Risk factors for ocular toxoplasmosis in Brazil

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SUMMARY

The aim of this study was to investigate risk factors for ocular toxoplasmosis (OT) in patients who received medical attention at a public health service. Three hundred and forty-nine consecutive patients, treated in the Outpatient Eye Clinic of Hospital de Base, São José do Rio Preto, São Paulo state, Brazil, were enrolled in this study. After an eye examination, enzyme-linked immunosorbent assay (ELISA) was used to determine anti-*Toxoplasma gondii* antibodies. The results showed that 25·5% of the patients were seronegative and 74·5% were seropositive for IgG anti-*T. gondii* antibodies; of these 27·3% had OT and 72·7% had other ocular diseases (OOD). The presence of cats or dogs [odds ratio (OR) 2·22, 95% confidence interval (CI) 1·24–3·98, $P = 0·009$] and consumption of raw or undercooked meat (OR 1·77, 95% CI 1·05–2·98, $P = 0·03$) were associated with infection but not with the development of OT. Age (OT 48·2 ± 21·2 years *vs*. OOD: 69·5 ± 14·7 years, $P < 0·0001$) and the low level of schooling/literacy (OT *vs*. OOD: OR 0·414, 95% CI 0·2231–0·7692, $P = 0·007$) were associated with OT. The presence of dogs and cats as well as eating raw/undercooked meat increases the risk of infection, but is not associated with the development of OT.

Key words: Eye infection, ocular toxoplasmosis, risk factors, *Toxoplasma gondii*, toxoplasmic retinochoroiditis, toxoplasmosis.

INTRODUCTION

Toxoplasmosis, a disease resulting from infection by the protozoan, *Toxoplasma gondii*, can be congenital or acquired [1]. *T. gondii* is a versatile parasite for which the prevalence of infection varies between countries, geographical areas and ethnic groups living within a specific region [2]. In Brazil, infection rates range from 50% to 83% of the population depending on the location of the study [3]; the high prevalence worldwide is correlated to environmental risk factors and socioeconomic factors [3, 4].

There are several transmission routes with a large proportion of acquired infections related to the

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ingestion of cysts in raw or undercooked meat and contact with sporulated oocysts in the environment [3, 5].

Manifestations of infection by *T. gondii* are often subclinical, i.e. asymptomatic, or with non-specific symptoms [6]. Ocular toxoplasmosis (OT), associated with both congenital and acquired infections, is one of the most common manifestations of the disease [3, 7]. OT is the main cause of posterior uveitis, a disease that can cause serious sequelae including complete loss of vision [8].

The prevalence of OT varies in different regions around the world; in Brazil about 30% of patients with eye diseases have OT [3, 9, 10]. Several studies have investigated risk factors for congenital toxoplasmosis [11, 12]; however, few studies have evaluated factors related to the occurrence, severity and recurrence of OT.

The aim of this study was to investigate risk factors contributing to infection by *T. gondii* and to the development of OT in patients who received medical attention in an outpatient eye clinic of a tertiary teaching hospital of a public health service.

MATERIALS AND METHODS

Patient selection

Over a 2-year period (2009–2010), 349 consecutive male and female patients treated in the Outpatient Eye Clinic of Hospital de Base, Fundação Faculdade Regional de Medicina (HB-FUNFARME), a public health service in São José do Rio Preto, São Paulo state, Brazil were enrolled in this study. Each participant signed an informed consent form after receiving details about the objectives of the study and the procedures involved. This study was approved by the Research Ethics Committee of the Medical School in São José do Rio Preto, Brazil (FAMERP; no. 050/2009 dated 9 March 2009).

Epidemiological data

Participants completed a questionnaire on epidemiological data related to *T. gondii* infection, including social, environmental and economic factors and housing.

Diagnosis of OT

The clinical evaluation of patients was conducted by two experienced physicians (F.B.F. and G.C.A.Jr.),

using an indirect binocular ophthalmoscope (Binocular Ophthalmoscope ID10, Topcon Corporation, USA). Subsequently, patients were allocated to either the OT group or the other ocular diseases (OOD) group.

Blood sampling

Serum, obtained for each patient from a peripheral blood sample collected without anticoagulant by venepuncture, was stored at −20°C until use.

Identification of IgG class anti-*T. gondii* antibodies

After an eye examination, IgG class anti-*T. gondii* antibodies were investigated by enzyme-linked immunosorbent assay (ELISA) as described previously [13].

Statistical analysis

The results were analysed using GraphPad version 3.1 software (GraphPad Software Inc., USA). Fisher's exact test, odds ratio (OR) and χ^2 test were used to compare independence between proportions, and ages were compared using Student's t test. Differences were considered statistically significant at $P \leqslant 0.05$.

RESULTS

The mean age of the 349 patients in this study was $56 \cdot 9 \pm 17 \cdot 0$ years (median 61, minimum 18, maximum 88 years); 187 (53·6%) were male and 16 (46·4%) were female. Ninety-eight percent ($n = 342$) resided in São Paulo state; 15·5% ($n = 53$) in São José do Rio Preto and 84·5% ($n = 289$) in neighbouring towns. Four patients (1·1%) resided in the state of Minas Gerais and three (0·9%) in the state of Mato Grosso do Sul. Based on the data, 73·4% ($n = 256$) owned a house and only 0·3% ($n = 1$) lived in wooden shacks.

Table 1 lists the socioeconomic parameters and environmental risk factors of patients who were seronegative and seropositive for IgG anti-*T. gondii* antibodies.

Table 2 shows the socioeconomic parameters and environmental risk factors of seropositive patients with OT and with OOD.

DISCUSSION

This study investigated the risk factors for *T. gondii* infection and for the development of OT. Several

Table 1. *Socioeconomic parameters and environmental risk factors of patients who are seropositive or seronegative for IgG anti-*Toxoplasma gondii *antibodies in an outpatient eye clinic, São José do Rio Preto, São Paulo state, Brazil**

	Seropositive		Seronegative				
	n	%	*n*	%	OR	95% CI	*P* value
Total (*n* = 349)	260	74·5	89	25·5			
Gender							
Female	125	48·1	37	41·6	1·301	0·7997–2·117	0·325
Male	135	51·9	52	58·4			
Mean age (years)	57·1		56·4				0·721
(s.d.)	17·5		15·3				
Median (range)	61 (18–88)		58 (18–87)				
Years at school							
0–7	203	78·1	67	75·3	1·169	0·6651–2·056	0·660
⩾8	57	21·9	22	24·7			
Family income, minimum salary (Reais, R$)							
⩽1	82	31·5	31	34·8			0·839
1·5–3·5	152	58·5	50	56·2			
⩾4	26	10·0	8	9·0			
Blood transfusion recipient	64	24·6	17	19·1	1·383	0·7595–2·518	0·312
Direct contact with cat or dog	223	85·8	65	73·0	2·225	1·242–3·988	0·009
Work with soil	93	35·8	22	24·7	1·696	0·9840–2·923	0·067
Drink unpasteurized milk	142	54·6	52	58·4	0·8563	0·5260–1·394	0·540
Consumption of raw/undercooked meat	110	42·3	26	29·2	1·777	1·057–2·986	0·032
Does not wash vegetables well	6	2·3	3	3·4	0·6772	0·1657–2·767	0·699
Live in the countryside	18	6·9	8	9·0	0·7531	0·3154–1·798	0·492
Drink tap water	134	51·5	53	59·5	0·7224	0·4433–1·177	0·219
Lack of basic sanitary conditions	26	10·0	11	12·3	0·7879	0·3721–1·668	0·552
Rats, cockroaches or flies in the home	94	36·15	31	34·83	1·059	0·6399–1·754	0·898

OR, Odds ratio; CI, confidence interval.
* Univariate analysis; 95% confidence interval, $P \leqslant 0.05$.

studies have been conducted to ascertain the risk factors contributing to *T. gondii* infection in pregnant women [14–16]; however, there are few studies in outpatient eye clinics that address risk factors, in particular in patients who developed OT.

ELISA was used to detect IgG anti-*T. gondii* antibodies as recommended, as it is frequently used to determine whether an individual has anti-*T. gondii* antibodies [3, 17].

Patients were examined for the presence of ocular lesions by ophthalmoscopy. The high rate of patients infected by *T. gondii* in this study is in agreement with the literature reporting that this percentage can be as high as 80% in Brazil [3], including the region of this study [18–20].

The frequency of OT in seropositive patients demonstrates that eye diseases caused by *T. gondii* are common in individuals with ocular diseases in the study region. Dubey *et al.* report a lower rate of

ocular lesions suggestive of toxoplasmosis than that found in the present study [3]. Thus the results reported here are not consistent with the findings of Dubey *et al.*, probably due to differences in the sample population and the study region.

Indeed, the prevalence of ocular disease caused by *T. gondii* in Brazil varies greatly from 1·1% to 27·3% depending on the region but mainly the study population (Table 3). Most research was conducted as population-based studies, whereas in the current study the patients were selected in a referral eye clinic. Similar rates were reported by Gouveia *et al.* [28] in a study at a centre specializing in uveitis care in São Paulo city.

The parameters of age, gender, schooling and family income were evaluated in relation to the risk of infection and the development of OT. Comparing the two groups, no statistically significant differences were found between women and men.

Table 2. *Socioeconomic parameters and environmental risk factors of patients seropositive for anti-*Toxoplasma gondii *IgG antibodies with ocular toxoplasmosis (OT) or other ocular diseases (OOD), in an outpatient eye clinic, São José do Rio Preto, São Paulo state, Brazil**

	OT		OOD				
	n	%	n	%	OR	95% CI	P value
Total (n = 260)	71	27·3	189	72·7			
Gender							
Female	35	49·3	90	47·6	1·069	0·6195–1·846	0·889
Male	36	50·7	99	52·4			
Mean age	48·2		60·5				<0·001
(s.d.)	21·2		14·7				
Median (range)	50 (18–87)		62 (18–88)				
25th percentile (range)	29 (18–49)		53 (18–62)				
75th percentile (range)	67 (54–87)		72 (62–88)				
Normal distribution	Yes		No				
Years of schooling							
0–7	47	66·2	156	82·5	0·4143	0·2231–0·7692	0·007
≥8	24	33·8	33	17·5			
Family income, minimum salary (Reais, R$)							0·402
≤1	21	29·6	61	32·3			
1·5–3·5	40	56·3	112	59·2			
≥4	10	14·1	16	8·5			
Blood transfusion recipient	17	23·9	47	24·9	0·9511	0·5029–1·799	1·000
Direct contact with cat or dog	57	80·3	164	86·8	0·6206	0·3019–1·276	0·241
Work with soil	30	42·2	63	33·3	1·463	0·8361–2·561	0·194
Drink unpasteurized milk	38	53·5	104	55·0	0·9411	0·5443–1·627	0·889
Consumption of raw/undercooked meat	34	47·9	76	40·2	1·366	0·7890–2·366	0·324
Does not wash vegetables well	1	1·4	5	2·6	0·5257	0·06032–4·582	1·000
Live in the countryside	6	8·4	12	6·3	1·362	0·4907–3·778	0·586
Drink tap water	37	52·1	97	51·3	1·032	0·5978–1·782	1·000
Lack of basic sanitary conditions	10	14·1	16	8·5	1·773	0·7633–4·116	0·244
Rats, cockroaches or flies in the home	27	38·0	67	35·4	1·117	0·6354–1·965	0·772

OR, Odds ratio; CI, confidence interval.

* Univariate analysis; 95% confidence interval, $P \leqslant 0.05$.

Aleixo et al. [27] found an association between ocular lesions suggestive of toxoplasmosis and women, while Jones et al. [29] reported a higher prevalence of OT in men, possibly due to greater contact with soil on farms and rural settings. These data suggest that the prevalence of OT by gender may be dependent on environmental and biological risk factors that were not present in our population.

No association was found between *T. gondii* infection and the mean age or the socioeconomic conditions, but the difference in mean age was statistically significant between seropositive patients with OT and those with OOD. Patients with OT had a lower mean age than those with OOD; OT can affect individuals at any stage of life, with 70–90% of cases being due to congenital infections and 2–30% due to infections acquired after birth [30].

Additional analysis in OT patients showed lower values for the 25th percentile regarding age compared to those with OOD. This observation is consistent with previous reports and suggests that the high incidence of eye disease in congenital toxoplasmosis in Brazil causes patients to seek ophthalmology consultations early [3, 17, 23, 31, 32].

These findings could provide some background for public policies towards maternal fetal and neonatal screening in São Paulo state and postnatal monitoring for the early identification and characterization of toxoplasmosis in children, thereby avoiding later complications and delays in the

Table 3. *Prevalence of ocular disease caused by* Toxoplasma gondii *in Brazil**

Year of sampling	Age group (yr)	Source of patients	Municipality (state)	Positive for T. gondii antibodies (%)	No. of patients	OT (%)	Reference
1990	All ages	Population-based household survey	Erechim (Rio Grande do Sul)	74·8	1042	17·7	[21]
1996–1997	5–78	Residents in rural area	Jaguapitã (Paraná)	66	345	2·6	[22]
1998	All ages	Population-based household survey	Venda Nova do Imigrante (Espírito Santo)	Not stated	1074	11·7	[23]
1999	All ages	Rural area	Governador Valadares (Minas Gerais)	49·5	414	7·0	[24]
1998–2000	All ages	Urban slums	Campos dos Goytacazes (Rio de Janeiro)	90	110	8	[25]
		Rural area, poor community			104	14	
2001	5–21	Students	Natal (Rio Grande do Norte)	46	959	1·1	[26]
2004	All ages	Rural	Santa Rita de Cássia, Barra Mansa (Rio de Janeiro)	65·9	1071	3·8	[27]
2002	4–88	Uveitis outpatient clinic	São Paulo city (São Paulo)	Not stated	262	22	[28]
2009–2010	18–80	Outpatient eye clinic	São José do Rio Preto (São Paulo)	74·5	349	27·3	This study

* Adapted from Table Supplement S3 of Dubey et al. [3], with permission.

characterization of OT in children, adolescents and young adults.

In a study conducted in the High-Risk Pregnancy Clinic of Hospital de Base in São José do Rio Preto, the rate of congenital transmission in the region proved to be 2·3% [33] and the acute infection rate in 556 pregnant women was 3·4% [18].

However, there is no government programme of maternal fetal and neonatal screening in the São Paulo state nor is there postnatal monitoring for the early identification and characterization of toxoplasmosis in children, which complicates and delays the characterization of OT in children, adolescents and young adults.

There was no association between seropositive and seronegative patients and the level of schooling. A lower percentage of OT was observed in those stating that they were illiterate or had few years of schooling. This finding seems paradoxical since less schooling implies greater exposure to risk due to not adopting appropriate measures, such as good hygiene practice, to prevent infection [34]. It would be expected that individuals with a higher level of schooling would have a lower risk of developing OT. It is possible that individuals with better schooling go to eye clinics more frequently and therefore the diagnosis in this group is higher.

The association observed in this study between infection with T. gondii and direct contact with cats and dogs has been reported previously [12, 35]. This observation is plausible because the cat is the definitive host of the parasite and thus eliminates oocysts in stools, which after maturation, contaminate the environment [36]. It is believed that consumption of canine meat may contribute to the spread of the parasite in humans, as has been demonstrated in other diseases [37]; however, Brazilians do not have the habit of eating canine meat. However, there is the possibility of contamination due to oocysts in dog fur, as well explained by Frenkel & Parker [38].

It was noted in the current study that infection is strongly associated with the consumption of raw or undercooked meat, a habit believed to be the main infection route of T. gondii. An association was also found in this study, albeit insignificant, between T. gondii infection and regular contact with soil. Direct contact with earth/soil suggests that infection may also occur in this way [11, 39]; and industrialized processed meat and poor hygiene habits also contribute to infection with T. gondii [40–42].

No association was found with other factors evaluated and *T. gondii* infection. No statistically significant differences were observed when environmental risk factors associated with *T. gondii* infection were analysed in relation to the development of OT. As the risk factors for infection are not associated with OT, it is possible that the risk of developing OT is dependent on the pathogenicity of the infecting strain and/or the immune competence of the host coupled with immunogenetic factors that determine susceptibility. However, these factors are inherent in patients and the parasite requires further study.

In conclusion, the results of this study confirm that the presence of dogs and cats, as well as the consumption of raw or undercooked meat increases the risk of infection, but does not influence the development of OT.

DECLARATION OF INTEREST

None.

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