

High Prevalence of *Toxoplasma gondii* Infection in Miners: A Case-Control Study in Rural Durango, Mexico

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Abstract

Background: Very little is known about the seroepidemiology of infection with the parasite *Toxoplasma gondii* (*T. gondii*) in miners. We determine the association of *T. gondii* infection and the occupation of miner, and the association of seropositivity for *T. gondii* with the socio-demographic, clinical, work and behavioral characteristics of the miners.

Methods: Through a case-control study, 125 miners working in Durango State, Mexico and 250 age- and gender-matched non-miner subjects were examined for the presence of anti-*T. gondii* IgG and IgM antibodies using enzyme-linked immunoassays. In addition, the presence of *T. gondii* DNA in miners was determined using polymerase chain reaction. Bivariate and multivariate analyses were used to determine the association of socio-demographic, work, clinical and behavioral characteristics of miners with *T. gondii* infection.

Results: Anti-*T. gondii* IgG antibodies were detected in 75 (60.0%) of 125 miners and in 55 (22.0%) of 250 controls (odds ratio (OR) = 5.31; 95% confidence interval (CI): 3.33 - 8.47; $P < 0.001$). Among IgG seropositive subjects, the frequency of anti-*T. gondii* IgM antibodies was significantly higher in miners (39/75, 52%) than in controls (8/55, 14.5%) ($P < 0.001$). All *T. gondii* seropositive miners referred themselves as healthy. Multivariate analysis of socio-demographic, housing, and behavioral characteristics of miners showed that *T. gon-*

dii seropositivity was positively associated with being born in Durango State (OR = 3.44; 95% CI: 1.09 - 10.7; $P = 0.03$), consumption of boar meat (OR = 5.53; 95% CI: 1.49 - 20.3; $P = 0.01$), living in an overcrowded home (OR = 5.83; 95% CI: 1.49 - 22.8; $P = 0.01$), and was negatively associated with cleaning cat excrement (OR = 0.33; 95% CI: 0.11 - 0.90; $P = 0.03$) and consuming goat meat (OR = 0.16; 95% CI: 0.03 - 0.76; $P = 0.02$).

Conclusions: Surprisingly, our results indicate that miners represent a risk group for *T. gondii* infection. This is the first age- and gender-matched case-control study on the association of *T. gondii* infection and the occupation of miner. Further studies to identify the exact cause of high seropositivity in miners in rural Durango are needed.

Keywords: *Toxoplasma gondii*; Infection; Seroprevalence; Miners; Case-control study; Epidemiology; Mexico

Introduction

Infections with the parasite *Toxoplasma gondii* (*T. gondii*) are common in humans around the world [1]. These infections may lead to toxoplasmosis characterized by lymph node enlargement, chorioretinitis, or neuropsychiatric manifestations [2, 3]. Immunocompromised subjects infected with *T. gondii* may develop severe to life-threatening symptoms, most often toxoplasmic encephalitis [4]. In addition, a primary infection with *T. gondii* in pregnant women may lead to fetal infection and congenital toxoplasmosis [2, 5]. Infection with *T. gondii* is typically acquired by ingestion of raw or undercooked meat containing viable tissue cysts [6], or by ingestion of food or water contaminated with oocysts shed by cats [7]. Other routes of *T. gondii* infection are thought to be rare including organ transplantation [8] and blood transfusion [9].

The epidemiology of *T. gondii* infection in miners has been scanty studied, and we are not aware of any study of this infection in miners in Mexico. The epidemiological link between miners and *T. gondii* may be the close contact with soil and water that could be contaminated with oocyst shed by cats or other felids. In addition, miners work in rural areas where hunting of wild animals is common, and the risk for acquiring infection by eating raw or undercooked meat from *T. gondii*-infected animals is high. The seroprevalence of infection with *T. gondii* and its

Manuscript accepted for publication October 14, 2016

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doi: <http://dx.doi.org/10.14740/jocmr2789w>

association with risk factors for infection in miners in Mexico are largely unknown. Therefore, we sought to determine the seroprevalence of *T. gondii* infection in miners in a municipality in rural Durango, Mexico, and to determine the association of seropositivity for *T. gondii* with the socio-demographic, clinical, work and behavioral characteristics of the miners.

Materials and Methods

Study design and population groups studied

We performed a case-control seroprevalence study of 125 miners (cases) and 250 age- and gender-matched non-miner subjects (controls). Cases and controls were examined for the presence of anti-*T. gondii* IgG and IgM antibodies. Miners were enrolled from December 2015 to August 2016 in a mine located in the San Dimas Municipality, in the northern Mexican state of Durango. Inclusion criteria for the miners were as follows: 1) working in the mine for at least 3 months, 2) 18 years and older, and 3) willing to participate in the study. All cases included in the study were males and had been working from 3 months to 47 years (mean: 11.4 ± 9.5 years) as miners. They were 20 - 87 (mean: 43.8 ± 14.6) years old. Controls were subjects randomly selected from the general population in rural Durango [10]. Controls were matched with cases by gender and age. Controls were males aged 20 - 87 (mean: 43.85 ± 14.5) years old and their age did not differ from that in cases ($P = 0.99$).

Socio-demographic, housing, clinical, work, and behavioral data of miners

Socio-demographic, clinical, work, and behavioral characteristics of the miners were recorded with the aid of a questionnaire. Socio-demographic items included age, gender, birthplace, residence, and socioeconomic level. Housing conditions items included availability of potable water, form of elimination of excretes, years of education of the head of the family, type of flooring at home, and crowding. Assessment of crowding was performed by dividing the number of people by the number of bedrooms in a house. "Semi-crowded" was considered when 1.6 - 3.5 people were living in a single bedroom. "Overcrowded" was considered when 3.6 or more people were living in a single room. The socioeconomic level of participants was self-reported. A "low socioeconomic status" was considered when a participant lived in poverty, whereas a "medium socioeconomic status" was considered when a participant did not live in poverty or wealth but in an intermediate status.

Clinical data included presence of any illness, history of lymphadenopathy, surgery, transplant, or blood transfusion, impairments in memory, reflexes, hearing and vision, frequent abdominal pain or headache, and dizziness. We recorded the duration of the activity as a miner for each participant. Behavioral items were contact with cats or other animals, cleaning cat feces, foreign traveling, type of meat consumed, frequency of meat consumption, eating raw or undercooked meat, animal brains, beef liver, and dried or cured meat, untreated water or

unpasteurized milk, and unwashed raw vegetables or fruits. In addition, behavioral items included washing hands before eating, frequency of eating out of home (in restaurants or fast food outlets), alcoholism, tobacco smoking, and soil contact.

Detection of *T. gondii* IgG and IgM antibodies

Serum samples of miners 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 result ≥ 8 IU/mL was considered positive. Serum samples with anti-*T. gondii* IgG antibodies were further tested for anti-*T. gondii* IgM antibodies by the commercially available enzyme immunoassay "Toxoplasma IgM" kit (Diagnostic Automation Inc.). All assays were performed according to the manufacturer's instructions. Positive and negative controls included in the kits were included in each run.

Extraction of DNA and detection of *T. gondii* DNA

Miners with *Toxoplasma*-specific IgG antibodies by EIA were further examined for *T. gondii* DNA by nested-polymerase chain reaction (PCR). Extraction of DNA from whole blood was performed following a protocol described by Iranpour and Esmailzadeh (<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>). PCR amplification was performed with primers directed against the B1 gene of *T. gondii* and following the protocol described by Roth et al [11]. Amplification products were analyzed with 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized by ultraviolet transillumination.

Statistical analysis

Results were analyzed with the aid of the software Microsoft Excel 2010, Epi Info version 7 (Centers for Disease Control and Prevention: <http://wwwn.cdc.gov/epiinfo/>) and SPSS version 15.0 (SPSS Inc. Chicago, IL). For calculation of the sample size, we used a 95% confidence level, a power of 80%, a 1:2 proportion of cases and controls, a reference seroprevalence of 23.8% [10] as the expected frequency of exposure in controls, and an odds ratio (OR) of 2. The result of the sample size calculation was 115 cases and 229 controls. We compared the age of cases and controls using the Student's *t*-test. The Pearson's Chi-square test and the Fisher exact test (when values were small) were used to determine the association of *T. gondii* seropositivity with the characteristics of miners. As a strategy for multivariate analysis, only characteristics of miners with a P value ≤ 0.10 obtained in the bivariate analysis were included in the analysis. OR and 95% confidence interval (CI) were calculated using logistic regression analysis with the Enter method. A P value < 0.05 was considered as statistically

Table 1. Socio-Demographic and Housing Characteristics of Miners and Prevalence of *T. gondii* Infection

Characteristics	Total subjects	Prevalence of <i>T. gondii</i> infection		P value
		No.	%	
Age groups (years)				
30 or less	26	16	61.5	0.73
31 - 50	59	37	62.7	
> 50	40	22	55.0	
Birth place				
Durango State	98	64	65.3	0.02
Other Mexican State	27	11	40.7	
Educational level				
No education	16	9	56.3	0.72
1 - 6 years	68	42	61.8	
7 - 12 years	36	20	55.6	
> 12 years	5	4	80.0	
Socio-economic level				
Low	61	42	68.9	0.04
Medium	64	33	51.6	
Flooring at home				
Ceramic or wood	5	3	60.0	0.87
Concrete	92	54	58.7	
Soil	28	18	64.3	
Availability of potable water				
In home	2	1	50.0	0.34
In land	3	3	100.0	
In street	120	71	59.2	
Toilet facilities				
Sewage pipes	5	3	60.0	1.00
Latrine or another	120	72	60.0	
Crowding at home				
No	41	26	63.4	0.05
Semi-crowded	55	27	49.1	
Overcrowded	29	22	75.9	
Education of the head of family				
7 years or more	17	10	58.8	0.52
4 - 6 years	53	29	54.7	
Up to 3 years	55	36	65.5	

significant.

participant.

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 miners examined. A written informed consent was obtained from each

Results

Anti-*T. gondii* IgG antibodies were detected in 75 (60.0%) of 125 miners and in 55 (22.0%) of 250 controls. Seroprevalence of anti-*T. gondii* IgG antibodies was significantly higher in miners than in controls (OR = 5.31; 95% CI: 3.33 - 8.47;

Table 2. Bivariate Analysis of Selected Behavioral Characteristics and Infection With *T. gondii* in Miners

Characteristics	No. of subjects tested	Prevalence of <i>T. gondii</i> infection		P value
		No.	%	
Cleaning cat excrement				
Yes	43	21	48.8	0.06
No	82	54	65.9	
Raising farm animals				
Yes	99	61	61.6	0.47
No	26	14	53.8	
National trips				
Yes	77	43	55.8	0.23
No	48	32	66.7	
Goat meat consumption				
Yes	101	56	55.4	0.03
No	24	19	79.2	
Sheep meat consumption				
Yes	59	30	50.8	0.04
No	66	45	68.2	
Boar meat consumption				
Yes	94	61	64.9	0.05
No	31	14	45.2	
Chicken meat consumption				
Yes	124	75	60.5	0.40
No	1	0	0.0	
Turkey meat consumption				
Yes	65	32	49.2	0.01
No	60	43	71.7	
Pigeon meat consumption				
Yes	72	48	66.7	0.07
No	53	27	50.9	
Quail meat consumption				
Yes	39	26	66.7	0.30
No	86	49	57.0	
Rabbit meat consumption				
Yes	51	27	52.9	0.18
No	74	48	64.9	
Armadillo meat consumption				
Yes	60	38	63.3	0.46
No	65	37	56.9	
Iguana meat consumption				
Yes	24	16	66.7	0.45
No	101	59	58.4	
Badger meat consumption				
Yes	24	17	70.8	0.22
No	101	58	57.4	

Table 2. Bivariate Analysis of Selected Behavioral Characteristics and Infection With *T. gondii* in Miners - (continued)

Characteristics	No. of subjects tested	Prevalence of <i>T. gondii</i> infection		P value
		No.	%	
Degree of meat cooking				
Undercooked	6	5	83.3	0.39
Well done	117	68	58.1	
Raw dried meat				
Yes	85	46	54.1	0.06
No	39	28	71.8	
Chorizo consumption				
Yes	124	75	60.5	0.40
No	1	0	0.0	
Brain of cow consumption				
Yes	48	24	50.0	0.07
No	77	51	66.2	
Unwashed raw vegetables				
Yes	85	54	63.5	0.24
No	40	21	52.5	
Untreated water				
Yes	107	66	61.7	0.34
No	18	9	50.0	
Frequency of eating out of home				
Never	1	1	100.0	0.19
1 - 10 times a year	84	46	54.8	
> 10 times a year	40	28	70.0	
Alcohol consumption				
Yes	67	45	67.2	0.07
No	58	30	51.7	
Tobacco smoking				
Yes	57	39	68.4	0.07
No	68	36	52.9	

$P < 0.001$). Of the 75 anti-*T. gondii* IgG positive miners, 30 (40.0%) had anti-*T. gondii* IgG antibody levels higher than 150 IU/mL, 10 (13.3%) between 100 and 150 IU/mL, and 35 (46.7%) between 8 and 99 IU/mL. All seropositive controls had ≥ 8 IU/mL of anti-*T. gondii* IgG antibodies as determined by the qualitative test. However, we were unable to quantitate further for the specific IgG level in all 55 seropositive controls. IgG levels could be determined in only 36 of 55 seropositive controls; of these 27 (75.0%) had anti-*T. gondii* IgG antibody levels higher than 150 IU/mL, two (5.6%) between 100 and 150 IU/mL, and seven (19.4%) between 8 and 99 IU/mL. The frequency of individuals with high IgG levels was significantly higher in the controls compared to the cases group ($P = 0.003$).

Of the 75 miners seropositive for anti-*T. gondii* IgG antibodies, 39 (52.0%) were also positive for anti-*T. gondii* IgM antibodies compared to only eight (14.5%) of the 55 controls

seropositive to anti-*T. gondii* IgG antibodies ($P < 0.001$). DNA of *T. gondii* was detected in 13 miners, and eight (61.5%) of them were positive for anti-*T. gondii* IgM antibodies.

The frequency of IgG was similar ($P = 0.47$) in participants working less than 1 year as a miner (62.5%) and those with 1 - 5 years (68.8%) or > 5 years (56.5%); the frequency of IgM was comparable ($P = 0.92$) in participants with less than 1 year of working as a miner (25.0%), those with 1 - 5 years (31.3%) or those with > 5 years (31.8%).

Concerning socio-demographic and housing characteristics (Table 1), bivariate analysis showed three characteristics potentially (P values ≤ 0.10) associated with *T. gondii* infection: birth place ($P = 0.02$), socioeconomic status ($P = 0.04$), and crowing at home ($P = 0.05$). Other socio-demographic and housing characteristics of miners including age, educational level, flooring at home, availability of potable water, form of elimination of excretas, and years of education of the head of

Table 3. Multivariate Analysis of Selected Characteristics of Miners and Their Association With *T. gondii* Infection

Characteristics	Odds ratio	95% confidence interval	P value
Birth place (Durango State)	3.44	1.09 - 10.7	0.03
Socioeconomic level (low)	2.05	0.73 - 5.71	0.17
Cleaning cat excrement (yes)	0.33	0.11 - 0.90	0.03
Goat meat consumption (yes)	0.16	0.03 - 0.76	0.02
Sheep meat consumption (yes)	1.10	0.34 - 3.48	0.88
Boar meat consumption (yes)	5.53	1.49 - 20.3	0.01
Turkey meat consumption (yes)	0.35	0.12 - 1.00	0.05
Pigeon meat consumption (yes)	2.20	0.80 - 5.98	0.12
Raw dried meat (yes)	0.45	0.15 - 1.32	0.15
Brain of cow consumption (yes)	0.50	0.18 - 1.32	0.16
Alcohol consumption (yes)	0.83	0.27 - 2.51	0.75
Tobacco use (yes)	2.40	0.85 - 6.73	0.10
Crowding			
Semi-crowded	2.78	0.86 - 8.93	0.09
Overcrowded	5.83	1.49 - 22.8	0.01

the family had P values > 0.10.

With respect to the clinical characteristics, all seropositive miners referred themselves as healthy. The frequency of *T. gondii* seropositivity was higher in miners without memory impairment (56/85, 65.9%) than in miners with memory impairment (18/39, 46.2%) (P = 0.03). Other clinical characteristics including history of lymphadenopathy, surgery, blood transfusion, impairments in reflexes, hearing and vision, frequent abdominal pain or headache, and dizziness showed no association with seropositivity. None of the miners had received an organ transplant.

Of the behavioral characteristics of the miners examined (Table 2), 10 variables had P values ≤ 0.10 in the bivariate analysis: cleaning cat excrement (P = 0.06), consumption of meat from goat (P = 0.03), sheep (P = 0.04), boar (P = 0.05), turkey (P = 0.01), and pigeon (P = 0.07), consumption of raw dried meat (P = 0.06), cow's brains (P = 0.07), alcohol consumption (P = 0.07), and tobacco smoking (P = 0.07).

Multivariate analysis of socio-demographic, housing, and behavioral characteristics of miners with P values ≤ 0.10 in the bivariate analysis (Table 3) showed that *T. gondii* seropositivity was positively associated with being born in Durango State (OR = 3.44; 95% CI: 1.09 - 10.7; P = 0.03), consumption of boar meat (OR = 5.53; 95% CI: 1.49 - 20.3; P = 0.01), and overcrowded homes (OR = 5.83; 95% CI: 1.49 - 22.8; P = 0.01), and seropositivity was negatively associated with cleaning cat excrement (OR = 0.33; 95% CI: 0.11 - 0.90; P = 0.03), and consumption of goat meat (OR = 0.16; 95% CI: 0.03 - 0.76; P = 0.02).

Discussion

Very little is known about the epidemiology of *T. gondii* infec-

tion in miners. To the best of our knowledge, the correlation of *T. gondii* infection with the occupation of miner has not been assessed by an age- and gender-matched case-control study design. Therefore, we performed an age- and gender-matched case-control study to investigate the association of *T. gondii* infection with the occupation of miner in the northern Mexican State of Durango.

Remarkably, we found that the prevalence of *T. gondii* exposure was significantly higher in miners than in controls. The seroprevalence found in miners in Durango, Mexico is higher than those reported in miners in other countries. A 7.7% prevalence of infection was found in coal miners in China using the indirect hemagglutination test [12]. In Ukraine, 37.7% of miners were seropositive for *T. gondii* using complement-fixation, passive hemagglutination, and intradermal toxoplasmin tests [13]. Furthermore, the high seroprevalence of *T. gondii* exposure found in miners (60.0%) is the highest seroprevalence reported in population groups in Durango State so far. Thus, seroprevalence found in miners is higher than the seroprevalences of *T. gondii* infection reported in adults in rural communities in Durango State (23.8%) [10], in schizophrenic patients (20%) [14], in waste pickers (21.1%) [15], inmates (21.1%) [16], and ethnic groups living in rural communities including Tepehuanos (22.4%) [17] and Huicholes (33.2%) [18]. In addition, the seroprevalence found in miners is higher than the weighted mean (19.27%) national seroprevalence of *T. gondii* infection found in Mexico [19]. It is not clear why miners had a higher seroprevalence of *T. gondii* exposure than age- and gender-matched controls which were also obtained from rural settings. Seroprevalence of infection with *T. gondii* increases with age as reported in general populations in rural [10] and urban [20] Durango. However, in the present study, no such increase in *T. gondii* exposure with age was observed, and a surprisingly high (61.5%) seroprevalence of *T. gondii* infec-

tion was already observed in the youngest miners aged 18 - 30 years old.

We searched for socio-demographic, work, housing and behavioral characteristics to investigate the high seroprevalence of *T. gondii* in miners. Duration in the activity did not correlate with *T. gondii* exposure. Even miners with less than 1 year as a miners had a high seroprevalence of infection with *T. gondii*. Multivariate analysis showed that *T. gondii* exposure was positively associated with being born in Durango State. This result was unexpected since *T. gondii* exposure has been repeatedly associated with the characteristic of being born out of Durango State in diverse cohorts including the general population in Durango City [20], inmates [16], patients with vision and hearing impairments, cancer, HIV, or undergoing hemodialysis [21], female sex workers [22], elderly people [23], patients with heart diseases [24], and people applying for medical certificates [25]. The association of *T. gondii* infection with being born in Durango State found in this study likely indicates that infection was acquired in Durango State. In fact, traveling did not increase the prevalence of infection with *T. gondii* in miners. Furthermore, multivariate analysis showed that infection with *T. gondii* was associated with consumption of boar meat, and living in an overcrowded home. These characteristics may have contributed to the high seroprevalence of *T. gondii* infection in miners. Consumption of boar meat was associated with *T. gondii* seropositivity in several populations in the region with lower seroprevalence than miners including patients with work accidents [26], elderly people [23], and the general population in Durango City [20]. Concerning the association of infection with *T. gondii* and living in an overcrowded home, this is the first time we found such association in our studies of infection with *T. gondii* in the region. Living in an overcrowded area has been considered as a contributing factor for infection with *T. gondii* in pregnant women in Nigeria [27]. It is not clear why overcrowding influenced the seroprevalence of *T. gondii* infection in that study, but other factors for infection including poor sanitation and contamination of environment with cat excrement were also present [27]. In addition, in the Third National Health and Nutrition Examination Survey in the USA (1988 - 1994), researchers found that risk for *T. gondii* infection increased in those who lived in crowded conditions [28]. On the other hand, we found that infection with *T. gondii* was negatively associated with cleaning cat excrement, and consumption of goat meat. These factors have been suggested as risks for infection with *T. gondii* exposure by others [29, 30].

The present study has some limitations. The sample size is small, and we studied miners working in only one mine. Further studies should include a larger sample size and sample miners of more than one mine. A high frequency (61.5%) of positive *T. gondii* PCR assays among miners seropositive for anti-*T. gondii* IgM antibodies was found. Therefore, further research on the epidemiology of acute cases of *T. gondii* infection in miners should be conducted.

We conclude that miners represent a risk group for infection with *T. gondii*. This is the first age- and gender-matched study on the association of *T. gondii* infection and the occupation of miner. Further studies to confirm our results are needed.

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