Seroepidemiologic profile of antibodies against *Toxoplasma gondii* in veterinary medicine students

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**Abstract**

The state of Pará, due to its location in equatorial region, presents climatic and epidemiologic characteristics propitious to the maintenance of the *Toxoplasma gondii* in the environment. The objective of the present study is verifying the occurrence of anti-*T. gondii* antibodies in Veterinary Medicine students from the Federal University of Pará and serologically following them up for three consecutive semesters assessing their hygiene-food habits and behaviors. 51 veterinary students (22 women and 29 men) participated on the study All the volunteers signed an informed consent and answered an epidemiologic questionnaire. The anti-*T. gondii* antibodies research was performed by indirect immunofluorescence reaction. From the 51 students 22 (43.14%) were reactive to anti-*T. gondii* antibodies. The variables with strong statistical association were: the presence of cats at home (p=0.002), cats from the neighborhood with access to the backyard (p=0.046), the consumption of water or juice of unknown provenance in the streets (p=0.026), the consumption of raw salad in restaurants (p=0.031), and the consumption of sandwiches containing raw vegetables in the streets (p=0.009). It can be concluded that eating food in places without hygienic standards of sanitation favors the contamination by *T. gondii*.

**Keywords:** toxoplasmosis; serology; college students; Pará.

**Perfil soroepidemiológico de anticorpos contra Toxoplasma gondii em estudantes de medicina veterinária**

O Estado do Pará por se localizar em região equatorial apresenta características climáticas e epidemiológicas propícias para a manutenção do *Toxoplasma gondii* no meio ambiente. O objetivo do presente estudo é verificar a ocorrência de anticorpos anti-*T. gondii* em alunos de Medicina Veterinária da Universidade Federal do Pará e acompanhá-los sorologicamente durante três semestres consecutivos avaliando os seus hábitos e comportamentos higiênico-alimentares. Participaram do estudo 51 alunos de Medicina Veterinária (22 mulheres e 29 homens). Todos os voluntários assinaram um termo de consentimento livre e esclarecido e respondem a um questionário epidemiológico. A pesquisa de anticorpos anti-*T. gondii* foi realizada pela reação de imunofluorescência indireta. Dos 51 alunos 22 (43.14%) foram reagentes a anticorpos anti-*T. gondii*. As variáveis que tiveram forte associação estatística foram: a presença de gatos em casa (p=0.002), gatos dos vizinhos tinham acesso ao quintal (p=0.046), o consumo de água ou suco na rua (p=0.062), o consumo de salada crua em restaurantes (p=0.031) e o consumo de sanduíches contendo legumes crus na rua (p=0.009). Conclui-se que a alimentação realizada em locais sem padrão higiênico-sanitário favorece a infecção pelo *T. gondii*.

**Palavras-chave:** toxoplasmoses; sorologia; estudantes de medicina veterinária.

**Introduction**

Toxoplasmosis is a parasitical disorder spread worldwide which has *Toxoplasma gondii* as etiological agent. The main forms of transmission are through the ingestion of tissue cysts in rare or raw meat, or through the ingestion of food or water contaminated with oocysts (DUBEY, 2010). The infection by *T. gondii* varies much from one region to another (DUBEY, 2010). In serological inquiry performed in Malaysia, for the research of anti-*T. gondii* antibodies in 312 professionals directly working with animals, seropositivity was observed in 62 (19.9%) people, being 18.4% (7/38) reactant veterinarians, 33.5% (15/45) veterinarian technicians, 14.9% (29/194) veterinary students, and 31.4% (11/35) animal owners (BRANDON-MONG et al., 2015).

In Brazil, 145 students of Veterinary Medicine from the University of Campo Grande, state of Mato Grosso do Sul, with positivity of 30.34% (44/145) for anti-*T. gondii* antibodies through Indirect Hemagglutination method were assessed (ARAÚJO et al., 2000). In research performed at State University of São Paulo, state of São Paulo, 208 samples of undergraduate and graduate students from the veterinary course with positivity of 9.2% were analyzed by Indirect Immunofluorescence Reaction (VASCONCELOS, 2003). Moraes et al. (2008) performed study in 62 students and 10 professors from the Veterinary Medicine course of the Federal University of Pará, by Direct Agglutination Test (DAT), where 70% (7/10) of the professors and 48.39% (30/62) of the students were reactant.

The state of Pará, due to its location in equatorial region, presents climatic and epidemiologic characteristics propitious to the maintenance of the *T. gondii* in the environment, favoring the transmission and consequent record of high seroprevalence indexes and the occurrence of disease outbreaks. In the year of 2004, a breakout of toxoplasmosis occurred in the city of Almeirim, state of Pará, which after serological assessment by Enzyme-Linked Immunosorbent Assay (ELISA) in 186 individuals, including symptomatic patients and their family members, 21.5% (49/186) presented serologic profile of acute toxoplasmosis, presenting high titers for IgM and IgG (CARMO et al., 2010).

Toxoplasmosis is considered a public health issue in Brazil and, as an occupational zoonosis, veterinary professionals and students, due to their activities, have higher changes of being infected, making necessary a study about this issue for the implementation of preventive measures, which can be used for the control of this disease in this professional group. Thus, the objective of the present study was...
Materials and Methods

The research was performed from the year of 2009 to 2010, with the authorization of the committee of human ethics from the Federal University of Pará, according to official letter N°: 071/10 with CAE protocol: 0046.0.073.000-10. The samples of the study were compounded by students from the Veterinary Medicine course of the UFPA, from both sex and from different age groups. The proposal of the investigation included three blood collections during three consecutive semesters, and the adhesion of students to the research was voluntary. The inclusion criteria the study was to be veterinary medicine students. The exclusion criteria was the veterinary students did not participate of first blood sample.

At the occasion of the first blood collection, all the volunteers involved in the study signed a consent term and answered an epidemiologic questionnaire, which was presented with the objective of getting information on the occupational and environmental variables, investigating epidemiologic factors related to toxoplasmosis.

The blood samples of the students were collected by a qualified professional of the nursing area. The material collection was performed in an aseptic way by puncture of the cephalic vein (5mL/individual) in tubes without anticoagulant identified, stored in cool box transported to the laboratory. The samples were centrifuged (2500 rpm. per 10 minutes) and later, the serums obtained were conditioned in plastic microtubes and kept at -20°C until the performance of the serological test.

The anti-\textit{T. gondii} antibody research was performed at the Laboratory of Toxoplasmosis from Instituto Evandro Chagas/Ananindeua, state of Pará, using the Indirect Immunofluorescence Test (IIFT). The antigenic source used was obtained from tachyzoites present in peritoneal fluid from Swiss albino mice (\textit{Mus musculus}), previously infected with RH strain of \textit{T. gondii}. The technique was developed according to description of Camargo (1974). Briefly, the samples of serums were diluted in series (1:10 to 1:20.40) and deposited in slides sensitized with \textit{T. gondii} antigen. After incubation and washing steps, the reaction was revealed with anti-antibody (anti-IgG or anti-IgM) marked with fluorescein (biaMérieux®). For both immunoglobulins, the cutting point established was 1:40.

For the statistical analysis, the prevalence of toxoplasmosis among the variables of the study (sex, home area, cats at home, cats in the neighborhood with access to the backyard, potable water at home, consumption of non-pasteurized milk, consumption of non-pasteurized cheese, consumption of homemade yogurt, consumption of rare meat, washing fruits and vegetables before eating them, consumption of water or juice from unknown provenance in the streets, consumption of raw salad in restaurants, consumption of sandwiches containing vegetables in the streets) was confronted against Chi-squared or Fisher tests, with the aid of PROC FREQ from the Statistical Analysis Software – SAS version 9.3 (SAS, 2011). The level of significance of 5% (\(p < 0.05\)) was adopted.

Results

From 2009 to 2010, 51 students from the Veterinary Medicine Course of the Federal University of Pará – UFPA were included in the study, 29 (56.86%) women and 22 (43.14%) men. The age of the participants ranged from 17 to 42 years old (average: 21 years old ± 4). With regard to the provenance, 88.2% (45/51) of the students lived in urban zone and 11.8% (06/51) lived in mixed zone, i.e. they went to the city for studying, but they returned daily or in the weekends to the rural zone, where they lived.

Although the proposal of following-up the students has been elaborated for three consecutive semesters, due to the fact that the participation was voluntary, it was only possible performing the serological follow-up in 24 students, where 14 students were followed in two serologies and 10 were followed in three serological examinations. In the first serological assessment from the 51 students submitted to serology, 22 (43.14%) were reactant to anti-\textit{T. gondii} antibodies, 13 men and 09 women (Table 1).

![Table 1. Univariate investigation of risk factors for Toxoplasmosis in veterinary medicine students from the Federal University of Pará, campus of Castanhal, state of Pará.](biota-amazonia-issn-2179-5746)
Among the 24 students serologically followed-up (performing two and/or three serologies) 10 (41.67%) were reactive to *T. gondii* antibodies, where six kept the titers stable, two students presented decrease, and two increased the titers of antibodies in the next serologies (Table 2). The 14 seronegative students in the first collection remained in this condition in the next collections.

Table 2. Description of the anti-*T. gondii* antibody titers of four veterinary medicine students performed during biannual serology.

<table>
<thead>
<tr>
<th>Students</th>
<th>1st Serology</th>
<th>2nd Serology</th>
<th>3rd Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>320</td>
<td>1280</td>
<td>-</td>
</tr>
<tr>
<td>04</td>
<td>1280</td>
<td>320</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>1280</td>
<td>1280</td>
<td>320</td>
</tr>
<tr>
<td>26</td>
<td>80</td>
<td>320</td>
<td>5120</td>
</tr>
</tbody>
</table>

With regard to the analysis of the variables investigated in the epidemiological questionnaire, it was observed that 94.1% (48/51) of the students used to wash fruits and vegetables before consuming them, 86.3% (44/51) had portable water at home, 78.4% (40/51) did not consume non-pasteurized milk, 66.7% (34/51) did not consume non-pasteurized cheese, 84.3% (43/51) did not consume homemade yogurt with non-pasteurized milk, and 70.6% (36/51) of the students answered they did not consume raw meat (Table 1). The variables previously mentioned, when related to the positivity for anti-*T. gondii* antibodies, did not present statistical association (p=0.05) (Table 1).

However, other variables had strong statistical association, they were: consumption of water or juice from unknown provenance in the streets (p=0.026), the consumption of raw salad in restaurants (p=0.031), and the consumption of sandwiches containing raw vegetables in the streets (p=0.009) (Table 1).

Discussion

The professionals in close touch with animals presented higher risk of being infected by *T. gondii* (BRANDON-MONG et al., 2015), and the veterinary students, besides being in this group, pass through big changes when leaving home and starting a life in another city, not worrying about their food habits when comparing to the habits they had at their parents’ home. The results found in this study showed that 43.5% of the students presented anti-*T. gondii* antibodies. Seropositivity rate superior to the one obtained in Veterinary Medicine students from Campo Grande, state of Mato Grosso do Sul, 30.3% (ARAUJO et al., 2000) and from Botucatu, state of São Paulo, 9.3% (VASCONCELOS, 2003).

Pilot study performed by Moraes et al. (2008) in another group of students of Veterinary Medicine from the Federal University of Pará had already demonstrated a relatively similar index, 48.39% (30/62), to the one observed in the present investigation. In both studies, it is suggested that the high number of reagent students is due to the fact that the region presents equatorial hot-wet climate, favoring the oocysts sporulation, increasing the presence of the infectious form of the parasite in the environment.

The titration of the 10 reactive students serologically followed-up by titre, three of the examinations remained stable in 60% of the students, however, in four students, there were significant changes in their anti-*T. gondii* antibodies titers. Similar results were observed in study performed with 112 veterinary students, where most of the students remained with stable titration, however, three students presented serocconversion in serology performed with a six-month interval (RODRIGUES et al., 2015).

Students 01 and 26 had abrupt elevation of the titers (Table 2), and these students presented similar behavior when their answers to the epidemiological questionnaire were assessed. The students observed in both students which could cause the exposure to *T. gondii* were: contact with cats, presence of neighborhood cats in their backyard, consumption of non-pasteurized cheese, consumption of water or juice from unknown provenance in the streets, consumption of raw salad in restaurants and the consumption of sandwich containing raw vegetables in the streets. From the six variables mentioned above, five could be considered, according to the statistical analysis performed, risk factors for possible contraction of the infection by *T. gondii* in veterinary medicine students.

The variables presence of cats at the students’ house (p=0.002) and the presence of neighborhood cats at the students’ backyard (p=0.046) presented strong statistical association with the contraction of the infection by *T. gondii*, fact justified since the felines are this parasite’s definitive hosts, which eliminates them in the feces during the infection phase (15 days), and the oocysts, after sporulation in the environment, become infectious and remain viable for a long time (DUBEY, 2010). In compliance with the study performed in veterinary medicine students of São Paulo, which observed that the contact with cats out of the university had significant statistical association with the infection by *T. gondii* (RODRIGUES et al., 2015). However, in study performed with university students in Jordan, there was no association with the infection by *T. gondii* and the presence of cats at home (OBAIDAT et al., 2015).

Some common food habits in the academic routine of the investigated students presented significant statistical association with the acquisition of the infection by *T. gondii*; they were: the consumption of water or juice from unknown provenance in the streets (p=0.026), the consumption of raw salad in restaurants (p=0.031), and the consumption of sandwiches containing raw vegetables in the streets (p=0.009) (Table 1).

The consumption of water or juice from unknown provenance in the streets is presented strong statistical association with the infection (p=0.046). This parasite’s definitive hosts, which eliminates them in the feces (p=0.046), presented strong statistical association with the contraction of the infection by *T. gondii*; they were: the consumption of water or juice from unknown provenance in the streets (p=0.026), the consumption of raw salad in restaurants (p=0.031), and the consumption of sandwiches containing raw vegetables in the streets (p=0.009) (Table 1).

**Conclusion**

It can be concluded that the prevalence of anti-*T. gondii* antibodies in veterinary medicine students from UFPA is high. With regard to the fact that seronegative students remain with this profile in the subsequent serological analyzes, it indicates that the continuous contact with animals during the academic course must not be an important source of infections. Unlike, in the present study, the contact with cats at home and eating food and drinking beverages in the streets without proper hygiene, with unknown sanitary state may be possible risk factors for the infection by *T. gondii* in these students.

**References**


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