

# BMJ Open Seroprevalence and correlates of *Toxoplasma gondii* infection in Yoremes (Mayos) in Mexico: a cross-sectional study

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## ABSTRACT

**Objectives:** We sought to determine the prevalence of anti-*Toxoplasma gondii* antibodies in Yoremes and to identify associations of *T. gondii* exposure with sociodemographic, clinical and behavioural characteristics of Yoremes.

**Design:** A cross-sectional survey.

**Setting:** Yoremes were enrolled in the locality of Tierra Blanca in the municipality of Navjoa in Sonora State, Mexico.

**Participants:** We studied 200 Yoremes (Mayos); they are an indigenous ethnic group living in a coastal region in northwestern Mexico.

**Primary and secondary outcome measures:** We assessed the prevalence of anti-*Toxoplasma* IgG and IgM antibodies in participants using enzyme-linked immunoassays. We used a standardised questionnaire to obtain the characteristics of Yoremes. The association of *T. gondii* exposure and Yoremes' characteristics was assessed by bivariate and multivariate analyses.

**Results:** Of the 200 Yoremes studied (mean age: 31.50±18.43 years), 26 (13.0%) were positive for anti-*T. gondii* IgG antibodies and 19 (73.1%) of them were also positive for anti-*T. gondii* IgM antibodies. Seroprevalence of *T. gondii* infection did not vary with sex, educational level, occupation or socioeconomic status. In contrast, multivariate analysis of sociodemographic and behavioural characteristics showed that *T. gondii* exposure was associated with increasing age (OR=1.02; 95% CI 1.00 to 1.04; p=0.03) and consumption of squirrel meat (OR=4.99; 95% CI 1.07 to 23.31; p=0.04). Furthermore, seroprevalence of *T. gondii* infection was significantly higher in Yoremes with a history of lymphadenopathy (p=0.03) and those suffering from frequent abdominal pain (p=0.03). In women, *T. gondii* exposure was associated with a history of caesarean sections (p=0.03) and miscarriages (p=0.02).

**Conclusions:** We demonstrate, for the first time, serological evidence of *T. gondii* exposure among Yoremes in Mexico. Results suggest that infection with *T. gondii* might be affecting the health of Yoremes.

## Strengths and limitations of this study

- This is the first cross-sectional study of *Toxoplasma gondii* infection in the Mexican ethnic group of Yoremes (Mayos).
- The seroprevalence of *T. gondii* infection was determined in Yoremes.
- Prevalence association with sociodemographic, clinical and behavioural characteristics of Yoremes was determined.
- The sample size was small and the seropositivity rate was low to perform a wider analysis of the association of *T. gondii* exposure and characteristics of Yoremes.

Results may be useful for an optimal design of preventive measures against *T. gondii* infection.

## INTRODUCTION

*Toxoplasma gondii* is a ubiquitous intracellular parasite.<sup>1 2</sup> This parasite is currently infecting about one-third of humanity.<sup>3</sup> Infection with *T. gondii* is usually asymptomatic.<sup>2 4</sup> However, *T. gondii* disseminates after infection to many organs and may lead to disease in the eyes, lymph nodes and central nervous system.<sup>4-6</sup> Furthermore, primary infection with *T. gondii* in pregnant women is a threat for congenital disease.<sup>4 7</sup> Infection with *T. gondii* may lead to a life-threatening disease in immunocompromised patients.<sup>4 8</sup> The main routes of *T. gondii* infection are ingestion of food or water contaminated with oocysts shed by cats and eating undercooked or raw meat containing tissue cysts.<sup>2 4</sup>

The epidemiology of *T. gondii* infection in ethnic groups in Mexico has been poorly studied. Serological evidence of *T. gondii* infection has been demonstrated in

Mennonites,<sup>9</sup> Tepehuanos<sup>10</sup> and Huicholes<sup>11</sup> in the northern Mexican State of Durango. However, there is a lack of knowledge about the seroepidemiology of *T. gondii* infection in Yoremes or Mayos (an indigenous ethnic group living in a coastal region in the northwestern Mexican states of Sonora and Sinaloa). Yoremes live in rural communities and work mainly in agriculture and fishing. They live in a region with a climate that is different from those in other regions where other population groups in Mexico were studied for the seroepidemiology of *T. gondii* infection. The climate in the Yoremes' region is desert-like or subtropical, and it is unclear whether this climate (or the food habits among Yoremes) may influence the seroprevalence of *T. gondii*. Indigenous people in Mexico, including the Yoremes, usually live in rural areas with a limited coverage of health services. The aims of the present study were to determine the seroprevalence of *T. gondii* in Yoremes and the association of *T. gondii* prevalence with the sociodemographic, clinical and behavioural characteristics of Yoremes.

## MATERIALS AND METHODS

### Study design and Yoremes' population studied

Through a cross-sectional survey, we studied Yoremes in Sonora, Mexico, from January to June 2015. Yoremes were enrolled in the locality of Tierra Blanca in the municipality of Navojoa in Sonora State, Mexico. Tierra Blanca (27°19'N 109°34'W) is situated at an altitude of 25 m above sea level and has a desert-like climate and a mean annual temperature of 25.4°C. It Blanca has a mean annual rainfall of 266 mm. Inclusion criteria for the study participants were: (1) Yoremes' ethnicity (people who speak the Yoremes' language and identify themselves as Yoremes); (2) aged 12 years and older and (3) that they voluntarily accepted to participate.

### Sample size and sampling method

We calculated the sample size using a two-sided confidence level of 95%, a power of 80%, a ratio of unexposed: exposed=1, a reference *T. gondii* seroprevalence of 22.4%<sup>10</sup> in unexposed participants, and an OR of 2.6. The result of the calculation was 182 participants. We added a 5% for refusals and the final sample size was 198 participants. Sampling of Yoremes was performed by a convenience method. First, the authors met the Yoremes leaders to provide information about the study. After obtaining permission from the leaders, they invited the people they lead. Yoremes who accepted to participate in the study were gathered in two public places (a health centre and a high school) to provide a blood sample and submit a questionnaire. Since this strategy was not enough to reach the sample size, the authors visited houses in the community to enrol participants until the sample size was reached. This new strategy is not likely to influence the results since a minority of cases was obtained by this type of sampling. In total, 200 Yoremes were included in the study.

## Sociodemographic, clinical and behavioural data

Data from the participants were obtained with the aid of a standardised questionnaire. This questionnaire included sociodemographic, clinical and behavioural items. Sociodemographic items were age, sex, birthplace, residence, education, occupation and socioeconomic status. Clinical items included current health status, history of lymphadenopathy, frequent presence of headache and abdominal pain, dizziness, impairments of memory, reflexes, hearing and vision, and a history of blood transfusion, transplants or surgery. In women, obstetric history was also obtained. Behavioural items included contact with animals, food consumed, travelling, frequency of eating away from home (in restaurants or fast food outlets), contact with soil (gardening or agriculture) and type of flooring at home. Data about food were type of meat consumed, frequency of meat consumption, consumption of raw or undercooked meat, dried or processed meat, and consumption of unwashed raw vegetables and fruits, unpasteurised milk or untreated water.

### Serological tests for anti-*T. gondii* antibodies

We obtained a blood sample from each participant. Blood samples were centrifuged and serum samples were obtained. Sera were stored at -20° C until analysed. Serum samples were tested for anti-*T. gondii* IgG antibodies with the commercially available 'Toxoplasma IgG' (Diagnostic Automation Inc, Calabasas, California, USA) enzyme immunoassay (EIA). Anti-*T. gondii* IgG antibody levels were expressed as IU/mL, and a value ≥8 IU/mL was used as a cut-off for seropositivity. Sera positive for anti-*T. gondii* IgG antibodies were further analysed for anti-*T. gondii* IgM antibodies by the commercially available 'Toxoplasma IgM' (Diagnostic Automation Inc) EIA. The cut-off for anti-*T. gondii* IgM seropositivity for each assay was obtained by multiplying the mean cut-off calibrator optical density by a correction factor ( $f=0.35-0.40$ ) printed on the label of the calibrator. All assays were performed following the manufacturer's instructions, and positive and negative controls were included in each run.

### Statistical analysis

Data were analysed with the aid of the software Epi Info V.3.5.4 and SPSS V.15.0. To avoid bias in the measure of associations, care was taken in obtaining all data about the characteristics of participants, and there were no missing data. We used Pearson's  $\chi^2$  test and Fisher's exact test (when values were small) for initial comparison of the frequencies among groups. Multivariate analysis was used to assess the association between the sociodemographic and behavioural characteristics of Yoremes and the seropositivity to *T. gondii*. Only variables with a p value ≤0.10 obtained in the bivariate analysis were included in the multivariate analysis. This strategy allowed us to substantially reduce the number of variables in the analysis. OR and 95% CI were calculated by logistic regression using the stepwise backward method.

We used the Hosmer-Lemeshow goodness of fit test to assess the fitness of the regression model. Statistical significance was set at a p value <0.05.

## RESULTS

Yoremes participating in the study had a mean age of  $31.50 \pm 18.43$  years (range 12–83 years). Of the 200 Yoremes studied, 26 (13.0%) were positive for anti-*T. gondii* IgG antibodies. Of these 26 IgG-seropositive patients, 19 (73.1%) were also positive for anti-*T. gondii* IgM antibodies. Of the 26 anti-*T. gondii* IgG-positive Yoremes, 16 (61.5%) had IgG levels higher than 150 IU/mL, and 10 (38.5%) between 24 and 45 IU/mL. A correlation of the sociodemographic characteristics of Yoremes and *T. gondii* seroprevalence is shown in table 1. Seroprevalence of *T. gondii* infection did not vary with sex, birthplace, residence, educational level, occupation or socioeconomic status of Yoremes (table 1). In contrast, seroprevalence increased significantly with age ( $p=0.005$ ). With respect to anti-*T. gondii* IgM seropositivity among the 26 IgG-seropositive Yoremes, seroprevalence did not vary with age ( $p=0.54$ ), and seropositivity was found in 6 of 10 males and 13 of 16 females ( $p=0.36$ ).

With respect to clinical characteristics (table 2), seroprevalence of *T. gondii* infection was significantly higher in Yoremes with a history of lymphadenopathy ( $p=0.03$ ) and those suffering from frequent abdominal pain ( $p=0.03$ ). In women, *T. gondii* exposure was associated with a history of caesarean sections ( $p=0.03$ ) and miscarriages ( $p=0.02$ ). Some clinical variables associated with *T. gondii* exposure may interact with each other, and no further regression analysis with these clinical variables was performed. The frequencies of other clinical characteristics including the presence of underlying diseases, suffering from frequent headaches, impairments in reflexes, hearing and vision, and a history of surgery, blood transfusion or transplant were similar among *T. gondii*-positive and *T. gondii*-negative Yoremes.

Concerning behavioural characteristics, a number of variables showed p values  $\leq 0.10$  in the bivariate analysis including consumption of goat and squirrel meat, raw dried meat, beef intestines and beef brains, and alcoholism. Other behavioural characteristics of Yoremes including contact with animals, travelling, consumption of meat other than that of goat and squirrel, frequency of meat consumption, degree of meat cooking, consumption of untreated water, unpasteurised milk, processed meat, unwashed raw vegetables or fruits, frequency of eating out of home, contact with soil, and type of flooring at home showed p values higher than 0.10 in the bivariate analysis. Multivariate analysis of sociodemographic and behavioural variables showed that *T. gondii* exposure was associated only with increasing age (OR=1.02; 95% CI 1.00 to 1.04;  $p=0.03$ ) and consumption of squirrel meat (OR=4.99; 95% CI 1.07 to 23.31;  $p=0.04$ ). An acceptable fit ( $p=0.37$ ) of our regression model was obtained in the Hosmer-Lemeshow test.

## DISCUSSION

The epidemiology of *T. gondii* infection among ethnic groups in Mexico has been scantily studied. This work aimed to determine the seroprevalence and correlates of *T. gondii* infection in an indigenous ethnic group (Yoremes) in northwestern Mexico. We found a 13.0% seroprevalence of *T. gondii* infection in Yoremes. To the best of our knowledge, there are no previous reports of *T. gondii* exposure in this ethnic group. The seroprevalence found in Yoremes is lower than seroprevalences of *T. gondii* infection reported in other ethnic groups in the northern Mexican state of Durango: seroprevalences of 22.4%, 30.3% and 33.2% have been reported in Tepehuanos,<sup>10</sup> Mennonites<sup>9</sup> and Huicholes,<sup>11</sup> respectively. The lower prevalence of *T. gondii* exposure in Yoremes than in Tepehuanos, Mennonites and Huicholes might be explained by differences in their environment or behavioural difference. Seroprevalence of *T. gondii* infection may be influenced by environment conditions with a high seroprevalence in humid regions<sup>12</sup> and a low seroprevalence in dry and hot regions.<sup>13</sup> Tepehuanos and Huicholes live in remote communities in a mountainous region (Sierra Madre Occidental) and Mennonites in a Valley region, whereas Yoremes live in a desert region at low altitude. Very little is known about the seroprevalence of *T. gondii* infection in population groups living in a desert climate. In a study in Niamey, Niger researchers showed that prevalence of toxoplasmosis was higher in humid coastal regions than in dry desert areas.<sup>12</sup> Seroprevalence of *T. gondii* infection increased with age. This finding might be related to differences in sanitation and hygiene among generations. Poor sanitation and hygiene have been linked to *T. gondii* infection in the indigenous population in Brazil.<sup>14</sup> Improvement of these epidemiological factors may result in the lowering of seroprevalence of *T. gondii* exposure in younger generations. We did not include minor (younger than 12 years) participants in this study because the frequency of *T. gondii* infection in young people is usually very low. We also analysed associations with factors other than the environment. Seroprevalence was found to increase with age, consistent with previous reports in rural<sup>15</sup> and urban<sup>16 17</sup> populations in northern Mexico. The mean age (31.50 years) in Yoremes was similar to that in Tepehuanos (31.03 years).<sup>10</sup> However, the mean age in Yoremes was lower than the one (37.98 years) in Huicholes<sup>11</sup> and that (38.4 years) in Mennonites.<sup>9</sup>

Multivariate analysis also showed an association of *T. gondii* exposure with consumption of squirrel meat. In two previous studies in the general population in rural<sup>15</sup> and urban<sup>16</sup> Durango, consumption of squirrel meat was also associated with *T. gondii* exposure. These findings show the importance of consumption of squirrel meat in the transmission of *T. gondii* infection in the region. Although squirrel meat is usually cooked before eating, failure to obtain a thorough cooking may occur specially for thick pieces of meat. Yoremes usually grill the

**Table 1** Sociodemographic characteristics of Yoremes and seroprevalence of *Toxoplasma gondii* infection

Characteristic	Participants tested Number	Prevalence of <i>T. gondii</i> infection		p Value
		Number	Percentage	
Age groups (years)				
30 or less	124	9	7.3	0.005
31–50	38	7	18.4	
>50	38	10	26.3	
Sex				
Male	77	10	13.0	0.99
Female	123	16	13.0	
Birthplace				
Sonora state	198	26	13.1	1
Other Mexican state or abroad	2	0	0.0	
Residence area				
Rural	184	22	12.0	0.13
Urban	16	4	25.0	
Educational level				
No education	6	1	16.7	0.33
1–6 years	32	7	21.9	
7–12 years	144	15	10.4	
>12 years	18	3	16.7	
Occupation				
Labourer*	43	8	18.6	0.21
Non-labourer†	157	18	11.5	
Socioeconomic level				
Low	111	19	17.1	0.15
Medium	88	7	8.0	
High	1	0	0.0	

\*Labourer: agriculture, business, construction, livestock raising, professional, other.

†Non-labourer: student or housekeeping.

squirrel meat, and this process may result in an uneven cooking. In addition, tasting of raw or undercooked meat while grilling might occur. Tasting of fresh raw meat was linked to toxoplasmosis in Italy.<sup>18</sup> Serological evidence of *T. gondii* infection has been demonstrated in squirrels.<sup>19</sup> In addition, *T. gondii* has been detected in organs of Korean squirrels (*Tamias sibericus*)<sup>20</sup> and grey squirrels (*Sciurus carolinensis*)<sup>21</sup> with fatal toxoplasmosis. We previously investigated the presence of *T. gondii* in animals in Durango but were unable to detect anti-*T. gondii* antibodies in 69 squirrels (*Spermophilus variegatus*) collected.<sup>22</sup> However, we cannot rule out *T. gondii* infection in squirrels in the region because the sample size was small and infection might occur in other squirrel species than the one studied. Further research about the epidemiological link of *T. gondii* infection and consumption of squirrel meat including the search for *T. gondii* in squirrels should therefore be conducted.

Intriguingly, in this study, we found an association of *T. gondii* exposure with abdominal pain, history of lymphadenopathy, caesarean sections and miscarriages. It is well known that *T. gondii* infection is a cause of lymph node enlargement and miscarriages.<sup>2 4</sup> In contrast, *T. gondii* infection is not typically associated with abdominal pain, but abdominal pain has been reported in gastric toxoplasmosis in patients with AIDS.<sup>23 24</sup> We

also found an association of *T. gondii* infection with a history of caesarean section. It is not clear why women with caesarean sections had a higher seroprevalence of *T. gondii* infection than those without this history. Interestingly, in a study of women with stillbirths in Durango, Mexico, *T. gondii* exposure was associated with a history of surgery.<sup>25</sup> It raises the question whether a specific type of surgery as caesarean section or a specific population group as women might have a higher risk of *T. gondii* exposure than others. We did not investigate the indications for the caesarean sections or the health status of the children born by this surgical procedure, and this was a limitation of the study. Several factors could be considered to explain *T. gondii* infections in women with caesarean sections. Congenital toxoplasmosis may precipitate not just early delivery or induction of delivery, but it may also prompt caesarean section.<sup>26–28</sup> In addition, the use of contaminated surgical instruments or materials during caesarean sections cannot be ruled out. Blood transfusion is relatively common in surgical patients, and infection with *T. gondii* by blood transfusion may also occur.<sup>29</sup> Further research about the association of *T. gondii* infection and caesarean section and other surgical procedures should be conducted.

In this work, anti-*T. gondii* IgM antibodies were present in a relatively high number of anti-*T. gondii* IgG-positive



**Table 2** Bivariate analysis of clinical data and infection with *Toxoplasma gondii* in Yoremes

Characteristic	Participants tested Number	Prevalence of <i>T. gondii</i> infection		p Value
		Number	Percentage	
Clinical status				
Healthy	170	19	11.2	0.08
Ill	30	7	23.3	
Lymphadenopathy ever				
Yes	57	12	21.1	0.03
No	143	14	9.8	
Abdominal pain frequently				
Yes	51	11	21.6	0.03
No	149	15	10.1	
Headache frequently				
Yes	54	10	18.5	0.15
No	146	16	11	
Memory impairment				
Yes	28	5	17.9	0.37
No	172	21	12.2	
Dizziness				
Yes	46	6	13	0.99
No	154	20	13	
Reflexes impairment				
Yes	23	5	21.7	0.19
No	177	21	11.9	
Hearing impairment				
Yes	16	1	6.3	0.70
No	184	25	13.6	
Visual impairment				
Yes	45	8	17.8	0.27
No	155	18	11.6	
Surgery ever				
Yes	55	10	18.2	0.18
No	145	16	11	
Blood transfusion				
Yes	15	4	26.7	0.11
No	185	22	11.9	
Pregnancies				
Yes	71	12	16.9	0.17
No	52	4	7.7	
Deliveries				
Yes	51	8	15.7	0.45
No	72	8	11.1	
Caesarean sections				
Yes	23	6	26.1	0.03
No	100	10	10	
Miscarriages				
Yes	16	5	31.3	0.02
No	107	11	10.3	
Stillbirths				
Yes	6	1	16.7	0.57
No	117	15	12.8	

Yoremes compared with previous studies. This finding should be interpreted with caution because positive results in IgM tests may indicate persistent IgM antibodies rather than acute infection.<sup>30</sup> We did not test all participants for anti-*T. gondii* IgM antibodies. Only IgG-positive patients were tested because a high number of false-positive results for IgM have been reported when using immunoassays.<sup>30</sup> Therefore, a positive IgM test

with a negative IgG test has a limited usefulness for drawing diagnostic and epidemiological conclusions.

The small sample size and the low rate of seropositivity were limitations of the study. These factors did not allow us to perform a wider analysis of the association of *T. gondii* exposure and the characteristics of Yoremes. Reaching the sample size of Yoremes was challenging. However, the strategy to enrol participants by visiting

them at their houses allowed us to include participants who were unable to get out of home for sampling because of illnesses or other conditions.

## CONCLUSIONS

We demonstrate, for the first time, serological evidence of *T. gondii* exposure among Yoremes in Mexico. Results suggest that infection with *T. gondii* may be associated with specific food habits and health conditions. The optimal design of preventive measures against *T. gondii* infection should take our findings into consideration.

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**Contributors** CA-E, AR-C, MAGC-M and MLA-M designed the study protocol and participated in the coordination and management of the study. AR-C, MAGC-M, GJA-B and ADN-A obtained blood samples, submitted the questionnaires and performed the data analysis. CA-E performed the laboratory tests. CA-E, JH-T, LFS-A, AR-C, MAGC-M, MLA-M and OL performed the data analysis and wrote the manuscript.

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**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** The Institutional Ethical Committee of the University of Sonora, Mexico approved this study. The purpose and procedures of the survey were explained to all Yoremes. Participation in the study was voluntary. Written informed consent was obtained from all participants and from the next of kin of minor participants.

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