

## Occurrence of anti-*Toxoplasma gondii* antibodies in caprines from Pitanga City, Paraná State, Brazil

Célia Rosimarie dos REIS<sup>1</sup>  
 Fabiana Maria Ruiz LOPES<sup>1</sup>  
 Daniela Dib GONÇALVES<sup>1</sup>  
 Roberta Lemos FREIRE<sup>2</sup>  
 João Luis GARCIA<sup>2</sup>  
 Itamar Teodorico  
 NAVARRO<sup>2</sup>

1 - Post-Graduation Program – Animal Science, Department at Preventive Veterinarian Medicine of Universidade Estadual de Londrina, Londrina-PR, Brazil  
 2 - Department at Preventive Veterinarian Medicine of Universidade Estadual de Londrina, Londrina-PR, Brazil

### Abstract

The aim of this study was to verify the prevalence of anti-*Toxoplasma gondii* antibodies in sera from naturally infected milk-producing caprines using indirect immunofluorescence assay (IFA) and modified agglutination test (MAT), determine specificity and sensibility of MAT and to evaluate the agreement of results between the tests. For this study, 282 caprine serum samples were collected from farms in the city of Pitanga, Paraná State, a region with a high pecuary of small ruminants. Prevalence found by IFA was of 44.68% and 23.05% by MAT. There was statistically significant difference ( $p=0.0086$ ) in relation to the gender of animals, being females more seropositive. Sensitivity and specificity results for MAT were, respectively, 42.2% and 94.9%. By comparing results, a moderate agreement was observed ( $Kappa = 0.42$ ). The high prevalence for the infection, associated to high titers found by IFA and clinical signs presented in the herds suggest toxoplasmosis as one of the causes of reproductive problems in caprines. Sensitivity and specificity results for MAT obtained in this study allow this test to be recommended as a confirmatory test for the caprine species.

### Key words:

*Toxoplasma gondii*.  
 Indirect immunofluorescence.  
 Modified agglutination test.  
 Caprines.  
 Reproductive problems.

### Correspondence to:

Prof. Dr. Itamar Teodorico Navarro -  
 e-mail: itamar@uel.br – tel. fax: (43) 3371  
 4766 - Universidade Estadual de Londrina  
 (UEL) - Departamento de Medicina  
 Veterinária, Preventiva; Centro de Ciências  
 Agrárias; 86051-990, Londrina, Paraná,  
 CP 6001.

Recebido para publicação: 10/04/2006  
 Aprovado para publicação: 23/03/2007

### Introduction

*Toxoplasma gondii* has the ability of infecting a great majority of homeothermic animals and frequently causes abortion in humans and other species, including caprines.<sup>1</sup> It is a cosmopolitan distributed zoonosis and is considered one of the most common parasitary infections in the world.<sup>2</sup>

Feldman and Miller studied caprine herds in the state of New York and found the first evidence of toxoplasmosis in this species.<sup>3</sup> Munday and Mason, in Australia, were the first to describe toxoplasmosis as an important cause of reproductive problems in caprines.<sup>4</sup> In this species, the infection showed episodes of abortion, stillborns, premature births, weak kids, deaths in young and adult female goats<sup>5</sup>, leading to great economic losses<sup>1</sup>. Transplacental transmission was proven

through experimental infection by Dubey<sup>6</sup>, Obendorf et al.<sup>7</sup> and Vitor et al.<sup>8</sup> Caprines are an important source of infection for humans since transmission may occur by two means: through “in natura” milk containing tachyzoites<sup>8</sup> and through meat infected with bradyzoites<sup>9, 10</sup>.

In Brazil, seroprevalence of toxoplasmosis in goats varies from 16.6% to 65.0%.<sup>11, 12, 13, 14, 15</sup> In Paraná, Sella et al.<sup>16</sup> verified a 30.71% prevalence in dairy caprines (herds) from the region of Londrina, north of Paraná State<sup>16</sup>. An epidemiological study conducted in Belo Horizonte region, MG, has shown the association between *T. gondii* seropositivity in humans and ingestion of goat milk.<sup>17</sup> Goat milk is widely used in feeding children who are allergic to cow milk<sup>10</sup>, therefore the necessity of knowing the prevalence of this illness in the caprine herd, by means of a fast, feasible, sensitive

and specific serological test.

Different serological tests are indicated in order to determine the agent prevalence in a variety of animal species infected by *T. gondii*. Among these, indirect immunofluorescence assay (IFA) is considered a standard test in many laboratories. Modified agglutination test (MAT) can also be used as a simplified and low-cost serological technique because it does not need specific conjugates for each animal species and can be used both on domestic and wild animals.<sup>18</sup>

Considering the raising of goats a developing culture in Brazil and particularly in the Central and South Region of Parana State, joined to the scarcity of data on toxoplasmosis prevalence in goats and the incidence of reproductive problems in herds of the region, the present paper aimed to verify the occurrence of anti-*Toxoplasma gondii* antibodies in sera from naturally infected dairy goats using IFA and MAT and to determine specificity and sensitivity of MAT to evaluate agreement of results between tests.

## Material and Method

### Population and sampling

According to data from IBGE<sup>19</sup> the city of Pitanga, state of Paraná, is 1,664 km<sup>2</sup> wide and is situated between latitude 23°45'21S and longitude 51°45'41W, with mesothermic humid subtropical climate, average temperature of 21°C, mild summers, severe and frequent frosts in the winters, not presenting in the dry seasons. Data from IBGE<sup>19</sup> estimate herd in Pitanga in approximately 1,530 goats.

A total of 282 blood samples were collected from milk-producing goats from properties in the city of Pitanga, PR. The size of the sample was calculated using the program Epi Info 6.04<sup>20</sup>, having the sampling been performed random and stratified by age group.

### Epidemiological Questionnaire

For each farm a form was filled, including data from the property (location,

main activity) and epidemiological aspects of the herd (gender, age, type of feed, incidence of reproductive problems and type of problem).

### Material collection

Blood samples were collected by jugular venipuncture, with the animal in station, using 40X12 disposable needles and assay tubes properly identified. After retraction of clot, serum samples were stored in Eppendorf tubes, labeled and kept at a temperature of -18°C until the performance of serological assays.

### Serological assays

The serological techniques used were IFA, performed according to Camargo's description<sup>21</sup>, using the dilution 1:64<sup>22</sup> as a cut off point, anti-caprine IgG conjugate (Sigma Immuno Chemicals, Catalog 1990, Product F9012), previously standardized in the dilution 1:2100 and MAT, according to Desmonts and Remington<sup>23</sup> and modified by Dubey and Desmonts<sup>24</sup>, also using as a cut off point the dilution of 1:64.

### Statistical analysis

Chi-square test was used to analyze the variables studied, considering a significance level of 5%. Sensitivity, specificity<sup>25</sup> and Kappa agreement calculus were used<sup>26</sup>. Epi Info 6.04 program was used to tabulate and analyze data.<sup>20</sup>

## Results

From 282 serum samples collected and analyzed, 126 (44.68%) were considered positive by IFA and 65 (23.05%) by MAT. When these results were compared, only 57 (20.21%) from the 282 samples were seropositive for both tests and 148 (52.48%) were seronegative (Table 1). The titer with the highest frequency in IFA was 64 (42.85%), following by 4096 (30.95%), 256 (14.28%) and 1024 (11.90%) (Table 2). Seropositive distribution of IFA results according to the age group and to gender can be observed in table 3.

**Table 1** - Seropositive and seronegative results obtained by indirect immunofluorescence assay (IFA) and by modified agglutination test (MAT) in caprine from the city of Pitanga, Paraná State, Brazil, 2004

		IFA		
		Positivo	Negativo	
MAT	Positivo	57	08	65
	Negativo	69	148	217
Total		126	156	282

*Kappa* = 0.42

Sensitivity (S): 42.2% (CI 95%: 36.4 – 54.3)

Specificity (E): 94.9% (CI 95%: 89.8 – 97.6)

Positive Predictive Value (PPV): 87.7% (CI 95%: 76.6 – 94.2)

Negative Predictive Value (NPV): 68.2% (IC 95%: 61.5 – 74.3)

**Table 2** - Distribution of seropositive titer frequencies obtained by indirect immunofluorescence assay (IFA) in caprines from Pitanga, Paraná State, Brazil, 2004

	Antibody - Titers			
	64	256	1024	4096
Number of samples/Total	54/126	18/126	15/126	39/126
Percentage (%)	42.85	14.28	11.90	30.95

**Table 3** - Distribution of seropositive and seronegative samples by indirect immunofluorescence assay (IFA) according to age group and to gender, in caprines from Pitanga, Paraná State, Brazil, 2004

Age group (months) *	Seropositive / Total (%)
0 - 6	20 / 44 (45.45)
6 - 12	20 / 61 (32.78)
12 - 24	30 / 69 (43.47)
> 24	56 / 108 (51.85)
<b>Total</b>	126 / 282 (44.68)
Gender **	
male	15 / 54 (27.77)
female	111 / 228 (48.68)
<b>Total</b>	126 / 282 (44.68)

p = 0.1223 \* p=0.0086 \*\*

All the properties studied that showed positive animals had a semi-extensive handling and feed had pasture as a basis, supplemented with hay during winter. Incidence of reproductive problems in the herd was noticed in every property, such as: abortions, birth of weak kids, with bad

formation and/or neurological problems, and also of females in anestrus and repetitive heat.

## Discussion

The prevalence obtained by IFA

(44.68%) can be considered high when compared to the prevalence of 30.71% observed by Sella et al.<sup>16</sup> in the region of Londrina, north of Paraná State. The highest frequencies observed were found in at dilutions of 1:64 (42.85%), compatible with the chronic phase of the infection, and 42.85% above 1:1024 suggesting the acute phase of the illness, which could be represented by the reproductive problems observed in the properties. Similar symptoms were observed in ovine herds in Scotland, UK, by Buxton<sup>27</sup> and in USA by Dubey and Kirkbride<sup>28</sup>. The occurrence of toxoplasmosis in small ruminant herds depends essentially on the presence of felines which were observed in all the properties and with free access to the installations.

In the seropositive distribution by IFA according to age group, no statistically significant differences were observed. However, according to gender, the frequency of infection was higher in females (48.68%) than in males (27.77%). Such difference was also observed by Silva et al.<sup>14</sup> while studying milk-producing caprines in Pernambuco State, where the percentage of seroreagent females (43.88%) was significantly higher than the males (21.21%). The authors pointed the handling of animals as the cause of this difference, since it is more common to confine or gather the females in milk-producing properties.

The seropositivity obtained by MAT (23.05%) in the present work, although lower than the one obtained by IFA (44.68%), was similar to that found by Dubey and Adams<sup>5</sup>, who analyzed sera in caprine herds in the northwest USA and obtained a seropositivity of 22.1%.<sup>5</sup>

When the results obtained in the IFA and MAT tests were compared, only 57 (20.21%) from the 282 samples were seropositive for both tests. *Kappa* value, established in 0.42, shows that the agreement between the techniques was moderate, according to criteria proposed by Landis and Koch<sup>26</sup>. Only one comparative study between the IFA and MAT techniques has been made in Brazil, in the state of São Paulo, by Silva et al.<sup>29</sup> using groups of 100 animals from four different species, ovines (*Kappa*=0.84), canines (*Kappa*=0.70), felines (*Kappa*=0.76) and caprines (*Kappa*=0.59)<sup>29</sup>. The agreement between the results obtained ranged from substantial to great for all the species but caprine, where agreement was moderate, similar to the one found in this present study (*Kappa*=0.56). Minho et al.<sup>30</sup> compared both techniques in swines experimentally infected and observed an excellent agreement between results, with *Kappa*=0.93<sup>30</sup>.

Despite the low sensitivity obtained by MAT (55.3%), there was a high specificity (96.6%) what assurance the positive results, enabling it to be used as a confirmatory test.

## Conclusion

From the analysis of the results it can be concluded that the high prevalence of seroreagents allied to the high serological titers found by IFA and the clinical signs observed suggest toxoplasmosis as one of the causes for reproductive problems in the caprine herds studied. The sensitivity and specificity results obtained for MAT in the present study permit its recommendation as a confirmatory test.

## Ocorrência de anticorpos anti-*Toxoplasma gondii* em caprinos de Pitanga, Paraná, Brasil

### Resumo

Objetivou-se verificar a prevalência de anticorpos anti-*Toxoplasma gondii* em soros de caprinos de produção leiteira naturalmente infectados utilizando os testes de imunofluorescência indireta (IFI) e o de aglutinação modificada (MAT), determinar a especificidade e a

### Palavras-chave:

*Toxoplasma gondii*.  
Imunofluorescência indireta.  
Teste de aglutinação modificada.  
Caprinos.  
Problemas reprodutivos.

sensibilidade do MAT e avaliar a concordância dos resultados entre os testes. Para o estudo foram coletadas 282 amostras de soro caprino, de propriedades do município de Pitanga, Paraná, região com uma grande pecuária de pequenos ruminantes. A prevalência encontrada pela IFI foi de 44,68% e 23,05% pelo MAT. Houve diferença estatisticamente significativa ( $p=0,0086$ ) em relação ao sexo dos animais, sendo que as fêmeas foram mais soropositivas. Os resultados de sensibilidade e especificidade para o MAT foram, respectivamente, 42,2% e 94,9%. Na comparação dos resultados foi observada uma concordância moderada ( $Kappa = 0,42$ ). A alta prevalência da infecção, aliada aos altos títulos obtidos pela IFI e aos sinais clínicos presentes nos rebanhos sugere a toxoplasmose como uma possível causa de problemas reprodutivos em caprinos. Os resultados de sensibilidade e especificidade para o MAT obtidos neste estudo permitem recomendá-lo como teste confirmatório para a espécie caprina.

## References

- 1 DUBEY, J. P. Toxoplasma – induced abortion in dairy goats. **Journal of American Veterinary Medical Association**, v. 178, n. 7, p. 671-674, 1981.
- 2 ESTEBAN-REDONDO, I. et al. Detection of *Toxoplasma gondii* in tissues of sheep and cattle following oral infection. **Veterinary Parasitology**, v. 86, n. 3, p. 155-171, 1999.
- 3 FELDMAN, H.; MILLER, I. Serological study of toxoplasmosis prevalence. **American Journal of Hygiene**, v. 64, n. 3, p. 320-335, 1956.
- 4 MUNDAY, B. L.; MASON, R. W. Toxoplasmosis as a cause in perinatal death in goats. **Australian Veterinary Journal**, v. 55, n. 10, p. 485-487, 1979.
- 5 DUBEY, J. P.; ADAMS, D. S. Prevalence of *Toxoplasma gondii* antibodies in dairy goats from 1982 to 1984. **Journal of American Veterinary Medical Association**, v. 196, n. 2, p. 295-296, 1990.
- 6 DUBEY, J. P. Lesions in transplacentally induced toxoplasmosis in goats. **American Journal of Veterinary Research**, v. 49, n. 6, p. 905-909, 1988.
- 7 OBENDORF, D. L. et al. Resistance to *Toxoplasma* abortion in female goats previously exposed to *Toxoplasma* infection. **Australian Veterinary Journal**, v. 67, n. 6, p. 233-234, 1990.
- 8 VITOR, R. W. A. et al. Eliminação de *Toxoplasma gondii* através de urina, saliva e leite de caprinos experimentalmente infectados. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 42, n. 2, p. 147-154, 1991.
- 9 DUBEY, J. P. Persistence of encysted *Toxoplasma gondii* in caprine livers and public health significance of toxoplasmosis in goats. **Journal of American Veterinary Medical Association**, v. 177, n. 12, p. 1203-1207, 1980.
- 10 FIGUEIREDO, J. F. et al. Seroprevalence of *Toxoplasma gondii* infection in goats by the indirect haemagglutination, immunofluorescence and immunoenzymatic tests in the region of Uberlândia, Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 96, n. 8, p. 687-692, 2001.
- 11 FIGLIUOLO, L. P. C. et al. Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in goats from São Paulo State Brazil. **Journal of Small Ruminant Research**, v. 55, n. 1-3, p. 29-32, 2004.
- 12 MACHADO, T. M. M.; LIMA, J. D. Frequência de anticorpos anti-*Toxoplasma gondii* em caprinos criados sob diferentes formas de exploração no Estado de Minas Gerais. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 39, n. 2, p. 255-264, 1987.
- 13 MAINARDI, R. S. et al. Soroprevalência de *Toxoplasma gondii* em rebanhos caprinos no Estado de São Paulo. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 36, n. 6, p. 759-761, 2003.
- 14 SILVA, A.V. et al. Toxoplasmose em ovinos e caprinos em duas regiões do Estado de Pernambuco, Brasil. **Revista Ciência Rural**, v. 33, n. 1, p. 115-119, 2003.
- 15 STACCHISSINI, A. V. M. *Toxoplasma gondii* e *Neospora caninum* em caprinos do estado de São Paulo: perfis soro-epidemiológicos e co-infecção com o vírus da artrite-encefalite caprina, 2005. 120 f. Tese (Doutorado em Medicina Veterinária – Vigilância Sanitária) – Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, 2005.
- 16 SELLA, M. Z. et al. Epidemiologia da toxoplasmose caprina, levantamento sorológico do *Toxoplasma gondii* em caprinos leiteiros na micro região de Londrina, Paraná, Brasil. **Revista Brasileira de Parasitologia Veterinária**, v. 3, n. 1, p. 13-16, 1994.
- 17 CHIARI, C. A. et al. Soro-epidemiologia da toxoplasmose caprina em Minas Gerais, Brasil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 39, n. 4, p. 587-609, 1987.
- 18 DUBEY, J. P. et al. Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows. **American Journal of Veterinary Research**, v. 56, n. 8, p. 1030-1036, 1995.

- 19 IBGE. **Instituto Brasileiro de Geografia e Estatística**. Cidade de Pitanga. Cidade @, Produção de Pecuária Municipal, 2002. Disponível em: <<http://www.ibge.gov.br/cidadesat/default.php>>. Acesso em: 8 set. 2005.
- 20 DEAN, G. A. et al. **Epi Info, Version 6**: a word processing, database, and statistic program for epidemiology on microcomputers. Atlanta: Center for Diseases Control and Prevention, 1994.
- 21 CAMARGO, M. E. Introdução às técnicas de imunofluorescência. **Revista Brasileira de Patologia Clínica**, v. 10, n. 4, p. 143-171, 1973.
- 22 RIEMANN, H. P. et al. Survey for *Toxoplasma* antibodies among sheep in Western United States. **Journal of American Veterinary Medical Association**, v. 171, n. 12, p. 1200-1264, 1977.
- 23 DESMONTS, G.; REMINGTON, J. S. Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. **Journal of Clinical Microbiology**, v. 11, n. 6, p. 562-568, 1980.
- 24 DUBEY, J. P.; DESMONTS, G. Serological responses of equids fed with *Toxoplasma gondii* oocysts. **Equine Veterinary Journal**, v. 19, n. 4, p. 337-339, 1987.
- 25 GART, J. J.; BUCK, A. A. Comparison of screening test a reference test in epidemiologic studies. **American Journal of Epidemiology**, v. 83, n. 1, p. 593-602, 1966.
- 26 LANDIS, J. R.; KOCH, G. G. The measurement of observer agreement the categorical data. **Biometrics**, v. 33, p. 59, 1977.
- 27 BUXTON, D. Ovine toxoplasmosis – a review. **Journal Research Society Medicine**, v. 83, n. 8, p. 509-511, 1990.
- 28 DUBEY, J. P.; KIRKBRIDE, C. A. Epizootics of ovine abortion due to *Toxoplasma gondii* in north central United States. **Journal of the American Veterinary Medical Association**, v. 184, n. 6, p. 657-660, 1984.
- 29 SILVA, A. V. et al. Comparação da reação de imunofluorescência indireta e do método de aglutinação direta na detecção de anticorpos anti – *Toxoplasma* em soros de ovinos, caprinos, caninos e felinos. **Arquivo do Instituto Biológico**, v. 69, n. 1, p. 7-11, 2002.
- 30 MINHO, A. P. et al. Evaluation of indirect fluorescent antibody test and modified agglutination test for detection of antibodies against *Toxoplasma gondii* in experimental infected pigs. **Pesquisa Veterinária Brasileira**, v. 4, n. 24, p. 199-202, 2004.