Brief Report



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# Environmental Risk Factors Associated with Retinochoroiditis Caused by *Toxoplasma gondii*

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**Abstract:** To evaluate which risk factors, contribute directly or indirectly to *Toxoplasma gondii* infection in patients with ocular toxoplasmosis. Between 2018-2020, a total of 306 patients were selected, and treated at the Retinology Outpatient Clinic of the Specialty Hospital of São José do Rio Preto, in the state of São Paulo. After the fundoscopy test, the Enzyme Linked Fluorescent Assay (ELFA) was used to determine IgG and IgM class anti-*Toxoplasma gondii* antibodies. The results showed that individuals aged 60 years or older, and women who had a previous pregnancy, ($p$=0.000, OR= 8.137 and 95 % CI = 2.562 – 25.844), ($p$=0.016, OR= 7.395 and CI 95 % = 1,449 – 37,725) respectively, were 48.6 % more likely to develop ocular toxoplasmosis. In this way, previous pregnancy is a risk factor for the reactivation of toxoplasma, as well as for the generation of retinochoroiditis in previously infected women.

**Keywords:** eye infection; ocular toxoplasmosis; risk factors; toxoplasmosis; retinochoroiditis; *Toxoplasma gondii*

## Introduction

Toxoplasmosis is caused by the protozoan *Toxoplasma gondii*, which was first described in 1908 by Nicolle and Manceaux.[1] It is an obligate intracellular parasite, with worldwide distribution and capable of infecting warm-blooded animals, including mammals and birds.[2]

Its life cycle is heteroxenous, i.e., it occurs both sexually and asexually, with felines as definitive hosts, where sexual reproduction takes place, and vertebrates as intermediate hosts, where asexual reproduction occurs.[3,4]

Both animals and humans become infected from the consumption of raw or undercooked meat containing *T. gondii* cysts, by ingestion of food and water contaminated with oocysts of the parasite, or the transmission can be transplacental from mother to child during pregnancy.[5–8] There are reports of other transmission routes such as accidental inoculation, blood transfusion, and organ transplantation.[6,9,10]

Toxoplasmosis is asymptomatic in 80 % of cases due to the efficiency of the immune system, which is able to limit the spread of multiplying tachyzoites.[11] However, in immunocompromised patients (such as HIV+ and transplant recipients), the cysts can rupture, releasing bradyzoites, which can seriously compromise health status.[12–15]

Ocular toxoplasmosis (OT) is an infection caused by toxoplasma and is the most common cause of posterior uveitis that variety is directly related to the immune system of each individual. [16,17]

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Lesions caused by the parasite usually heal in two to four months in immunocompetent patients and a study reveals that in about 70 % of cases of patients who seek an ophthalmologist, the lesions resulting from OT that heal are associated with other lesions. [17,18] The study carried out by our research group revealed that OT represents 27 % of eye diseases affecting patients in the northwest region of the state of São Paulo, Brazil.[19]

Infection by the parasite is recognized as the most common zoonotic disease in the world, due to its worldwide distribution and the large number of intermediate hosts. Studies indicate that 25 to 30 % of the world population is infected by *T. gondii*. However, infection rates can vary significantly between and within countries, depending on socioeconomic, environmental, and cultural conditions such as living, eating, and housing habits.[1,7] The aim of this study was to evaluate which risk factors contribute directly or indirectly to *T. gondii* infection in patients with ocular toxoplasmosis.

## Methods

Between the years 2018-2020, a total of 306 patients with positive and negative serology for toxoplasmosis were selected. These individuals were divided into four groups as follows: G1 = 53 patients with active lesion resulting from toxoplasmosis and positive serology; G2 = 29 patients who presented ocular scarring due to toxoplasmosis and positive serology; G3 = 135 patients without lesion or scar resulting from toxoplasmosis and with positive serology; and G4 = 89 patients without lesion or scar resulting from toxoplasmosis and with non-reactive serology. All individuals recruited in this study received care at the Retinology Outpatient Clinic of the Specialty Hospital of São José do Rio Preto – SP, where they underwent clinical evaluation by fundoscopy.

Two skilled doctors evaluated patients clinically, using the previously mentioned indirect binocular ophthalmoscope (Binocular Ophthalmoscope ID10, Topcon Corporation, USA).[19,20] All patients underwent thorough eye exams that included measuring intraocular pressure using Goldmann applanation tonometry, measuring visual acuity using the logMAR Early Treatment Diabetic Retinopathy Study [ETDRS] chart with the best correction in accordance with ETDRS standards, performing biomicroscopy with a slit lamp, and performing stereoscopic biomicroscopy using a 78-diopter lens (Volk) and classifying the results in accordance with ETDRS.[21]

Patients who agreed to participate in the study signed the Free and Informed Consent Form (FICT) and after medical evaluation of these individuals, peripheral blood collection was performed to identify IgG and IgM anti-*Toxoplasma gondii* antibodies, using the ELFA method (Enzyme Linked Fluorescent Assay), according to the manufacturer's instructions (Biomeriux-France) and also answered a questionnaire with basic epidemiological data such as gender, age, eating habits (consumption of raw or undercooked meat, unpasteurized milk, adequate food hygiene); housing conditions (area in which they live, frequent presence of mechanical vectors and domestic animals); socioeconomic conditions; and history of blood transfusion.

*Statistical analysis:*

The collected data were analyzed using the Statistical Package For Social Sciences (SPSS, IBM, version 24.0), GraphPad Instat 3.10 (2009), and Prisma 6.07 (2015) statistical programs.

Descriptive statistical analysis was performed from the calculations of measures of central tendency, dispersion, and frequency counts. In univariate analysis, frequency comparisons were performed using Pearson's Chi-square test. Multivariate analysis of Binary Logistic Regression was used to verify the predictor variables of the outcome (Ocular Toxoplasmosis). Variables that presented P values ≤ 0.20 in the bivariate analysis were included in the regression model, which used the Backward Stepwise selection method (Likelihood Ratio).

In all analyses, a $p$ value ≤ 0.05 was considered statistically significant.

## Results

Binary logistic regression analysis was performed using the Backward Stepwise method (Likelihood Ratio), and only variables that presented $p \leq 0.20$ in the univariate analysis were included

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in the model (Table 1). The percentage of correctness of the model was 70.1 %, with Nagelkerke's $R^2$ of 48.6 % and Hosmer and Lemeshow's Test with chi-square ($X^2$) = 4.403, gl = 4, and $p$=0.3540. These values indicate that the model constructed has a high probability of being correct (70.1%) and a moderate association of the variables that remained in the model at the outcome (Nagelkerke's $R^2$ of 48.6 %).

The variables that remained in the model and presented $p \leq 0.05$ were: age ≥ 60 years ($p$=0.000, OR= 8.137 and 95 % CI = 2.562 – 25.844) and previous pregnancy ($p$=0.016, OR= 7.395 and 95 % CI = 1.449 – 37.725). Based on the OR (Odds Ratio) values it was possible to verify the existence of a significant association between these variables present in the regression model, and the occurrence of ocular toxoplasmosis in the samples evaluated in the present study (Table 1).

**Table 1.** Risk factors associated with *Toxoplasma gondii* infection in ocular toxoplasmosis patients by univariate logistic regression analysis, Sao Jose do Rio Preto, Sao Paulo state, 2022.

| Variables | | G1 | | G2 | | G3 | | G4 | | Univariate |
|---|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | N | % | p- value |
| Age | ≥60 years | 7 | 13.2 | 6 | 20.7 | 104 | 77 | 21 | 23.6 | p = 0.000 |
| | <60 years | 46 | 86.7 | 23 | 79.3 | 31 | 23 | 68 | 76.4 | |
| Gender | Male | 36 | 67,9 | 18 | 62 | 77 | 57 | 53 | 59,5 | p= 0.894 |
| | Female | 17 | 32,1 | 11 | 38 | 58 | 43 | 36 | 40,5 | |
| Blood transfusion | Yes | 12 | 22.6 | 2 | 6.9 | 17 | 12.6 | 6 | 6.7 | p = 0.000 |
| | No | 41 | 77.3 | 27 | 93.1 | 118 | 87.4 | 83 | 93.3 | |
| Domestic animal | Yes | 45 | 84.9 | 22 | 75.8 | 86 | 63.7 | 69 | 77.5 | p = 0.118 |
| | No | 8 | 15.1 | 7 | 24.2 | 49 | 36.3 | 20 | 22.5 | |
| Have a cat at home | Yes | 15 | 28.3 | 5 | 17.2 | 33 | 24.4 | 20 | 22.5 | p = 0.716 |
| | No | 38 | 71.7 | 24 | 82.8 | 102 | 75.6 | 69 | 77.5 | |
| Go barefoot | Yes | 20 | 37.7 | 15 | 51.7 | 44 | 32.6 | 39 | 43.8 | p = 0.192 |
| | No | 33 | 62.3 | 14 | 48.3 | 91 | 67.4 | 50 | 56.2 | |
| Drink unpasteurized milk | Yes | 30 | 56.6 | 10 | 34.4 | 73 | 54 | 26 | 29.2 | p = 0.000 |
| | No | 23 | 43.4 | 19 | 65.6 | 62 | 46 | 63 | 70.8 | |
| Eat raw or undercooked beef | Yes | 26 | 49 | 6 | 20.7 | 38 | 28.1 | 23 | 25.8 | p = 0.268 |
| | No | 27 | 51 | 23 | 79.3 | 97 | 71.9 | 66 | 74.2 | |
| Eat raw or undercooked pork | Yes | 5 | 9.4 | 2 | 6.9 | 12 | 8.9 | 2 | 2.2 | p = 0.041 |
| | No | 48 | 90.6 | 27 | 93.1 | 123 | 91.1 | 87 | 97.3 | |
| Wash the food | Yes | 51 | 96.2 | 27 | 93.1 | 132 | 97.8 | 83 | 93.3 | p = 0.181 |
| | No | 2 | 3.8 | 2 | 6.9 | 3 | 2.2 | 6 | 6.7 | |
| Had a previous pregnancy | Yes | 13 | 24.5 | 10 | 34.4 | 54 | 40 | 22 | 24.7 | p = 0.000 |
| | No | 40 | 75.5 | 19 | 65.6 | 81 | 60 | 67 | 75.3 | |
| Had a premature child | Yes | 5 | 9.4 | 3 | 10.3 | 8 | 6 | 5 | 5.6 | p = 0.479 |
| | No | 48 | 90.6 | 26 | 89.7 | 127 | 94 | 84 | 94.4 | |
| Had an abortion | Yes | 5 | 9.4 | 1 | 3.4 | 24 | 17.8 | 4 | 4.5 | p = 0.055 |

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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | 48 | 90.6 | 28 | 96.6 | 111 | 82.2 | 85 | 95.5 | |
| **Housing area** | Rural | 4 | 7.5 | 3 | 10.3 | 14 | 10.4 | 4 | 4.5 | p = 0.098 |
| | Urban | 49 | 92.5 | 26 | 89.7 | 121 | 89.6 | 85 | 95.5 | |
| **Type of housing** | Masonry | 47 | 88.6 | 29 | 100 | 132 | 97.8 | 87 | 97.3 | p = 0.285 |
| | Wood | 6 | 11.4 | 0 | 0 | 3 | 2.2 | 2 | 2.2 | |
| **Own home** | Yes | 35 | 66 | 20 | 69 | 105 | 77.8 | 65 | 73 | p = 0.900 |
| | No | 18 | 34 | 9 | 31 | 30 | 22.2 | 24 | 27 | |
| **Has a sewer network** | Yes | 51 | 96.2 | 25 | 86.2 | 124 | 91.9 | 87 | 97.3 | p = 0.066 |
| | No | 2 | 3.8 | 4 | 13.8 | 11 | 8.1 | 2 | 2.2 | |
| **Consume artesian well water** | Yes | 8 | 15 | 2 | 6.9 | 14 | 10.4 | 1 | 1.1 | p = 0.004 |
| | No | 45 | 85 | 27 | 93.1 | 121 | 89.6 | 88 | 98.9 | |
| **Consume tap water** | Yes | 19 | 35.8 | 12 | 41.4 | 34 | 25.2 | 20 | 22.5 | p = 0.184 |
| | No | 34 | 64.2 | 17 | 58.6 | 101 | 74.8 | 69 | 77.5 | |
| **Consume filtered water** | Yes | 23 | 43.4 | 15 | 51.7 | 86 | 63.7 | 68 | 76.4 | p = 0.002 |
| | No | 30 | 56.6 | 14 | 48.3 | 49 | 36.3 | 21 | 23.6 | |
| **Garbage destination** | Burnt | 1 | 1.8 | 1 | 3.4 | 4 | 3 | 3 | 3.4 | p = 0.240 |
| | Public collection | 52 | 98.2 | 28 | 96.6 | 131 | 97 | 86 | 96.6 | |
| **There are rats where subject lives** | Yes | 7 | 13.2 | 10 | 34.4 | 9 | 6.7 | 8 | 9 | p = 0.449 |
| | No | 46 | 86.8 | 19 | 65.6 | 126 | 93.3 | 81 | 91 | |

## Discussion

This study analyzed environmental risk factors for retinochoroiditis caused by *T. gondii*. The results of our analysis showed that individuals aged 60 or over had a risk factor for toxoplasma reactivation ($p$=0.000, OR= 8.137 and 95 % CI = 2.562 – 25.844). Studies show that extremes of age are considered a risk factor for increased severity of clinical manifestations; therefore, older patients appear to be in a more risky situation.[2] In the elderly, several clinical variations of ocular toxoplasmosis are likely to be present, such as atypical necrotizing disease.[22,23] The weakening of the immune system can impair the immune response to such an extent that it can trigger fulminant ocular toxoplasmosis. Possibly, the reduction of the immune response allows the large-scale proliferation of the parasite in the retinal cells, triggering severe lesions in atypical, extensive, or multiple areas of retinochoroiditis.[24] This information cited above is in agreement with our study on risk factors.

The idea that patients aged 60 years or older are more likely to develop toxoplasma retinochoroiditis is supported by Sadighi (2018)·[25] In the elderly, a higher frequency of severe) manifestations related to toxoplasmosis is observed due to changes that occur in the innate and adaptive immune system in their senescent organisms,[25] which is in agreement with our study. When in contact with the organism, the toxoplasma triggers responses from macrophages, lymphocytes, and cytokines, all in association for the complete defense of the infected organism. As elderly individuals have a modified immune system that defense becomes deficient, allowing greater aggression of the parasite in target organs.

The hypothesis found in this study suggests that this increase in people aged 60 years or older is due to the decline of the immune system that occurs over the years, as well as that over the years, the patient is exposed to the parasite for a longer time, and over that time vision impairment occurs.

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Likewise, associated with the results obtained from our study on a higher risk in women with previous pregnancy, the authors Florença Robert-Gangneux and Marie-Laure Dardéc agree in saying that when the primary infection occurs in the first trimester of pregnancy, the chance of the placental barrier protecting the fetus is ninety percent, so the mother remains with the parasitemia in her body, allowing more maternal cells to be affected by the parasite; as the eye is a target organ of toxoplasma, the chance of it being lodged in this site increases.[3] Thus, there is the possibility of re-acceleration of toxoplasmosis in these chronically infected patients, causing retinochoroiditis.[6,22,26] Our study shows that women who had a previous pregnancy are more likely to reactivate the infection ($p$=0.016, OR= 7.395 and 95 % CI = 1.449 – 37.725).

In addition, ocular toxoplasmosis, also called uveitis, is characterized by severe inflammation in the uvea, which can lead to visual impairment and blindness due to eye complications.[20,21] Studies have shown that the microglia present in the retina plays a key role in the onset of uveitis, and the activation of peripheral immune system cells results in apoptosis of retinal cells, promoting tissue inflammation. This indicates that microglia underlie the regulation of retinal inflammation as an immune response.[21] Many studies corroborate that cytokines such as TNF-$\alpha$, IL-1$\beta$, IL-6, and CCL2, and toxic mediators such as NO are directly involved in the regulation of the immune system in ocular toxoplasmosis, some of which act on vascular permeability, recruitment of leukocytes, and retinal inflammation.[27–33]

The reactivation of eye infection caused by *T. gondii* is frequent because it is an immunologically favorable environment [26,29,31,34–36] and pregnancy is a facilitating factor for its reactivation due to the immunotolerance that it confers on the host.[26,37–39] The *T. gondii* parasite, when penetrating the human cell, activates several cytokine genes such as IL-10, IL-1$\beta$, IFN-$\gamma$, which are associated with the development of ocular toxoplasmosis.[3,33,40–42] A study shows that the appearance of eye lesions is a consequence of the susceptibility of the host gene associated with exposure to more virulent strains.[43–45]

The studies by Kalogeropoulos and collaborators[46], as well as by Naranjo-Galvis and collaborators[47], describe the relationship of predisposition to susceptibility to the occurrence of ocular impairment, and indicate that the immune response triggered by genetic control is considerable for the pathogenesis of *T. gondii* retinochoroiditis. The patient's immunological status is decisive for the pathogenesis of ocular toxoplasmosis, as well as the status of the patient's immune system is closely related to the effectiveness of infection control, as its success is closely linked to the correct function of immune system T cells.[3,47–51]

The patients answered a questionnaire with basic epidemiological data such as gender, eating habits (consumption of raw or undercooked meat, unpasteurized milk, adequate food hygiene); housing conditions (area in which they live, frequent presence of mechanical vectors, and domestic animals); socioeconomic conditions; and history of blood transfusion, which did not present a statistically significant difference. Another study carried out by our research group, which evaluated the risk factors for the development of toxoplasmosis in Brazil, revealed that the presence of dogs and puppies and the consumption of raw or undercooked meat are associated with infection caused by the parasite *T. gondii*, but not with the development of ocular toxoplasmosis.[19,52]

Previous pregnancy and age over 60 years confer a risk factor for Toxoplasma reactivation.

**Author Contributions:** CCB: FBF, PMC were responsible for the concept and design of the study. RCS, FBF, GHP, AAACF, MP, GMFJ performed the inclusion of patients with ocular toxoplasmosis, sample collection, and developed the ophthalmological clinical evaluation and clinical analyses. GMFJ, APO, ALG, FHM performed the laboratorial tests. CGFG, GMFJ, LC, LCM, CCB performed the data analysis. CCB, LCM, GMFJ, CGFG, PMC wrote the manuscript. All authors read and approved the final manuscript.

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**Ethical approval:** Written informed consent was obtained from each enrolled patient. This study was approved by the Ethical in Human Research Committee of the Faculty of Medicine of São José do Rio Preto (CAAE: 96386518.2.0000.5415).

**Conflicts of interest:** None declared.

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