

# Association between *Toxoplasma gondii* infection and history of blood transfusion: a case-control seroprevalence study

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## Abstract

**Objectives:** This study was performed to determine the association between seropositivity to *Toxoplasma gondii* and a history of blood transfusion.

**Methods:** Patients who had undergone blood transfusion (n = 410) and age- and sex-matched controls who had not undergone blood transfusion (n = 1230) were examined for anti-*T. gondii* IgG and IgM antibodies using enzyme-linked immunoassays.

**Results:** Anti-*T. gondii* IgG antibodies were detected in 57 (13.9%) patients and in 129 (10.5%) controls with a borderline difference [odds ratio (OR) = 1.37, 95% confidence interval

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(CI) = 0.98–1.92]. High anti-*T. gondii* IgG antibody levels (>150 IU/mL) were found in 27 (47.4%) of the 57 anti-*T. gondii* IgG-positive patients and in 37 (28.7%) of the 129 anti-*T. gondii* IgG positive controls with a significant difference (OR = 2.23, 95% CI = 1.17–4.26). Anti-*T. gondii* IgM antibodies were found in 13 (22.8%) of the 57 seropositive patients and in 37 (28.7%) of the 129 seropositive controls, but the difference was not significant (OR = 0.73, 95% CI = 0.35–1.52). Seroprevalence was significantly higher in patients aged >50 years than in controls of the same age and in female patients than in female controls.

**Conclusions:** These findings indicate that a history of blood transfusion is a risk factor for *T. gondii* infection.

## Keywords

Seroprevalence, blood transfusion, case-control study, Mexico, *Toxoplasma gondii*, risk factor

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## Introduction

Infection with the parasite *Toxoplasma gondii* is prevalent in humans and animals worldwide.<sup>1,2</sup> Most infections with *T. gondii* are asymptomatic.<sup>3</sup> However, individuals who develop toxoplasmosis, the disease caused by *T. gondii*, may present several well-recognized clinical features: lymphadenopathy, chorioretinitis, and meningoencephalitis.<sup>4,5</sup> In addition, infection with *T. gondii* has been associated with heart disease,<sup>6,7</sup> schizophrenia,<sup>8,9</sup> depression,<sup>10,11</sup> and mixed anxiety and depression.<sup>12</sup> Immunocompromised individuals infected with *T. gondii* may develop disseminated and life-threatening disease.<sup>13</sup> Furthermore, a primary infection with *T. gondii* during pregnancy is a risk factor for infection of the fetus and consequent congenital toxoplasmosis.<sup>14</sup>

Infections with *T. gondii* are usually acquired by ingestion of raw or undercooked meat containing tissue cysts or by ingestion of food or water contaminated with oocysts shed by cats.<sup>15</sup> Transmission of *T. gondii* can also occur by organ transplantation.<sup>16</sup> In addition, blood transfusion has been associated with *T. gondii*

infection.<sup>17,18</sup> After infection, *T. gondii* disseminates from the blood to the host tissues.<sup>19–21</sup> In fact, *T. gondii* parasitemia has been diagnosed by examination of blood smears<sup>22</sup> and by polymerase chain reaction.<sup>23</sup> Infection with *T. gondii* has been detected in blood donors.<sup>24–26</sup> However, the extent of the association between blood transfusion and infection with *T. gondii* remains unclear. To the best of our knowledge, no case-control study has been performed to investigate this issue. Therefore, we sought to determine the association between infection with *T. gondii* and blood transfusion in a sample of people in the northern Mexican city of Durango.

## Materials and methods

### Study design and study population

This case-control seroprevalence study included patients with a history of blood transfusion (cases) and age- and sex-matched subjects without a history of blood transfusion (controls). The cases were enrolled in a public family health care center (Institute of Security and

Social Services of State Workers) in Durango City, Mexico. All participants were examined for the presence of anti-*T. gondii* IgG and IgM antibodies. This study was performed from August 2015 to May 2016 in Durango City, Mexico. The inclusion criteria for the cases were a history of blood transfusion, age of  $\geq 16$  years, and agreement to participate in the study.

### Sample size calculation

We calculated the sample size using a 95% confidence level, a power of 80%, a 1:3 proportion of cases and controls, a reference seroprevalence of 6.1%<sup>27</sup> as the expected frequency of exposure in the controls, and an odds ratio (OR) of 2. The result of this calculation was 295 cases and 883 controls.

### Clinical data of cases

The clinical characteristics of the subjects with a history of blood transfusion were recorded with the aid of a questionnaire. The clinical items were the presence of any illness, frequent headache or dizziness, history of surgery, lymphadenopathy, transplantation, or impairments in memory, reflexes, hearing, or vision.

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

A sample of blood was obtained from each participant. Sera were obtained by centrifugation of whole blood and stored at  $-20^{\circ}\text{C}$  until analyzed. The sera of participants were analyzed for anti-*T. gondii* IgG antibodies. For this purpose, we used the commercially available enzyme immunoassay kit “*Toxoplasma* IgG” (Diagnostic Automation Inc., Woodland Hills, CA, USA). Anti-*T. gondii* IgG antibody levels were expressed as international units (IU)/mL, and a cut-off of  $\geq 8$  IU/mL was used for seropositivity. Sera with anti-*T. gondii* IgG antibodies were further tested

for anti-*T. gondii* IgM antibodies. For this purpose, we used the commercially available enzyme immunoassay “*Toxoplasma* IgM” kit (Diagnostic Automation Inc.). All tests were performed in accordance with the manufacturer’s instructions. We analyzed the positive and negative controls included in the kits in each run.

### Statistical analysis

We statistically analyzed the data using the software SPSS version 15.0 (SPSS Inc. Chicago, IL, USA) and Epi Info version 7 (Centers for Disease Control and Prevention: <http://wwwn.cdc.gov/epiinfo/>). We compared the age of the cases and controls using Student’s *t* test. The frequency of blood transfusion among seropositive and seronegative individuals was assessed with Student *t* test and the Mann–Whitney *U* test. Pearson’s chi-square test was used to determine the association of *T. gondii* seropositivity with transfusional data. The ORs and 95% confidence intervals (CIs) were calculated, and statistical significance was set at a *P* value of  $< 0.05$ .

### Ethics statement

This project was approved by the Institutional Ethics Committee of the General Hospital of the Secretary of Health in Durango City, Mexico. Before enrollment, all participants were informed about the purpose and procedures of this study, and written informed consent was obtained from all subjects.

### Results

This study included 410 cases and 1230 age- and sex-matched controls. The cases comprised 57 male and 353 female patients. This study included a higher number of female than male participants because a history of blood transfusion was more frequently observed in women than in men among the attendees of the family health

care center surveyed. The cases were 16 to 80 years old (mean,  $41.90 \pm 11.58$  years). The controls were randomly selected from the general population in the same city and matched with the cases by sex and age. Thus, the controls included 171 male and 1059 female participants aged 16 to 80 years (mean,  $41.92 \pm 11.55$  years). The age of the controls was not significantly different from that of the cases.

Anti-*T. gondii* IgG antibodies were detected in 57 (13.9%) of the 410 cases and in 129 (10.5%) of the 1230 controls (OR = 1.37, 95% CI = 0.98–1.92,  $P = 0.05$ ). Of the 57 anti-*T. gondii* IgG-positive cases, 27 (47.4%) had anti-*T. gondii* IgG antibody levels of >150 IU/mL and 30 (52.6%) had lower antibody levels. Of the 129 anti-*T. gondii* IgG-positive controls, 37 (28.7%) had anti-*T. gondii* IgG antibody levels of >150 IU/mL and 92 (71.3%) had lower antibody levels. Significantly more subjects had high IgG antibody levels among the cases than controls (OR = 2.23, 95% CI = 1.17–4.2,  $P = 0.01$ ). Of the 57 cases seropositive for anti-*T. gondii* IgG antibodies, 13 (22.8%) were also positive for anti-*T. gondii* IgM antibodies compared with 37 (28.7%) of the 129 controls seropositive to

anti-*T. gondii* IgG antibodies (OR = 0.73, 95% CI = 0.35–1.52).

Stratification by age and sex showed that the seroprevalence of *T. gondii* infection was significantly higher in cases aged  $\geq 51$  years than in controls of the same age and in female cases than in female controls ( $P < 0.05$  for both) (Table 1).

With respect to the number of blood transfusions in the cases, seropositive subjects underwent 1 to 7 transfusions (mean,  $1.87 \pm 1.43$ ; median, 1), whereas seronegative cases underwent 1 to 24 transfusions (mean,  $1.80 \pm 2.09$ ; median, 1). No statistically significant difference in the number of blood transfusions was found between cases and controls (Mann–Whitney *U* test and Student's *t* test).

With respect to the clinical characteristics, the frequency of *T. gondii* seropositivity was similar in cases regardless of the presence of any illness, frequent headache, history of surgery, lymphadenopathy, transplantation, or impairments in memory, reflexes, hearing, or vision. Cases with dizziness had a higher seroprevalence of *T. gondii* infection than cases without this clinical characteristic [30/168 (17.9%) vs 27/242 (11.2%), respectively; borderline significance:  $P = 0.05$ ].

**Table 1.** Comparison of seroprevalence of *Toxoplasma gondii* infection between cases and controls according to age and sex.

Characteristics	Cases			Controls			P value
	Subjects tested	Seroprevalence of <i>T. gondii</i> infection		Subjects tested	Seroprevalence of <i>T. gondii</i> infection		
	n	n	%	n	n	%	
Age, years							
$\leq 30$	72	9	12.5	210	22	10.5	0.63
31–50	236	28	11.9	716	79	11.0	0.72
$> 50$	102	20	19.6	304	28	9.0	0.005
Sex							
Male	57	7	12.3	171	29	17.0	0.40
Female	353	50	14.2	1059	100	9.4	0.01

## Discussion

After infection, *T. gondii* enters the bloodstream and disseminates into the infected host.<sup>28</sup> Experiments in mice infected with *T. gondii* have shown that intracellular tachyzoites in the blood circulation mainly disseminate throughout the body, while extracellular tachyzoites hardly contribute to parasite dissemination.<sup>29</sup> Therefore, the presence of *T. gondii* in blood can be a source of infection for patients receiving a blood transfusion and for laboratory workers, physicians, and nurses handling blood. However, this risk factor for *T. gondii* infection has been neglected. In fact, screening for *T. gondii* infection is not currently performed in blood banks. Epidemiological studies have shown considerable numbers of blood donors infected with *T. gondii*. For instance, a 7.4% to 13.5% seroprevalence of *T. gondii* infection in blood donors has been reported in Mexico<sup>25,26</sup> and Taiwan<sup>30</sup>; a 19.5% to 28.8% seroprevalence has been reported in Turkey,<sup>31</sup> India,<sup>32</sup> and Iran<sup>33</sup>; and a 59.6% and 79.0% seroprevalence has been reported in Egypt<sup>34</sup> and Brazil,<sup>35</sup> respectively. Although the current epidemiological knowledge suggests that *T. gondii* can be transmitted by blood transfusion, the magnitude of the association between *T. gondii* infection and blood transfusion has been poorly studied. To the best of our knowledge, no age- and sex-matched case-control study on the association of blood transfusion and infection with *T. gondii* has been performed. Therefore, in the present study, we aimed to determine this association in subjects from the northern Mexican city of Durango. In general, we found a slightly higher seroprevalence of *T. gondii* infection in subjects with than without a history of blood transfusion. The association between infection and blood transfusion was of borderline statistical significance ( $P=0.05$ ); however, stratification by age and sex showed a significant

association between *T. gondii* infection and blood transfusion in subjects aged >50 years and in females. In addition, the frequency of high IgG antibody levels was significantly higher in subjects with than without a history of transfusion. Therefore, these results suggest that infection with *T. gondii* is associated with blood transfusion. Why older and female cases had a higher seroprevalence than their matched controls remains unclear. Older and female cases likely had a higher rate of exposure to *T. gondii* than their controls. Why cases had a higher frequency of high IgG levels than their matched controls also remains unclear. High levels of specific anti-*T. gondii* IgG antibodies can be present in patients with recent infections.<sup>36</sup> In addition, high IgG levels may persist due to frequent contact with the parasite. We arbitrarily considered high levels of anti-*T. gondii* IgG antibodies when an IgG titer of >150 IU/mL was found. This category was selected because it is higher than the highest calibrator (150 IU/mL) of IgG included in the diagnostic kit used. The results of the present study, including the higher seroprevalence of *T. gondii* infection and higher frequency of high anti-*T. gondii* IgG antibody levels in cases than controls, suggest that *T. gondii* infection can be transmitted by blood transfusion in Durango, Mexico.

We selectively tested only anti-*T. gondii* IgG antibody-positive samples for anti-*T. gondii* IgM antibodies because anti-*T. gondii* IgG seropositivity appears early after infection.<sup>37</sup> This fact supports the use of this marker in epidemiological studies; a single IgM result without an IgG result has limited value for the diagnosis of *T. gondii* infection because IgM enzyme immunoassays to detect anti-*T. gondii* IgM antibodies yield a high frequency of false-positive results.<sup>38,39</sup>

The present study has some limitations. First, we studied the association of *T. gondii* infection with a history of blood

transfusion, but we did not determine the association between acute *T. gondii* infection and recent blood transfusion. Second, the median number of blood transfusions per individual was low; therefore, the association between *T. gondii* infection and a high median number of transfusions remains unclear. Third, we did not control for confounding factors. Finally, we used controls from the general population rather than controls from the same family health care center surveyed.

## Conclusions

We conclude that individuals with a history of blood transfusion have a higher risk of infection with *T. gondii* than those without a history of blood transfusion. To the best of our knowledge, no previous age- and sex-matched case-control study of the association of infection with *T. gondii* and blood transfusion has been performed. Further studies to determine the magnitude of this epidemiological association should be conducted.


## Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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