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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 reactant 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

RESUMO

O Estado do Pará por se localizar em região equatorial apresenta características climáticas e epidemiológicas propicias 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 responderam 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. gondi*. As variáveis que tiveram forte associação estatística foram: a presença de gatos em casa (p=0.002), se gatos dos vizinhos tinham acesso ao quintal (p=0.046), o consumo de água ou suco na rua com procedência desconhecida (p=0.026), 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: toxoplasmose, sorologia, universitários, Pará.

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% (07/38) reactant veterinarians, 33.3% (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 serological 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

verifying the occurrence of anti-*T. gondii* antibodies in students from the School of Veterinary Medicine of the Federal University of Pará, and serologically following them up during three semesters of the course, assessing the hygiene-food habits and behaviors of the same through epidemiologic inquiry and associating them to the serological results.

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 sample of the study was 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 participle 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 epidemiological questionnaire, which was presented with the objective of getting information on the occupational and environmental variables, investigating epidemiological 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 r.p.m. 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-*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 albine mice (*Mus musculus*), previously infected with RH strain of *T. gondii*. The technique was developed according to description of Camargo (1974). Briefly, the samples of serum were diluted in series (1:10 to 1: 10.240) and deposited in slides sensitized with *T. gondii* antigen. After incubation and washing steps, the reaction was revealed with anti-antibody (anti IgG or anti-IgM) marked with fluorescein (bioMérieux®). 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% ($\alpha = 0.05$) 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-*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.

Variables	Total	Positive, N (%)	P-Value
Sex			
Male	22	13 (59.1)	0.053
Female	29	09 (31.0)	
Home area			
Urban zone	45	17 (37.8)	0.073
Mixed zone	06	05 (83.3)	
With a cat at home			
Yes	26	17 (65.4)	0.002
No	25	05 (20.0)	
Cats accessing the backyard			
Yes	28	16 (57.1)	0.046
No	23	06 (26.1)	
The house has potable water			
Yes	44	17 (38.6)	0.216
No	07	05 (71.4)	
Consumption of non -pasteurized milk			
Yes	11	05 (45.4)	1.00
No	40	17 (42.5)	
Consumption of non -pasteurized cheese			
Yes	17	09 (52.9)	0.377
No	34	13 (38.2)	
Consumption of homemade yogurt			
Yes	08	06 (75.0)	0.063
No	43	16 (37.2)	
Consumption of rare meat			
Yes	15	08 (53.3)	0.371
No	36	14 (38.9)	
Washing fruits and vegetables before eating them			
Yes	48	22 (45.8)	0.249
No	03	0 (0.0)	
Drinking water or juice from unknown provenance in the streets			
Yes	23	14 (60.9)	0.026
No	28	08 (28.6)	
Consumption of raw salad in restaurant			
Yes	35	19 (54.3)	0.031
No	16	03 (18.7)	
Consumption of sandwiches containing vegetables in the streets			
Yes	28	17 (60.7)	0.009
No	23	05 (21.7)	

Among the 24 students serologically followed-up (performing two and/or three serologies) 10 (41.67%) were reactant to anti-*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* antibodies titration of four veterinary medicine students whose titers ranged during biannual serologies

Students	1st Serology	2nd Serology	3rd Serology
01	320	1280	-
04	1280	320	-
25	1280	1280	320
26	80	320	5120

With regard to the analysis of the variables investigated in the epidemiologic 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 potable 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 rare 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: 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 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 caring about their food habits when comparing to the habits they had at their parents' home. The results found in this study showed that 43.14% 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, 30.34% (ARAÚJO 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 reagent students serologically followed-up by two or three 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 seroconversion 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 epidemiologic questionnaire were assessed. The variables 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 student's 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 mainly referred to the ones prepared from the pulp of fruits and commercialized in snack bars, or by street vendors of snacks, common in the city of Castanhal. This habit was shown as an important mean for the contraction of this protozoan's infection since the students that reported the ingestion of water or juice from unknown provenance were highly infected (60.9%) in comparison to the ones which did not consume beverages from unknown origin (28.6%) with value of p=0.026. Such hypothesis is supported by the fact that toxoplasmosis has also been characterized as an important infection of hydric propagation, including with outbreaks recorded in Brazil and related to this way of transmission (BOWIE et al., 1997; MOURA et al., 2006).

The consumption of raw salad in restaurants and the consumption of sandwiches containing raw vegetables in the streets (p=0.009) were other variables associated to the infection by *T. gondii* in students. The ones that consumed raw salad in restaurants presented higher index of infection by *T. gondii* (54.3%) than the ones that reported they do not consume raw salad in commercial establishments (18.7%) with value of p=0.031. As well as the students that consumed sandwiches containing raw vegetables in the streets, who presented higher prevalence (60.7%) for anti-*T. gondii* antibodies than the ones that did not report this habit (21.7%) with value of p=0.009. This association is probably due to the access of stray cats to vegetables gardens and plantations, and the lack of hygienic measures on the preparation of food to be served to clients in some commercial establishments, such as the proper washing of the vegetables and greenery (OBAIDAT et al., 2015).

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 might not be an important source of infection by the parasite. 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 relevant risk factors for the infection by *T. gondii* in these students.

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