doi: 10.5216/rpt.v52i3.73539

ORIGINAL ARTICLE

# SEROLOGICAL AND MOLECULAR DIAGNOSIS OF *Toxoplasma gondii* IN BLOOD AND CEREBROSPINAL FLUID SAMPLES FROM HOSPITALIZED PATIENTS WITH NEUROLOGICAL BRAIN DISORDERS: A CASE SERIES STUDY

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## ABSTRACT

The aim of this study was to perform the laboratory and molecular diagnosis of *Toxoplasma gondii* in patients with neurological brain disorders in the city of Jataí, GO, Brazil as well as highlighting the toxoplasmosis' risk behavior. To achieve this aim, a questionnaire was applied to these patients, and it was associated with the blood for serum and cerebrospinal fluid (CSF) collection. The samples were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) and by Polymerase Chain Reaction (PCR). The results indicated contact with stray cats, fresh vegetables, raw meat and meat products consumption. Seroreactivity for IgG in serum samples was 100% (14/14) and in CSF, 6.7% (1/14). In the molecular diagnosis, the presence of *T. gondii* DNA was not detected. Our data suggests that educational actions on toxoplasmosis prophylaxis are recommended using several tools, such as social media, to raise public awareness about this.

KEY WORDS: Neurological infectious disorders; *T. gondii* serology; *T. gondii* molecular diagnosis; *Toxoplasma gondii*.

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Received for publication: 1/ 8/ 2022. Reviewed: 15/ 12/ 2022. Accepted: 4/ 7/ 2023.

## INTRODUCTION

*Toxoplasma gondii* is an obligate intracellular parasite that causes toxoplasmosis. This disease has a high worldwide serological prevalence, and the estimated global prevalence is 25.7% (95% CI: 25.6–25.8%) (Kovačević et al., 2023). The population sociocultural and socioeconomic factors, such as hygiene and eating habits, in addition to health system access, education, and basic sanitation favor the high prevalence of this parasitosis in people. However, cases that present symptoms in immunocompetent individuals are less frequent (Taila et al., 2011; Robert-Gangneux & Darde, 2012; Lijeskić et al., 2021).

In immunocompromised individuals, especially patients living with HIV/ AIDS condition without antiretroviral therapy, in failure or in poor adherence to it, with low $CD4^+$ T lymphocytes, the immunosuppression may lead to reactivation of the infection or a latent toxoplasmosis. This occurs when the quiescent cysts rupture, releasing bradyzoites that convert into tachyzoites (Abgrall et al., 2001; Coelho et al., 2016). In this sense, the exacerbation of the disease contributes to an immune reactivation and an inflammatory action, mainly affecting the retina and the Central Nervous System (CNS) (Weiss & Kim, 2000; Montoya & Liesenfeld, 2004; Cerutti et al., 2020).

Neurotoxoplasmosis is the main cause of CNS infection among people living with HIV (Coelho et al., 2016). The clinical condition may occur subacutely, lasting from two to three weeks, or even suddenly, accompanied by fever, headache, and ataxia. The most frequent signs and symptoms are sensory changes, hemiparesis or hemihypoesthesia, which may be accompanied by other focal signs of meningeal irritation (convulsions, mental confusion, and cerebrovascular accidents) (Sacktor et al., 2001; San-Andres et al., 2003; Nogui et al., 2009; Mesquita et al., 2010).

Due to the possible clinical forms of toxoplasmosis aggravation, it becomes crucial to conduct an assertive diagnosis as well as the infection prevention. Therefore, this study aims to perform the laboratory and molecular diagnosis of *T. gondii* in patients with neurological brain disorders in the city of Jataí, GO, Brazil as well as highlighting the risk behavior for toxoplasmosis.

## MATERIAL AND METHODS

### *Ethical Aspects and Study Group*

This is a case series study on the identification of toxoplasmosis in patients with suspected neurological brain disorders. This study was approved by the Research Ethics Committee from the Federal University of Jataí (UFJ), under the protocol number 3.440.231.

Patients who were admitted into the Hospital das Clínicas Dr. Serafim de Carvalho (HCSC) in Jataí with a complain of neurological alterations investigated by the HCSC medical team, from August 2019 to October 2020 were included in this study. The patients were invited to take part in the study when a member of the project team explained the goal of the research. The participants have signed an Informed Consent Form (ICF) and were interviewed based on a standardized questionnaire in order to obtain data on general risk factors related to contact and infection by *T. gondii*. No questions about ocular toxoplasmosis were asked for these patients.

Individuals younger than 18 years old who despite having a neurological brain disorder in the hospital care would not take part of this research due to being underage.

*Collection of Biological Samples*

An aliquot of serum (approximately 500 µL) obtained by separation of venous blood and an aliquot of CSF (approximately 200 µL) obtained by puncture of CSF were stored at -20 °C and later it was sent to serological procedures for detection of antibodies against *T. gondii*.

*Antibody Detection*

The ELISA test was performed to detect IgM and IgG antibodies against *T. gondii*, using SERION ELISA® classic *T. gondii* IgG/ IgM brand kit according to the manufacturer instructions. In a 96-well flat-bottomed microwell plates, previously sensitized with *T. gondii* antigens, 100 µL of serum or CSF samples previously treated with RF-absorbent (overnight, refrigerator, 1:100) were added to remove IgM derived from rheumatoid factor that interfere with the assay. In the same way, 100 µL of negative and positive control serum and standard serum, provided by the kit, were added. The plate was incubated at 37 °C (±1 °C) for 60 minutes (± 5 minutes) in a humid chamber. After, the microplates were washed four times with the washing solution. Then, 100 µL of APC conjugate provided by the kit were added (except for the blank well) and the plate was incubated at 37 °C (± 1 °C) for 30 minutes (± 5 minutes) in a humid chamber. Then, the plate was subjected to another washing step, and 100 µL of pNPP substrate solution was added. The plate was incubated again at 37 °C (± 1 °C) for 30 minutes (± 5 minutes) in a humid chamber and then 100 µL of the stop solution was added to stop the reaction and stabilize the color. The reaction reading was performed by using a microplate reader, Thermo Fisher Scientific® at a wavelength of 405 nm with subtraction of the reading obtained in the blank well.

All samples, including the controls standard and blank sera, were tested in duplicates, and the arithmetic mean of the optical density was used as a result criterion. The final readings were sent to the quality control criteria provided by the manufacturer for the conclusion of the reactivity threshold value (cut-off), and the cut-off for ELISA IgM and IgG obtained were 0.900 and 0.150, respectively.

*Molecular diagnosis by polymerase chain reaction (PCR)*

Initially, the extraction of genetic material was performed using the commercial kit BIOPUR®. To start the reaction, 0.5 mM of each deoxynucleotides (dATP/ dTTP/ dGTP/ dCTP, Sigma®), 17.3 µL of sterile Mili-Q $H_2O$, 1.0 µL of $MgCl_2$, 2.5 µL of 10× buffer (Invitrogen®), 0.2 µL of Taq DNA polymerase (Invitrogen®), 50 pmoles of each reaction primer (Invitrogen®) and 2 µL of the extracted DNA were added to the reaction (Rezende, 2019). Then, the combination of these materials was sent to the thermocycler in order to carry out the amplification step for each sample. This material underwent cycles of temperature variation, with a first denaturation at 94 ºC (5 min), 35 cycles of denaturation at 94 ºC (1 min), annealing at 62 ºC (1 min) and extension at 72 ºC (1 min) followed by extension at 72 ºC for 10 minutes (Rezende, 2019). The amplified products were observed by 6% agarose gel electrophoresis and developed with silver (Santos et al., 1993).

## RESULTS

*Socioepidemiological Characterization of the Patients*

A total of 15 blood and CSF samples were obtained from a total of 14 patients. Of these samples, a woman participant presented again to the HC with complaints suggestive of neurological alterations, at an interval of one year. Thus, there was a duplication of the sample in the database of this study coming from the same patient. The participants' clinical data are described in Table 1.

Out of the 14 patients in this study, the majority were female (57.1%), with an age average of 51.6 years old (ranging from 18 to 77 years of age) and the predominant age group between 48 and 77 years of age. Regarding having general knowledge about the disease "toxoplasmosis," the majority (64.3%) reported not knowing what the disease was and 85.7% did not know about the types of transmission (Table 2).

*Table 1.* Clinical aspects of the patients.

| Case | First diagnosis | Comorbidities | Immunosuppressive drugs |
|---|---|---|---|
| 1 | Migraine | No | No |
| 2 | Epilepsy | Diabetes | No |
| 3 | Head trauma | Hypertension | No |
| 4 | Aneurysm | No | Dexamethasone |
| 5 | Brain stroke | No | No |
| 6 | Brain stroke | Hypertension | No |
| 7 | Epilepsy | No | No |
| 8 | Brain stroke | Diabetes | Cyclosporine |
| 9 | Dystonia | Hypertension | No |
| 10 | Brain stroke | Hypertension, diabetes and CRF* | No |
| 11 | Person living with HIV | No | No |
| 12 | Migraine | No | No |
| 13 | Epilepsy | No | No |
| 14 | First diagnosis Migraine | No | No |

*CRF: Chronic Renal Failure

All the patients included in this study, reported having access to basic sanitation. The majority (64.3%) having their sewage being disposed in cesspools, and the garbage disposal from their neighborhood done through the public collection with trucks (92.9%). Only the resident of a rural area reported that burns the waste.

When patients were asked about the presence of cats as pets at home, the majority (78.6%) said they did not have cats at home; however, 72.7% reported that stray cats sometimes appear in their homes´ backyard. When it comes to the 21.4% of patients who reported having cats at home, they declared that their cats eat raw or undercooked meat, and they often go outside and return to their home. Of the 14 patients, the majority (57.14%) did not report handling sand or clay.

Regarding their eating habits and therefore, variables that may be related to the transmission of *T. gondii*, through the ingestion of oocysts or protozoan cysts, data in Table 3 shows that most of them reported eating raw fruits and vegetables, raw or undercooked meat, artisanal sausages and drinking milk without boiling. Half of the patients occasionally eat in restaurants. And on hygiene aspects, 64.3% reported that they do not wash their hands before meals or that they wash them sometimes, and most of them wash cutting boards with soap and water after use.

*Table 2.* Epidemiological description of the patients.

| Variable | Number of samples | % |
|---|---|---|
| Gender | | |
| Male | 6 | 42.9 |
| Female | 8 | 57.1 |
| Age | | |
| 18-33 | 1 | 7.1 |
| 33-48 | 5 | 35.7 |
| 48-63 | 4 | 28.6 |
| 63-77 | 4 | 28.6 |
| Place of Residence | | |
| Urban area | 13 | 92.9 |
| Countryside | 1 | 7.1 |
| Education level | | |
| Complete Elementary Degree | 4 | 28.6 |
| Incomplete Elementary Degree | 7 | 50.0 |
| Complete High School Degree | 2 | 14.3 |
| Incomplete High School Degree | 1 | 7.1 |
| Monthly family income | | |
| ≥ 1 minimum wage | 4 | 28.6 |
| 1 – 2 minimum wages | 4 | 28.6 |
| 2 – 3 minimum wages | 1 | 7.1 |
| > 3 minimum wages | 1 | 7.1 |
| not answered | 4 | 28.6 |
| Knowledge about toxoplasmosis | | |
| Yes | 5 | 35.7 |
| No | 9 | 64.3 |
| Knowledge about *T. gondii* transmission | | |
| Yes | 2 | 14.3 |
| No | 12 | 85.7 |

*Table 3*. Risk factors for *T. gondii* infection among the participants.

| Variable | Number of samples | % |
|---|---|---|
| Do you have a vegetable garden at home? | | |
| Yes | 2 | 14.3 |
| No | 12 | 85.7 |
| Do you eat fruit? | | |
| Often | 7 | 50.0 |
| Occasionally | 4 | 28.6 |
| No | 3 | 21.4 |
| Do you eat vegetables or raw vegetables? | | |
| Often | 6 | 42.8 |
| Occasionally | 4 | 28.6 |
| No | 4 | 28.6 |
| Cleaning cutting boards after use?* | | |
| Wash only with water | 4 | 28.5 |
| Wash with soap and water | 6 | 42.8 |
| Do not wash | 1 | 7.1 |
| Eat meat? | | |
| Yes | 13 | 92.8 |
| No | 1 | 7.2 |
| Do you eat raw or undercooked meat? | | |
| Yes | 8 | 57.1 |
| No | 6 | 42.9 |
| Do you eat raw kibbeh?* | | |
| Yes | 5 | 35.7 |
| No | 8 | 57.1 |
| Do you eat sausage (artisanal or redneck)? | | |
| Yes | 10 | 71.4 |
| No | 4 | 28.6 |
| Do you drink milk?* | | |
| Yes | 8 | 57.1 |
| No | 6 | 42.9 |
| Boil the milk? | | |
| Yes | 3 | 21.4 |
| No | 4 | 28.6 |
| Do you eat fresh cheese? | | |
| Yes | 8 | 57.1 |
| No | 3 | 21.4 |
| Do you eat at a restaurant often? | | |
| Often | 0 | 0 |
| Occasionally | 7 | 50.0 |
| No | 7 | 50.0 |
| Do you regularly wash your hands before meals? | | |
| Yes | 4 | 28.6 |
| Occasionally | 5 | 35.7 |
| No | 5 | 35.7 |

*Some data were not informed.

*Determination of Antibodies against* T. gondii

Regarding serological data, neither serum nor CSF sample showed IgM class antibodies and only one CSF sample showed IgG antibody against *T. gondii*. However, the seroreactivity for IgG in serum samples was 100%.

*Detection of* T. gondii *DNA*

When performing the PCR, it did not detect the presence of *T. gondii* DNA in any of the 15 CSF samples.

## DISCUSSION

Between the 60's and 70's, with the description of the evolutionary cycle of *T. gondii*, the cat was then presented as a participant in the transmission of toxoplasmosis, through the elimination of oocysts in its feces (Hutchinson, 1965; Hutchinson et al., 1970). With the expansion of knowledge about the disease in the 90s, studies showed that the cat is not the main transmitter of the disease. Only 1% of the cat population releases oocysts at some point in their lives, and this environmental contamination occurs in the primary infection (Tenter et al., 2001; Hill & Dubey, 2002; Dubey, 2005).

A minority of the participants in this study reported having cats at home; however, approximately 80% of them reported that stray cats sometimes appear in their homes' backyard. It is known that it is not the fact of having domestic cats that increases the risk of transmission, but the fact of having stray cats, which can become contaminated, when they feed on contaminated carcasses in the peridomiciliary region, associated with the poor handling of these feces and hygiene habits (Dubey et al., 2012; Igreja et al., 2021).

Here we show that most patients reported the consumption of raw and undercooked meat and artisanal sausages. The city of Jataí is located in the southwest region of Goiás, being economically attractive for agriculture and livestock, with appropriate relief, hydrography, and climates. Furthermore, it has constant migration and circulation of several people in Brazil, especially individuals from the southern region of the country, coming for the production of soy, corn, sorghum, and sugar cane, besides cattle and swine farming (Belluco et al., 2018). Therefore, the southwest of Goiás is a mixed region between social, cultural habits and customs, including the consumption of undercooked meat in meals and the traditional barbecue that may hold *T. gondii* cysts, posing risks to consumers.

Given these eating habits, most meat in Brazilian slaughterhouses are treated in brine, a procedure capable of ending *T. gondii* tissue cysts. Even so, the consumption of certain types of meat presents a quite contamination risk. Seroprevalence studies in different Brazilian regions, North, Midwest,

Southeast, and South, where the consumption of undercooked meat is common, have revealed seroreactivity of 90%, 67%, 48.1%, 73.6%, respectively (Bóia et al., 2008; Mareze et al., 2019; Santos et al., 2019; Nakashima et al., 2020).

In addition, patients in this study also reported consumption of raw fruits and vegetables, ingestion of untreated milk, and failure to routinely wash their hands before meals. As all Brazilian states present, at a certain point in the season, hot and humid weather, they become favorable for the sporulation of oocysts released by cats (Yan et al., 2016). Contamination by oocysts in water, poorly washed food and on hands from handling soil or contaminated food without the protection of gloves is an important source of infection which was already reported by Brazilian studies (Bahia-Oliveira et al., 2003; Heukelbach et al., 2007). For example, in the city of Santa Maria, RS, Brazil, where the largest outbreak of toxoplasmosis occurred in 2018, the main form of infection was through contaminated water (Minuzzi et al., 2021).

All patients in the present study presented seroreactivity to *T. gondii* protozoan, showing that they had an infection with the protozoan at some point of their lives. Most participants were immunocompetent, which was shown by clinical evaluation and physical examination performed by the HCSC healthcare team, together with negative serology for HIV. Even the only HIV-seropositive patient in this study had a good $CD4^+$ T lymphocyte count and no other clinical manifestations.

Even though neurotoxoplasmosis predominantly affects immunocompromised individuals, there are still some descriptions of complications of the disease, including neurotoxoplasmosis in immunocompetent individuals (Ramachandran et al., 2014; Lima et al., 2021). Therefore, it is suggested that patients with neurological complaints, even with an apparently healthy immune system, should be investigated for *T. gondii* infection, since these patients had contact with the protozoan, and they are at risk of developing subsequent complications of possible immunosuppression and reactivation of the disease.

The only CSF reactive sample in this study was from a 39-years-old female patient who was apparently immunocompetent. However, a series of investigations is still needed regarding her immune status, as in the study by Martinot et al. (2020), in which a 31-years-old patient had a case of medullary toxoplasmosis infected with the type 2 strain. Several genetic and immunological tests were performed which showed she was immunocompetent.

Regarding the molecular detection of *T. gondii*, all CSF samples were negative. The study is composed mostly of immunocompetent individuals, which corroborates this result since the positive molecular diagnosis in CSF is more common in immunocompromised patients (Sterkes et al., 2010; de Melo et al., 2020). Furthermore, only one participant was HIV positive, who had a high $CD4^+$ T-lymphocyte count, which also corroborated with the negative CSF test.

The set of results of this study shows reactivity for *T. gondii* in serum and CSF samples in a group of patients who present neurological brain disorders and, some of them, comorbidities such as diabetes and hypertension. Toxoplasmosis is of great importance in public health, and due to its considerable impact, the World Health Organization (WHO) recommends the collection of epidemiological data, in addition to disease prevention and control measures (Robert-Gangneux & Dardé, 2012).

The seroreactivity of 100% for IgG alerts to the chances of reactivation in conditions of immunosuppression. However, there were some limitations in this study, such as the fact that the research was conducted during the SARS-CoV-2 pandemic period, which reduced the number of patients with neural symptoms at the HCSC, directly affecting sample collection and the number of participants in this study. The pandemic also made it impossible to accompany patients. Therefore, there was scarcity of clinical data to characterize the participants, absence of neuroimaging tests as well as CSF results and access to the final diagnosis.

In addition, the small sample size made it impossible to characterize the neurological disorders or represent society in general, as this was only a case series study. Thus, further studies still need to be addressed in order to define the associations of risk factors for neurotoxoplasmosis in these individuals.

From this study, we observed the risk factors for the development of toxoplasmosis in patients with neurological brain disorders in the city of Jataí, GO, Brazil. In general, participants reported contact with stray cats, consumption of vegetables, raw meat and derivatives *in natura*.

Regarding antibody detection tests, all patients showed IgG seroreactivity against *T. gondii* by ELISA, a single CSF sample showed IgG reactivity. The data obtained point to the need for educational actions that cover toxoplasmosis prophylaxis, using several tools, such as social media.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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