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TOXOPLASMA GONDII EXPOSURE AND NEUROLOGICAL DISORDERS: AN AGE- AND GENDER-MATCHED CASE-CONTROL PILOT STUDY

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Little is known about the association of *Toxoplasma gondii* infection and neurological disorders. We performed a case-control study with 344 patients with neurological diseases and 344 neurologically healthy age- and gender-matched subjects. Sera of participants were analyzed for anti-*T. gondii* IgG and IgM antibodies using commercially available immunoassays. Anti-*T. gondii* IgG antibodies were detected in 25 (7.3%) cases and in 35 (10.2%) controls (odds ratio [OR] = 0.69; 95% confidence interval [CI]: 0.40–1.18; $P = 0.17$). Anti-*T. gondii* IgM antibodies were found in 5 (14.3%) of the 25 IgG seropositive cases and in 13 (37.1%) of the 35 IgG seropositive controls ($P = 0.15$). Anti-*T. gondii* IgG antibodies were found in 8 (3.8%) of 213 female cases and in 23 (10.8%) of 213 female controls (OR = 0.32; 95% CI: 0.14–0.73; $P = 0.005$); and in 17 (13.0%) of 131 male cases and in 12 (9.2%) of 131 male controls ($P = 0.32$). No direct association between IgG seropositivity and specific neurological disorders was detected. We found no support for a role of latent *T. gondii* infection in the risk for neurological disorders in this setting. With respect to specific neurological disorders, further studies using larger patient cohorts will be required.

Keywords: *Toxoplasma gondii,* infection, seroprevalence, neurological disorders, case-control study, epidemiology, Mexico

Introduction

Infection with the parasite *Toxoplasma gondii (T. gondii)* occurs frequently worldwide [1]. The infection is commonly acquired by two routes: consumption of raw or undercooked meat containing parasite tissue cysts, as well as by ingestion of water or food containing parasite oocysts shed by the final hosts, the feline species [1, 2]. Other infection routes are infrequent and include organ transplantation [3] and blood transfusion [4]. Acute infections with this parasite are usually asymptomatic [5]; however, in some infected individuals, toxoplasmosis is characterized by lymphadenopathy, chorioretinitis, and central nervous system (CNS) involvement [5, 6]. Life-threatening toxoplasmosis, characterized by meningoencephalitis or disseminated disease, may develop in immunocompromised

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patients [5, 7]. On the other hand, primary *T. gondii* infection during pregnancy may lead to congenital toxoplasmosis [5, 8].

After infection, *T. gondii* disseminates throughout the body of infected host and reaches the immune privileged sites, such as the central nervous system [9, 10]. The parasite may be located anywhere in the brain [11]. Bradyzoite-containing cysts persist within neurons, where they remain for the whole lifetime of the hosts [12]. Acute infections with *T. gondii* may cause CNS and/or ocular damage, and chronic infections have been linked to behavioral changes [13]. Cysts in the brain are controlled by the immune system, resulting in an increased proinflammatory milieu [14]. Immune processes during chronic the infection may lead to changes in neuronal connectivity, synaptic plasticity, and altered brain function [15, 16].

The causal relationship between congenital toxoplasmosis and severe brain damage is a well-established clinical phenomenon. In Brazil, for example, approximately one third of children with congenital toxoplasmosis have been found to show severe neurological alterations, including hydrocephalus, microcephalia, and mental retardation [17]. In adulthood, latent infections with *T. gondii* have been associated with an increased risk for psychiatric disorders, e.g., schizophrenia [18, 19], mixed anxiety–depression disorder [20], personality disorders [21], or obsessive–compulsive disorder [22]. However, little is yet known about a potential link between latent *T. gondii* infection and neurological diseases, and results of studies are rather conflicting [23–32]. We are not aware of any matched case–control study about the association of *T. gondii* infection and the overall risk for neurological diseases. The present study was intended as a pilot study to pave the way for a future systematic investigation of the fragmentary and conflicting results about the link of *T. gondii* infection and neurological disease. To this end, we sought to determine the association between *T. gondii* infection and general neurological disorders in patients attended in a public hospital of Durango City, Mexico.

Materials and methods

Study design and study populations

We performed a case-control seroprevalence study in 344 patients suffering from various neurological diseases (cases) and 344 people without neurological diseases from the general population (controls) in Durango City, Mexico from April to December 2016. Inclusion criteria for the cases were as follows: (a) patients suffering from neurological diseases attended in the Neurology Department at the public Hospital "Dr. Santiago Ramón y Cajal" in Durango City; (b) aged 9 years and older; and (c) who voluntarily accepted to participate in the study. In total, 213 females and 131 males with neurological diseases were included in the study. Cases were 13–93 (mean = 53.01 ± 18.29) years old. Diagnoses of neurological diseases in patients were based on the International Statistical Classification of Diseases and related Health Problems version 2016 (ICD-10) (http://apps.who.int/classifications/icd10/browse/2016/en). *Table 1* shows the diagnoses of the studied patients. Patients unable to give informed consent to participate in the study were not enrolled. With respect to the control group, 344 subjects without neurological diseases were randomly selected from the general population

Table 1. Diagnoses of the studied patients

ICD-10 code	Diagnosis	No. of patients*
C69–C72	Malignant neoplasms of eye, brain, and other parts of central nervous system	1
F00–F09	Organic, including symptomatic, mental disorders	22
G00–G09	Inflammatory diseases of the central nervous system	2
G10–G14	Systemic atrophies primarily affecting the central nervous system	4
G20–G26	Extrapyramidal and movement disorders	33
G30–G32	Other degenerative diseases of the nervous system	5
G35–G37	Demyelinating diseases of the central nervous system	2
G40–G47	Episodic and paroxysmal disorders	179
G50–G59	Nerve, nerve root, and plexus disorders	15
G60–G64	Polyneuropathies and other disorders of the peripheral nervous system	28
G70–G73	Diseases of myoneural junction and muscle	1
G80–G83	Cerebral palsy and other paralytic syndromes	6
G90–G99	Other disorders of the nervous system	4
H46–H48	Disorders of optic nerve and visual pathways	3
I60–I69	Cerebrovascular diseases	57
Q00–Q07	Congenital malformations of the nervous system	1

*Sum is more than 344 because some patients had more than one diagnosis

in Durango City. Controls were matched with cases by age (±4 years) and gender. Controls were 9–88 (mean = 52.65 ± 17.89) years old and included 213 females and 131 males. There was no difference in age between cases and controls ($P = 0.79$ by the student t test).

Detection of T. gondii *antibodies*

Serum samples from participants were analyzed for anti-*T. gondii* IgG antibodies with the commercially available enzyme immunoassay kit "*Toxoplasma* IgG" (Diagnostic Automation Inc., Woodland Hills, CA, USA). This test was used to determine the presence and levels of IgG antibodies. As indicated in the kit insert, seropositivity was considered when a value of ≥8 IU/mL of specific anti-*T. gondii* IgG antibody was detected. This cut-off has been used in other epidemiological studies in Durango, Mexico [4, 19, 20]. All IgG seropositive serum samples were additionally analyzed for anti-*T. gondii* IgM antibodies by the commercially available enzyme immunoassay "*Toxoplasma* IgM" kit (Diagnostic Automation Inc.). All tests were performed following the manufacturer's instructions, and positive and negative controls were included in each run.

Statistical analysis

Data were analyzed with the Microsoft Excel 2010, Epi Info version 7 (Centers for Disease Control and Prevention: http://wwwn.cdc.gov/epiinfo/) and SPSS version 15.0 (SPSS Inc. Chicago, Illinois) software. For calculation of the sample size, we used a 95% confidence level, a power of 80%, a 1:1 proportion of cases and controls, a reference seroprevalence of 6.1% [33] as the expected frequency of exposure in controls, and an odds ratio of 2.1. The result of the sample size calculation was 328 cases and 328 controls. We used the paired student's t test to compare age values among the groups. Bivariate analysis was used to compare the seropositivity rates between cases and controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated, and statistical significance was set at a P value <0.05.

Ethical considerations

This study was approved by the Ethical Committee of the Institute of Security and Social Services for State Workers in Durango City, Mexico. The purpose and procedures of the study were explained to all participants, and a written informed consent was obtained from all of them and from the next of kin of minor participants.

Results

Anti-*T. gondii* IgG antibodies were detected in 25 (7.3%) of the 344 cases and in 35 (10.2%) of the 344 controls. There was no significant difference in seroprevalence of *T. gondii* infection between cases and controls (OR = 0.69; 95% CI: 0.40–1.18; $P = 0.17$). Among the 25 anti-*T. gondii* IgG-positive cases, 13 (52.0%) had anti-*T. gondii* IgG antibody levels higher than 150 IU/mL, and 12 (48.0%) had levels between 8 and 99 IU/mL. Among the 35 anti-*T. gondii* IgG-positive controls, 22 (62.9%) had anti-*T. gondii* IgG antibody levels higher than 150 IU/mL, 5 (14.2%) had levels between 100 and 150 IU/mL, and 8 (22.9%) had levels between 8 and 99 IU/mL. The frequency of high anti-*T. gondii* IgG levels (>150 IU/ml) was not significantly different between cases and controls ($P = 0.40$ by χ^2). Anti-*T. gondii* IgM antibodies were found in 5 (14.3%) of the 25 IgG seropositive cases and in 13 (37.1%) of the 35 IgG seropositive controls ($P = 0.15$ by χ^2). Anti-*T. gondii* IgG antibodies were detected in 8 (3.8%) of the 213 female cases and in 23 (10.8%) of the 213 female controls (OR = 0.32; 95% CI: 0.14–0.73; $P = 0.005$ by χ^2). In males, on the other hand, anti-*T. gondii* IgG antibodies were detected in 17 (13.0%) of the 131 cases and in 12 (9.2%) of the 131 controls ($P = 0.32$ by χ^2). The frequency of high (>150 IU/mL) anti-*T. gondii* IgG antibody levels was similar in male (9/17: 52.9%) than in female (4/8: 50.0%) seropositive cases ($P = 1.0$ by Fisher exact test).

Bivariate analysis of IgG seropositivity to *T. gondii* and ICD-10 codes did not show an association between *T. gondii* infection and groups of specific neurological diseases *(Table 2)*.

Table 2. Correlation of a selection of ICD-10 diagnosis groups and seroprevalence of *T. gondii* infection

ICD-10 groups	Diagnosis		Prevalence of *T. gondii* infection		OR	95% confidence interval	*P* value
		No.	No.	%			
F00–F09	Organic, including systematic, mental disorders	22	1	4.5	–	–	–
G20–G26	Extrapyramidal and movement disorders	33	1	3.0	0.65	0.03–11.07	0.65
G40–G47	Episodic and paroxysmal disorders	179	13	7.3	1.64	0.20–13.21	0.97
G50–G59	Nerve, nerve root, and plexus disorders	15	1	6.7	1.50	0.08–26.01	0.64
G60–G64	Polyneuropathies and other disorders of the peripheral nervous system	28	4	14.3	3.50	0.36–33.81	0.50
I60–I69	Cerebrovascular diseases	57	5	8.8	2.01	0.22–18.33	0.87

Discussion

Despite the well-documented detrimental effects of congenital *T. gondii* infection on brain development and several studies suggesting a relationship between seropositivity and psychiatric disorders, there are, to our knowledge, thus far no reports of matched case-control studies on the association between *T. gondii* infection and the overall risk for neurological disorders. The present study was intended as a pilot study addressing this issue and was performed as an age- and gender-matched case-control study. To test for such an association in a naturalistic setting, aiming to reduce population stratification effects, we recruited patients attended in the Neurology Department in a public hospital in Durango City, Mexico.

We observed that the frequencies of anti-*T. gondii* IgG and IgM antibodies, and anti-*T. gondii* antibody levels in neurology patients were similar to those obtained in control subjects of the general population. Therefore, our results provide no evidence for an association between *T. gondii* infection and increased risk for neurological disorders in general. The 7.3% seroprevalence of *T. gondii* infection found in patients with neurological disease is also comparable to the 6.1% and 7.4% seroprevalences of *T. gondii* infection reported in the general population [33] and healthy blood donors in Durango City [34], respectively. In contrast, the seroprevalence found in neurology patients is lower than seroprevalences reported in some population groups in the same city, i.e., 15.4% in female sex workers [35], and 21.1% in inmates [36] and waste pickers [37]. In addition, the seroprevalence found in neurology patients is lower than the 18.2% seroprevalence reported in patients in a psychiatric hospital in the same city [38]. The fact that neurology patients had a seroprevalence rate comparable to those reported in healthy blood donors and subjects of the general population, and lower than rates reported in psychiatric patients, inmates, waste pickers, and female sex workers suggest that *T. gondii* exposure does not contribute for a higher risk of neurological diseases in general. It is likely that differences in contributing factors for infection among the groups accounted for differences in the seroprevalence rates. Besides this, the infection route and time point were not followed in the current study, which might also influence the magnitude how the infection may influence or even trigger certain neurological diseases.

Considering the potential pathogenic role of *T. gondii* in brain, the relatively low (7.3%) seroprevalence of *T. gondii* infection found in neurology patients was unexpected. Results indicate that factors other than *T. gondii* infection might be contributing for neurological diseases. Intriguingly, we observed even a lower frequency of *T. gondii* infection in female patients with neurological diseases (3.8%) than in female control subjects (10.8%) (OR = 0.32; 95% CI: 0.14–0.73; $P = 0.005$ by χ^2). This fact might be interpreted rather as a protective effect of *T. gondii* infection against neurological diseases in general, or that *T. gondii* infection did not play a substantial role in neurological diseases in the studied population. It is not clear why female patients had a significantly lower seroprevalence of *T. gondii* infection than their controls. We are not aware of any underlying mechanism of *T. gondii* infection that protects against neurological diseases. In a study based on behavioral and neurophysiological data obtained by means of a stop-change paradigm, researchers found that latent infection with *T. gondii* led to improved action control in healthy young humans [39]. This paradoxical improvement of cognitive control process of *T. gondii* infection contrasts with results of many reports where researchers found an impairment in neurocognitive and neurobehavioral functioning due to *T. gondii* infection [40–42]. For instance, latent toxoplasmosis was associated with neurocognitive impairment in young adults with and without chronic human immunodeficiency virus (HIV) infection [40]. In another study, seropositivity to *T. gondii* was associated with lower reading skills and memory capacities in school-aged children [41]. Moreover, immediate memory impairment was observed in older adults with latent toxoplasmosis [42, 43]. In addition, in a recent study in Germany, *T. gondii*-infected seniors showed reduced working-memory performance, which was associated with attenuated event-related potentials and frontal theta activity, suggesting neurotransmitter imbalance [44].

The association between *T. gondii* infection and neurological diseases has been controversial. In the Czech Republic, toxoplasmosis was strongly associated with neurological disorders [23]. Whereas in India, the seropositivity rate of IgM antibodies against *T. gondii* was high in neurosurgery patients [24]. In addition, infection with *T. gondii* has been associated with reflexes impairment [25], epilepsy [26, 27], Parkinson's disease [28], and Alzheimer's disease [29]. However, other studies found no association between *T. gondii* infection and Parkinson's disease [30, 31] and Alzheimer's disease [32]. Remarkably, in a murine model, neuroinflammatory response following chronic toxoplasmosis reduced the accumulation of β-amyloid plaques, ameliorating the hallmark of Alzheimer's disease [45].

During latent infection, *T. gondii* is located within neurons, inducing changes to the host cells and altering signature neurological signaling pathways [46, 47]. Experiments in mice showed an increase in levels of dopamine metabolites in the brain cortex and a decrease in serotonin levels in the amygdala and norepinephrine levels in the cortex and amygdala of *T. gondii*-infected mice; however, these data are under discussion [48, 49]. In addition, in a transcriptome analysis using RNA sequencing of mouse brain infected with *T. gondii*, researchers found positive correlations between the numbers of parasites and the expression levels of genes involved in immune responses, and a negative correlation between parasite numbers and the expression of genes involved in neurological functions [50]. Importantly, a very recent study that conducted transcriptomic analysis of monocytes from congenitally infected individuals and primary *T. gondii*-infected human

neuronal stem cells revealed effects on neurodevelopment and plasticity in neural and immune networks, highlighting altered pathways of neurodegeneration, motor disease, and epilepsy [51].

We did not find an association between *T. gondii* infection and ICD groups of specific neurological diseases in this setting. However, the sample size of specific groups was rather small, limiting the outcome of the study. Further investigation with larger case numbers should be conducted to determine the potential association between *T. gondii* infection and specific neurological diseases. In addition, further studies should focus in female populations to confirm or challenge the protective role of *T. gondii* infection against neurological disorders.

Conclusions

The age- and gender-matched case–control study served as a pilot study on the association between *T. gondii* infection and general neurological diseases. The results do not suggest a substantial role for *T. gondii* exposure in the risk for neurological diseases in general, but the results nevertheless demonstrate the feasibility of association studies with *T. gondii* infection in an outpatient clinic setting. However, the pilot character of this study must be pointed out, and the number of cases for each specific neurological disorder included was rather low. Thus, further studies should be performed to determine the association between *T. gondii* infection and specific neurological disorders like dementia or epilepsy, for which an underlying pathomechanism seems plausible.

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Authors' contributions

C.A.E. conceived and designed the study protocol, performed the laboratory tests, analyzed the data, and wrote the article. Y.R.R.A., G.Q.C., and J.T.G. obtained the blood samples and clinical data, and/or performed the data analysis. J.H.T. and L.F.S.A. performed the data analysis and wrote the article. B.S., O.L., and I.R.D. performed the data analysis and wrote the article.

Conflict of interest

The authors declare that they have no conflict of interest.

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