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Original Article

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Association Between *Toxoplasma gondii* Exposure and Heart Disease: A Case-Control Study

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Abstract

Background: The parasite *Toxoplasma gondii* causes infections all around the world. Infections with *T. gondii* are systemic and the parasite can persist in the heart muscle. Very little is known about the impact of *T. gondii* on patients with heart disease. We determined the association between *T. gondii* exposure and patients suffering from heart diseases attending in a public hospital in Durango, Mexico; the association of *T. gondii* exposure with socio-demographic, behavioral, and clinical characteristics of these patients was also investigated.

Methods: Through a case-control study, we examined the seroprevalence of anti-*T. gondii* IgG and IgM antibodies in 400 patients with heart diseases and 400 age- and gender-matched controls using enzyme-linked immunoassays. In addition, we analyzed the association of patient characteristics as determined by a standardized questionnaire with *T. gondii* exposure by bivariate and multivariate analyses.

Results: Fifty-five (13.8%) of 400 patients and 32 (8.0%) of 400 controls had anti-*T. gondii* IgG antibodies (odds ratio (OR) = 1.83; 95% confidence interval (CI): 1.15 - 2.90; P = 0.01). High anti-*T. gondii* IgG levels (> 150 IU/mL) were found in 28 (50.9%) of the 55 positive cases and in 14 (43.8%) of the 32 positive controls (P = 0.51). Anti-*T. gondii* IgM antibodies were found in 13 (23.6%) of the 55 anti-*T. gondii* IgG positive patients and in 19 (59.4%) of 32 anti-*T. gondii* IgG positive controls (OR = 0.21; 95% CI: 0.08 - 0.54; P = 0.0008). Multivariate analysis showed that *T. gondii* exposure was positively associated with being born out of Durango State (OR = 2.93; 95% CI: 1.40 - 6.13; P = 0.004), and with consumption of alcohol (OR = 2.04; 95% CI: 1.01 - 4.12; P = 0.04).

Conclusions: Results obtained in this study indicate that *T. gondii* infection is associated with heart disease, and suggest that heart disease might be related with a chronic infection. This is the first report of an association of *T. gondii* exposure with alcohol consumption in this population. Results warrant for further research to determine the epidemiological impact of *T. gondii* exposure on patients with heart diseases. Risk factors associated with *T. gondii* exposure are critical to design future prevention strategies against *T. gondii* exposure.

Keywords: *Toxoplasma gondii*; Seroprevalence; Heart disease; Case-control study; Epidemiology; Alcohol

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Introduction

The protozoan parasite *Toxoplasma gondii* causes infections all around the world [1]. Nearly one-third of the world population is infected with *T. gondii* [2]. Most infections with *T. gondii* occur by ingestion of food or water contaminated with oocysts shed by cats [3, 4] and eating undercooked or raw meat containing tissue cysts [3, 5]. The clinical spectrum of *T. gondii* infection varies from asymptomatic to severe systemic disease [3]. Most commonly, toxoplasmosis is a mild disease with lymphadenopathy. However, some *T. gondii*-infected individuals develop chorioretinitis that may progress to blindness [6]. Immunocompromised patients infected with *T. gondii* may develop severe neurological disease [7, 8]. In addition, primary infections with *T. gondii* during pregnancy may lead to congenital disease [3, 8]. Infections with *T. gondii* may manifest in the heart in humans [9-13] and animals [14-17] with myocarditis [18-20], pericarditis with myocarditis [21, 22], and acute heart failure [23, 24]. Patients with *T. gondii* myocarditis may present with pericardial effusion, constrictive pericarditis, congestive heart failure, and arrhythmias [11].

The seroepidemiology of infection with *T. gondii* in patients suffering from heart diseases has been poorly studied. We are not aware of any data about the epidemiology of *T. gondii* infec-

Table 1. Socio-Demographic Characteristics of Patients With Heart Diseases and Seroprevalence of *T. gondii* Infection

Characteristic	No. of subjects tested	Prevalence of *T. gondii* infection		P value
		No.	%	
Age groups (years)				
30 or less	13	2	15.4	0.92
31 - 50	103	13	12.6	
51 - 70	284	40	14.1	
Gender				
Male	156	23	14.7	0.64
Female	244	32	13.1	
Birth place				
Durango State	333	39	11.7	0.008
Other Mexican State	67	16	23.9	
Residence place				
Durango State	395	54	13.7	0.52
Other Mexican State	5	1	20.0	
Residence area				
Urban	294	43	14.6	0.55
Suburban	79	10	12.7	
Rural	27	2	7.4	
Educational level				
No education	37	8	21.6	0.06
1 - 6 years	151	25	16.0	
7 - 12 years	140	18	12.9	
> 12 years	72	4	5.6	
Occupation				
Laborer[a]	183	23	12.6	0.52
Non-laborer[b]	217	32	14.7	
Socio-economic level				
Low	19	5	26.3	0.22
Medium	379	50	13.2	
High	2	0	0.0	

[a]Agriculture, business, employee, cattle raising, factory worker, professional, other. [b]Housewife, student, or none occupation.

tion in these patients in Mexico. Therefore, we determined the association between *T. gondii* exposure and patients with heart disease attending in a public hospital in northern Mexico, and the association of seropositivity to *T. gondii* with socio-demographic, behavioral, and clinical characteristics of these patients.

Methods

Study design and study population

Through a case-control study, we enrolled 400 patients suffering from heart diseases attending in a public Hospital in Durango City, Mexico and 400 control subjects without heart diseases of the same city. All heart patients were enrolled from June to November 2014. Inclusion criteria for the cases were: 1) inpatients with heart disease attending in the Cardiology Department at the General Hospital of the Secretary of Health in Durango City; 2) aged 11 years and older; and 3) that voluntarily accepted to participate. Control subjects were randomly selected and were matched with cases by age and gender. Inclusion criteria for the control subjects were: 1) people without heart diseases from the general population of Durango City; and 2) who voluntarily accepted to participate in the study. Patients included 156 (39%) males and 244 (61%) females with a mean age of 58.87 ± 14.59 years (range 11 - 93 years). Controls included 156 males and 244 females with a mean age

Table 2. Correlation of *T. gondii* Seroprevalence and Clinical Characteristics of Patients With Heart Diseases

Characteristic	No. of patients tested	Prevalence of *T. gondii* infection		P value
		No.	%	
Hypertensive disease				
Yes	249	31	12.4	0.33
No	151	24	15.9	
Ischemic disease				
Yes	156	26	16.7	0.17
No	244	29	11.9	
Valvulopathy				
Yes	19	1	5.3	0.49
No	381	54	14.2	
Myocardiopathy				
Yes	19	2	10.5	1.00
No	381	53	13.9	
Electrical heart disease				
Yes	75	12	16.0	0.53
No	325	43	13.2	
Congestive heart failure				
Yes	27	4	14.8	0.77
No	373	51	13.7	
Congenital heart disease				
Yes	1	0	0	1.00
No	399	55	13.8	
Pericardial disease				
Yes	17	2	11.8	1.00
No	383	53	13.8	
Pulmonary heart disease				
Yes	2	0	0.0	1.00
No	398	55	13.8	
Functional classification				
I	252	35	13.9	0.78
II	110	14	12.7	
III	35	5	14.3	
IV	3	1	33.0	
Response to treatment				
Good	309	45	14.6	0.64
Regular	74	8	10.8	
Bad	11	2	18.2	
Memory impairment				
Yes	235	37	15.7	0.17
No	164	18	11	
Blood transfusion				
Yes	87	16	18	0.15
No	312	39	12.5	

of 58.76 ± 14.54 years (range 9 - 91). Age was comparable between cases and controls (P = 0.91).

Ethical aspects

This study was approved by the Ethical Committee of the General Hospital of the Secretary of Health in Durango City, Mexico. The purpose and procedures of the study were explained to all patients. Participation in the study was voluntary. A written informed consent was obtained from all participants and from the next of kin of minor participants.

Sample size

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% [25] as the expected frequency of exposure in controls, and an odds ratio (OR) of 2.1. The result of the sample size calculation was 370 cases and 370 controls.

Socio-demographic, clinical, and behavioral characteristics of patients

The socio-demographic, clinical, and behavioral characteristics of the patients were obtained with the aid of a standardized questionnaire. Socio-demographic data included age, sex, birthplace, residence, educational level, occupation, and socioeconomic status. Clinical data included diagnosis of the heart disease, evolution time (years) of the heart disease, functional classification of the heart disease, and response to treatment. To evaluate the functional class of heart disease, we used the criteria of the New York Heart Association [26]. In addition, we obtained clinical data about the presence of underlying diseases, history of lymphadenopathy, surgery, blood transfusion or transplants, presence of frequent headaches, dizziness, and impairments of memory, reflexes, hearing, and vision. Obstetric data in women were also obtained. Behavioral data included contact with animals, cleaning cat excrement, foreign traveling, consumption of meat (pork, beef, goat, lamb, boar, chicken, turkey, pigeon, duck, rabbit, venison, squirrel, horse, opossum, or other), frequency of meat consumption, consumption of raw or undercooked meat, unpasteurized milk, dried or processed meat (ham, sausages or chorizo), consumption of unwashed raw vegetables or fruits, untreated water, frequency of eating away from home (in restaurants or fast food outlets), contact with soil (gardening or agriculture), hand washing before eating, and type of flooring at home.

Detection of *T. gondii* antibodies

A blood sample (about 3 mL) was obtained from each participant. Blood was centrifuged and serum samples were obtained. Serum samples were kept frozen at -20 °C until analyzed. Sera were analyzed for anti-*T. gondii* IgG antibodies with a commercially available enzyme immunoassay "*Toxoplasma* IgG" (Diagnostic Automation Inc., Calabasas, CA, USA). Anti-*T. gondii* IgG antibody levels were expressed as International Units (IU)/mL. A cut-off of ≥ 8 IU/mL was used for seropositivity. In addition, sera of patients positive for anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* IgM antibodies by a commercially available enzyme immunoassay "*Toxoplasma* IgM" kit (Diagnostic Automation Inc., Calabasas, CA, USA). All assays were performed following the manufacturer's instructions. We included positive and negative controls in each run.

Statistical analysis

The statistical analysis was performed with the software: Epi Info version 7 and SPSS version 15.0. We used the Student's *t*-test to compare age among cases and controls. The Pearson's Chi-square test and the Fisher exact test (when values were small) were used for comparison of the frequencies among groups. Multivariate analysis was used to assess the association between the characteristics of the patients and the seropositivity to anti-*T. gondii* antibodies. Socio-demographic and behavioral characteristics of the patients were included in the multivariate analysis if they had a P value ≤ 0.20 in the bivariate analysis. OR and 95% confidence interval (CI) were calculated by multivariate analysis with the Enter method. A P value < 0.05 was considered statistically significant.

Results

Anti-*T. gondii* IgG antibodies were detected in 55 (13.8%) of 400 patients with heart diseases and in 32 (8.0%) of 400 controls. Seroprevalence of anti-*T. gondii* IgG antibodies was significantly higher in cases than in controls (OR = 1.83; 95% CI: 1.15 - 2.90; P = 0.01). High anti-*T. gondii* IgG levels (> 150 IU/mL) were found in 28 (50.9%) of the 55 positive cases and in 14 (43.8%) of the 32 positive controls (P = 0.51). Anti-*T. gondii* IgM antibodies were found in 13 (23.6%) of the 55 anti-*T. gondii* IgG positive patients and in 19 (59.4%) of 32 anti-*T. gondii* IgG positive controls. Seroprevalence of anti-*T. gondii* IgM antibodies was significantly lower in cases than in controls (OR = 0.21; 95% CI: 0.08 - 0.54; P = 0.0008).

General socio-demographic characteristics of the 400 patients studied and their correlation with *T. gondii* seroprevalence are shown in Table 1. Seroprevalence of *T. gondii* infection was not associated with age, sex, residence, educational level, occupation or socioeconomic level of the patients by bivariate analysis. In contrast, patients born out of Durango State had a significantly higher seroprevalence of *T. gondii* infection than patients born in Durango State (P = 0.008).

With respect to the clinical characteristics, the variables "ischemic disease", "memory impairment", and "blood transfusion" showed P values ≤ 0.20 by bivariate analysis. Whereas other diagnoses of heart disease, functional stages of heart disease, response to treatment, presence of underlying diseases,

Table 3. Bivariate Analysis of Selected Putative Risk Factors for Infection With *T. gondii* in Patients With Heart Disease

Characteristic	No. of subjects tested	Prevalence of *T. gondii* infection		P value
		No.	%	
Raising farm animals				
Yes	137	15	10.9	0.24
No	263	40	15.2	
National trips				
Yes	310	47	15.2	0.12
No	90	8	8.9	
Squirrel meat consumption				
Yes	51	11	21.6	0.08
No	349	44	12.6	
Opossum meat consumption				
Yes	11	4	36.4	0.05
No	389	51	13.1	
Armadillo meat consumption				
Yes	11	3	27.3	0.18
No	389	52	13.4	
Iguana meat consumption				
Yes	10	3	30.0	0.14
No	390	52	13.3	
Snake meat consumption				
Yes	83	15	18.0	0.19
No	317	40	12.6	
Frequency of meat consumption				
Never	2	1	50.0	0.16
Up to three times a week	315	46	14.6	
4 - 7 times a week	83	8	9.6	
Degree of meat cooking				
Undercooked	16	5	31.3	0.05
Well done	384	50	13.0	
Unwashed raw vegetables				
Yes	46	9	19.6	0.22
No	354	46	13.0	
Unwashed raw fruits				
Yes	55	12	21.8	0.06
No	345	43	12.5	
Untreated water				
Yes	180	21	11.7	0.27
No	220	34	15.5	
Frequency of eating out of home				
Never	16	5	31.3	0.07
1 - 10 times a year	153	17	11.1	
> 10 times a year	231	33	14.3	
Alcohol				

Table 3. Bivariate Analysis of Selected Putative Risk Factors for Infection With *T. gondii* in Patients With Heart Disease - *(continued)*

Characteristic	No. of subjects tested	Prevalence of *T. gondii* infection		P value
		No.	%	
Yes	86	18	20.9	0.02
No	314	37	11.8	
Floor at home				
Ceramic or wood	226	34	15.0	0.20
Concrete	158	17	10.8	
Soil	16	4	25.0	

history of lymphadenopathy, surgery or transplants, presence of frequent headaches, dizziness, and impairments of memory, reflexes, hearing, and vision showed P values > 0.20 by bivariate analysis. A selection of clinical characteristics of the patients and their correlation with *T. gondii* seroprevalence is shown in Table 2. In female patients, seropositivity to *T. gondii* did not correlate with obstetric history.

Among the behavioral characteristics, a number of variables showed P values ≤ 0.20 in the bivariate analysis including national trips (P = 0.12), consumption of meat from squirrel (P = 0.08), opossum (P = 0.05), armadillo (P = 0.18), iguana (P = 0.14), and snake (P = 0.19), degree of meat cooking (P = 0.05), consumption of unwashed raw fruits (P = 0.06), frequency of eating out of home (P = 0.07), frequency of meat consumption (P = 0.16), and alcohol consumption (P = 0.02). A selection of behavioral characteristics of the patients and their correlation with *T. gondii* seroprevalence are shown in Table 3. Other behavioral characteristics of patients including contact with cats, cleaning cat excrement, raising animals, consumption of meat other than those from squirrel, opossum, armadillo, iguana and snake, consumption of unpasteurized milk, processed meat, unwashed raw vegetables, and untreated water, and contact with soil showed P values > 0.20 in the bivariate analysis. Further analysis by using logistic regression showed that *T. gondii* exposure was positively associated with being born out of Durango State (OR = 2.93; 95% CI: 1.40 - 6.13; P = 0.004), and with consumption of alcohol (OR = 2.04; 95% CI: 1.01 - 4.12; P = 0.04) (Table 4).

Discussion

The seroepidemiology of *T. gondii* infection in patients with heart diseases is largely unknown. The present study was performed to investigate the association of *T. gondii* infection with patients suffering from heart diseases attending in

Table 4. Multivariate Analysis of Selected Characteristics of Patients and Their Association With *T. gondii* Infection

Characteristic	Odds ratio	95% confidence interval	P value
Born out of Durango State	2.93	1.40 - 6.13	0.004
Low educational level	0.72	0.47 - 1.12	0.14
Ischemic disease	1.49	0.78 - 2.87	0.22
Memory impairment	1.66	0.85 - 3.27	0.13
Blood transfusion	1.81	0.91 - 3.60	0.09
National trips	1.11	0.57 - 2.15	0.75
Consumption of:			
Squirrel meat	1.75	0.74 - 4.13	0.19
Opossum meat	2.15	0.47 - 9.90	0.32
Armadillo meat	2.14	0.39 - 11.61	0.37
Iguana meat	0.75	0.12 - 4.47	0.75
Snake meat	1.24	0.59 - 2.57	0.56
Unwashed raw fruits	1.81	0.79 - 4.13	0.15
Undercooked meat	2.91	0.71 - 11.82	0.13
Alcohol	2.04	1.01 - 4.12	0.04
Consumption of meat 4 - 7 days a week	0.53	0.20 - 1.35	0.18
Eating out of home	0.35	0.09 - 1.27	0.11

a public hospital in Durango City, Mexico and to determine the correlates of infection in these patients. We found a 13.8% seroprevalence of anti-*T. gondii* IgG antibodies in patients suffering from heart disease. This seroprevalence is higher than the 8.0% seroprevalence found in controls, and the 6.1% seroprevalence of *T. gondii* infection reported in the general population in Durango City [25]. Results indicate that patients with heart diseases represent a risk group for *T. gondii* infection. Seroprevalence of *T. gondii* infection increases with age in our region [25, 27]. Therefore, we matched cases and controls by age and this strategy allowed us to evaluate properly the association between *T. gondii* exposure and patients with heart disease. It is hypothesized that patients with *T. gondii* infection have a higher risk for heart disease caused by the presence of cysts in the heart muscle. The interaction of *T. gondii* within skeletal muscle cells has been nicely described recently [28]. However, to the best of our knowledge, the interaction of *T. gondii* within heart muscle cells has not been studied. It is unclear how *T. gondii* may affect the function of heart muscle.

With respect to IgM seropositivity to *T. gondii*, it is remarkable that seroprevalence of anti-*T. gondii* IgM antibodies was significantly lower in cases than in controls. This finding suggests that heart disease might be related with a chronic rather than a recent infection with *T. gondii*. However, the high frequency of IgM antibodies should be interpreted with caution since IgM ELISA kits have a high rate of false positive results [29]. Seroprevalence of *T. gondii* infection varied among heart diseases. However, this difference was not statistically significant. This might be due to a small sample size of some subgroups of specific diagnosis.

We searched for factors associated with the *T. gondii* exposure in patients. Logistic regression showed that seropositivity was positively associated with being born out of Durango State, and with consumption of alcohol. The characteristic "born out of Durango State" has been associated with *T. gondii* seropositivity in some previous epidemiological studies in Durango including adults [25] and elderly people of the general population [27]. On the other hand, the association of *T. gondii* exposure with consumption of alcohol was unexpected. To the best of our knowledge, this association has not been reported in alive people. It is unclear why subjects with alcohol consumption had a higher seroprevalence of *T. gondii* infection than those without alcohol consumption. It is uncertain whether subjects with alcohol consumption have behaviors that might favor *T. gondii* exposure. In a recent study of postmortem examinations in people who died suddenly in Warsaw, Poland, the frequency of *T. gondii* IgG antibodies was higher in subjects with positive blood alcohol test than that in subjects with negative test [30]. Researchers also found the highest seroprevalence of *T. gondii* infection among persons who died because of suicide and with positive alcohol test [30]. Interestingly, ethanol has been involved in *T. gondii* invasion to and egress from the host cells [31], and ethanol produces a dose-dependent stimulation of microneme secretion leading to adhesion of *T. gondii* to host cells [32]. Furthermore, exposure of *T. gondii* tachyzoites to ethanol induced conoid extrusion - an event of cell invasion - without affecting parasite viability [33]. These facts support the association of *T. gondii* exposure and alcohol consumption found in the present study. Further research to elucidate the role of alcohol consumption with *T. gondii* exposure should be conducted.

The current study was limited for the scanty number of certain heart diseases among the studied population. This limitation did not allow properly assessing differences in *T. gondii* seroprevalence among the diagnoses and functional stages of heart diseases.

Conclusion

Our results indicate that *T. gondii* infection is associated with heart disease and suggest that heart disease might be related with a chronic infection. Results warrant for further research to determine the epidemiological impact of *T. gondii* exposure on heart diseases. The association of *T. gondii* infection with alcohol consumption deserves further research. Risk factors associated with *T. gondii* exposure found in the present study may help to design future prevention strategies against *T. gondii* infection.

Conflict of Interest

None.

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