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SEROPREVALENCE AND FACTORS ASSOCIATED WITH *Toxoplasma gondii* INFECTION IN HUMANS AND ITS RELATIONSHIP WITH CONTACT WITH DOMESTIC CATS (*Felis catus*) IN SOUTHERN RIO GRANDE DO SUL

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ABSTRACT

Toxoplasma gondii is the agent of a protozoan zoonotic disease of public health importance, toxoplasmosis, which may have serious consequences on the infected species. Cats are definitive hosts of the protozoan, and once infected, shed oocysts that sporulate in the environment and may become a source of infection for humans. The results reported by different authors about contact with pet cats as a risk factor for human infection are controversial. In this study, serum samples from 108 individuals who own or owned cats, and from 110 individuals who do not like cats and never owned one, were analyzed for the presence of IgG antibodies to *Toxoplasma gondii* through Indirect Immunofluorescence (IFI). An epidemiological questionnaire was also applied. The seroprevalence was 39.8% for the 108 individuals who owned cats and 26.4% for the 110 individuals who never owned cats. The analysis of risk factors showed significant association between seropositivity and the variables: aged over 31 years (p=0.0000), contact with cats (p=0.0143), and handling of raw meat (p=0.0290). Our findings indicate that even in a population with a high education level, contact with cats may double the chances of infection with *T. gondii,* when the animals are not kept indoors and under adequate sanitary conditions.

KEY WORDS: Toxoplasmosis; risk factors; cats; epidemiology.

RESUMO

Soroprevalência e fatores associados à infecção por *Toxoplasma gondii* em humanos e sua relação com o convívio com gatos (*Felis catus*) de estimação no sul do Rio Grande do Sul

Toxoplasma gondii é o agente da toxoplasmose, uma protozoonose de importância em saúde pública e que pode causar graves consequências nas espécies infectadas. Os felídeos são hospedeiros definitivos do protozoário que, ao se infectarem, eliminam oocistos que esporulam no ambiente e

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podem tornar-se fonte de infecção para humanos. Existem controvérsias nos resultados encontrados por diferentes autores em estudos sobre o convívio com gatos de estimação como fator de risco para a infecção humana. Neste estudo, em amostras de soro de 108 pessoas que têm/tiveram gatos e de 110 pessoas que não gostam e nunca tiveram gatos foram analisados anticorpos IgG para *T. gondii* por meio do teste de Imunofluorescência Indireta (IFI). Um questionário epidemiológico também foi aplicado. Os resultados indicam soroprevalência de 39,8% para as 108 pessoas que convivem com gatos e 26,4% para as 110 pessoas que nunca tiveram esses animais. Na análise dos fatores de risco, foi constatada associação significativa entre a soropositividade e as variáveis: idade superior a 31 anos (p = 0,0000), convívio com gatos (p = 0,0143) e manuseio de carne crua (p = 0,0290). Conclui-se que, mesmo em uma população com escolaridade elevada, conviver com gatos pode aumentar duas vezes a chance de infecção humana por *T. gondii*, desde que esses animais não sejam mantidos no interior do domicílio e haja condições higiênico-sanitárias adequadas.

DESCRITORES: Toxoplasmose; fatores de risco; felídeos; epidemiologia.

INTRODUCTION

Toxoplasma gondii (Nicolle and Manceaux, 1909) is a protozoan and obligate intracellular parasite of the phylum Apicomplexa, class Sporozoa, subclass Coccidia, suborder Eimeriina, family Sarcocystidae, distributed worldwide (Costa et al., 2007).

This parasite is described as the etiological agent of toxoplasmosis, a protozoan zoonotic disease of public health importance, as it may cause serious consequences in human and homeothermic animals (Dubey et al., 1995).

Felids are essential in the biological cycle of *T. gondii*, as they shed thousands of oocysts in their feces. Oocysts become infectious after sporulation, contaminating water and food for many months due to their high survival rates in the habitat (Vidotto, 1992; Dubey, 2010).

In acquired forms, humans may be infected through the ingestion of sporulated oocysts in the water or vegetables and tissue cysts present in raw or undercooked meat (Dubey, 1986; Sparkes, 1998). In congenital transmission, infection coincides with pregnancy, when tachyzoites diffuse to fetal tissues, causing serious damage (Frenkel, 1973; Langoni et al., 2001).

In Brazil, the prevalence rates of IgG antibodies to *T. gondii* in adult individuals range from 50% to 80% (Dubey et al., 2012). The variation in prevalence rates in different regions of the world depends on various factors, such as cultural patterns of the population, food habits, age, rural or urban origin, type of employment, geographic climate, socioeconomic and religious factors (Frenkel, 1996; Almeida-Filho, 2002; Rouquayrol, 2002).

In Pelotas, state of Rio Grande do Sul, seroprevalence was 54.8% (233/425) among pregnant women in Health Basic Units (Cademartori et al., 2008). In the Specialized Health Service (Serviço de Atendimento Especializado – SAE) of the same municipality, 80% (200/250) of HIV positive patients were seroreactive to *T. gondii* (Xavier et al., 2013), while among blood donors of the Regional Blood Bank, seroprevalence was 57.5% (115/200) (Loges et al., 2012).

Contact with felines as a potential risk factor for human infection with *T. gondii* has been controversial in the literature. Some authors found that contact with cats increases the risks of human infection with the protozoan (Kapperud et al., 1996; Ramírez et al., 1999; López-Castillo et al., 2005; Lopes et al., 2009; Figueiredo et al., 2010), while others reported that direct contact with these animals is not considered a risk factor for this disease (Dubey, 2000; Bobić et al., 2003; Cademartori et al., 2008; Pôrto et al., 2008; Blos, 2009; Elmore et al., 2010; Loges et al., 2012; Xavier et al., 2013).

This study was aimed at examining the importance of contact with cats as domestic animals for human infection with *T. gondii*, through seroepidemiological evaluation.

MATERIAL AND METHODS

Two groups were set up: one with 108 samples of serum from persons who owned or had owned cats and another group with 110 samples from people who did not like and had never owned cats in their homes.

Sampling was carried out by convenience, including residents of the urban area of Pelotas, RS, Brazil (31º 45' S, 52º 21' O).

Blood was drawn at the Laboratory of Clinical Analysis of the School of Medicine of the Federal University of Pelotas and at the residences of some participants by trained personnel from May to December 2011. An epidemiological survey to assess the risk of *T. gondii* infection was applied during the collection.

The epidemiological variables examined through the survey were: age, gender, geographic location of the residency, education level, knowledge about the disease and transmission modes, contact with cats, hygiene-sanitary practices regarding contact with cats, handling of raw meat, consumption of raw vegetables, raw or undercooked meat, homemade cold meats, untreated water and non-pasteurized milk, meals in restaurants, hand washing before meals, direct contact with soil, history of blood transfusions.

Serum samples were stored in *eppendorf*® tubes and frozen at -20 ºC until being processed at the Laboratory of Parasitology of the Institute of Biology of the Federal University of Pelotas.

The qualitative and quantitative analyses of IgG antibodies to *T. gondii* in serum samples were carried out through Indirect Immunofluorescence (IFI), with the "kit" WAMA® Diagnóstica[2].

Independent variables were described based on their frequency distributions. Differences in infection rates between groups were analyzed with a Chi-square test (x^2). Logistic regression (univariate and multivariate) models were used to evaluate the association between seropositivity to *T. gondii* and demographic

2 Dilutions were considered reactive from 1:32, according to the manufacturer's instructions.

and epidemiological factors. The data analysis was carried out with the software Statistix v. 9.0.

Ethical aspects: The protocol of this study was designed according to the Directives and Guidelines for Research involving humans (Resolution of the National Health Council # 196/96, 10 October 1996) and was approved by the Committee of Ethics in Research of the School of Medicine of the Federal University of Pelotas (OF. 51/11). The participants were previously informed about the study and those that agreed to participate signed a Term of Informed Consent about the use of the collected material.

RESULTS

Of the 218 samples analyzed, 33% exhibited IgG antibodies to *T. gondii*. The titers ranged from 32 to 64. The most common titer was 32 (98.6%). The seroprevalence of the 108 persons that had contact with pet cats and of the 110 persons that did not like and/or had never owned cats was 39.8% (43) and 26.4% (29), respectively.

Based on the results of the epidemiological survey, most interviewees were female (69.3%), with an average age of 35.5 years (SD 14.4), and secondary or higher education level (90.4%) (Table 1).

Table 1. Profile of residents of Pelotas, Rio Grande do Sul, based on cat ownership from May to December 2011.

VARIABLE	Own		Do not own	
	n	Frequency (%)	n	Frequency (%)
SEX				
Female	76	70.4	75	68.2
Male	32	29.6	35	31.8
AGE RANGE				
15-30 years	57	52.8	51	46.4
≥ 31 years	51	47.2	59	53.6
EDUCATION LEVEL				
Illiterate and Completed Primary Education	11	10.2	10	9.1
Completed Secondary Education	55	50.9	49	44.5
Completed Higher Education	42	38.9	51	46.4

Most interviewees (84.4%) had some knowledge about toxoplasmosis, but only 23.9% mentioned accurate sources of human infection with the protozoan.

Regarding hygiene-sanitary practices with cats, among the 108 persons that owned (or had owned) cats in their homes, 67% owned (or had owned) up to two cats simultaneously and 33%, more then three. In most cases, the cat(s) had access to all rooms of the house (84.3%), were frequently petted, as 98.1% were held in the lap and 69.4% slept in bed with the owners. Cats defecated in litter boxes

(71.3%) or outdoors (66.7%) and the litter boxes were cleaned and feces placed in the trash (67.6%). Only 15.7% of owners disposed of feces in the toilet.

The univariate analysis revealed a significant association ($p \leq 0.25$) between the seropositivity of owners and the lack of use of litter boxes for the animal's feces ($p=0.1645$).

In both groups examined, the univariate analysis of the risk factors associated with the presence of antibodies to *T. gondii* with $p \leq 0.25$ were: age over 31 years, poor hand hygiene before meals, contact with cats, lack of knowledge about toxoplasmosis, blood transfusions, handling of raw meat, female gender, consumption of unpasteurized milk without boiling, and consumption of raw vegetables (Table 2).

Table 2. Frequency of possible risk factors for *Toxoplasma gondii* infection and their relationship with seropositivity among persons with and without contact with cats, after univariate analysis.

Risk factors	Frequency		Seropositivity		P value
	n	%	n	%	
Age ≥ 31 years	110	50.5	51	46.4	0.0000*
Sometimes did not wash hands before meals	20	9.2	1	5.0	0.0052*
Contact with cats	108	49.5	43	39.8	0.0347*
Up to primary education	21	9.6	11	52.4	0.0458*
Unaware of toxoplasmosis	34	15.6	16	47.1	0.0583*
Blood transfusion	17	7.8	9	52.9	0.0690*
Handling of raw meat	151	69.3	45	29.8	0.1284*
Female gender	151	69.3	45	29.8	0.1284*
Consumption of unpasteurized raw milk	36	16.5	15	41.7	0.2277*
Consumption of raw vegetables	162	74.3	57	35.2	0.2493*
Contact with soil without gloves	117	53.7	42	35.9	0.3322
Consumption of raw or undercooked meat	72	33.0	21	29.2	0.3947
Meals in restaurant	75	34.4	22	29.3	0.4010
Unaware of transmission modes	65	29.8	24	36.9	0.4254
Presence of cats in the around the household	141	64.7	49	34.8	0.4639
Tasking seasoning of raw meat	59	27.1	18	30.5	0.6300
Sometimes did not wash fruits and vegetables	21	9.6	6	28.6	0.6479
Knowledge about incorrect transmission modes	133	61.0	44	33.1	0.9827
Consumption of untreated water	70	32.1	23	32.9	0.9707
Consumption of homemade cold meats	72	33.0	22	30.6	0.5858

*$p \leq 0.25$

After the multivariate logistic regression model analysis, the statistically significant variables ($p \leq 0.05$) were: age, contact with cats, handling of raw meat. Persons over 31 years of age were 3.99 times more likely to be infected, and those who had contact with cats were 2.18 times more likely to be seropositive. Persons who handled raw meat were 0.47 times more likely to be infected than those who did not (Table 3).

Table 3. Association ($p \leq 0.05$) of risk factors for *Toxoplasma gondii* infection and seropositivity in persons with and without contact with cats, after multivariate analysis.

Variables	OR (IC = 95%)	P value
Age ≥ 31 years	3.99	0.0000
Contact with cats	2.18	0.0143
Handling of raw meat	0.47	0.0290

*$p \leq 0.05$

DISCUSSION

The prevalence of 33% of IgG antibodies to *T. gondii* from the 218 samples is relatively low, since surveys in Brazil have shown that in adults, prevalence ranges from 50 to 80% (Camargo, 1996; Dubey et al., 2012).

The low prevalence observed in the present study might be associated with the education level of participants, as 90.4% had secondary or higher education, which possibly improved general prevention measures against exposure to the forms of toxoplasmosis transmission. The seroprevalence observed in this survey is similar to another study conducted in Campo Grande, Mato Grosso do Sul, where 39% (39/100) of college students were seroreactive to *T. gondii* (Figueiredo et al., 2010). In Porto Alegre – RS, education level (nine or more school years) of pregnant women tested had a protective effect against toxoplasmosis, supporting the hypothesis that higher education levels decreases the exposure to the disease (Varella et al., 2003). In Londrina, Paraná, pregnant women of secondary education level were at the highest risk of *T. gondii* infection (Lopes et al., 2009), as well as in Goiânia, Goiás, where women of childbearing age with lower education levels and low income were more likely to become infected (Avelino et al., 2004). In China, illiterate pregnant women were at a higher risk of *T. gondii* infection (Liu et al., 2009). Lower education level than secondary school was also considered a risk factor for infection in studies conducted in Pelotas – RS with HIV-positive patients (Xavier et al., 2013) and blood donors (Loges et al., 2012). The same was reported in Taubaté, São Paulo, in a study with pregnant women of low socioeconomic status (Kawasaki et al., 2006).

On the other hand, most interviewees (84.4%) stated they were aware of toxoplasmosis, but only 23.9% mentioned the actual sources of human infection with the protozoan. The lack of knowledge about toxoplasmosis and especially its transmission routes (participants cited for example cat urine, dog feces, contact with animals, etc), observed in the present study has also been reported in other groups of the local population (Cademartori et al., 2008; Loges et al., 2012; Xavier et al., 2013). In a study conducted in Cuiabá, Mato Grosso, 78.1% of pregnant women were unaware of toxoplasmosis (Leão et al., 2004). These results indicate the need to educate the population about the disease, especially food handling practices.

The low titer levels recorded in the present study (98.6% with titer of 1:32) are similar to results found in a previously study conducted in this municipality, in which 1:64 was the most frequent titer observed (46.5%), followed by 1:32 (41%) (Xavier et al., 2013). Low titers could suggest chronic infection (Camargo, 1996).

Regarding the age of participants, persons of 31 years and older were significantly correlated to seropositivity, supporting most seroepidemiological surveys in different groups of the general population (Amendoeira et al., 2003; Varella et al., 2003; Spalding et al., 2005; Cademartori et al., 2008; Figueiredo et al., 2010; Loges et al., 2012; Torgerson; Mastroiacovo, 2013). According to the literature, the older the individuals, the more exposure to sources of *T. gondii* infection, and consequently, the higher the risk of infection (Jamra, 1964; Sánchez et al., 1989; Frenkel, 1995).

Results in this research regarding the handling of raw meat demonstrated significant risk of infection, as it facilitates the contamination of hands and kitchen utensils, in addition to other food items, by cysts in meat during food preparation. This supports the importance of properly handling raw meat, adopting useful preventative measures, such as the use of soap and water to wash hands and utensils after use (Kapperud et al., 1996; Silva et al., 2014).

Similar results were observed by other authors: in São Paulo, comparing the frequency of seropositive persons based on occupation (jobs), homemakers and housemaids had higher seropositivity levels, suggesting that the manipulation of meat and the habit of tasting raw meat during the preparation of meals are risk factors (Jamra, 1964). The manipulation of pig carcasses and viscera by slaughterhouse workers put them at higher risk of *T. gondii* infection (Ishizuka, 1978). A study conducted in Pato Branco – PR revealed that the high prevalence in slaughterhouse workers is correlated to poor hygiene while handling cow's meat (mainly for eviscerators and inspectors) (Daguer et al., 2004). In studies conducted in Palmas – PR with workers from a cold storage facility for pork, the authors concluded that the manipulation of carcasses may have contributed to the transmission of the parasite (Millar et al., 2007).

In the present study, people who owned cats were 2.18 times more susceptible to the protozoan parasite, thus contact with cats was significantly associated with *T. gondii* seropositivity. This might be explained by the hygiene-sanitary practices that owners have with their animals. Of the 108 people who owned (or had owned) cats in their homes, a significant association between seropositivity and free access to the outdoors by cats was found, as 84.3% (91/108) of cat owners allowed them to go outside. This may facilitate hunting of small prey, making animals more susceptible to infection with the parasite and consequently causing contamination with oocytes of this habitat, whereas the humid climate characteristic of the study area influenced survival of oocysts in the environment for long periods of heat (Lucas et al., 1999; Moura et al., 2007; Robert-Gangneux; Dardé, 2012).

The results are similar to those found in Guadalajara – Jalisco (Mexico), where the seropositivity found in cat owners (64% - 38/59) was higher than the seroprevalence in the population of the state (38%) (Ramírez et al., 1999). In Armenia - Quindío (Colombia), contact with 6-month old or younger kittens was a significant risk factor (p=0.01), but with undefined OR and CI 95% (López-Castillo et al., 2005). A study conducted in Norway revealed that cleaning the litter box and contact with cat's feces increased the risk of *T. gondii* infection (OR=5.5 and p=0.02) (Kapperud et al., 1996).

In Brazil, Londrina – PR, the authors reported that the presence of cats in the homes of pregnant women was significantly associated (p=0.004) with the risk of *T. gondii* infection (Lopes et al., 2009); as well as in RS, it was observed that contact with cats influenced the prevalence of toxoplasmosis in pregnant women who live in urban areas (proportion of etiologic impact of 5.1%) (Spalding et al., 2005); in Campo Grande – MS, of the 39% (39/100) college students who were IgG reactive for toxoplasmosis, 72.97% declared they owned or had owned cats in their homes, while of the seronegative participants, only 50% reported they owned or had owned cats (Figueiredo et al., 2010); in Minas Gerais, the presence of cats in the neighborhood (OR=2.27), ownership or visiting homes with domestic cats (OR=1.90), represented the most likely route to congenital toxoplasmosis (Carellos et al., 2014); in Fortaleza, Ceará, 256 of the 995 participants mentioned having contact with cats, and of these 60% were seropositive, against 51% of those with no contact (p=0.01) (Rey; Ramalho, 1999).

Contrary to these data, other studies such as those conducted in Pelotas – Rio Grande do Sul (Cademartori et al., 2008; Loges et al., 2012; Xavier et al., 2013), Porto Alegre – RS (Blos, 2009), Recife – PE (Pôrto et al., 2008), Mérida Yucatán – Mexico (Jiménez-Coello et al., 2011), United States (Dubey, 2000; Elmore et al., 2010), Serbia (Bobić et al., 2003) and in six large European cities (Cook et al., 2000), reported that contact with cats did not pose a risk of infection. These controversial results may be associated with cat feeding and hygiene practices. In our study, risk was higher to owners of cats with access to the outdoors (higher risk of infection), and those who did not use or clean the litter box daily.

From the results obtained in this study, the population needs to be informed regarding the main forms of contamination and prevention of toxoplasmosis. Regarding the risk of having cats as pets, the results reported by different studies are uncertain. In the present study, the seroprevalence of cat owners was higher than in those who did not own cats. However, further studies are needed to increase the sample size (cat owners and non-cat owners) and overall different sociocultural levels. It was also clear from the results that it is possible reduce the risk of infection by keeping cats exclusively indoors with adequate hygiene-sanitary practices, such as daily cleaning of the litter box, removing feces, and feeding the animal with cat food, avoiding raw meats.

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