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## Human Toxocariasis: Prevalence and Factors Associated with Biosafety in Research Laboratories

Gabriela Torres Mattos,<sup>1\*</sup> Paula Costa dos Santos,<sup>1</sup> Paula de Lima Telmo,<sup>1</sup>  
Maria Elisabeth Aires Berne,<sup>2</sup> and Carlos James Scaini<sup>1</sup>

<sup>1</sup>Laboratory of Parasitology, Faculty of Medicine (FAMED), Area Interdisciplinary Biomedical Sciences (AICB), Federal University of Rio Grande (FURG), Rio Grande do Sul, Brazil; <sup>2</sup>Laboratory of Parasitology, Department of Microbiology and Parasitology, Institute of Biology, Federal University of Pelotas, Rio Grande do Sul, Brazil

**Abstract.** Human toxocariasis is a neglected parasitic disease worldwide. Researchers studying this disease use infectious strains of *Toxocara* for experiments. Health workers are at risk in the course of their daily routine and must adhere to biosafety standards while carrying out the activities. Researchers on biosafety concerning working with these parasites are insufficient. The aim of this study was to determine the rate of seroprevalence of *Toxocara* species among health-care research laboratory workers (professors, technicians, and students), and to investigate the risk factors of *Toxocara* infection associated with laboratory practices. This cross-sectional study involved 74 researchers at two federal universities in southern Brazil from February 2014 to February 2015; 29 researchers manipulated infective strains of *Toxocara canis* (test group) and 45 did not (control group). Serum samples were examined using enzyme-linked immunosorbent assay. Epidemiological data were obtained via a questionnaire containing information about laboratory routine, eating behavior, and contact with dogs. The seroprevalence of anti-*T. canis* IgG was 14.9% (11/74; 13.8% [4/29] in the test group and 15.6% [7/45] in the control group). Most individuals in the test group correctly understood the primary mode of infection; however, 13.8% did not use gloves while manipulating *T. canis* eggs. Knowledge of biosafety must be well understood by health-care professionals doing laboratory work with biological agents. To our knowledge, this is the first study to investigate the rate of seroprevalence of IgG against *Toxocara* spp. among professionals and students who handle infective forms of the nematode *T. canis*.

### INTRODUCTION

Human toxocariasis is a neglected, worldwide parasitic disease with an underestimated prevalence<sup>1</sup> due to difficulties in clinical laboratory diagnosis.<sup>2</sup> The disease is most prevalent in tropical, developing countries.<sup>3</sup> In Brazil, the prevalence rates recorded for *Toxocara* spp. in the adult population range from 8.7% to 46.3%.<sup>4,5</sup> The nematode *Toxocara canis*, an intestinal parasite of dogs, is the etiological agent most associated with toxocariasis.<sup>2</sup>

In recent decades, there has been an increase in researchers related to the diagnosis and control of this disease.<sup>2,6–8</sup> Activities that involve handling of the infective forms of *T. canis* include larval culture for the production of excretion and secretion antigen of *T. canis*,<sup>9</sup> inoculation of eggs hatched in experimental models,<sup>10–12</sup> and manipulation of the agent in vitro tests.<sup>13</sup>

Laboratory accidents linked to parasitic infections have been associated with a lack of knowledge about modes of transmission, pathogenicity, treatment, and specific prophylactic measures.<sup>14</sup> The parasites most often responsible for laboratory-acquired infections are the protozoans *Trypanosoma cruzi*, *Toxoplasma gondii*, and *Leishmania* spp.<sup>15–17</sup> There is a shortage of laboratory accidents involving parasites. This is because helminthic infections generally are less likely than protozoan infections to be acquired in the laboratory. Usually, helminthic infections are asymptomatic. Moreover, some infected laboratorians have not recalled the discrete accident suggests que subtle exposures such as exposure through aerosolization in obtaining the antigen of *Ascaris*.<sup>14</sup>

This study aimed to determine the prevalence rates of *Toxocara* spp. infection and to investigate the risk factors of and the level of knowledge about toxocariasis and the laboratory practices performed by professionals and students in health research laboratories.

### MATERIALS AND METHODS

**Type of study.** In the present cross-sectional study, we investigated the prevalence of and knowledge about human toxocariasis in a convenience sample of 29 professionals and students who handle infectious forms of *T. canis* (G1) and 45 people who do not perform experimental studies with this parasite (G2, control). The sample size was calculated using the Epi Info software (Centers for Disease Control and Prevention, Atlanta, GA) version 3.5.2.

The professionals (teachers and technicians) and students (undergraduate and postgraduate) who participated in the study work in the Interdisciplinary Area Laboratory of Biomedical Sciences, Faculty of Medicine, Federal University of Rio Grande (FURG), and the Department of Microbiology and Parasitology, Institute of Biology (IB), Federal University of Pelotas. The study was conducted from February 2014 to February 2015.

**Participation in the study.** Participation by the research subjects consisted of responding to a self-administered epidemiological questionnaire and consenting to blood collection for *Toxocara* spp. serological test and blood count. The professionals and students who agreed to participate in the study signed the Informed Consent Form. This study was approved by the Research Ethics Committee of the Health Area, FURG (opinion no. 102/2012).

**Epidemiological data.** The self-administered, structured questionnaire was designed to collect demographic and epidemiological data (contact with dogs and cats, nail biting, and eating habits) and knowledge about toxocariasis and

\* Address correspondence to Gabriela Torres Mattos, Laboratory of Parasitology, Faculty of Medicine (FAMED), Area Interdisciplinary Biomedical Sciences (AICB), University of Rio Grande, Rua General Osório S/N, 96200-190, Centro, Rio Grande, Rio Grande do Sul, Brazil. E-mail: gastgabi.mattos@gmail.com

biosafety. The questionnaires were double entered using the EpiData program, version 3.1 (Odense, Denmark).

**Hemogram.** Hemograms were generated using a Pentra 80 (Montpellier, France) hematology analyzer at the Clinical Analysis Laboratory of the University Hospital Dr. Miguel Riet Corrêa Júnior, FURG.

**Excretory-secretory antigen production.** *Toxocara canis* eggs were collected from the oviducts of female adult parasites after treatment of young dogs (4 to 8 weeks old) with pyrantel pamoate (12.5 mg/kg). The eggs were incubated for 30 days in 2% formalin solution at 28°C, with oxygen and humidity greater than 80%.<sup>12</sup> The larvae derived from embryonated eggs were then incubated in RPMI-1640 medium with antibiotics and antifungals at 37°C with 5–8% CO<sub>2</sub><sup>19</sup> to obtain excretory-secretory antigens (*T. canis* excretory-secretory antigen [TES]).<sup>20</sup> The protein concentration was determined using the bicinchoninic acid (BCA) method.<sup>21</sup>

**Somatic antigen of *Ascaris suum*.** This antigen was produced from adult female *Ascaris suum* nematodes acquired from a slaughterhouse in the city of Pelotas, Rio Grande do Sul, Brazil, following the methodology described by Souza and others<sup>3</sup> to avoid cross-reactions with the antigen TES. The concentration of TES protein antigen was determined using the BCA method.<sup>21</sup>

**Enzyme-linked immunosorbent assay (ELISA)–TES: IgG detection of *Toxocara* spp.** In brief, a 96-well flat-bottom plate (Kasvi®, Curitiba, Brazil) was sensitized with TES (2 µg/mL) in carbonate/bicarbonate buffer at a pH of 9.6 to 9.8 and incubated overnight in a humid chamber at 4°C. Blocking of free sites was performed with 5% casein in phosphate-buffered saline (PBS)-Tween-20 0.05% (PBS-T) at a pH of 7.2 for 1 hour in a humid chamber at 37°C. Sera previously adsorbed with somatic antigen of *A. suum* antigen were tested in duplicate at a dilution of 1:50 in PBS-T, and the conjugate (anti-human Fc-specific peroxidase-conjugated IgG; Sigma® Aldrich, San Diego, CA) was used at a dilution of 1:6,000 in PBS-T. Both the sera and conjugate were incubated for 45 minutes in a humid chamber at 37°C. The plates were washed with PBS-T five times for 10 minutes between different phases. All the reagents were used at a volume of 100 µL. Orthophenylenediamine at a concentration of 0.5 mg/mL in citrate-phosphate buffer at a pH of 4.0 plus 0.1% hydrogen peroxide was applied as the chromogen, and the absorbance at 450 nm was measured after 15 minutes. The cutoff (0.903) was calculated as the mean absorbance of seven serum samples negative for *Toxocara* spp. plus two standard deviations. Serum was considered negative if no blood eosinophilia was present (< 2%) and the participant had no dog at home, no contact with puppies, and no onychophagia.<sup>22</sup>

**ELISA-TES: IgE detection of *Toxocara* spp.** The test was performed as described in the previous section, except using anti-human Fc-specific peroxidase-conjugated IgE (Sigma) diluted 1:5,000. The cutoff point was calculated as the mean absorbance of seven serum samples negative for *Toxocara* spp. plus two standard deviations.

**ELISA-TES: IgG4 detection of *Toxocara* spp.** The test was performed as described in the previous section, except that preadsorption with somatic *A. suum* antigen was not performed and whole serum was used. Anti-human Fc-specific peroxidase-conjugated IgG4 was used (Sigma) at 1:5,000 dilution. We did not previously adsorb the serum because IgG4-ELISA has higher specificity compared with the IgG-ELISA.<sup>18</sup> The cutoff point

TABLE 1

Seroprevalence (IgG) in professionals and students who handle (G1) and do not handle (G2, control) infectious forms of *Toxocara canis* in the laboratories of two federal universities in southern Brazil ( $n = 74$ )

Group	N	Positive	%	Prevalence ratio	Confidence interval	P value
Group 1	45	60.8	7	15.6	1	
Group 2	29	39.2	4	13.8	1.13	0.362–3.51
Total	74		11	14.9		0.556

was calculated as the mean absorbance of seven serum samples negative for *Toxocara* spp. plus two standard deviations.

**Statistical analysis.** Descriptive data analysis consisted of calculations of absolute and relative frequencies for categorical variables and means and 95% confidence intervals for continuous variables. The prevalence ratio was calculated for each variable, and a significant difference was considered at  $P < 0.05$ . All the analyses were performed using the SPSS (Chicago, IL) and Epi Info, version 3.5.2 programs.

## RESULTS

Among the 74 workers and students, the recorded prevalence was 14.9% (11/74) for IgG specific for *Toxocara* spp.; the rate of G1 was 13.8% (4/29), and that of G2 (controls) was 15.6% (7/45) (Table 1). None of the study subjects was positive for specific IgE.

Among the 11 individuals seropositive for IgG, only one student in G2 showed IgG4 positivity for *Toxocara* spp., which was accompanied by an increase in eosinophils (8.5%).

Table 2 shows the results of the investigation of *T. canis* infection risk factors in the studied population, and none of the variables was significant as a risk factor.

Regarding the identification of biological symbols, the symbols for biohazard, toxic, fire, and explosion were identified by more than 90% of the respondents in G1 and G2 (control),

TABLE 2

Seropositivity (IgG) of professionals and students who handle (G1) and do not handle (G2, control) infectious forms of *Toxocara canis* and risk factors

Variable	Group 1 ( $n = 29$ )		Group 2 ( $n = 45$ )	
	Sample N (%)	Positivity N (%)	Sample N (%)	Positivity N (%)
Domicile				
House	14 (48.3)	2 (14.3)	23 (51.1)	2 (8.7)
Apartment	15 (51.7)	2 (13.3)	21 (46.6)	5 (23.8)
House in countryside	0 0	0 0	1 (2.3)	0 0
Domiciled dog				
Yes	22 (75.6)	4 (18.2)	29 (64.4)	2 (6.9)
No	7 (24.4)	0 (0)	16 (35.5)	5 (31.2)
Contact with puppy (< 6 months)				
Yes	6 (20.7)	1 (16.7)	15 (33.3)	3 (20.0)
No	23 (79.3)	3 (13.0)	30 (66.7)	4 (13.3)
Onychophagia				
Yes	10 (34.5)	0 (0)	11 (24.5)	0 (0)
No	19 (65.5)	4 (21.0)	34 (75.5)	6 (16.6)
Consumption of raw and/or undercooked meat				
Yes	10 (34.5)	1 (10.0)	17 (37.8)	1 (5.9)
No	19 (65.5)	3 (15.8)	28 (62.2)	6 (21.4)
Consumption of processed food (sausage)				
Yes	23 (79.3)	2 (8.7)	32 (71.2)	5 (15.6)
No	6 (20.7)	2 (33.3)	13 (28.8)	2 (15.4)
Consumption of raw vegetables				
Yes	26 (89.6)	4 (15.4)	43 (95.5)	7 (16.3)
No	3 (10.4)	0 (0)	2 (4.5)	0 (0)

TABLE 3

Biosafety risk symbol identification by professionals and students who handle (G1) and do not handle (G2, control) infectious forms of *Toxocara canis* (n = 74)

Symbols	Group 1 N (%)	Group 2 N (%)
Biological	27 (93.1)	41 (91.1)
Toxic	29 (100)	44 (97.8)
Forbidden to use water on fire	23 (79.3)	32 (71.1)
Electric shock	26 (89.6)	37 (82.2)
Corrosion	26 (89.6)	34 (75.5)
Explosion	28 (96.5)	41 (91.1)
Fire	29 (100)	44 (97.8)
Radiation	25 (86.2)	42 (93.3)

and the symbol for “forbidden to use water on fire” presented was not identified in 79.3% and 71.1%, respectively (Table 3).

With regard to knowledge of roundworms, 89.7% (24/29) of G1 properly rated this zoonosis as a neglected parasitic disease. Most of the G1 respondents answered correctly that the main mode of infection occurs via ingestion of *T. canis* eggs and that there are other modes of infection.

With regard to laboratory practices, 100% (29) of the G1 respondents performed activities involving the handling of *T. canis*. Of these subjects, 93.1% (27/29) responded that they perform airings of the eggs for embryonation, 62.1% (18/29) perform inoculations in experimental models, and 31.0% (9/29) execute incubation procedures in vitro using parasite larvae. However, 13.8% (4/29) responded that they do not use gloves to handle the incubation greenhouse, and 7.4% (2/27) do not use gloves when handling glassware containing incubating *T. canis* eggs. Not all researchers manipulate all infective forms of the parasite. In addition, 20.7% reported that they do not always use a biological safety cabinet. Biosecurity serves to protect the researcher, the environment, and the experiment, and often the researcher does not identify in his laboratory routine the risks to which it is exposed daily and is only concerned with the experiment, neglecting good laboratory practices and not using the equipment protection.

## DISCUSSION

This is the first study to investigate the rate of seroprevalence of IgG against *Toxocara* spp. among professionals and students who handle infective forms of the nematode *T. canis*. The overall prevalence rate in the entire study population was 14.9% (11/74), which was similar to that recorded in adults in Goiania (14.7%),<sup>23</sup> greater than that observed in São Paulo (8.7%)<sup>5</sup> and less than that observed in Salvador (46.3%).<sup>4</sup>

It is worth emphasizing that prevalence rates vary according to the region and population studied. A higher level of education has also been associated with a lower positivity rate,<sup>23,24</sup> and our study confirms this. Hence, this study demonstrated to work in the laboratory is not a risk factor; in addition, professional handling infective forms have more knowledge and therefore has attitudes prevention.

Dattoli and others<sup>4</sup> reported significant association between *T. canis*, eosinophilia and IgE production; however, in our work, one student seropositive for specific IgG presented eosinophilia and seropositivity for IgG4 specific, although no present IgE positive. In agreement, Noordin and others<sup>18</sup> showed that IgG4 has increased the specificity of the ELISA, recommended that use in the *Toxocara* serodiagnosis.

Specific IgE was not detected in this study, which could be explained by immunoglobulins having a lower sensitivity than IgG for diagnosing toxocariasis and specific IgE generally not being detected in asymptomatic patients.<sup>25</sup>

Contact with a young dog is an important risk factor for infection with *Toxocara* spp.<sup>26–28</sup> However, this variable was not significant in our study, despite higher rates in both groups: 16.7% (1/6) in G1 and 20% (3/15) in G2.

Laboratory areas should be marked with the international symbol for “biohazard,”<sup>29</sup> and the ability to interpret laboratory symbols should be required knowledge, as these symbols combine color, form, and shape.<sup>30</sup> In this study, 63.5% of the interviewees correctly identified all of the laboratory symbols, unlike the findings of Carvalho and Medeiros,<sup>31</sup> who reported that only 21% of researchers correctly identified symbols. The bottles with chemicals and solutions should be appropriately labeled, as these are used in various procedures in the laboratory, thus reducing chemical risk.

Laboratory biosafety is practiced by most professionals and students, who in fact wear gloves and use biological safety cabinets, and misuse of this equipment increases the risk of infection.<sup>32</sup>

With regard to knowledge about toxocariasis, the majority of respondents (89.7%) correctly answered that human toxocariasis is a neglected parasitic disease, in agreement with other research.<sup>3,33,34</sup> The main mode of toxocariasis infection is ingestion of embryonated *T. canis* eggs,<sup>7,35</sup> and 89.6% of researchers identified this form of transmission. In recent decades, toxocariasis has been linked to consumption of viscera and/or undercooked meat and to natural paratenic hosts<sup>36–40</sup>; 72.4% of researchers identified this mode of infection.

## CONCLUSION

Despite no significant differences in serology for the group handling infectious forms of *Toxocara* spp. or for other professionals and students, there is a need for greater understanding of this parasite. Knowledge of biosafety must be communicated to all laboratory personnel, and the necessary measures should be adopted in the case of an accident.

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Authors' addresses: Gabriela Torres Mattos, Paula Costa dos Santos, Paula de Lima Telmo, and Carlos James Scaini, Laboratory of Parasitology, Faculty of Medicine, Area Interdisciplinary Biomedical Sciences (AICB), Federal University of Rio Grande (FURG), Rio Grande do Sul, Brazil, E-mails: gastgabi.mattos@gmail.com, paulavet1@hotmail.com, paulatelmo@gmail.com, and cjscaifurg@gmail.com. Maria Elisabeth Aires Berne, Departamento de Microbiologia e Parasitologia, Universidade Federal de Pelotas, Rio Grande do Sul, Brazil, E-mail: bernemea@gmail.com.

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