Concordance of *Toxoplasma gondii* Infection Markers Among Heterosexual Couples

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Abstract

Background: Very little is known about concordance of *Toxoplasma gondii* (*T. gondii*) infection markers among couples. Through a cross-sectional study, we sought to determine the correlation of *T. gondii* infection in a sample of 119 heterosexual couples in Durango State, Mexico.

Methods: Participants were examined for the presence of anti-*T. gondii* IgG and IgM antibodies using enzyme-linked immunoassays. IgG and IgM seropositive couples were further analyzed for the presence of *T. gondii* DNA by using polymerase chain reaction (PCR).

Results: Anti-*T. gondii* IgG antibodies were found in 71 (59.7%) men and in 63 (52.9%) women (odds ratio (OR) = 1.31; 95% confidence interval (CI): 0.78 - 2.19; P = 0.29). Of the 71 seropositive men, 40 (56.3%) had a seropositive couple; in contrast, of the 63 seropositive women, 40 (63.5%) had a seropositive couple (OR = 0.74; 95% CI: 0.37 - 1.48; P = 0.39). In total, 65 (54.6%) couples had concordant results (both IgG positive or both IgG negative), and 54 (45.4%) had discordant results (a seropositive man with a seronegative woman, or a seropositive woman with a seronegative man) (kappa index = 0.08; 95% CI: -0.09 - 0.26). With respect to high (> 150 IU/mL) levels of anti-*T. gondii* IgG antibodies, 79 (66.4%) of the 119 couples had concordant results and 40 (33.6%) had discordant results (kappa index = -0.15; 95% CI: -0.03 - 0.33). Forty couples were positive for anti-*T. gondii* IgM antibodies. Of them, 21 (52.5%) had concordant results, and 19 (47.5%) had discordant results (kappa index = -0.01; 95% CI: -0.39 - 0.28). Concerning PCR, eight (50%) of 16 couples were positive for *T. gondii* DNA. Of them, 11 (68.8%) had concordant results, and five (31.2%) had discordant results (kappa index = 0.31; 95% CI:

-0.17 - 0.79).

Conclusions: Results suggest a poor concordance of serological and molecular markers of *T. gondii* infection among heterosexual couples. Further studies to confirm our results should be conducted.

Keywords: *Toxoplasma gondii*; Infection markers; Seroprevalence; Couples; Concordance; Epidemiology; Mexico

Introduction

The parasite *Toxoplasma gondii* (*T. gondii*) causes infections in humans and animals all around the world [1]. The disease caused by *T. gondii* is called toxoplasmosis, and it is characterized by the presence of chorioretinitis, lymphadenopathy, or meningoencephalitis [2]. A severe and life-threatening toxoplasmosis may occur in immunocompromised individuals infected with *T. gondii* [3]. Furthermore, infection with *T. gondii* in susceptible pregnant women may result in fetal infection leading to congenital toxoplasmosis [4]. Major routes for *T. gondii* infection include ingestion of food or water contaminated with oocysts shed by cats [2, 5], and by ingestion of raw or undercooked meat containing tissue cysts [2, 6]. Less frequently, *T. gondii* infection may be acquired by blood transfusion [7], and organ transplantation [8].

It is unclear whether infection with *T. gondii* may be sexually transmitted. Studies by Janitschke and Nurnberger in humans and animals showed that sexual transmission of *T. gondii* did not have epidemiological significance [9]. In contrast, Flegr et al have hypothesized that toxoplasmosis can be a sexually transmitted infection [10]. Light-microscopic evidence of *T. gondii* in testes of autopsy cases of patients with acquired immunodeficiency syndrome and systemic opportunistic infections was provided [11]. In a study of psychiatric patients, sexual promiscuity was shown to be associated with *T. gondii* infection [12]. In addition, a high seroprevalence of *T. gondii* infection was found in female sex workers in an age- and gender-matched case-control study [13]. In a recent study, Singh et al found a significantly higher prevalence of toxoplasmosis in married women than in unmarried women [14]. On the other hand, experimental infections with *T. gondii* in animals have shown conflicting results on sexual transmission. Lopes et al

demonstrated sexual transmission of *T. gondii* in sheep with vertical transmission to their lambs [15]. In contrast, experiments in mice showed that toxoplasmosis could not be transmitted to female mice and their offspring due to mating with infected male mice [16]. We are aware of only one study that determined the seroconcordance of *T. gondii* infection in human couples. In an article published in 1969, Price reported a poor seroconcordance of anti-*T. gondii* antibodies among 43 married couples using the dye test [17]. In the present study, we sought to determine the correlation of *T. gondii* infection among heterosexual couples in Durango, Mexico using serological and molecular methods.

Materials and Methods

Study design and couples studied

Through a cross-sectional study design, 119 heterosexual couples who were either married or living together were examined. All participants were inhabitants of the rural San Dimas Municipality, in Durango State, Mexico. Couples were enrolled consecutively when attending medical consultations provided by a rural mobile medical unit of the Secretary of Health. Couples were enrolled in the study from December 2015 to August 2016. Inclusion criteria were couples either married or living together, each participant aged 18 years and older, and who accepted to participate in the study. Participants were 15 - 80 (mean: 41.1 ± 14.1) years old. Men were 20 - 78 (mean: 43.36 ± 14.2) years old, whereas women were 15 - 80 (mean: 38.6 ± 13.6) years old. Duration of cohabitation in the 119 couples studied was: less than 3 years in 31 (26.1%), between 3 and 6 years in 48 (40.3%), and more than 6 years in 40 (33.6%).

Detection of *T. gondii* IgG and IgM antibodies

Participants were firstly examined for the presence of anti-*T. gondii* IgG and those who were positive for this test were further examined for IgM antibodies. Sera of 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). Levels of anti-*T. gondii* IgG antibody were expressed as International Units (IU)/mL, and a cutoff ≥ 8 IU/mL was considered for seropositivity. Anti-*T. gondii* IgM antibodies were detected by the commercially available enzyme immunoassay "*Toxoplasma* IgM" kit (Diagnostic Automation Inc.). All assays were performed following the instructions of the manufacturer. Positive and negative controls included in the kits were run in each assay.

DNA extraction and detection of *T. gondii* DNA

Couples with *T. gondii*-specific IgG and IgM antibodies by enzyme immunoassay were further examined for *T. gondii* DNA by nested-polymerase chain reaction (PCR). DNA ex-

traction from whole blood of seropositive participants was performed following a protocol described elsewhere (http://www.protocol-online.org/prot/Protocols/Rapid-Extraction-of-High-Quality-DNA-from-Whole-Blood-Stored-at-4-C-for-Long-Period-4175.html). Amplification of DNA was carried out with primers directed against the B1 gene of *T. gondii* and following the protocol described by Roth et al [18]. Amplification products were run in a 2% agarose gel electrophoresis, then stained with ethidium bromide, and visualized with an ultraviolet transilluminator.

Statistical analysis

We performed the statistical analysis with the software SPSS version 15.0 (SPSS Inc. Chicago, IL). We measured the agreement between seropositivity rates among couples by the Cohen test using the kappa index. Statistical significance was set at a P value < 0.05.

Ethics statement

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

Results

In the 119 couples studied, anti-*T. gondii* IgG antibodies were found in 71 (59.7%) men and in 63 (52.9%) women (odds ratio (OR) = 1.31; 95% confidence interval (CI): 0.78 - 2.19; P = 0.29). Of the 71 seropositive men, 40 (56.3%) had a seropositive couple; in contrast, of the 63 seropositive women, 40 (63.5%) had a seropositive couple (OR = 0.74; 95% CI: 0.37 - 1.48; P = 0.39). Thus, in 40 (33.6%) of the 119 couples, both men and women were seropositive for anti-*T. gondii* IgG antibodies. Whereas in 25 (21%) of the 119 couples studied, both men and women were seronegative for anti-*T. gondii* IgG antibodies. In total, of the 119 couples, 65 (54.6%) had concordant results (both IgG positive or both IgG negative), and 54 (45.4%) had discordant results (a seropositive man with a seronegative woman, or a seropositive woman with a seronegative man). Analysis of concordance of IgG seropositivity among couples showed a poor agreement (kappa index = 0.08; 95% CI: -0.09 - 0.26) (Table 1). The frequency of anti-*T. gondii* IgG antibodies was similar (P = 0.89) in couples with less than 3 years of relationship (14/31, 45.2%) to those with 3 - 6 years (9/48, 18.8%) or with more than 6 years (17/40, 42.5%).

Of the 119 couples studied, 52 (43.7%) had at least one member with high (> 150 IU/mL) anti-*T. gondii* IgG antibody levels. In 12 couples both members had high antibody levels, whereas in 67 both members did not have high antibody levels. Because of this increase in the number (n = 67) of couples with antibody levels equal to or lower than 150 IU/mL,

Table 1. Extent of Agreement of Seropositivity to Anti-*T. gondii* IgG Antibodies Among Couples

Men	Women		Total	Kappa index	95% CI	P-value
	Exposed (*T. gondii* IgG positive)	Unexposed (*T. gondii* IgG negative)				
Exposed (*T. gondii* IgG positive)	40	31	71			
Unexposed (*T. gondii* IgG negative)	23	25	48			
Total	63	56	119	0.08	-0.09 - 0.26	0.36

Table 2. Extent of Agreement of High (> 150 IU/mL) Anti-*T. gondii* IgG Antibody Levels Among Couples

Men	Women		Total	Kappa index	95% CI	P-value
	Exposed (high levels of *T. gondii* IgG)	Unexposed (no high levels of *T. gondii* IgG)				
Exposed (high levels of *T. gondii* IgG)	12	16	28			
Unexposed (no high levels of *T. gondii* IgG)	24	67	91			
Total	36	83	119	0.15	-0.03 - 0.33	0.09

the rate of concordance increased. Thus, 79 (66.4%) of the 119 couples had concordant results (both with high antibody levels or both with lower antibody levels or seronegative). Whereas 40 (33.6%) of the 119 couples had discordant results (a man with high antibody levels with a woman with lower antibody levels or seronegative; or a woman with high antibody levels with a man with lower antibody levels or seronegative). Analysis of concordance of high IgG antibody levels among couples showed a poor agreement (kappa index = -0.15; 95% CI: -0.03 - 0.33) (Table 2). The frequencies of high anti-*T. gondii* IgG antibody levels were similar (P = 0.49) in couples with less than 3 years of relationship (6/31, 19.4%) and those with 3 - 6 years (10/48, 20.8%) or with more than 6 years (12/40, 30.0%).

Anti-*T. gondii* IgM antibodies were found in 51 (38.1%) of the 134 individuals with anti-*T. gondii* IgG antibodies. Of them, 28 (54.9%) were found in men and 23 (45.1%) in women. In total, one or two members of 40 couples were positive for anti-*T. gondii* IgM antibodies. Of these 40 seropositive couples, 21 (52.5%) had concordant results (both IgM positive or both IgM negative), and 19 (47.5%) had discordant results (a seropositive man with a seronegative woman, or a seropositive woman with a seronegative man). Analysis of concordance of IgM seropositivity among IgG seropositive couples showed a poor agreement (kappa index = -0.01; 95% CI: -0.39 - 0.28) (Table 3). Among the 40 IgG seropositive couples, the frequency of anti-*T. gondii* IgM antibodies was similar (P =

0.77) in couples with less than 3 years of relationship (5/14, 35.7%) to those with 3 - 6 years (4/9, 44.4%) or with more than 6 years (7/17, 41.2%).

Detection of *T. gondii* DNA by PCR was performed in the 16 couples with anti-*T. gondii* IgM antibodies. *T. gondii* DNA was found in a man or a woman or both in eight (50%) of these 16 couples. Of them, 11 (68.8%) had concordant results (both PCR positive or both PCR negative), and five (31.2%) had discordant results (a PCR positive man with a PCR negative woman, or a PCR positive woman with a PCR negative man). Analysis of concordance of *T. gondii* DNA positivity among these couples showed a fair agreement (kappa index = 0.31; 95% CI: -0.17 - 0.79).

Discussion

Whether *T. gondii* infection can be transmitted among couples is poorly understood. Therefore, we sought to determine the concordance of *T. gondii* infection markers among heterosexual couples. We found poor agreements in seropositivity of anti-*T. gondii* IgG antibodies (Cohen's kappa = 0.08), in high anti-*T. gondii* IgG antibody levels (Cohen's kappa = -0.15), and in seropositivity of anti-*T. gondii* IgM antibodies (Cohen's kappa = -0.01) among couples. In addition, a fair agreement in positivity to *T. gondii* DNA among IgM seropositive cou-

Table 3. Extent of Agreement of Anti-*T. gondii* IgM Antibodies in Anti-*T. gondii* IgG Seropositive Couples

Men	Women		Total	Kappa index	95% CI	P-value
	Exposed (Anti-*T. gondii* IgM positive)	Unexposed (Anti-*T. gondii* IgM negative)				
Exposed (anti-*T. gondii* IgM positive)	16	7	23			
Unexposed (anti-*T. gondii* IgM negative)	12	5	17			
Total	28	12	40	-0.01	-0.39 - 0.28	0.94

ples was found (kappa index = 0.31; 95% CI: -0.17 - 0.79). Thus, we found a poor concordance of serological (presence of IgG, high levels of IgG, and presence of IgM) and molecular (PCR) markers of *T. gondii* infection among couples. None of the serological markers correlated with the duration of the relationship among couples. Our results suggest that clustering of infection with *T. gondii* among couples within the community studied may not be attributable to intrafamilial transmission. Several routes for *T. gondii* infection might occur in people living in the same home including cleaning cat feces, contact with soil, and consumption of water or food. In addition, heterosexual intercourse might be an additional intrafamilial route for *T. gondii* infection. The lack of concordance in markers of *T. gondii* infection among couples was unexpected because several previous observations suggest a likely sexual transmission of *T. gondii* infection. For instance, a light-microscopic evidence of testicular infection with *T. gondii* in autopsy cases with acquired immunodeficiency syndrome was provided [11]. It is likely that the presence of *T. gondii* in testes occur only in immunocompromised individuals. In the present study, apparently healthy individuals were enrolled and *T. gondii*-infected subjects were likely immunocompetent and perhaps no parasite dissemination to genital organs occurred. In a recent age- and gender-matched case-control study, a high rate of *T. gondii* infection was found in female sex workers [13]. It is possible that a higher frequency of sexual contacts and diversity of partners in female sex workers than those in women of the present study might account for an increase in the likelihood for a *T. gondii* sexual transmission. In another study, married women had a higher seroprevalence of *T. gondii* infection than single women in India [14]. However, researchers found an increase of seroprevalence with age in the studied women [14]. Perhaps differences in ages might explain differences in seroprevalences between married and single women. Married women could be older than single women and further studies, i.e. matched case-control studies, may confirm an association between *T. gondii* exposure and a married status. A hypothesis was made recently that toxoplasmosis is transmitted from infected men to non-infected women during unprotected sexual intercourse [10]. Results of the present study cannot support this hypothesis because no agreement in serological and molecular markers of *T. gondii* infection among couples was found. In addition, no difference in seroprevalence among men and women was found. In a further study, infection with *T. gondii* was associated with sexual promiscuity in psychiatric patients [12]. It is possible that, as occurs in female sex workers, a higher frequency of sexual contacts and diversity of partners in psychiatric patients with sexual promiscuity than in subjects of the present study might account for an increase in the likelihood for a sexual transmission of *T. gondii*. In a study in animals, *T. gondii* was isolated from semen of *T. gondii* infected sheep and researchers demonstrated a sexual transmission of *T. gondii* in sheep with consequent vertical transmission to their lambs [15]. In contrast, a study in mice found a lack of transmission of *T. gondii* to female mice and their offspring due to mating [16]. In addition, in a study using a mouse inoculation test, *T. gondii* could not be cultured from ejacula and biopsies specimens of the testicles of men from childless marriages [9].

This study has some limitations. The sample size is rela-

tively small, and we studied people from only one community. No genotyping of *T. gondii* DNA was performed. Further studies should include a larger sample size of couples, of several communities, and perform genotyping of *T. gondii* DNA.

We conclude that there is poor concordance of serological and molecular markers of *T. gondii* infection among heterosexual couples in the northern Mexican state of Durango. Further studies to confirm our results should be conducted.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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