothermic wet temperate climate, with an average temperature of 21°C. A total of 710,463 animal was estimated for the studied herds.

The Epi Info program (6.04 version, CDC-Atlanta) was used to calculate the sample size (confidence interval 95%, precision of 5% and expected frequency of 50%). The 385 serum samples were stratified and systematically selected from the total of 3233 cattle according to the representative percentage in each county (Table 1, 2). The blood samples were collected from September to December 1999, by venous puncture in test tubes without anti coagulant.

Table 1. Dairy-cow *Neospora caninum* antibody seroprevalence in the counties of the northern region of Paraná State, 2000

Counties	Farms	Total of animals	Samples	Positive samples (%)
Astorga	5	227	20	3 (15)
Centenário do Sul	7	241	38	2 (5)
Colorado	6	191	49	6 (12)
Flórida	7	231	31	2 (6)
Guaraci	6	126	35	2 (6)
Jaguapitã	11	366	34	5 (15)
Lobato	11	481	28	3 (11)
Londrina	6	149	25	3 (12)
Lupionópolis	9	296	30	8 (27)
Munhoz de Melo	7	232	34	3 (9)
Santa Fé	9	387	41	5 (12)
Santo Inácio	6	306	20	3 (15)
Total	90	3233	385	45 (12)

Table 2. Dairy-cow *Toxoplasma gondii* antibody seroprevalence in the counties of the northern region of Paraná State, 2000

Counties	Farms	Total of animals	Samples	Positive samples (%)
Astorga	5	227	20	9 (45)
Centenário do Sul	7	241	38	3 (8)
Colorado	6	191	49	12 (25)
Flórida	7	231	31	16 (52)
Guaraci	6	126	35	7 (20)
Jaguapitã	11	366	34	2 (6)
Lobato	11	481	28	7 (25)
Londrina	6	149	25	11 (44)
Lupionópolis	9	296	30	6 (20)
Munhoz de Melo	7	232	34	6 (18)
Santa Fé	9	387	41	14 (34)
Santo Inácio	6	306	20	9 (45)
Total	90	3233	385	102 (26)

The samples were firstly analyzed by the indirect fluorescent antibody test (IFAT) as reported by Conrad et al. (1993) for the detection of anti-*N. caninum* antibodies. The *N. caninum* (JPA-1 strain) was produced and prepared for the serological examinations according to Yamane et al. (1997). The sera were screened in a 1:200 dilution and the positive samples were tested again (in sequential two fold dilutions) until 1:3200. Anti-bovine IgG conjugate<sup>1</sup> was used and the slides were viewed using an epifluorescence microscope<sup>2</sup>. Reactions with titers equal or greater than 200 were considered positive (Dubey and Lindsay, 1996). Detection of IgG antibody to *T. gondii* was undertaken by IFAT as described by Camargo (1964). Slides containing the standard strain (RH) of the parasite and control sera with previously known titers were used. The initial dilution of the sera was 1:64, and sequential dilutions were made until 1:4096. The slides were viewed under an epifluorescence microscope<sup>3</sup>, and the serum samples with titers equal or greater than 64 were considered positive (Costa et al., 1977).

Variables related to epidemiological aspects and cattle management of the properties were collected in a questionnaire applied to their owners on the blood samples collection day. The questions were about the type of management in the properties, the frequency of reproductive problems, milk production, feed, presence of dogs and other animals.

<sup>1</sup> Sigma Chemical, USA, F-7887

<sup>2</sup> Olympus, BX50

<sup>3</sup> Nikon, Labophot, YB-EFA

Chi-Square test was performed to test for significant associations between seroprevalence and the variables described above, with 5% as a significant level.

## RESULTS AND DISCUSSION

Forty-five (12%) positive samples to *N. caninum* were observed. From the 45 positive samples 19 had titer of 200, 10 had titer of 400, 7 had titer of 800, and 9 had titer of 1600. This value was similar to reports from dairy herds present in other regions of Brazil. Gondim et al. (1999b) in Bahia State and Sartor et al. (1999) in the region of Avaré, São Paulo State, used IFAT with serological titer (cutoff) of 200, obtained 14.1% and 16.3% seropositive animals, respectively. However, Rezende et al. (1999) and Belo et al. (1999) in the northeastern region of São Paulo State obtained 22.2% and 62.9% positive animals by IFAT, respectively. These higher rates of prevalence may be explained by the small cutoff (100) of the reaction, which may have overestimated the number of seropositive animals.

One hundred and two (26%) samples were positive to *T. gondii*. Four animals showed antibody titers for both coccidia. They showed serum antibody titers of 64 for *T. gondii*, while for *N. caninum*, one animal showed a titer of 200 and two showed titers of 1600. This percentage is in agreement with the reported by Garcia et al. (1999) who obtained 25.8% cattle with anti-*T. gondii* antibodies, using IFAT with a cutoff of 64 in the same region. Gondim et al. (1999b) also reported the simultaneous occurrence of antibodies against both the coccidia in IFAT. These authors observed that only three (4.8%) of 63 *N. caninum* seropositive cows (titers of 200, 400 and 800) were seropositive to *T. gondii* (titer of 64).

Animals positive to *N. caninum* were detected in all counties studied and no significant difference regarding rates of prevalence among the counties was found (Table 1). This similarity can be explained by the fact that the counties share similar ambient conditions and management practices. Mainar-Jaime et al. (1999) used the ELISA test to analyze 889 sera from cattle on 43 dairy farms in three counties in Asturia, Spain, and they also did not find differences concerning prevalence among counties.

No significant difference was observed between the *N. caninum* serology and any of the variables studied, such as dairy cattle management, milk production, reproductive problems, feeding and presence of dogs, cats and rodents. Similarly, Paré et al. (1998) did not detect significant differences regarding milk production, animal purchase, food management, access to pasture and calf management by the Chi-Square, Fisher's exact or Mann-Whitney tests. However, Paré et al. (1998) found significant difference for the presence and number of dogs on the dairy farms. Dogs were most frequently observed in greater number in the herds with cases of abortions caused by *N. caninum* than in the control herds.

The transmission rate after birth and the persistence of *N. caninum* oocysts in the environment has not been established yet and a possible participation of the transplacental transmission is suggested in the herds studied. Several authors have considered that transplacental transmission of *N. caninum* is a major route of infection in cattle, collaborating significantly to infection maintenance within the herd (Davison et al., 1999). Björkman et al. (1996) did not observe evidence of infection after birth in the dairy herd studied and suggested that the infection was introduced by infected cows coming from other farms.

Once the animals become infected, the infection may remain for lifetime, and may transmit *N. caninum* to their descendents in different pregnancies (Björkman et al., 1996; Wouda et al., 1998). The antibody titers may vary in these animals from negative values, generally after an abortion, and may increase after the occurrence of the abortion (Conrad et al., 1993; Paré et al., 1997). Only a single serum sample was analyzed from cows in the present study, pregnant or not, so the number of animals truly infected could have been underestimated, creating false negative animals.

The data show that exposure to *N. caninum* is present in the dairy herds in the northern region of Paraná state. The low simultaneous antibody frequency for *N. caninum* and *T. gondii* (1%) shows their independent occurrence in dairy cows and, epidemiological aspects and cattle management of the farms studied did not influence the anti-*N. caninum* antibody occurrence in dairy cows. However, there should

be further more detailed epidemiological studies of the region, to indicate the possible variables for the outbreak of *N. caninum* infection in dairy cattle.

#### ACKNOWLEDGEMENTS

For the financial support for this project provided by grants from the CPG-UEL/CAPES/CNPq/EMATER-PR.

#### REFERENCES

- BARTELS, C.J.M.; WOUDA, W.; SCHUKKEN, Y.H. Risk factors for *Neospora caninum*-associated abortion storms in dairy herds in The Netherlands (1995 to 1997). *Theriogenology*, v.52, p.247-257, 1999.
- BELO, M.A.A.; REZENDE, P.C.B.; SOUZA, L.M. et al. Presença de anticorpos anti-*Neospora caninum* em bovinos com histórico de abortos não diagnosticados etiologicamente. In: SEMINÁRIO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 11., 1999, Salvador-BA. *Anais...* Salvador, 1999. p.228 (Resumo).
- BJÖRKMAN, C.; JOHANSSON, O.; STENLUND, S. et al. *Neospora* species infection in a herd of dairy cattle. *J. Am. Vet. Med. Assoc.*, v.208, p.1441-1444, 1996.
- BRAUTIGAM, F.E.; HIETALA, S.K.; GLASS, R. Resultados de levantamento sorológico para a espécie *Neospora* em bovinos de corte e leite. In: CONGRESSO PANAMERICANO DE CIÊNCIAS VETERINÁRIAS, 15., 1996, Campo Grande-MS. *Anais...* Campo Grande, 1996. p.284 (Resumo).
- CAMARGO, M.E. Improved technique of indirect immunofluorescence for serological diagnosis of toxoplasmosis. *Rev. Inst. Med. Trop.*, v.6, p.117-118, 1964.
- CONRAD, P.A.; SVERLOW, K.W.; ANDERSON, M.L. et al. Detection of serum antibody responses in cattle with natural or experimental *Neospora* infections. *J. Vet. Diagn. Invest.*, v.5, p.572-578, 1993.
- COSTA, A.J.; ARAÚJO, F.G.; COSTA, J.O. et al. Experimental infection of bovines with oocysts of *Toxoplasma gondii*. *J. Parasitol.*, v.63, p.212-218, 1977.
- DAVISON, H.C.; OTTER, A.; TREES, A.J. Significance of *Neospora caninum* in British dairy cattle determined by estimation of seroprevalence in normally calving cattle and aborting cattle. *Int. J. Parasitol.*, v.29, p.1189-1194, 1999.
- DE MAREZ, T.; LIDDELL, S.; DUBEY, J.P. et al. Oral inoculation of calves with *Neospora caninum*

- oocysts from dogs: humoral and cellular immune responses. *Int. J. Parasitol.*, v.29, p.1647-1657, 1999.
- DUBEY, J.P. A review of toxoplasmosis in cattle. *Vet. Parasitol.*, v.22, p.177-202, 1986.
- DUBEY, J.P.; BEATTIE, C.P. Long-term persistence of *Toxoplasma gondii* in tissue of pigs inoculated with *T. gondii* oocysts and effect of freezing on viability of tissue cysts in pork. *Am. J. Vet. Res.*, v.49, p.910-913, 1988.
- DUBEY, J.P.; LINDSAY, D.S. A review of *Neospora caninum* and neosporosis. *Vet. Parasitol.*, v.67, p.1-59, 1999.
- FUJII, T.U.; KASAI, N.; NISHI, S.M. et al. Seroprevalence of *Neospora caninum* in female water buffaloes (*Bubalus bubalis*) from the southeastern of Brazil. *Vet. Parasitol.*, v.99, p.331-334, 2001.
- GARCIA, J.L.; NAVARRO, I.T.; OGAWA, L. et al. Soroprevalência do *Toxoplasma gondii*, em suínos, bovinos, ovinos e eqüinos, e sua correlação com humanos, felinos e caninos, oriundos de propriedades rurais do norte do Paraná-Brasil. *Ciê. Rural*, v.29, p.91-97, 1999.
- GENNARI, S.M.; YAI, L.E.O.; D'ÁURIA, S.N.R. et al. Occurrence of *Neospora caninum* antibodies in sera from dogs of the city of São Paulo, Brazil. *Vet. Parasitol.*, v.106, p.177-179, 2002.
- GONDIM, L.F.P.; SARTOR, I.F.; HASEGAWA, M. et al. Seroprevalence of *Neospora caninum* in dairy cattle in Bahia, Brazil. *Vet. Parasitol.*, v.86, p.71-75, 1999b.
- GONDIM, L.F.P.; SARTOR, I.F.; MONTEIRO JR., L.A. et al. *Neospora caninum* infection in an aborted bovine foetus in Brazil. *N. Zeal. Vet. J.*, v.47, p.35, 1999a.
- HASEGAWA, M.Y.; SARTOR, I.F.; GONDIM, L.F.P. et al. Ocorrência de anticorpos contra *Neospora caninum* em vacas de corte na região de Avaré, SP- resultados preliminares. In: SEMINÁRIO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 11., 1999, Salvador-BA. *Anais...* Salvador, 1999. p.227 (Resumo).
- HIETALA, S.K.; THURMOND, M.C. Postnatal *Neospora caninum* transmission and transient sorologic responses in two dairies. *Int. J. Parasitol.*, v.29, p.1669-1676, 1999.
- MAINAR-JAIME, R.C.; THURMOND, M.C.; BERZAL-HERRANZ, B. et al. Seroprevalence of *Neospora caninum* and abortion in dairy cows in northern Spain. *Vet. Rec.*, v.145, p.72-75, 1999.
- MARANA, E.R.M.; NAVARRO, I.T.; VIDOTTO, O. et al. Ocorrência de anticorpos anti-*Toxoplasma gondii* em rebanhos de bovinos de leite no norte do Paraná-Brasil. *Semina*, v.16, p.40-42, 1995.
- MCALLISTER, M.M.; DUBEY, J.P.; LINDSAY, D.S. et al. Dogs are definitive hosts of *Neospora caninum*. *Int. J. Parasitol.*, v.28, p.1473-1478, 1998.
- MELO, C.B.; LEITE, R.C. *Neospora caninum* em Minas Gerais: Dados preliminares. In: SEMINÁRIO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 11., 1999, Salvador-BA. *Anais...* Salvador, 1999. p.225 (Resumo).
- PARÉ, J.; FECTEAU, G.; FORTIN, M. et al. Seroepidemiologic study of *Neospora caninum* in dairy herds. *J. Am. Vet. Med. Assoc.*, v.213, p.1595-1598, 1998.
- PARÉ, J.; THURMOND, M.; HIETALA, S. Congenital *Neospora caninum* infection in dairy cattle and associated calthood mortality. *Can. J. Vet. Res.*, v.60, p.133-139, 1996.
- PARÉ, J.; THURMOND, M.; HIETALA, S. *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. *J. Parasitol.*, v.83, p.82-87, 1997.
- REZENDE, P.C.B.; BELO, M.A.A.; MARCHIORI FILHO, M. et al. Anticorpos anti-*Neospora caninum* em bovinos leiteiros da região nordeste do Estado de São Paulo, Brasil. In: SEMINÁRIO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 11., 1999, Salvador-BA. *Anais...* Salvador, 1999. p.229 (Resumo).
- SARTOR, I.F.; HASEGAWA, M.Y.; GONDIM, L.F.P. et al. Prevalência de anticorpos contra *Neospora caninum* em rebanhos leiteiros do município de Avaré, SP. Resultados preliminares. In: SEMINÁRIO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 11., 1999, Salvador-BA. *Anais...* Salvador, 1999. p.225 (Resumo).
- SOUZA, S.L.P.; GUIMARÃES Jr, J.S.; FERREIRA, F. et al. Prevalence of *Neospora caninum* antibodies in dogs from dairy cattle farms in Paraná, Brazil. *J. Parasitol.*, v.88, p.408-409, 1997.
- THILSTED, J.P.; DUBEY, J.P. Neosporosis-like abortions in a herd of dairy cattle. *J. Vet. Diagn. Invest.*, v.1, p.205-209, 1989.
- UGGLA, A.; STENLUND, S.; HOLMDAHL, O.J.M. et al. Oral *Neospora caninum* inoculation of neonatal calves. *Int. J. Parasitol.*, v.28, p.1467-1472, 1998.
- WOUDA, W.; MOEN, A.R.; SCHUKKEN, Y.H. Abortion risk in progeny of cows after a *Neospora caninum* epidemic. *Theriogenology*, v.49, p.1311-1316, 1998.
- YAMANE, I.; KOKUHO, T.; SHIMURA, K. et al. *In vitro* isolation and characterization of bovine *Neospora* species in Japan. *Res. Vet. Sci.*, v.63, p.77-80, 1997.