A seroepidemiological survey of the frequency of *Toxoplasma gondii* in corneal donors from Volta Redonda eye bank*

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ABSTRACT. Seabra M.G., Aleixo A.L.Q. do C., Pereira P.F., Pinheiro J. & Amendoeira M.R.R. **A seroepidemiological survey of the frequency of *Toxoplasma gondii* in corneal donors from Volta Redonda eye bank.** [Levantamento soroepidemiológico da frequência de *Toxoplasma gondii* em doadores de córnea do Banco de Olhos de Volta Redonda, RJ, Brasil.] *Revista Brasileira de Medicina Veterinária, 38(supl. 3):188-194, 2016.* Programa de Pós-graduação em Ciências Veterinárias, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR 465, km 7, *Campus* Seropédica 23897-970, Seropédica, RJ, Brazil. E-mail: jairopinheirodasilva@gmail.com

Toxoplasma gondii is a protozoan parasite that infects up to a third of the world's population. Infection is mainly acquired by ingestion of food or water that is contaminated with oocysts shed by cats or by eating undercooked or raw meat containing tissue cysts and by blood transfusion or organ transplantation. Primary infection is usually subclinical but in some patients cervical lymphadenopathy or ocular disease can be present. Infection acquired during pregnancy may cause severe damage to the fetus. In immunocompromised patients, reactivation of latent disease can cause life-threatening encephalitis. Diagnosis of toxoplasmosis can be established by direct detection of the parasite or by serological techniques. The aim of the present study was verify the seroprevalence of toxoplasmosis in cornea donors of Rio de Janeiro, Brazil. IgM and IgG anti-*T. gondii* antibodies were investigated in 426 sera of corneal donors by using the indirect fluorescent antibody test (IFAT) and immunoenzymatic assay (ELISA) techniques. The participants were selected by convenience sampling. Demographic information of study subjects including their gender, age, cause of death and home region were recorded. Out of 426 serum samples, 338 (79.34%) and 17 (3.99%) were positive regarding anti-*T. gondii* IgG and IgM antibodies ELISA and/or IFAT, respectively. These data demonstrate the importance of regional and national seroepidemiological inquiries to define public health strategies that can revert and reduce the serological toxoplasmosis prevalence in Brazil.

KEY WORDS. Cornea donors, *Toxoplasma gondii*, IgG, IgM, epidemiology.

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RESUMO. *Toxoplasma gondii* é um parasito protozoário que infecta até um terço da população mundial. A infecção é adquirida principalmente pela ingestão de alimentos ou água que é contaminada com oocistos eliminados por gatos infectados ou por comer carne crua ou mal cozida contendo cistos de tecido e por transfusão de sangue ou transplante de órgãos. O diagnóstico de toxoplasmose pode ser estabelecido pela detecção direta do parasito ou por técnicas sorológicas. O objetivo do presente estudo foi verificar a soroprevalência da toxoplasmose em doadores de córnea do Rio de Janeiro, através de material obtido do Banco de Olhos de Volta Redonda, RJ, Brasil. Os anticorpos IgM e IgG anti-*T. gondii* foram investigados em 426 soros de dadores de córnea, utilizando o teste de anticorpos fluorescentes indiretos (IFAT) e técnicas de ensaio imunoenzimático (ELISA). Os participantes foram selecionados por amostragem de conveniência. As informações demográficas dos sujeitos do estudo, incluindo seu sexo, idade, causa de morte e região de origem, foram registradas. Das 426 amostras de soro, 338 (79,34%) e 17 (3,99%) foram positivas em relação ao anti-*T. gondii* IgG e IgM ELISA e/ou IFAT, respectivamente. Esses dados demonstram a importância dos inquéritos soroepidemiológicos regionais e nacionais para definir estratégias de saúde pública que possam reverter e reduzir a prevalência de toxoplasmose sorológica no Brasil.

PALAVRAS-CHAVE. Doação de córnea, *Toxoplasma gondii*, IgG, IgM, epidemiologia.

INTRODUCTION

Toxoplasma gondii, the causative agent of toxoplasmosis is a parasitic protozoan which infects a wide range of warm-blooded vertebrates.

The human infections are prevalent in many countries around the world, with an average prevalence varying between 16 - 80% (Hill & Dubey 2005). In children, the prevalence is relatively low, increasing with age and with more exposure to risk factors during the course of life. In Brazil, the seroprevalence of *T. gondii* infection is high, around 20 and 97% of the general population (Dubey et al. 2012).

Human infection is mainly developed by either oral ingestion of water and foods contaminated with parasite oocysts excreted in cat feces as final host, or eating raw and undercooked meat of intermediate hosts containing tissue cysts. Moreover, the infection can be transmitted through placenta, milk, organ transplantation, and blood transfusion (Montoya et al. 2009, Asgari et al. 2011). Thus, the sources of infection by *T. gondii* are varied and widespread. Maternal infection leads to the risk of a transplacental passage of *Toxoplasma* parasites to the fetus and may cause congenital toxoplasmosis, resulting potentially in abortion or fetal death (Amendoeira & Camillo-Coura 2010). Severe symptoms, such as hydrocephalus, microcephaly or encephalitis, or sequelae, such as visual impairment, intracranial calcifications, or mental retardation, can be observed in affected infants (Daguer et al. 2004). The severity and percentage of infection depend on the date of contamination during pregnancy. It is crucial to determine the immunological status of the pregnant woman. Although in most newborns the infection is asymptomatic, these children when deprived of treatment may develop late symptoms, with recurrent ocular toxoplasmosis, which can lead to blindness and neurological problems in childhood and adolescence, including schizophrenia, bipolar disorder, depression and suicide attempts (Hill & Dubey 2005).

In immunocompetent individuals, this parasitic disease is asymptomatic in its majority; however retinitis toxoplasmosis is often a cause of a serious ocular disease in healthy adults. While in immunocompromised individuals, infection can be severe or even lead to death when associated with reactivation of cysts in cases of congenital and acquired infection (Luft & Remington 1992, Robert-Gangneux & Dardè 2012).

The factors that control the occurrence, severity and recurrence of ocular toxoplasmosis are not well understood. Although a variety of components, including genetic susceptibility of the host, nutritional status, immune system, parasitic load and the parasite genotype has been suggested as possibly involved in the development of the infection.

The routine diagnosis is based on the immune response of patients by serological tests to detect antibodies specific anti-*T. gondii*. Knowledge of the prevalence of infection in different population groups, as well as the risk factors that may be operating in the region, is of paramount importance so that we can implement prevention.

The study aimed to determine the occurrence of anti-*T. gondii* antibodies, IgG and IgM, by ELISA and IFAT in human serum, from cornea donors of Pedro Sélmo Thiesen Eye Tissue Bank, Volta Redonda, and Rio de Janeiro, Brazil.

MATERIAL AND METHODS

Study area and Sampling

A descriptive study with a convenience sample was carried out among 426 corneal donors, according to the

number of eyes captured between February 2013 and April 2015, The study was conducted at Pedro Sélmo Thiesen (Volta Redonda Eye Bank) eye tissue bank opened in St. John the Baptist Hospital (HSJB), 130 km from the City of Rio de Janeiro.

Identification of potential donors was done through passive reporting and active searching. Every deceased patient aged 2 - 80 years was a potential donor of eye tissue for transplantation up to 6 hours after cardiac arrest, or 24 hours if the entire body was kept in a cold room; brain death was not necessary. The donor's eyelids should be kept closed to prevent the corneas from drying due to light exposure. The maximum time for extracorporeal preservation of corneas was 14 days. Notification of potential donors is required by law and should be reported to the Organ Notification, Collection and Distribution Unit (CNCDO) and the eye bank operating in the region.

All the 426 donors' families filled out a free and informed consent statement. Serum samples were collected from cornea donors by Volta Redonda eye bank medical staff.

Serum was submitted to IgM and IgG anti-*T. gondii* analysis by immunoenzymatic assay - ELISA (BIOLISA Toxoplasmose®) commercial kit (Bioclin, Belo Horizonte) and indirect fluorescent antibody test (IFAT) performed at the Laboratory of Toxoplasmosis and others protozoosis – LabTOXOP, Oswaldo Cruz Institute - IOC/ Fiocruz.

Results from ELISA were obtained by comparison with a cut-off value measured at 450nm absorbance, according to the manufacture´s recommendations. In the IFAT, the samples were serially diluted 1:16 to 1: 4096 and was used anti-Human IgG (Y-chain specific) FITC, antibody produced in goat and IgG conjugate, and anti-Human IgG (μ-chain specific) FITC, antibody produced in goat (Sigma-Aldrich). The samples were observed under an immunofluorescent microscopy (Nikon-Labophot-2, objective E PLAN 40× magnification and ocular lens CFWE 10xA/18). It was performed Latex agglutination in the IgM reagents sera for the detection of rheumatoid factor (Imuno-Látex FR, Wama Diagnostica).

Information such as age, gender, cause of death and home region were collected.

Statistical analysis

Associations between gender, age group and locality were determined by means of the chi-square test using R software (R Core Team, 2014). The level of significance was fixed at 0.05. The copositivity and conegativity rates and the concordance of the tests were determined as described by Teva et al. (2009). The Kappa index was used to measure the real agreement between the serological techniques.

Ethical approval

This study was approved by the Research Ethics Committee (CEP) from Instituto Oswaldo Cruz/Fiocruz with protocol # 11201212.3.0000.5248.

RESULTS

The Kappa value for concordance between the two techniques for detection of IgG against *T. gondii* was 0.68, which reflects substantial agreement between the two techniques, as described by Teva et al. (2009). Furthermore, for detection of IgM against *T. gondii*, the *Kappa* was 0.14 which is considered to be a fair level of agreement.

In the data analysis, samples that were reactive in the IFAT and/or ELISA test were taken into consideration, thus totaling 338 (79.34%) and 17 (3.99%) samples that were reactive to IgG and IgM anti- *T. gondii*, respectively After performing the serological tests ELISA and IFAT to analyze the frequency of IgM antibodies anti- *T. gondii*, it was found that 23 donors were seropositive. Because of this outcome was performed latex agglutination test for the presence of rheumatoid factor and with that, 6 individuals had rheumatoid factor. So, 17 individuals were considered IgM seropositive and that they would be in their prime infection.

Among the 262 men, 212 (80.91%) were IgG seropositive and 12 (4.58%) were IgM seropositive. And 126 (76.82%) women donors were IgG seropositive and 5 (3.04%) were IgM seropositive. The Chi-squared test did not show a significant difference in anti-*T. gondii* IgG and IgM prevalence between men and women (p=0.310; p=0.432, respectively). (Table 1 and Table 2)

The average donors' age was 52.6 ± 16.96 years (10-80 years). The age group that obtained the highest number of cornea donors was 51 to 60 years, with 106 individuals. A progressive increase in frequency of seropositivity was observed with the age group 11 to 20 years, and the age group 71 to 80 had the highest prevalence in IgG anti-*T. gondii* (90.16%). While the 61-70 age group had the majority cases of IgM anti-*T. gondii* (13.11%), as shown in figure 1. Statistical differences were observed in IgG and IgM anti-*T. gondii* prevalence between age groups (Tables 1 and 2).

The main causes of death were cerebral vascular accident (34.5%), followed by traumatic brain injury (30.6%), cancer (27.2%) and other causes (7.7%). Distribution observed in the present study was similar in other studies (Adán et al. 2008, Flegr 2002), with a high prevalence of external causes.

Donors were from 37 Municipalities of the State of Rio de Janeiro as Angra dos Reis, Araruama, Barra Mansa, Belford Roxo, Búzios, Casimiro de Abreu, Campos dos Goytacazes, Duque de Caxias, Guapimirim, Itaboraí, Itaguaí, Itatiaia, Japeri, Macaé, Magé, Maricá, Mesquita, Miguel Pereira, Ni-

Table 1. Results of distribution of ELISA and/or IFAT for anti-*Toxoplasma gondii* antibodies of the IgG class in serum of corneal donors from different regions in the State of Rio de Janeiro.

Variables	ELISA and/or IFAT IgG						p Value	χ^2
	Non reactive		Reactive		Total			
	n	%	n	%	n	%		
Gender							0.310	1.02
Female	38	23.17	126	76.82	164	100		
Male	50	19.01	212	80.91	262	100		
Total	88	20.60	338	79.34	426	100		
Age Groups							<0.001	42.17
0-10	1	100	0	0	1	100		
11-20	14	50	14	50	28	100		
21-30	13	40.62	19	59.37	32	100		
31-40	4	11.76	30	88.23	34	100		
41-50	7	10.76	58	89.23	65	100		
51-60	26	24.52	80	75.47	106	100		
61-70	17	17	82	82.82	99	100		
71-80	6	9.84	55	90.16	61	100		
Total	88	20.60	338	79.34	426	100		
Regions							0.198	8.58
Baixadas Litoraneas	2	40	3	60	5	100		
Center South Fluminense	0	0	3	100	3	100		
Green Coast	3	50	3	50	6	100		
Middle Paraíba	17	16.83	84	83.16	101	100		
Metropolitan	66	21.86	236	78.14	302	100		
North Fluminense	0	0	2	100	2	100		
Montain	0	0	7	100	7	100		
Total	88	20.60	338	79.34	426	100		

IgG: immunoglobulin G; p: p value; χ^2: chi-square test, n: number of samples.

Table 2. Results of distribution of ELISA and/or IFAT for anti-*Toxoplasma gondii* antibodies of the IgM class in serum of corneal donors from different regions of the State of Rio de Janeiro.

Variables	ELISA and/or IFAT IgM						p Value	χ^2
	Non reactive		Reactive		Total			
	n	%	n	%	n	%		
Gender							0.432	0.617
Female	159	96.95	5	3.04	164	100		
Male	250	95.42	12	4.58	262	100		
Total	409	96.01	17	3.99	426	100		
Age groups							0.013	17.623
0-10	1	100	0	0	1	100		
11-20	28	100	0	0	28	100		
21-30	32	100	0	0	32	100		
31-40	33	97.06	1	2.94	34	100		
41-50	62	95.38	3	4.61	65	100		
51-60	104	98.11	2	1.88	106	100		
61-70	96	96.97	3	3.03	99	100		
71-80	53	86.89	8	13.11	61	100		
Total	409	96.01	17	3.99	426	100		
Regions							0.976	10.39
Baixadas Litorâneas	5	100	0	0	5	100		
Center South Fluminense	3	100	0	0	3	100		
Green Coast	6	100	0	0	6	100		
Middle Paraíba	96	95.05	5	4.95	101	100		
Metropolitan	290	96.03	12	3.97	302	100		
North Fluminense	2	100	0	0	2	100		
Montain	7	100	0	0	7	100		
Total	409	96.01	17	3.99	426	100		

IgM: immunoglobulin M; p: p value; χ^2: chi-square test, n: number of samples.

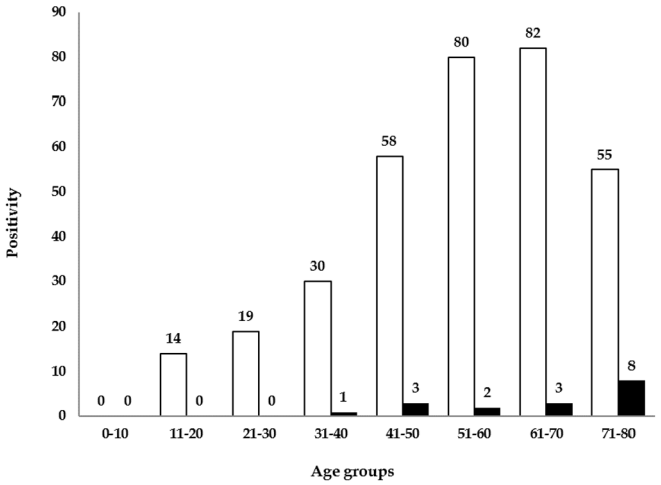

Figure 1. Positivity in ELISA and/or IFAT for anti-*Toxoplasma gondii* antibodies of the IgG and IgM classes, in serum of corneal donors according to their age (☐ IgG reactive; ■ IgM reactive).

lópolis, Niterói, Nova Iguaçu, Petrópolis, Pinheiral, Piraí, Queimados, Resende, Rio Claro, Rio de Janeiro, São Gonçalo, São João de Meriti, São José do Vale do Rio Preto, Silva Jardim, Tanguá, Três Rios, Valença, Vassouras and Volta Redonda. The regions of the State of Rio de Janeiro, Metropolitan and the Middle Paraiba focused the majority of donors (70.90% and 23.71%), respectively. Municipalities that had the highest number of donors and prevalence anti-*T.gondii* IgG were: Duque de Caxias (20.42%), Rio de Janeiro (20.19%) and Volta Redonda (18.78%). The regions from the State Rio de Janeiro did not show statistical difference in relation to anti- *T. gondii* IgG and IgM prevalence (p = 0.98; p = 0.976 respectively) (Tables 1 and 2).

DISCUSSION

By the first time an epidemiologic study about IgG and IgM anti-*T. gondii* antibodies was carried out among cornea donors in the Rio de Janeiro State, Brazil. The toxoplasmosis prevalence was confirmed by the positivity of IgG and IgM anti-*T. gondii* antibodies 79.34% and 3.99%, respectively.

The study showed a relatively high prevalence of primary *T. gondii* infection in cornea donors as a potential source for infection transmission.

In a study with blood donors in Iran, 34.04% and 1.17% were positive regarding anti-*T. gondii* IgG and IgM antibodies, respectively (Zainodini et al. 2014). A prevalence of 75% was found among the blood donors of HEMOPE (Fundação de Hematologia e Hemoterapia de Pernambuco), a Brazilian reference center in hematology and hemotherapy, localized in the State of Pernambuco Coelho et al. 2003). In a study with blood donors of five blood

banks in Natal, Rio Grande do Norte, Brazil the seroprevalence of *T. gondii* antibodies was 82.5% (Araujo et al. 1975).

The Chi-squared test did not show a significant difference in anti-*T. gondii* IgG and IgM prevalence between men and women. This is corroborated by findings of other authors (Horio et al. 2001, Daguer et al. 2004, Millar et al. 2007). Gender alone could not be considered a factor of resistance or susceptibility factor for this parasitosis (Sobral et al. 2005), only when associated with cultural habits differentiated by gender, such as in Indian tribes (Sobral et al. 2005, Amendoeira et al. 2003). A progressive increase in frequency of seropositivity was observed with the age group 11 to 20 years. This fact was expected because *T. gondii* has a complex life cycle that includes several risk factors (Araujo et al. 1975, Horio et al. 2001), and there is an increasing chance of contact with possible transmission mechanisms for toxoplasmosis with age. This fact was also observed by other researchers (Garcia et al. 1999, Souza et al. 1987, Silveira 2002, Spalding et al. 2005), including specific populations such as Indian tribes (Dubey 1998) and pregnant women (Spalding et al. 2005).

The metropolitan area had the most donors, and it is related that 74% of the population is concentrate in this region of the State Rio de Janeiro and also consists in the social pressure in space marked by great contradictions, because, often, economic growth does not go along with the basic needs of the population. These issues can be diagnosed in space from serious problems such as the unequal distribution of urban services and equipment; the growing demand for housing, marked by increased number of houses under poverty conditions and the expansion of slums; the intense environmental degradation and the consequent depletion of natural resources and public insecurity.

The Middle Paraíba region was the second area with corneal donors (78.14%), and this region is, after the Metropolitan, the most industrialized in the State of Rio de Janeiro. The industrialization of the region creates a number of problems, with the consequent loss of the population's quality of life, portrayed in expanding submoradias and under-served neighborhoods, as well as air and the Paraíba do Sul River pollution. The water supply can also be a source of infection by *T. gondii,* acting as a disseminator for oocysts, and contamination of municipal water tanks by the feces of infected cats can lead to outbreaks or epidemics (Silveira 2002, Bahia-Oliveira et al. 2003).

It is important to note that, apart from industry, agriculture also plays a prominent role in the Middle Paraíba. The region is one of the largest dairy belt in the State of Rio de Janeiro. Often this activity is practiced in traditional ways, with weak integration into the agricultural modernization process, with little coordinated with the big industrial capital, commercial and financial. Milk has been considered to be a potential vehicle for spreading toxoplasmosis in humans, since experimental work has shown that milk from infected animals contains tachyzoites and that this milk is capable of transmitting the infection to these animals' offspring. The habit of drinking raw (unpasteurized or untreated) milk may be a risk factor for infection with *T. gondii* during pregnancy. In the Municipality of Niteroí was found that 14.1% of the seropositive women had the habit of consuming untreated cow's or/and goat's milk (Moura et al. 2013). In Europe, 6 to 17% of pregnant women in different countries have been found to consume untreated milk or its derivatives (Qublan et al. 2001). In the State of Goiás, Brazil, a study showed that 18.6% of the pregnant women consumed unpasteurized goats' milk (Avelino et al. 2004). In Rio Grande do Sul State, 8.7% of the pregnant women reported consuming untreated milk (Cademartori et al. 2008). Aleixo et al. (2009) obtained seroprevalence of IgG anti-*T. gondii* of 65.9% inhabitants from the neighborhood of Santa Rita de Cássia, Barra Mansa in the Middle Region of Rio de Janeiro, showed that the *T. gondii* infection varied according to the region and the population studied due to environmental and socioeconomic factors.

In a recent multicenter retrospective study including 22 patients with acquired toxoplasmosis within a median time of 92 days post transplantation, mismatched transplants were documented for 9 patients and the donor's serology was unknown for 8 other negative recipients (Fernandez-Sabé et al. 2012). Twelve of 22 cases were heart transplant patients. The incidence of donor-acquired toxoplasmosis is less frequent in other patients, and only 9 and 16 cases were reported for liver- and kidney--mismatched patients, respectively, supported by solid serologic evidence. A case of disseminated toxoplasmosis following small bowel transplantation was also described, but the serostatus of the donor was unknown, making the source of infection uncertain (Campbell et al. 2006).

Chorioretinitis has also been reported following transplantation and may be the result of reactivation of latent infection in the host or disseminated

infection in a seronegative recipient. Toxoplasmosis occurred in 0.97% of 4,231 allogeneic transplants, most likely due to the use of trimethoprim sulfamethaxazole prophylaxis after engraftment in these patients in the majority of institutions (Martino et al. 2000).

CONCLUSION

Toxoplasmosis remains one of the most severe opportunistic infections occurring after transplantation, with a high mortality rate in cases of delayed diagnosis. It should be stressed that at least 75% of the cases occur in patients who have not received prophylaxis, emphasizing the need to make available updated information concerning the pathogenesis of toxoplasmosis, the procedures for identification of risk factors and the measures for prevention. The combination of serological screening of donors and recipients, chemoprophylaxis in patients susceptible to reactivation or exposed to organ transmission of *T. gondii* and Absolute prevention of the transmission of donor-derived infections in organ transplantation is not possible. However, improvements in screening technologies will enhance the safety of transplantation in the future.

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