

# *Toxoplasma gondii* Infection and Mixed Anxiety and Depressive Disorder: A Case-Control Seroprevalence Study in Durango, Mexico

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## Abstract

**Background:** The parasite *Toxoplasma gondii* (*T. gondii*) may invade the brain and might induce behavioral changes. We sought to determine the association of *T. gondii* infection and mixed anxiety and depressive disorder.

**Methods:** Through an age- and gender-matched case-control seroprevalence study, we examined 65 patients suffering from mixed anxiety and depressive disorder (WHO ICD-10 code: F41.2) attending in a public hospital of mental health and 260 control subjects without this disorder from the general population. Sera of participants were analyzed for anti-*Toxoplasma* IgG and IgM antibodies using enzyme-linked immunoassays.

**Results:** Fifteen (23.1%) of the 65 patients and 18 (6.9%) of the 260 controls had anti-*T. gondii* IgG antibodies (odds ratio (OR): 4.03; 95% confidence interval (CI): 1.90 - 8.53;  $P < 0.001$ ). The frequency of high ( $> 150$  IU/mL) anti-*T. gondii* IgG levels was similar in cases and controls (OR: 0.25; 95% CI: 0.05 - 1.06;  $P = 0.05$ ). Seroprevalence was similar in male cases and controls ( $P = 1.0$ ); however, se-

rovalence was significantly higher in female cases than in female controls (OR: 7.08; 95% CI: 2.83 - 17.67;  $P < 0.00001$ ). Patients aged 31 - 50 years old had a significantly higher seroprevalence of *T. gondii* infection than controls of the same age group (OR: 21.04; 95% CI: 5.22 - 84.80;  $P < 0.00001$ ). Anti-*T. gondii* IgM antibodies were found in four (26.7%) of the 15 anti-*T. gondii* IgG seropositive cases and in 10 (55.6%) of the 18 anti-*T. gondii* IgG seropositive controls ( $P = 0.15$ ).

**Conclusions:** Results support for the first time an association between seropositivity to *T. gondii* and mixed anxiety and depressive disorder. Further research to confirm this association and to determine the seroepidemiology of *T. gondii* infection in patients with this disorder is needed.

**Keywords:** *Toxoplasma gondii*; Seroprevalence; Mixed anxiety and depressive; Psychiatric patients; Case-control

## Introduction

*Toxoplasma gondii* (*T. gondii*) is a coccidian parasite causing infections worldwide [1]. About 30% of humanity is currently infected with *T. gondii* [2]. Infection with this parasite is usually acquired by ingestion of raw or undercooked meat containing tissue cysts, and water or food contaminated with oocysts shed by cats [3]. Other routes of *T. gondii* infection include congenital transmission [4], organ transplantation [5], and blood transfusion [6]. Although infections with *T. gondii* are usually asymptomatic, some infected individuals develop a symptomatic disease known as toxoplasmosis with involvement of lymph nodes, central nervous system or eyes [2, 7, 8]. Some psychiatric disorders have been associated with infection with *T. gondii*, i.e. schizophrenia [9, 10], bipolar disorder [11], obsessive compulsive disorder [11, 12], depression [13], and generalized anxiety disorder [14]. However, to the best of our knowledge, the association of infection with *T. gondii* and mixed anxiety and depressive disorder has not been reported. In this disorder, with the code F41.2 according to the classification of mental and behavioral disorders of the ICD-10 ([Manuscript accepted for publication May 03, 2016](http://</a></p>
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**Table 1.** Comparison of Seropositivity Rate to *T. gondii* According to Sex and Age Between Cases (F41.2) and Controls

Characteristics	Cases			Controls			P value
	No. tested	Seroprevalence of <i>T. gondii</i> infection		No. tested	Seroprevalence of <i>T. gondii</i> infection		
		No.	%		No.	%	
Sex							
Male	23	2	8.7	92	8	8.7	1.0
Female	42	13	31.0	168	10	6.0	< 0.0001
Age (years)							
30 or less	24	4	16.7	96	7	7.3	0.2
31 - 50	26	10	38.5	104	3	2.9	< 0.0001
> 50	15	1	6.7	60	8	13.3	0.7

apps.who.int/classifications/icd10/browse/2016/en#/F40-F48), anxiety and depression are both present in an individual, but neither is predominant, and neither symptom is present to an extent that justifies a separated diagnosis. Given the lack of information about the link of infection with *T. gondii* and this specific diagnosis, we attempted to determine the association between *T. gondii* infection and mixed anxiety and depressive disorder in a sample of patients attending in a public hospital of mental health in the northern Mexican city of Durango.

## Materials and Methods

### Study design and populations studied

We performed an age- and gender-matched case-control study of 65 psychiatric patients suffering from mixed anxiety and depressive disorder attending in a public hospital of mental health in Durango City, Mexico and 260 control subjects without mixed anxiety and depressive disorder from the general population of the same city. This case-control study was performed from August 2015 to February 2016. Inclusion criteria for cases were: 1) patients suffering from mixed anxiety and depressive disorder attending in the Hospital of Mental Health “Dr. Miguel Vallebuena” of the Secretary of Health in Durango City; 2) aged 18 years and older; and 3) who accepted to participate in the study. Diagnosis of mixed anxiety and depressive disorder was made by psychiatrists and was classified as ICD-10 code F41.2. Of the 65 cases, 42 (64.6%) were females and 22 (35.4%) were males. Mean age in cases was  $39.43 \pm 14.05$  (range 18 - 77) years old. Control subjects were randomly selected from the general population of Durango City and matched with cases for age ( $\pm 3$  years) and gender. Inclusion criteria for controls were: 1) subjects of the general population of Durango City without anxiety and depression; 2) aged 18 years and older; and 3) who accepted to participate in the study. Of the 260 controls, 168 (64.6%) were females and 92 (35.4%) were males. Mean age in controls was  $39.45 \pm 13.98$  (range 18 - 78) years old. No statistically significant differences in age ( $P = 0.99$ ) and gender ( $P = 1.0$ ) between cases and controls were found.

### Detection of anti-*T. gondii* IgG and IgM antibodies

A blood sample from each participant was collected. Blood samples were centrifuged and serum samples were obtained and stored at  $-20$  °C until analyzed. Sera were analyzed for anti-*T. gondii* IgG antibodies with the commercially available enzyme immunoassay (EIA) kit “*Toxoplasma* IgG” (Diagnostic Automation/Cortez Diagnostics Inc., Woodland Hills, CA, USA). A cut-off of  $\geq 8$  International Units (IU)/mL of anti-*T. gondii* IgG antibody was used. Sera of patients seropositive for anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* IgM antibodies by the commercially available EIA “*Toxoplasma* IgM” kit (Diagnostic Automation/Cortez Diagnostics Inc.). All IgG and IgM assays were performed following the manufacturer’s instructions.

### Statistical analysis

We used the software Epi Info version 7 and the SPSS version 15.0 (SPSS Inc. Chicago, IL) to perform the statistical analysis. For calculation of the sample size, we used a 95% confidence interval (CI) level, a power of 80%, a 1:4 proportion of cases and controls, a reference seroprevalence of 6.1% [15] as the expected frequency of exposure in controls, and an odds ratio (OR) of 3.5. The result of the sample size calculation was 60 cases and 237 controls. We used the Student’s *t*-test to compare the age among cases and controls. The association of *T. gondii* infection and mixed anxiety and depressive disorder was assessed with the two-tailed Pearson’s Chi-squared test. OR and 95% CI were calculated, and a  $P$  value  $< 0.05$  was considered as statistically significant.

### Ethical aspects

The Ethics Committee of the General Hospital of the Secretary of Health in Durango City, Mexico approved this project. The purpose and procedures of this study were explained to all participants. In addition, a written informed consent was obtained from each participant.

## Results

Fifteen (23.1%) of the 65 patients with mixed anxiety and depressive disorder and 18 (6.9%) of the 260 controls had anti-*T. gondii* IgG antibodies. The difference in seroprevalence of *T. gondii* infection between cases and controls was statistically significant (OR: 4.03; 95% CI: 1.90 - 8.53;  $P < 0.001$ ). Of the 15 anti-*T. gondii* IgG positive cases, five (33.3%) had IgG levels higher than 150 IU/mL, one (6.7%) between 100 and 150 IU/mL, and nine (60.0%) between 8 and 99 IU/mL. In contrast, of the 18 anti-*T. gondii* IgG positive controls, 12 (66.7%) had IgG levels higher than 150 IU/mL, one (5.5%) between 100 and 150 IU/mL, and five (27.8%) between 8 and 99 IU/mL. The frequency of high (> 150 IU/mL) anti-*T. gondii* IgG levels was similar in cases and controls (OR: 0.25; 95% CI: 0.05 - 1.06;  $P = 0.05$ ).

Seroprevalence of *T. gondii* infection was stratified by sex and age (Table 1). We observed that seroprevalence was similar in male cases and male controls ( $P = 1.0$ ); however, seroprevalence was significantly higher in female cases than in female controls (OR: 7.08; 95% CI: 2.83 - 17.67;  $P < 0.00001$ ). Patients aged 31 - 50 years old had a significantly higher seroprevalence of *T. gondii* infection than controls of the same age group (OR: 21.04; 95% CI: 5.22 - 84.80;  $P < 0.00001$ ). Seroprevalence did not vary between cases and controls in the age groups of 30 years and younger, and older than 50 years.

With respect to IgM seropositivity, anti-*T. gondii* IgM antibodies were found in four (26.7%) of the 15 anti-*T. gondii* IgG seropositive cases and in 10 (55.6%) of the 18 anti-*T. gondii* IgG seropositive controls. No statistically significant difference in the frequency of IgM seropositivity among cases and controls was found ( $P = 0.15$ ).

## Discussion

The association of *T. gondii* infection and mixed anxiety and depressive disorder is largely unknown. This case-control seroprevalence study aimed to determine whether *T. gondii* seropositivity is associated with mixed anxiety and depressive disorder in Durango City, Mexico. Results of the present study show that psychiatric patients suffering from mixed anxiety and depressive disorder have a significantly higher frequency of anti-*T. gondii* IgG antibodies than age- and gender-matched control subjects without this disorder of the same city. Remarkably, the seroprevalences found in women (31.0%) and patients aged 31 - 50 years old (38.5%) suffering from mixed anxiety and depressive disorder in our study are very high in Durango City, compared with the 6.1% seroprevalence of *T. gondii* exposure reported in the general population in the same city [15]. To the best of our knowledge, this is the first report of an association of *T. gondii* infection and mixed anxiety and depressive disorder. There are some reports of the association of *T. gondii* infection with either anxiety or depression but not about the association of infection with the presence of both anxiety and depression in the same patients. In a recent study, generalized anxiety disorder was associated with both seroprevalence of *T. gondii* infection and high levels of anti-

*T. gondii* IgG antibodies in subjects drawn from the Detroit Neighborhood Health Study in the USA [14]. In a study of pregnant women, those with serological evidence of *T. gondii* exposure showed positive correlations between IgG levels and the profile of mood scales depression and anxiety subscales [16]. In a study of male rats with experimental infection with *T. gondii*, researchers found that infected rats with *T. gondii* cysts in the forebrain showed an increased anxiety-related behavior [17]. There are conflicting results about the association of *T. gondii* infection and depression. Duffy and coworkers found a relationship between chronic *T. gondii* infection, depression and dysphoric mood in a military veteran women population [13]. On the other hand, in a study in mice, reactivation of chronic *T. gondii* infection by an immunosuppressive regimen caused induction of depressive-like behaviors [18]. In contrast, in a meta-analysis of 50 studies reporting seroprevalence of *T. gondii* infection in any psychiatric disorder compared with control subjects, researchers found no association of anti-*T. gondii* antibodies with major depression [11]. Differences in the finding of an association between *T. gondii* infection and depression could be due to differences in the severity of depression among the studies. Depression in mixed anxiety and depressive disorder is mild. Therefore, it is likely that *T. gondii* infection can be rather associated with mild depression than with severe depression. Further studies including patients suffering from minor and major depression to elucidate the link of *T. gondii* exposure and depression should be conducted. Very little is known about the mechanisms that *T. gondii* uses to cause anxiety and depression. It is well known that *T. gondii* invades the brain [12], and this invasion might result in behavioral changes. Mood disorders associated with *T. gondii* can be due to changes in the concentrations of some neurotransmitters in brain including dopamine and serotonin [19]. In the present study, the association of *T. gondii* infection with mixed anxiety and depressive disorder was related to IgG seropositivity but not to IgM seropositivity. This finding suggests that this disorder is associated with latent infection rather than with acute infection.

We observed that the association of infection with *T. gondii* and mixed anxiety and depressive disorder was influenced by gender and age. A strong association was found in females and in patients aged 31 - 50 years old. It is not clear why the association was influenced by these gender and age groups. Seroprevalence of *T. gondii* infection in Durango is similar in male and females, and increases with age [15]. On the other hand, women suffer from anxiety and depression more frequently than men. In a recent study in a treatment-seeking population, women had a higher prevalence of anxiety disorders than men [20]. Similarly, in the population-based Hispanic Community Health Study/Study of Latinos in the USA, the prevalences of depression and anxiety were higher in women than in men, and in the age group of 45 - 64 years than in the groups of 18 - 44 years and 65 - 74 years [21]. Therefore, the fact that the association of *T. gondii* infection and mixed anxiety and depressive disorder was stronger in women and in people of middle age than in men and in younger and older age groups in our study further supports a contributing role of *T. gondii* infection and this disorder. Our findings have also epidemiological importance; if this association is confirmed in other studies, the ben-



efit of treatment against *T. gondii* infection should be evaluated in patients suffering from anxiety and depressive disorder. Patients with bipolar disorder seropositive to *T. gondii* showed more depressive episodes when treated by drugs without anti-*T. gondii* activity than patients who received drugs with anti-*T. gondii* activity [22].

A limitation of the present study was a small sample size of patients suffering from anxiety and depressive disorder. Further studies should have a larger sample size of patients.

## Conclusions

Results support for the first time an association between seropositivity to *T. gondii* and mixed anxiety and depressive disorder. Further research to confirm this association and to determine the seroepidemiology of *T. gondii* infection in patients with this disorder is needed.

## Conflicts of Interest

The authors declare that no conflict of interest exists.

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