

# FREQUENCY OF ANTI-*TOXOPLASMA GONDII* ANTIBODIES IN APPARENTLY HEALTHY DOGS OF THE CITY OF UBERLÂNDIA-MG.

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**SUMMARY:** Toxoplasmosis is one of the most important zoonosis with a worldwide distribution, affecting many species of domestic animals. Dogs are highly predisposed to *T. gondii* infection because of their alimentary habits (carnivorous) and the close contact with the soil contaminated by sporulated oocysts and infection rates ranging from 19.6 a 91.0% in various parts of the world. Because of lack of data of this infection in the canine population of Uberlândia, MG, it was determined the incidence of anti-*T. gondii* antibodies in apparently healthy dogs presented to anti-rabic virus vaccination from August to October 1994. A total of 218 serum samples were analyzed by the indirect hemagglutination test (IHA) and the titers  $\geq 1:64$  were considered as positive. A total of 115 (52.7%) samples were found positive and the more frequent titer was 1:256. No statistically significant differences were observed in relation to sex. It was observed a positivity of 59.6% for crossbred dogs, significantly higher than for those pure bred dogs (33.3%) ( $p < 0.05$ ). Higher seropositivities were detected in the groups with ages between 0-2 (38.9%) and  $> 10$  years (78.5%). Serum samples which presented titers  $\geq 1:512$  were analyzed again after treatment with 2-mercapthoethanol (2-ME), in order to verify the presence of specific IgM antibodies. From 35 samples tested in the presence of 2-ME, only three (8.5%) revealed the presence of specific IgM. In conclusion, the incidence of *T. gondii* infection in the studied canine population was 52.7% and the infection possibility increases with age. Also, the detection of specific IgM in apparently healthy dogs suggests the presence of assymptomatic active infection.

**KEY-WORDS:** *Toxoplasma gondii*, dogs, seroprevalence, indirect hemagglutination.

## INTRODUCTION

Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, is one of the most common zoonosis, affecting several animal species, including mammals, reptiles, amphibians and fowl.

Dogs are considered to be highly predisposed to infection with *T. gondii*, due to their feeding habits (carnivorous), which facilitate ingestion of cyst-contaminated tissues, and to the close contact with soil contaminated with sporulated oocysts (GERMANO, *et alii*, 1985).

Several seroepidemiological surveys conducted in dogs have demonstrated that *Toxoplasma* infection is very frequent, with positivity rates ranging from 19.6 to 91.0% (ISHIZUKA & YASUDA, 1981; GERMANO *et alii*, 1985;

JACKSON & HUTCHISON, 1987; 1989; UGGLA *et alii*, 1990; LINDSAY *et alii*, 1990; GUIMARÃES *et alii*, 1992), using diverse serological tests (indirect hemagglutination, indirect immunofluorescence and ELISA)

Although most infections in dogs are subclinic or show moderate symptoms, serious consequences may arise in immunodepressed patients or in immunodepressing conditions, as seen in infectious diseases (distemper) or chemotherapy (DUBEY, 1985).

Some authors believe that because domestic animals coinhabit with humans, they might act as source of infection for the latter, who could become contaminated by direct contact with their secretions and/or ingestion of contaminated meat, milk and eggs (FRENKEL & RUIZ, 1980; RIEMANN *et alii*, 1975). Then, infection within the canine population means that the involved area represents an ecological niche for the parasite

and a potential transmission risk for humans (GERMANO *et alii*, 1985).

Due to the close contact of dogs and humans, to the medical-sanitary and preventive importance of toxoplasmosis and aiming to establish the actual prevalence of this infection in the canine population of the region, this study has the objective of determining the frequency of anti-*Toxoplasma gondii* antibodies in apparently healthy dogs of the city of Uberlândia, MG, by means of the indirect hemagglutination test.

## MATERIALS AND METHODS

Two hundred and eighteen serum samples of apparently healthy dogs were analyzed. Those dogs were submitted for anti-rabies vaccination from August through October, 1994, and proceeded from various districts of the city of Uberlândia, MG. The animals were classified according to sex (males and females), age (0 to 14 years) and breed (purebred or crossbred).

Blood was collected without anticoagulant, properly identified and after the sera were harvested, they were stored at  $-20^{\circ}\text{C}$  at the Immunology Laboratory of the Uberlândia Federal University, until tests were performed.

The indirect hemagglutination test was performed using the commercially available kit (HAP-Toxoplasmose, Salck Ind. e Com. Prod. Biológicos Ltda. – São Paulo, SP), following the instructions of the manufacturer.

After inactivating sera at  $56^{\circ}\text{C}$  for 30 minutes, a preliminary serological screening (qualitative test) was carried out at dilutions 1:16 and 1:32, and the reagent sera at the 1:32 dilution were submitted to serial double dilutions from 1:16 to 1:2048 (semi-quantitative test).

Positive serum samples in the dilutions equal or superior to 1:512 were tested again after treatment with 2-mercapthoethanol (2ME), to detect antibodies of the IgM class.

Results were statistically analyzed by the  $\chi^2$  method, with  $p < 0.05$ , according to GOLDSTEIN (1965).

## RESULTS

From 218 serum samples analyzed, 115 (52.7%) were positive to a dilution of 1:64 or higher (Table 1).

The distribution of frequencies of reagent sera, according to anti-*Toxoplasma* antibody titres, is expressed in Figure 1, the most frequent titre being 1:256. The distribution of the positivity of samples in relation to sex, breed and age is presented in Table 1:

Table 1 – Seroprevalence of *Toxoplasma gondii* in dogs of the city of Uberlândia, MG (1994), in relation to sex, breed and age, using the indirect hemagglutination test.

		Nº of samples tested	positive (%) ≥ 1:64	negative (%) < 1:64
<b>Sex</b>	Male	113	62 (54.8)	51 (45.1)
	Female	105	53 (50.4)	52 (49.6)
<b>Breed</b>	CB	161	96 (59.6)*	65 (40.4)
	PB	57	19 (33.3)*	38 (66.7)
<b>Age (years)</b>	0 - 2	90	35 (38.9)*	55 (61.1)
	2 - 4	45	23 (51.1)	22 (48.9)
	4 - 6	19	11 (57.9)	8 (42.1)
	6 - 8	12	7 (58.3)	5 (41.6)
	8 - 10	10	6 (60.0)	4 (40.0)
	> 10	42	33 (78.5)	9 (21.5)
<b>Total</b>		<b>218</b>	<b>115 (52.7)</b>	<b>103 (47.3)</b>

\*  $p < 0.05$

CB - crossbred

PB - purebred

**Sex:** A positivity rate of 54.8% for males and 50.4% for females was observed, and no statistically significant differences ( $p < 0.05$ ) were found ( $\chi^2 = 0.0012$ ).

**Breed:** 96 (59.6%) positive samples were observed among the crossbred (CB) dogs and 19 (33.3%) seropositive samples for purebred (PB) dogs. These data showed statistically significant differences ( $\chi^2 = 3.96$ ;  $p < 0.05$ ).

**Age:** The statistical analysis of positivity in relation to age groups revealed statistically significant differences ( $\chi^2 = 5.516$ ;  $p < 0.05$ ). Figure 2 illustrates the frequency of positive samples, as well as the geometric mean of titres (GMT) of anti-*Toxoplasma* antibodies, calculated for each age group. A profile of increasing positivity was observed as age increased, and significant differences were found between the groups of 0-2 and >10 years of age ( $\chi^2 = 8.55$ ;  $p < 0.05$ ). The GMT did not show any statistically significant differences, remaining between the titres of 256 and 512 in all age groups.

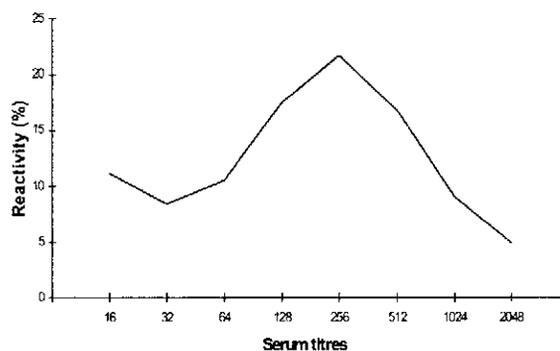


Fig. 1 - Distribution of anti-*Toxoplasma gondii* antibody titres in dogs from Uberlândia-MG (1994), using the indirect hemagglutination test.

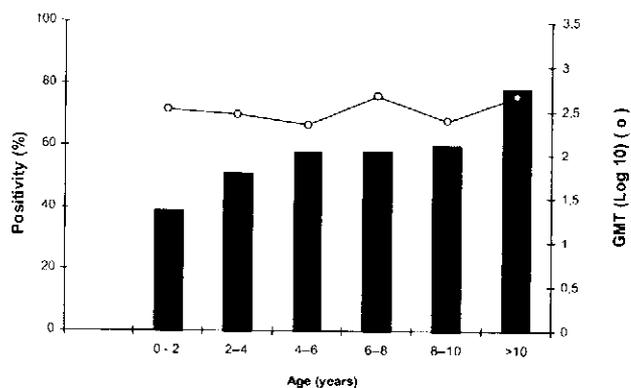


Fig. 2 - Positivity and geometric mean of titres (GMT) of anti-*Toxoplasma gondii* antibodies in dogs of the city of Uberlândia, MG (1994), using the indirect hemagglutination test.

**Assay for IgM class antibodies:** From the 35 samples that had titres equal to or higher than 512, tested before and after treatment with 2-mercapthoethanol (2ME), 3 (8.5%) samples had significant decreases in titres: serum sample n.º 1: from 1024 (without 2ME) to 256 (with 2ME); sample n.º 2: from 1024 (without 2ME) to 256 (with 2ME); sample n.º 3: from 1024 (without 2ME) to non-reagent (with 2ME).

## DISCUSSION

This study demonstrated that the frequency of anti-*T. gondii* antibodies in apparently healthy dogs, from the city of Uberlândia, MG, was 52.7%, by using the indirect hemagglutination test and considering the 1:64 dilution as the positive threshold. These data were slightly higher than those of AGANGA *et alii* (1984), MAYER *et alii* (1987), ULÓN *et alii* (1990), who found 37.0%, 37.2% and 43.6% of positive samples ( $\geq 1:64$ ) by the IHA test, respectively.

SVOBODA & SVOBODOVÁ (1987) and JACKSON & HUTCHISON (1987), by the reaction of Sabin and Feldman, found seropositive rates of 50.0% and 19.6%, respectively. ISHIZUKA & YASUDA (1981), GERMANO *et alii* (1985) and GUIMARÃES *et alii* (1992), demonstrated frequencies of anti-*T. gondii* antibodies in dogs of 63.8%, 91.0%, 47.3% respectively, by the indirect immunofluorescence reaction, considering 16 as the initial positive titre. UGGLA *et alii* (1990) reported a 23.0% rate of positive samples ( $\geq 1:100$ ), by means of an immunoenzymatic test (ELISA), while frequencies of 25.0% were observed by LINDSAY *et alii* (1990), using the direct agglutination test.

As it can be observed, there is considerable variation in the reported frequencies of anti-*T. gondii* antibodies in dogs, that may reflect either the assay used, or the positive threshold

determined for each test and consequently its sensitivity. The most frequent anti-*T. gondii* antibody titre found in this study was 256, being considered higher when compared to those obtained by ISHIZUKA & YASUDA (1981): 64; ULÓN *et alii* (1990): 64; LINDSAY *et alii* (1990): 25; GUIMARÃES *et alii* (1992): 16; and inferior to those obtained by GERMANO *et alii* (1990): 1000.

Regarding to sex, significant differences were not found in the frequency of seropositives, observations that are in agreement with ISHIZUKA & YASUDA (1981), AGANGA *et alii* (1984), GERMANO *et alii* (1985), SVOBODA & SVOBODOVÁ (1987), but disagreeing with JACKSON & HUTCHISON (1987).

In regard to breed, 59.6% seropositives were found in the CB group, while the PB group showed 33.3% of positive samples. These differences were significant, disagreeing with the findings of AGANGA *et alii* (1984) and SVOBODA & SVOBODOVÁ (1987). These observations might be justified by the influence of the different management to which the animals are submitted, both sanitary and nutritional. After analysis of the frequencies of anti-*T. gondii* antibodies in relation to age (Figure 2), significant differences were found when age groups of 0-2 and >10 years were compared, suggesting a behavior in which seropositivity increases along with age. These findings partially agree with those of JACKSON & HUTCHISON (1987), who found significant differences in positivity, comparing two age groups: younger (2.0%) and older (27.9%) than six months of age. However, AGANGA *et alii* (1984), GERMANO *et alii* (1985), SVOBODA & SVOBODOVÁ (1987) and GUIMARÃES *et alii* (1992) did not find significant differences in relation to age.

The geometric mean of the serological titres, in the different age ranks, remained relatively constant, between titres of 256 and 512 in all age groups.

In the assay for IgM class antibodies, 8.5% reagent samples were found, suggesting the presence of active asymptomatic infection in apparently healthy dogs. It is also noticed that most seropositive animals with high titres ( $\geq 512$ ) are either chronically infected or did not present specific IgM due to the sensitivity of the test used.

Although infection is common in this species and the epidemiological importance of dogs in the maintenance of the anthropological niche of *T. gondii* is not completely yet clear (CAMARGO *et alii* 1995), the results obtained in this study indicate a relatively high prevalence of toxoplasmosis-infection in apparently healthy dogs of the city of Uberlândia, emphasizing the presence of active infection in asymptomatic animals as a potential risk of transmission to man.

## SUMÁRIO

A toxoplasmose é uma das zoonoses mais importantes, acometendo várias espécies de animais domésticos. Os cães são altamente predispostos à infecção por *Toxoplasma gondii*, devido ao hábito alimentar (carnivorismo) e ao estreito contato com o solo contaminado com oocistos esporulados, apresentando taxas de infecção que variam de 19,6 a 91,0% em várias regiões do mundo. Em virtude da ausência de dados desta infecção na população canina de Uberlândia-MG, determinou-se a frequência de anticorpos anti-*T. gondii* em cães aparentemente saudáveis, apresentados à campanha de vacinação anti-rábica desta cidade, no período de agosto a outubro de 1994. Foram analisadas 218 amostras de soros por meio do teste de hemaglutinação indireta (HAI), sendo os títulos  $\geq 1$ : 64 considerados como positivos. Foram encontradas 115 (52,7%) amostras positivas e o título mais freqüente foi de 256, não apresentando diferenças estatisticamente significantes quanto ao sexo. Observou-se maior positividade nos cães sem raça definida (59,6%) que nos cães com raça definida (33,3%) ( $p < 0,05$ ). Foram encontradas diferenças significativas na proporção de soropositivos entre os grupos etários de 0-2 (38,9%) e  $> 10$  anos (78,5%). Amostras de soros que apresentaram títulos  $\geq 1$ : 512 foram retestadas, após tratamento com 2-mercaptoetanol, a fim de verificar a presença de anticorpos IgM específicos. Das 35 amostras retestadas, 3 (8,5%) revelaram a presença de IgM específica. Conclui-se que a frequência da infecção por *T. gondii* na população canina em questão é de 52,7%, apresentando um perfil crescente de soropositividade com o avançar da idade. A detecção de IgM específica em cães clinicamente saudáveis sugere a presença da infecção ativa assintomática.

**PALAVRAS-CHAVE:** *Toxoplasma gondii*, cães, soroprevalência, hemaglutinação indireta.

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