SEROEPIDEMIOLOGY OF Toxoplasma gondii INFECTION

IN STUDENTS OF VETERINARY MEDICINE

AND OTHER COURSES OF PUBLIC UNIVERSITIES

IN RIO DE JANEIRO STATE, BRAZIL

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ABSTRACT

The aim of this study was to estimate the serum prevalence of *Toxoplasma* infection in two groups of university students: Veterinary Medicine (VM) and Other Courses (OC). Students from two public universities in the state of Rio de Janeiro were investigated to identify risk habits and behaviors for *Toxoplasma gondii* infection, and to guide primary prevention. All 839 students answered a questionnaire about habits and behaviors (347 OC and 492 VM). A serum prevalence of 21.8% was observed by indirect immunofluorescence (IFA) and ELISA. No statistically significant difference was observed between students of the universities. Serum prevalence (IFA and ELISA IgG) was 16.1% in VM and 29.9% in OC. Six students were IgM seropositive, of which five were only positive in ELISA and one was positive with both diagnostic techniques (ELISA and IFA). Among those students, four were in the control group from UFF and one in each group from UFRRJ. The prevalence of students seropositive for toxoplasmosis was low in both universities and the results obtained suggest that veterinary students are probably not exposed to a higher risk than the general population for acquiring toxoplasmosis. Age, contact with cats, consumption of undercooked or raw meat, contact with campus soil and ignorance of prophylactic measures for toxoplasmosis were positively associated with prevalence of infection by *T. gondii*.

KEY WORDS: Toxoplasma gondii; veterinary medicine; students; seroepidemiology.

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RESUMO

Soroepidemiologia da infecção toxoplásmica em alunos de Medicina Veterinária e de outros cursos de Universidades Públicas do Estado do Rio de Janeiro.

O objetivo deste estudo foi estimar a soroprevalência da infecção toxoplásmica em dois grupos de universitários, de Medicina Veterinária (MV) e de Outros Cursos (OC), de duas instituições públicas de ensino do Estado do Rio de Janeiro, buscando identificar hábitos e comportamentos de risco para infecção por este protozoário, e orientando sobre a prevenção primária. Dos 839 universitários, 492 eram da MV e 347 de OC. Todos os acadêmicos responderam a um questionário sobre seus hábitos e costumes. A soroprevalência (RIFI e ELISA IgG) foi de 16,1% na MV e 29,9% nos OC. Seis estudantes foram IgM soro reagentes, dos quais cinco eram apenas positivos no ELISA e um era positivo em ambas as técnicas de diagnóstico (ELISA e RIFI). Entre os estudantes, quatro eram do grupo de controle da UFF e um em cada grupo da UFRRJ. A prevalência de universitários soros reagentes para a toxoplasmose nas duas Universidades foi baixa e os resultados obtidos neste estudo sugerem que provavelmente os estudantes de Medicina Veterinária não estão expostos a um risco maior que os de outros cursos de adquirir a toxoplasmose. A idade, o contato com gatos, com o solo do campus, o consumo de carne crua ou mal-passada e o desconhecimento sobre a profilaxia da toxoplasmose influenciaram na prevalência da infecção por *T. gondii*.

DESCRITORES: Toxoplasma gondii; medicina veterinária; estudantes; soroepidemiologia.

INTRODUCTION

Toxoplasma gondii is the causal agent of toxoplasmosis, a protozoan infection with a worldwide distribution. The occurrence of infection in Brazil is very high, being found in several regions (2, 32). The parasite is capable of infecting a wide variety of mammals and avian species, both wild and domesticated, including human beings. In humans, most infections are asymptomatic; however, toxoplasmosis in patients with immunological impairment and in fetuses is a severe or even fatal disease (5). One of the primary transmission mechanisms is the ingestion of sporulated oocysts, which are shed unsporulated in the feces of infected felids (definitive hosts) and then disseminated throughout the environment, contaminating water, soil and food. Other important transmission mechanisms are the ingestion of cysts found in tissues of infected animals, direct contact with carcasses and viscera of parasitized animals, and transplacental transmission (4, 13, 25). Human infection through meat production animals is also extremely important from a medical and public health point of view. However, transmission pathways and potential hosts should be clarified. Viable forms of T. gondii have been isolated from a variety of meats, and serological studies have demonstrated a wide distribution of infection among production animals (13, 21, 22, 26, 27, 28). Studies have shown an increase in prevalence of *Toxoplasma* infection when there is frequent human contact with animals and their carcasses (13, 25). In this context, veterinarians and students of veterinary medicine, because of the close contact with animals and carcasses, could be at a higher risk of acquiring this infection. Thus, the aim of this study was to estimate the serum prevalence of T. gondii infection in two

groups of graduate students: Veterinary Medicine and controls (students from the Humanities and Exact Sciences). Subjects were drawn from two public universities in the metropolitan area of Rio de Janeiro state, aiming to identify risk habits and behaviors that favor infection by this parasite and giving orientation about primary prevention towards infection control.

MATERIALS AND METHODS

A sectional study was conducted to evaluate the serum prevalence of T. gondii infection in students of two universities in the state of Rio de Janeiro. The selected universities were Universidade Federal Fluminense (UFF), located in Niterói municipality, and the Universidade Federal Rural do Rio de Janeiro (UFRRJ), located in Seropédica municipality. Students were divided into two groups: Veterinary Medicine (VM) and Other Courses (OC). The latter was composed of students from the following courses: Administration, Portuguese Language and Literature, Physics, History, Domestic Economy, Philosophy, and Pedagogy. Serology was performed on a total of 839 adult volunteers, of which 435 were from UFF (228 from VM and 207 from OC) and 404 from UFRRJ (264 from VM and 140 from OC). Informed consent was obtained from all participants and the project was approved by the Ethical Commission in Research with human beings (CEP) from IPEC/Fiocruz with protocol number 0015.0.009.000-09. All participants filled in a questionnaire about habits and behaviors. Blood samples were collected by venous puncture in vacuum collection tubes without anticoagulant, and serum was obtained after clot centrifugation and stored in duplicate at -20 °C. Serum was submitted to IgM and IgG anti-T. gondii analysis by immunoenzymatic assay - ELISA (BioKit®) and Indirect Immunofluorescence assay (IFA) performed at the Laboratório de Toxoplasmose - LabTOXO, Instituto Oswaldo Cruz- IOC/ FIOCRUZ. After sampling, students were informed about primary prophylaxis for control of protozoa by means of lectures and folders. The number of samples used was calculated based on expected prevalences of 40% and 30% in VM and OC groups, respectively. In order to achieve a first type error of 5% and a potency of 80% a minimum of 376 students needed to be analyzed in each group. Prevalence and Chi-squared (X2) tests were performed with Stata 10.1 (36).

RESULTS

Toxoplasmosis serum prevalence in all participants, i.e. 839 graduate students from UFF and UFRRJ, was 21.8%. The prevalence of seropositivity was a little higher in UFF students when compared to UFRRJ students. When compared to the VM group, a higher occurrence of IgG anti-*T. gondii* was observed in the OC group in both universities. Six students were IgM seropositive, of which five were only positive in ELISA and one was positive in both diagnostic techniques (ELISA

and IFA). Among those students, four were in the control group from UFF and one in each group from UFRRJ.

Data analysis using the Chi-squared test showed significant difference in the variables VM and OC of both universities (Table 1). The Chi-squared test did not show a significant difference in IgG anti-*T. gondii* prevalence between men and women. When students were divided into age groups, a progressive increase in frequency of seropositivity was observed with age. Statistical difference was observed in IgG anti-*T. gondii* prevalence between age groups (Table 2).

Table 1. Results of distribution of ELISA/IFA for anti-*Toxoplasma gondii* antibodies of the IgG class in students from UFF and UFRRJ universities, according to studied groups.

ELISA/IFA IgG	Veterinary Medicine % (n)	Other Courses % (n)	Total % (n)
Negative	83.94 (413)	70.03 (243)	78.19 (656)
Positive	16.06 (79)	29,97 (104)	21.81 (183)
Total	100 (492)	100 (347)	100 (839)

(x2=23.10; p=0.001)

Table 2. Results of distribution of ELISA/IFA for anti-Toxoplasma gondii antibodies of the IgG class in students from UFF and UFRRJ universities according to age groups

ELISA/IFA IgG	<20 years N (%)	20-24 years N (%)	25-29 years N (%)	>30 N (%)	Total N (%)
Negative	110 (88.00)	422 (82.91)	91 (67.91)	33(46.48)	656 (78.19)
Positive	15 (12.00)	87 (17.09)	43 (32.09)	38 (53.52)	183 (21.81)
Total	125 (100.00)	509 (100.00)	134 (100.00)	71 (100.00)	839 (100.00)

 $(x^2 = 64.29 \text{ and } p = 0.001)$

Figure 1 shows how serology results vary with age, according to the studied groups (VM and OC). The results show that prevalence of IgG anti-*T. gondii* was higher in the OC group regardless of age. However, for the age 20-24 group it was possible to observe an increase in both groups studied, albeit more noticeable in the OC group.

The following variables did not show statistical differences in relation to IgG anti-*T. gondii* prevalence: contact with soil, animal manipulation during lectures, and lack of use of individual protection equipment (IPE) use. However, contact with cats, contact with campus soil, consumption of undercooked or raw meat, and ignorance of prophylactic measures were significantly associated with IgG anti-*T. gondii* prevalence (Table 3).

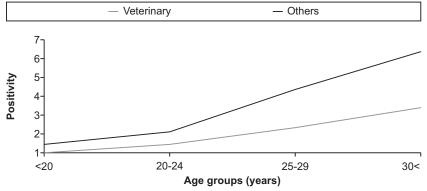


Figure 1. Positivity in IFA or ELISA for anti-Toxoplasma gondii antibodies of the IgG class, according to age and student groups from UFF and UFRRJ

DISCUSSION AND CONCLUSION

A prevalence of 21.8% was observed among UFF and UFRRJ students in both groups studied, which was smaller than in similar studies (17, 30) and most serum epidemiological studies conducted with adult populations not only in the State of Rio de Janeiro but also all over Brazil (1, 2, 6, 7, 32, 35). Regarding the IgM seropositive students, since there was no follow up of these students during this research to observe possible cases of serum conversion, it is not possible to state that the positivity was acquired during their time at university.

In general, university students come from more structured families, with better infrastructure and more access to information, which could have interfered with serological results in this study. Toxoplasmosis prevalence in veterinary medicine students of both universities was smaller than the numbers reported by Riemann et al. (1974) (31) (41.0%) in students of the same course in Brazil. However, similar results were reported by McCulloch et al. (1963) (23) in several locations in the USA, with low occurrences of infection in veterinary medicine students of public universities in Iowa (17.0%), Minnesota (20.1%), and Illinois (23.0%). UFF is a university located in an urban area, unlike UFRRJ, which is rural. Contrary to expectations, a higher prevalence of IgG anti-*T. gondii* was observed in UFF students when correlation between veterinary groups of both universities and serum positivity was tested. The same profile occurred in the OC group.

When comparing veterinary with other students at each university, a higher prevalence of seropositivity for *T. gondii* was observed in the OC group, despite the educational and practical classes imposed by several disciplines in the veterinary medicine curriculum. Despite no correlation between occupational risk and infection being observed in the present study, this risk exists for veterinary

students and could be related to a higher prevalence of IgG anti-*T. gondii*. Classroom practice, carcass and animal contact (13, 24), and misuse of IPE could be factors that explain the marked increase in IgG anti-*T. gondii* prevalence after the age of 20 in veterinary medicine students at both universities. In the OC group, this increase could be related to students' origins, based on socioeconomic and cultural factors prior to university enrollment. Studies analyzing population socioeconomic conditions demonstrated higher infection prevalence in places with worse conditions (6). However, the possibility of students from both groups being exposed to other infection sources outside university cannot be discounted, since the routes for *T. gondii* transmission are countless. Thus, it is not possible to state where and when the infection occurred, and only assumptions can be made regarding this matter.

Table 3. Results of distribution of ELISA/IFA for anti-Toxoplasma gondii antibodies of the IgG class in students from UFF and UFRRJ universities in relation to possible risk factors for *T. gondii* infection

Variables	Reactive n (%)	Non-reactive n (%)	Total n (%)	X2	P
Contact with cats		(11)			
Yes	50 (28.25)	127 (71.75)	177 (100.0)	5.032	0.025
No	133 (20.37)	520 (79.63)	653 (100.0)		
Total	183 (22.05)	647 (77.95)	830* (100.0)		
Contact with soil					
Yes	114 (24.05)	360 (75.95)	474 (100.0)	3.203	0.074
No	69 (18.90)	296 (81.10)	365 (100.0)		
Total	183 (21.81)	656 (78.19)	839 (100.0)		
Consumption of under	rcooked or raw meat				
Yes	55 (15.45)	301 (84.55)	356 (100.0)	15.024	0.001
No	126 (26.69)	346 (73.31)	472 (100.0)		
Total	181 (21.86)	647 (78.14)	828** (100.0)		
Contact with campus s	soil				
Yes	78 (26.53)	216 (73.47)	294 (100.0)	5.822	0.016
No	103 (19.29)	431 (80.71)	534 (100.0)		
Total	181 (21.86)	647 (78.14)	828** (100.0)		
Animal manipulation	during classes				
Yes	51 (17.53)	240 (82.47)	291 (100.0)	1.14	0.286
No	28 (13.93)	173 (86.07)	201 (100.0)		
Total	79 (100.0)	413 (100.0)	492*** (100.0)		
Use of Individual Prot	ection Equipment				
Yes	10 (10.87)	82 (89.13)	92 (100.0)	2.26	0.133
No	69 (17.25)	331 (82.75)	400 (100.0)		
Total	79 (16.06)	413 (83.94)	492*** (100.0)		
Prophylaxis knowledg	ge				
Yes	65 (18.31)	290 (81.69)	355 (100.0)	4.21	
No 0.040	117 (24.22)	366 (75.78)	483 (100.0)		
Total	182 (100.0)	656 (100.0)	838**** (100.0)		

^{*}Nine students did not answer this question. **Eleven students did not answer this question.

^{***} This question was only asked to veterinary students. ****One student did not answer this question.

A considerable increase in IgG anti-*T. gondii* prevalence was observed with age. This fact was expected because *T. gondii* has a complex life cycle that includes several risk factors (16), and there is an increasing chance of contact with possible transmission mechanisms for toxoplasmosis with age. This fact was also observed by other researchers (8, 19, 29, 34, 35), including specific populations such as Indian tribes (3, 32) and pregnant women (35).

Knowledge of prophylactic measures may have contributed to a decrease in *Toxoplasma* infection. In students with knowledge about *T. gondii* infection the prevalence was 18.3% and in those without such knowledge the prevalence was 24.2%. Students of courses outside health and agricultural areas, who do not know about transmission mechanisms of this parasitosis, could be more susceptible to *T. gondii* infection. Prophylactic knowledge about *Toxoplasma* infection, an interesting but little studied aspect, is one of the preventive measures for infection, as demonstrated in studies by Bonna (2007) (8) and Bueno (2008) (9). These studies also indicated the need for implementing primary prevention strategies against the disease.

No statistically significant differences were observed in relation to gender in the studied population. This corroborates findings of other authors (13, 20, 25, 32). Students were exposed, at least inside the university campus, to the same risk factors regardless of gender, suggesting an exposure in the same intensity and frequency. Gender alone could not be considered a resistance or susceptibility factor for this parasitosis (3), only when associated with cultural habits differentiated by gender, such as in Indian tribes (32). Domestic animals may be an important sentinel population for infection in the community (38).

Despite the contact with contaminated soil being considered a risk factor of great importance in protozoa transmission (12, 35), this variable did not contribute to infection prevalence in the present study. This could be explained by the different cultural characteristics, habits and behaviors of the studied population. This fact was also demonstrated by Millar et al. (2007) (25), when both groups analyzed did not have significant difference regarding soil contact. Contact with cats (10, 11, 17, 19, 29, 33) and the consumption of raw or undercooked meat are considered important infection sources (18, 33, 34), and direct relation between *T. gondii* antibodies and these variables was observed in the present study. This result was also observed in a study conducted with students and workers from a Veterinary University in Iowa (37). Socio-economic factors could influence these results, because a higher frequency of meat ingestion could increase the risk for infection, together with household quality and access to treated water (6).

Awareness of the importance of IPE use is increasing among educational and research facilities. Because of the scarcity of studies about this question, a correlation analysis between IPE use and *Toxoplasma* infection was conducted in the present study. Although it is known that the use of gloves and hand washing after animal manipulation could reduce the possibility of *T. gondii* (15) infection and other infections, no statistical difference was observed. A problem detected among the studied population was the inadequate use of IPE.

The prevalence of seropositive students for toxoplasmosis in both universities in Rio de Janeiro was low, and the results shown in this study suggest that veterinary medicine students are probably not exposed to a higher risk of acquiring toxoplasmosis than the general population. Age and knowledge about toxoplasmosis prophylaxis influenced *T. gondii* infection prevalence, which suggests that access to information about prophylaxis could contribute to a reduction in the chances of infection.

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