


Toxoplasma gondii exposure and epilepsy: A matched case-control study in a public hospital in northern Mexico

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Abstract

Objectives: This study aimed to determine the association between infection with *Toxoplasma gondii* and epilepsy in patients attended to in a public hospital in the northern Mexican city of Durango.

Methods: We performed an age- and gender-matched case-control study of 99 patients suffering from epilepsy and 99 without epilepsy. Sera of participants were analyzed for anti-*T. gondii* IgG and IgM antibodies using commercially available enzyme-linked immunoassays. Seropositive samples to *T. gondii* were further analyzed for detection of *T. gondii* DNA by polymerase chain reaction.

Results: Anti-*T. gondii* IgG antibodies were found in 10 (10.1%) of the 99 cases and in 6 (6.1%) of the 99 controls (odds ratio = 1.74; 95% confidence interval: 0.60–4.99; $p = 0.43$). High (> 150 IU/mL) levels of anti-*T. gondii* IgG antibodies were found in 6 of the 99 cases and in 4 of the 99 controls (odds ratio = 1.53; 95% confidence interval: 0.41–5.60; $p = 0.74$). Anti-*T. gondii* IgM antibodies were found in 2 of the 10 IgG seropositive cases, and in 2 of the 6 IgG seropositive controls (odds ratio = 0.50; 95% confidence interval: 0.05–4.97; $p = 0.60$). *T. gondii* DNA was not found in any of the 10 anti-*T. gondii* IgG positive patients. Bivariate analysis of IgG seropositivity to *T. gondii* and International Statistical Classification of Diseases and related Health Problems, 10th Edition codes of epilepsy showed an association between seropositivity and G40.1 code (odds ratio = 22.0; 95% confidence interval: 2.59–186.5; $p = 0.008$). Logistic regression analysis showed an association between *T. gondii* infection and consumption of goat meat (odds ratio = 6.5; 95% confidence interval: 1.22–34.64; $p = 0.02$), unwashed raw vegetables (odds ratio = 26.3; 95% confidence interval: 2.61–265.23; $p = 0.006$), and tobacco use (odds ratio = 6.2; 95% confidence interval: 1.06–36.66; $p = 0.04$).

Conclusions: Results suggest that *T. gondii* infection does not increase the risk of epilepsy in our setting; however, infection might be linked to specific types of epilepsy. Factors associated with *T. gondii* infection found in this study may aid in the design of preventive measures against toxoplasmosis.

Keywords

Toxoplasma gondii, infection, seroprevalence, epilepsy, case-control study, epidemiology, Mexico.

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Background

Toxoplasma gondii (*T. gondii*) is a ubiquitous parasite causing infections in humans and animals around the world.¹ Infection with *T. gondii* is common and about 30% of humans are chronically infected.² Several routes of *T. gondii* infection have been described including oral,^{2,3} vertical,⁴ blood transfusion,⁵ and organ transplantation.⁶ Most people infected with *T. gondii* are asymptomatic or may develop mild symptoms that are self-limited.⁷ However, toxoplasmic encephalitis can be a life-threatening disease in immunocompromised patients.⁸ Ocular toxoplasmosis is the most common form of posterior infectious uveitis.⁹ Children with congenital toxoplasmosis can have several clinical manifestations, including hepatomegaly, splenomegaly, jaundice, microcephaly, and chorioretinitis.¹⁰ The parasite forms cysts in brain¹¹ and persists lifelong in the host.^{12,13} New and emerging data about chronic infection with *T. gondii* in brain that associate with changes in neuronal architecture, neurochemistry, and behavior suggest that this infection is not without consequence.¹³ Infection with *T. gondii* has been associated with neuropsychiatric disorders.¹⁴ Epilepsy has been observed in patients with human immunodeficiency virus infection suffering from cerebral toxoplasmosis.^{15,16} High rates of *T. gondii* seropositivity and high levels of *T. gondii* antibodies have been found in cryptogenic epilepsy patients.^{17,18} In a Chinese study of patients with unknown central nervous system diseases, the highest seropositivity rate to *T. gondii* was found in patients with epilepsy.¹⁹ In two studies in sub-Saharan Africa, epilepsy was correlated to exposure to *T. gondii*.^{20,21} In a recent meta-analysis to estimate the risk of epilepsy due to toxoplasmosis, researchers found that toxoplasmosis should be regarded as an epilepsy risk factor.²² In contrast, a study in Iran showed that seropositive rate to *T. gondii* was significantly lower in epileptic patients than in healthy subjects.²³

To the best of our knowledge, there has not been a study about the link between *T. gondii* infection and epilepsy in Mexico. Therefore, this study aimed to determine the association between seropositivity to *T. gondii* and epilepsy in a public hospital in Durango City, Mexico. In addition, we determined the seroprevalence association with sociodemographic, clinical, and behavioral factors of the patients suffering from epilepsy.

Materials and methods

Study design and study populations

Through a case-control study design, we studied 99 patients suffering from epilepsy (cases) and 99 people without epilepsy (controls) from April 2016 to March 2017. Inclusion criteria for cases were as follows: (a) patients suffering from epilepsy attending the Department of Neurology at the public Hospital “Dr. Santiago Ramón y Cajal” of the Institute of Security and Social Services for State Workers in Durango

City, (b) aged 9 years and older, (c) any gender, and (d) who voluntarily accept to participate in the study. Epilepsy patients were recruited regardless of whether they had recent or past diagnosis of epilepsy. Diagnosis of epilepsy in patients was 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>). The ICD-10 uses the category G40 for epilepsy. This category is divided into 10 codes (G40.0-G40.9), and each code represents a specific and precise type of epilepsy. Codes G40.0-G40.2 include focal epilepsy types, G40.3 and G40.4 encompass generalized epilepsy types, G40.5 is used for special epileptic syndromes, while codes G40.6-G40.9 include unspecified or undetermined epilepsy types. Table 1 shows the types of epilepsy diagnosed in the study population. With respect to the control group, 99 subjects without epilepsy were randomly selected from the general population and enrolled in health care centers, shops, and Faculty of Medicine and Nutrition of Juarez University of Durango State in Durango City. Controls subjects were matched with cases by age (± 4 years) and gender.

Socio-demographic, clinical, and behavioral characteristics of patients with epilepsy

We obtained the socio-demographic, clinical, and behavioral characteristics of the patients suffering from epilepsy through a standardized questionnaire. Socio-demographic items were age, gender, birthplace, residence, occupation, and educational and socioeconomic statuses. Clinical data included history of lymphadenopathy, frequent headache, impairments in vision, memory, reflexes, and hearing, blood transfusion or organ transplantation. Behavioral items were as follows: contact with animals, washing hands before eating, type of meat consumed, ingestion of raw or undercooked meat, unpasteurized milk or untreated water, unwashed raw vegetables or fruits, consumption of alcohol, tobacco or drug use, type of flooring at home, and soil contact.

Detection of *T. gondii* antibodies

Serum samples of participants were obtained and kept frozen at -20°C until analyzed. We used a commercially available enzyme immunoassay kit “*Toxoplasma* IgG” (Diagnostic Automation Inc., Woodland Hills, CA, USA) to detect anti-*T. gondii* IgG antibodies in serum samples. Anti-*T. gondii* IgG antibodies were quantified, and a cut-off of ≥ 8 IU/mL was used for seropositivity. Serum samples with anti-*T. gondii* IgG antibodies of cases and controls were further analyzed for anti-*T. gondii* IgM antibodies by the commercially available enzyme immunoassay “*Toxoplasma* IgM” kit (Diagnostic Automation Inc.). All assays were performed following the instructions of the manufacturer.

Table 1. Diagnoses of epilepsy and frequency of *T. gondii* infection in the study population.

ICD-10 code	Diagnosis	No. of patients	Seropositivity to <i>T. gondii</i>		OR	95% CI	P value
			No.	%			
G40.1	Localization-related (focal) (partial) symptomatic Epilepsy and epileptic syndromes with simple Partial seizures	6	3	50	22.0	2.59–186.5	0.008
G40.2	Localization-related (focal) (partial) symptomatic Epilepsy and epileptic syndromes with complex Partial seizures	10	0	0	0.0	–	1.0
G40.3	Generalized idiopathic epilepsy and epileptic syndromes	12	2	16.7	4.4	0.55–35.10	0.18
G40.4	Other generalized epilepsy and epileptic syndromes	13	3	23.1	6.6	0.97–44.85	0.06
G40.5	Special epileptic syndromes	1	0	0	0.0	–	1.0
G40.7	Petit mal, unspecified, without grand mal seizures	1	0	0	0.0	–	1.0
G40.8	Other epilepsy	7	0	0	0.0	–	1.0
G40.9	Epilepsy, unspecified	46	2	4.3	Ref.		
G41.0	Grand mal status epilepticus	1	0	0	0.0	–	1.0
G41.2	Complex partial status epilepticus	1	0	0	0.0	–	1.0
G41.9	Status epilepticus, unspecified	1	0	0	0.0	–	1.0

DNA extraction and detection of *T. gondii* DNA

Whole blood of patients suffering from epilepsy diagnosed using anti-*T. gondii* IgG antibodies was analyzed for detection of *T. gondii* DNA by nested-polymerase chain reaction. DNA extraction was performed using a commercially available kit (QIAamp DNA Blood Mini kit; Qiagen, Germany). Amplification of DNA was carried out with primers directed against the B1 gene of *T. gondii* and following the protocol described by Burg et al.²⁴ Amplified products were run in a 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized with ultraviolet transillumination.

Statistical analysis

Statistical analyses were performed with the aid of the software Microsoft Excel 2010, SPSS version 20.0 (IBM Corp. Armonk, NY), and Epi Info version 7 (Centers for Disease Control and Prevention: <http://wwwn.cdc.gov/epiinfo/>). We calculated a sample size to determine the number of participants needed in our study for detection of differences in seroprevalences among the groups. For calculation of the sample size, we used a 95% two-sided confidence level, a power of 80%, a 1:1 ratio of cases and controls, a reference seroprevalence of 6.1%²⁵ as the percentage outcome in unexposed group, and an odds ratio (OR) of 4. The result of the sample size calculation was 85 cases and 85 controls. Age values among the groups were compared with the paired student's *t* test. The association between *T. gondii* seropositivity rate and characteristics of the cases was assessed by bivariate analysis and logistic regression. OR and 95% confidence intervals (CI) were calculated by logistic regression with the

Enter method. Only variables with $p < 0.05$ obtained in the bivariate analysis were included in the regression analysis. A $p < 0.05$ was considered statistically significant.

Ethics aspects

This case-control study was approved by the Ethics Committee of the Institute of Security and Social Services for State Workers in Durango City, Mexico. The purpose and procedures of this study were explained to all participants before sampling. All adult participants and minor patients' legally authorized representative provided a written informed consent.

Results

In total, 47 women and 52 men suffering from epilepsy were enrolled in the study. Patients were 12–80 (mean = 39.3 ± 16.2) years old. Control subjects were 9–79 (mean = 39.2 ± 15.9) years old. Age was similar in cases and in controls ($p = 0.98$).

Anti-*T. gondii* IgG antibodies were found in 10 (10.1%) of the 99 patients and in 6 (6.1%) of the 99 controls (OR = 1.74; 95% CI: 0.60–4.99; $p = 0.43$). High (> 150 IU/mL) levels of anti-*T. gondii* IgG antibodies were found in 6 of the 99 cases and in 4 of the 99 controls (OR = 1.53; 95% CI: 0.41–5.60; $p = 0.74$). Anti-*T. gondii* IgM antibodies were found in 2 of the 10 IgG seropositive patients, and in 2 of the 6 IgG seropositive controls (OR = 0.50; 95% CI: 0.05–4.97; $p = 0.60$). *T. gondii* DNA was not found in any of the 10 anti-*T. gondii* IgG positive patients. Bivariate analysis of IgG seropositivity to *T. gondii* and ICD-10 codes showed an association between seropositivity and G40.1 code

Table 2. Multivariate analysis of selected characteristics of epileptic patients and their association with *T. gondii* infection.

Patients and their association with <i>T. gondii</i> infection.	Odds ratio	95% confidence interval	p-value
Characteristic			
Educational status	0.56	0.22–1.43	0.23
Consumption of goat meat	6.51	1.22–34.64	0.02
Consumption of unwashed raw vegetables	26.33	2.61–265.23	0.006
Tobacco use	6.23	1.06–36.66	0.04

(Localization-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with simple partial seizures) (OR = 22.0; 95% CI: 2.59–186.5; $p = 0.008$) (Table 1).

With respect to sociodemographic, clinical, and behavioral factors of the patients suffering from epilepsy, bivariate analysis showed that the factors educational status, consumption of goat meat, and unwashed raw vegetables, and tobacco use had a likely association with *T. gondii* infection ($p < 0.05$). Other variables as described in the Methods section showed p values ≥ 0.05 by bivariate analysis. Logistic regression analysis of variables with $p < 0.05$ obtained by bivariate analysis showed an association between *T. gondii* infection and consumption of goat meat (OR = 6.5; 95% CI: 1.22–34.64; $p = 0.02$), unwashed raw vegetables (OR = 26.3; 95% CI: 2.61–265.23; $p = 0.006$), and tobacco use (OR = 6.2; 95% CI: 1.06–36.66; $p = 0.04$) (Table 2).

Discussion

Very little is known about the magnitude of the epidemiological impact of *T. gondii* exposure on epilepsy. Infection with *T. gondii* may produce epilepsy in some infected individuals;^{15–17} however, there is scarce information about how frequent people suffering from epilepsy have been exposed to *T. gondii*. In a recent study on the assessment of the association between postnatal toxoplasmosis and epilepsy in immunocompetent patients, researchers found that this association seemed possible, but only scanty and limited quality literature for the assessment was available.²⁶ To the best of our knowledge, there has not been any age- and gender-matched case-control study on the association between *T. gondii* infection and epilepsy in Mexico. Therefore, we sought to determine the association of *T. gondii* infection and epilepsy in patients attended at the Neurology Department in a public hospital in the northern Mexican city of Durango. We found that patients suffering from epilepsy had a similar IgG seropositivity rate to *T. gondii* infection (10.1%) than controls without epilepsy (6.1%). In addition, the frequency of high (>150 IU/mL) levels of specific anti-*T. gondii* IgG levels and the frequency of anti-*T. gondii* IgM antibodies were also similar in cases and in controls. These results can be interpreted as no association of *T. gondii* infection and epilepsy in the public hospital surveyed. None of the patients suffering from epilepsy and seropositive to *T. gondii* had detectable *T. gondii* DNA in their blood by nested-polymerase chain reaction. The

prevalence of *T. gondii* exposure found in patients with epilepsy is also comparable to a 6.1% prevalence of *T. gondii* exposure reported in the general population,²⁵ and lower than the 21.1% seroprevalence reported in inmates²⁷ in the same Durango City. Results thus suggest that *T. gondii* infection did not contribute substantially to a higher risk of epilepsy in our studied population. However, results do not rule out the possible role of *T. gondii* infection as a cause of epilepsy. In fact, a significant association between seropositivity to *T. gondii* and epilepsy of the ICD-10 G40.1 code (Localization-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with simple partial seizures) was found. We are not aware of any report of a correlation of *T. gondii* exposure and ICD-10 epilepsy codes. Further in-depth studies about the association between *T. gondii* infection and ICD-10 epilepsy codes should be conducted. Results thus indicate that infection with *T. gondii* is not frequent among epileptic patients in our setting but suggest that *T. gondii* infection could be associated with a specific type of epilepsy. Infection with *T. gondii* has been considered as a cause of epilepsy in several studies. In an American study, a statistically significant elevation (59% increase in optical density in the enzyme-linked immunoassay used) of *T. gondii* antibodies among cryptogenic epilepsy patients as compared to controls was found.¹⁷ In a Turkish study, the seropositive rate of anti-*T. gondii* antibodies in cryptogenic epilepsy patients was higher than in healthy volunteers and epilepsy patients with a known cause.¹⁸

We looked for sociodemographic, clinical and behavioral factors of patients suffering from epilepsy associated with *T. gondii* infection. We did not perform this analysis in control subjects because factors associated with *T. gondii* infection in subjects from the general population in Durango City have been previously reported.²⁵ We found that the variables consumption of goat meat, unwashed raw vegetables, and tobacco use were associated with *T. gondii* infection. This is the first time we found a positive association between *T. gondii* infection and consumption of goat meat in a population group in Durango. This association is supported by a previous demonstration of anti-*T. gondii* antibodies in 31% of goats surveyed in Durango State.²⁸ On the other hand, we previously reported an association between *T. gondii* infection and consumption of unwashed raw vegetables in migrant agricultural workers living in poverty in rural Durango.²⁹ Intriguingly, a

correlation between *T. gondii* seropositivity and tobacco use was found. We previously found this correlation in blood donors in Hermosillo City, Mexico.³⁰ It is unclear why patients with tobacco use had a higher seroprevalence of *T. gondii* infection than patients without tobacco use. This finding deserves further research.

Our study has some limitations. Few patients with some specific types of epilepsy were studied. Furthermore, we studied patients from only one hospital, and the majority of participants belonged to a medium socioeconomic level. The present study was not powered to detect a smaller difference than the one used in the calculation. Thus, studies with larger number of patients with specific types of epilepsy, of diverse socioeconomic statuses, attending several health care centers or hospitals to assess the association between *T. gondii* exposure and epilepsy are needed.

Conclusion

This is the first age- and gender-matched case control study on the association between *T. gondii* infection and epilepsy in Mexico. Results suggest that *T. gondii* seropositivity was not associated with epilepsy in general; however, *T. gondii* seropositivity was associated with a specific ICD-10 epilepsy code: G40.1 (localization-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with simple partial seizures). Factors associated with *T. gondii* infection found in this study may aid in the design of preventive measures against toxoplasmosis.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval for this study was obtained from Ethical Committee of the Institute of Security and Social Services for State Workers in Durango City, Mexico

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Informed consent

All adult participants and minor patients' legally authorized representative provided a written informed consent before the study

Trial registration

Not applicable *Toxoplasma gondii* exposure and epilepsy: a matched case-control study in a public hospital in northern Mexico

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