doi: 10.5216/rpt.v47i4.55976

ORIGINAL ARTICLE

EVALUATION OF TOXOPLASMOSIS RISK FACTORS AND SEROPREVALENCE RATES IN THE ELDERLY

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ABSTRACT

Toxoplasmosis is an infection caused by the protozoan *Toxoplasma gondii*, whose definitive hosts are cats and whose intermediate hosts include humans. This research aims to evaluate aspects related to seroprevalence rates of anti-*T. gondii* antibodies among elderly users of the Primary Health Care Service in the municipality of Aparecida de Goiânia, State of Goiás, Brazil. A total of 101 elderly people participated in the study, and a questionnaire was applied to examine sociodemographic factors related to risk factors for toxoplasmosis. Five mL of peripheral blood were collected from all the participants for serological tests. IgM and IgG antibody screening was performed using ELISA. The average age of the participants was 69.5 years, the seroprevalence rate was 75.2% (76/101) of reactive IgG, and 24.8% (25/101) were non-reactive. IgM and IgG antibodies were found in 2.9% (3/101) of the participants. An IgG avidity test was performed, which revealed that the antibodies in the three samples were of low avidity, thus indicating that these individuals were in the acute phase of the infection. It was suggested that, in terms of socioeconomic conditions, an income of one to three monthly minimum salaries is the predominant risk factor for toxoplasmosis in this group.

KEY WORDS: Primary health care; epidemiology; toxoplasmosis; elderly.

INTRODUCTION

Population aging is a worldwide phenomenon that has been occurring in the vast majority of countries around the world. In developing countries, this phenomenon has occurred more rapidly and is currently one of the greatest public health challenges (Camarano et al., 2013).

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Received for publication: 23/11/2018. Reviewed: 21/1/2019. Accepted: 24/1/2019.

Aging is a varied and complex process with biological, sociodemographic, psychological and even cultural factors. This phase of life presents a greater burden of diseases, disabilities and increased use of health services (Veras, 2009).

Toxoplasmosis is an infection caused by the protozoan *Toxoplasma gondii*, whose definitive hosts are cats and whose intermediate hosts include humans (Tender et al., 2000). Humans are infected by ingesting water and/or food contaminated with oocysts, and poorly cooked meat containing cysts, as well as through blood transfusions, organ transplants and congenital transmission. After the initial (acute) phase, the parasite enters the latent stage creating cysts with bradyzoites and is found predominantly in the nerve and muscle tissues of the infected host (Lebech et al., 1999; Schmidt et al., 2006). Toxoplasmosis is diagnosed through the detection of anti-*T. gondii* antibodies in serum or other body fluids. The presence of IgG antibodies characterizes the chronic phase of the infection; however, the detection of IgM antibodies is suggestive of acute infection. In many cases, patients have positive IgM, but are already in the chronic phase of infection. Therefore the IgG avidity test is required (Vaz, 2007).

T. gondii infection is significant in immunocompromised individuals. Therefore, senior citizens should undergo more frequent antibody screening tests for the diagnosis of toxoplasmosis, given that the disease may be more severe in the elderly (Engroff et al., 2014). The development of ocular toxoplasmosis during acute infection is more frequent among the elderly, probably due to the decline of cellular immunity in these individuals (Ronday et al., 1995; Montoya & Remington, 1996; Johnson et al., 1997).

T. gondii seroprevalence rates in Brazil and around the world vary greatly and depend on several factors that affect the epidemiology of this infection. Brazilian researchers suggest that elderly people are more likely to become infected by cysts in meat and meat products or by ingesting water contamined by oocysts (Engroff et al., 2014).

This research was carried out because there is a lack of information about toxoplasmosis prevalence on elderly people in Goiás State and it aims to evaluate aspects related to seroprevalence rates of anti-*T. gondii* antibodies among elderly users of the Primary Health Care Service in the municipality of Aparecida de Goiânia, State of Goiás, Brazil.

MATERIAL AND METHODS

This is a cross-sectional study, in which the participants were elderly people linked to two Basic Health Units (BHU) in the municipality of Aparecida de Goiânia, GO, Brazil. All the elderly patients that were present at the BHU on the days when visits for research project data collections were scheduled were invited to participate in the study.

The participants were selected randomly, and the criterion for inclusion was being aged 60 years or older, i.e., people under 60 years of age were excluded. The elderly who agreed to participate voluntarily signed an Informed Consent Form (ICF) after reading the document carefully and hearing an explanation of the objectives of the study.

The data were collected through interviews based on a questionnaire regarding information about the participants' eating, behavioral and social habits. Subsequently, 5 mL of venous blood were drawn from each participant into a tube containing gel without anticoagulant, to obtain the serum. Serological tests were performed in the Laboratory for Host-Parasite Relationship Studies at the Institute of Tropical Pathology and Public Health of the Federal University of Goiás in Goiânia, GO, Brazil.

IgG and IgM antibody tests were carried out using the ELISA. All the tests were performed with the SERION ELISA Classic® kit, as recommended by the manufacturer. The results were qualitative, expressed as reactive and non-reactive, for both IgG and IgM. Three samples were subjected to an IgG avidity test, using a Brazilian commercial kit, *Imunotoxo Bioclin-Quibasa*®, with a few modifications (Rahbari et al., 2012).

Statistical analyses were performed using the BioEstat® version 5.1 program to evaluate the prevalence rate of chronically and acutely infected individuals based on descriptive and exploratory analysis. Risk factors considered independent variables, were compared with the seroprevalence rates (reactive or non-reactive test). The odds ratio (OR) between the seroprevalence and the variables was analyzed, applying a 95% confidence interval (95% CI) and a 5% level of significance ($p < 0.05$).

To comply with the ethical and legal aspects of human research studies recommended by Resolution No. 466/12 of the National Health Council (*Ministério da Saúde*, 2013), the research was submitted to the Research Ethics Committee of the University of Rio Verde, and was approved under Opinion No. 1,121.012.

Upon conclusion of the analysis, the participants received a printed copy of their results.

RESULTS

A total of 101 elderly people participated in the study. Seventy-six (75.2%) of the participants, averaging 69.5 years of age, were found to be positive/reactive IgG. Positive IgG is a sign of previous infection by the parasite. In addition, 25 (24.8%) participants, with average age of 65.7 years, were negative IgG. In the cases of IgG (positive) and IgM (positive), an analysis of the biological sample (serum) revealed that three participants had antibody markers for the acute phase of toxoplasmosis. Based on this information, the three samples underwent an IgG avidity test, resulting in low avidity antibodies.

The analysis of socioeconomic and demographic characteristics revealed that the three IgM reactive participants, two women and one man, averaged 68 years old. All three were retired, married, living with their spouses in their own homes, and their income was one to three minimum monthly salaries. They all had a kitchen garden, and no cats or dogs, but all of them stated that such animals often appeared in their backyards.

In this work we highlight the importance of diagnosing three individuals with serology compatible with the acute phase of the infection, since at the moment of the collection all were assymptomatic. They reported no information regarding the forms of infection, making this much more difficult for that population to take the correct measures for prevention of toxoplasmosis.

The seroprevalence rate of 3% reactive IgG and IgM detected in the participants of this study was important, given that active infection in the elderly should be taken seriously since the disease may have a poor prognosis due to their weaker immune system. The lack of information in the studied population regarding toxoplasmosis was evident at the time of the interview with the elderly, suggesting that this contributes significantly to such a high percentage of reactive IgG.

Table 1. Evaluation of socioeconomic and demographic factors related to IgG seroprevalence in 101 elderly people in Aparecida de Goiânia, GO, Brazil.

Socioeconomic and demographic factors in 101 elderly people	N (positive IgG /total)	Percentage %	OR	CI 95%	p value
Marital status					
Married	31/44	73	0.9	0.4-2.1	0.961
Single	19/21	90	1.7	0.5-5.7	0.511
Divorced	13/16	81	1.5	0.4-5.8	0.771
Widowed	13/20	65	0.5	0.1-1.5	0.370
Total	76/101				
Educational level					
Elementary school, complete	45/60	76	1.0	0.5-2.5	0.850
Middle school, complete	10/12	83	1.7	0.4-8.5	0.737
Higher education, complete	1/5	20	0.07	0.01-0.6	0.016
Illiterate	20/24	83	1.9	0.6-6.1	0.435

Family income					
< 1 MS*	3/8	37	0.1	0.03-0.7	0.031
1-3 MS	71/89	79	5.5	1.5-19.4	0.011
≥4 MS	2/4	0.3	0.04-2.3	0.546	
Total	76/101				
Current occupation (field)					
Retired	63/82	78	2.0	0.7-6.0	0.289
Shopkeeper	2/4	50	0.3	0.1-2.3	0.546
Cleaner	7/11	63	0.5	0.1-1.9	0.565
Handcraft activities	3/3	100	*	*	*
Security	1/1	100	*	*	*
Occupation exercised during earlier life (field)					
Housewife	3/3	100	*	*	*
Administration	3/6	50	0.3	0.05-1.6	0.322
Health	4/5	80	1.3	0.1-12.5	0.780
Cleaning	25/35	74	0.9	0.3-2.3	0.936
Education	2/3	67	0.6	0.05-7.4	0.741
Customer service	4/8	50	0.3	0.06-1.2	0.194
Industry	9/11	81	1.5	0.3-7.6	0.869
Rural activities	12/15	80	1.4	0.3-5.3	0.890
Security	4/4	100	*	*	*
Shop keeping	6/7	85	2.0	0.2-17.9	0.832
Construction	4/4	100	*	*	*
No. of people living at home					
Lives alone	45/59	76	1.1	0.5-2.8	0.961
2 people	25/34	73	0.9	0.5-2.5	0.827
> 3 people	6/8	75	0.9	0.2-5.2	0.681
Type of House					
Own	60/81	74	0.7	0.2-2.3	0.794
Rented	4/6	66	0.6	0.1-3.7	0.988
Relatives	5/7	71	0.8	0.1-4.4	0.832
Loaned	7/7	100	*	*	*

Note: MS* = Minimum Salary

All the risk factors to which the elderly were exposed in their daily lives were listed and analyzed based on a questionnaire. A statistical analysis of the individuals with IgG reactive serology indicated that the most significant risk factor was family income. No other factors were statistically significant, as indicated in Table 1. An analysis of the occurrence of seroprevalence of IgG antibodies in 101 elderly individuals related to daily habits did not reveal statistical significance either (Table 2). No association was found between seroprevalence and food preparation in this study (Table 3).

Table 2. Analysis of the occurrence of IgG seroprevalence in 101 elderly people from the standpoint of sanitary conditions and living with animals

Sanitary Conditions and Behavior	N (positive IgG/total)	Percentage %	OR	CI 95%	p value
Source of water					
Treated (public water supply)	67/90	74.5	0.6	0.1-3.2	0.869
Untreated (other sources)	9/11	81.8	1.5	0.3-7.6	
Sewage Disposal System					
Public sewage disposal	3/5	60	0.4	0.1-3.0	0.780
Septic tank	73/96	76.1	2.1	0.3-13.4	
Contact with sand or soil	34/50	68	0.4	0.1-1.1	0.149
Swimming or fishing habits	19/23	82.6	0.5	0.1-1.8	0.511
Owns cats	11/15	73.3	0.8	0.2-3.0	0.890
Eats raw meat	3/3	100	*	*	*
Number of cats					
1 cat	5/7	57.1	0.8	*	0.667
2 cats	3/4	75	1.1	*	0.567
≥ 3 cats	3/4	75			
Cats up to one year old	3/4	75	1.1	*	0.567
Cat roams around the neighborhood	5/5	100	2.6	*	0.846
Other cats appear in the backyard/garden	44/59	74.6	0.9	0.3-2.3	0.961
Owns a dog	42/55	76.4	1.1	0.4-2.8	0.958
Dog roams around the neighborhood	5/7	71.4	0.7	0.1-4.3	0.883

Table 3. Analysis of positive IgG results in 101 elderly people informed about toxoplasmosis, from the standpoint of their eating habits and behaviors

Eating and Behavioral Habits	N (positive IgG /total)	Percentage %	OR	CI 95%	p value
Has a kitchen garden	23/33	69.7	0.6	0.2-1.6	0.512
Prepares food					
Always	54/73	74	0.7	0.2-2.2	0.824
Never	13/17	76.5	1.0	0.3-3.6	0.857
Occasionally	9/11	81.8	1.5	0.3-7.1	0.869
Eats meat	75/100	75	3.1	0.1-51.8	0.993
Undercooked or raw meat	11/13	84.6	2.1	0.4-10.5	0.512
Eats raw kibbeh	6/11	54.5	0.3	0.09-1.2	0.188
Eats undercooked barbecued meat	10/15	66,7	0.6	0.1-2.0	0.627
After using cutting board to cut meat, uses it to cut vegetables					
Washes cutting board with soap and water	74/97	76.3	3.2	0.4-24.1	0.546
Does not wash board	2/4	50	0.3	0.04-2.3	
Eats fruit					
Frequently	55/68	80.9	2.4	0.9-6.1	0.101
Occasionally	20/32	62.5	0.3	0.1-0.9	0.076
Does not eat fruit	1/1	100	*	*	*
Eats leafy vegetables and legumes					
Frequently	39/55	70.9	0.6	0.2-1.5	0.382
Occasionally	28/35	80	1.5	0.5-4.0	0.573
Does not eat vegetables	9/11	81.8	1.5	0.3-7.6	0.869
Drinks raw milk	23/31	74.2	0.9	0.3-2.4	0.931
Does not boil milk	21/29	72.4	2.6	0.1-47.1	0.896
Eats fresh white cheese	56/74	75.7	1.1	0.4-2.9	0.924

Eats homemade sausages	44/56	78.6	1.5	0.6-3.6	0.527
Has had worms	34/41	82.9	2.0	0.7-5.5	0.213
Has had tapeworms	13/16	81.3	1.5	0.4-5.8	0.771
Is knowledgeable about toxoplasmosis					
Yes	25/38	65.8	0.4	0.1-1.1	0.140
No	51/63	81	2.2	0.8-5.5	

DISCUSSION

It has been estimated that the seroprevalence rate of toxoplasmosis in the adult human population (not necessarily elderly) in Brazil is 50% to 80% (Bonfioli & Orefice, 2009). One of the few studies in the literature involving the elderly population was carried out in Panama, where 90% seroprevalence was found (Jones et al., 2001). According to a study by Engroff et al. (2014), the elderly served by Brazil's Family Health Strategy service in Porto Alegre, state of Rio Grande do Sul (RS), presented a correlation between lower levels of schooling and lower incomes and a higher seroprevalence of IgM and IgG for *Toxoplasma*. The aforementioned authors found an 88% seroprevalence rate in the population in their study. The seroprevalence rate of 75.2% detected in this study is consistent with the abovementioned data, confirming the high seroprevalence rate in the elderly population. The individuals whose monthly income was one to three minimum salaries presented IgG anti-*T. gondii*. Other studies have also associated low levels of schooling and low income with the prevalence of toxoplasmosis (Jones et al., 2001; Engroff et al., 2014).

The seroprevalence of *T. gondii* is higher in elderly people because, over the years, they are more likely to have been exposed to environments in which the parasite may be present (Studenicõva et al., 2006; Strhársky et al., 2009).

Owning a pet cat did not prove to be a risk factor in a survey carried out in Porto Alegre (Engroff et al., 2014), on the other hand this association has been found in other studies (Figueiredo et al., 2010; Chiang et al., 2012). However, the elderly who reported preparing their food presented seroprevalence for anti-*Toxoplasma gondii* IgM (Engroff et al., 2014).

Another significant factor reported by other researchers is the lack of care regarding the consumption of raw foods that may be contaminated by oocysts (Bahia-Oliveira et al., 2003; Bonfioli & Orefice, 2009; Amendoeira & Camilo-Moura, 2010; Figueiredo et al., 2010; Branco et al., 2012; Chiang et

al., 2012;). A water tank in the residence may be a protection factor, because fewer individuals living in houses with water tanks tested reactive for *T. gondii* (Araújo et al., 2011). Nevertheless this was not evidenced in the present study.

It was suggested that a monthly income of one to three minimum salaries posed the predominant risk factor for acquiring toxoplasmosis in the analyzed group. Seroprevalence rates are considered low among elderly people in the acute phase of the infection living in the metropolitan region of Goiânia, GO, Brazil when compared to those reported in other states of the country. The occurrence of new cases, even with a low percentage, is of great relevance since the transmission of toxoplasmosis is occurring in the region studied. It is known that the longer the person lives, the more likely they are to be in contact with one of the transmission mechanisms of toxoplasmosis. However, the seroprevalence of anti-*T. gondii* antibodies that are markers of the chronic phase of the infection remain significant, demonstrating that this protozoan is present in the state of Goiás.

Thus, overall monitoring of senior citizens' health is essential, given the great concern in preserving the well-being of this population to ensure they have a healthy and dignified old age.

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