

## Seroepidemiology of *Toxoplasma gondii* infection in Yoremes (Mayos) in Mexico

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Complete List of Authors:	Alvarado-Esquivel, Cosme; Universidad Juárez del Estado de Durango, Laboratorio de Investigación Biomédica Rascón-Careaga, Antonio ; University of Sonora. Mexico., Department of Chemical and Biological Sciences. Hernández-Tinoco, Jesús; Universidad Juárez del Estado de Durango, Instituto de Investigación Científica Corella-Madueño, Maria; University of Sonora. Mexico., Department of Chemical and Biological Sciences. Sánchez-Anguiano, Luis; Universidad Juárez del Estado de Durango, Instituto de Investigación Científica Aldana-Madrid, Maria; University of Sonora. Mexico., Department of Research and Postgraduate in Food. Almada-Balderrama, Gerardo ; University of Sonora. Mexico., Department of Chemical and Biological Sciences. Nuñez-Aguirre, Alan ; University of Sonora. Mexico., Department of Chemical and Biological Sciences. Liesenfeld, Oliver; Charité Medical School, Institute for Microbiology and Hygiene, Campus Benjamin Franklin
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# Seroepidemiology of *Toxoplasma gondii* infection in Yoremes (Mayos) in Mexico

Cosme Alvarado-Esquivel,<sup>1\*</sup> Antonio Rascón-Careaga,<sup>2</sup> Jesús Hernández-Tinoco,<sup>3</sup> María Alba Guadalupe Corella-Madueño,<sup>2</sup> Luis Francisco Sánchez-Anguiano,<sup>3</sup> María Lourdes Aldana-Madrid,<sup>4</sup> Gerardo Javier Almada-Balderrama,<sup>2</sup> Alan Daniel Nuñez-Aguirre,<sup>2</sup> Oliver Liesenfeld<sup>5#</sup>

<sup>1</sup>Biomedical Research Laboratory, Faculty of Medicine and Nutrition, Juárez University of Durango State. Avenida Universidad S/N. 34000 Durango, Mexico.

<sup>2</sup>Department of Chemical and Biological Sciences. University of Sonora. Mexico.

<sup>3</sup>Institute for Scientific Research “Dr. Roberto Rivera-Damm”, Juárez University of Durango State. Avenida Universidad S/N. 34000 Durango, Mexico.

<sup>4</sup>Department of Research and Postgraduate in Food. University of Sonora. Mexico.

<sup>5</sup>Institute for Microbiology and Hygiene, Campus Benjamin Franklin, Charité Medical School. Hindenburgdamm 27. D-12203 Berlin, Germany.

#Present address: Roche Molecular Diagnostics, Pleasanton, CA. USA.

\*Corresponding author: Cosme Alvarado-Esquivel. Biomedical Research Laboratory. Faculty of Medicine and Nutrition, Juárez University of Durango State, Avenida Universidad S/N, 34000 Durango, Dgo, Mexico. Tel and Fax: +52-618 8130527; e-mail: alvaradocosme@yahoo.com

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4 sectional study.  
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## ABSTRACT

**OBJECTIVES:** We sought to determine the prevalence of anti-*T. gondii* antibodies in Yoremes and to identify associations of *T. gondii* exposure with socio-demographic, clinical and behavioral characteristics of Yoremes.

**DESIGN:** A cross sectional survey.

**SETTING:** Yoremes were enrolled in the locality of Tierra Blanca in the municipality of Navojoa 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 standardized questionnaire to obtain the characteristics of the Yoremes. The association of *T. gondii* exposure and the Yoremes' characteristics was assessed by bivariate and multivariate analyses.

**RESULTS:** Of the 200 Yoremes studied (mean age:  $31.50 \pm 18.43$  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 socio-demographic and behavioral characteristics showed that *T. gondii* exposure was associated with increasing age (OR= 1.02; 95% CI: 1.00-1.04;  $P=0.03$ ) and consumption of squirrel meat (OR= 4.99; 95% CI: 1.07-23.31;  $P=0.04$ ). Furthermore, seroprevalence of *T. gondii* infection was significantly higher in Yoremes with history of lymphadenopathy ( $P=0.03$ ) and those suffering from frequent abdominal pain ( $P=0.03$ ). In women, *T. gondii*

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3 exposure was associated with history of cesarean sections ( $P=0.03$ ) and miscarriages  
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5 ( $P=0.02$ ).  
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8 **CONCLUSIONS:** We demonstrate for the first time serological evidence of *T. gondii*  
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10 exposure among Yoremes in Mexico. Results suggest that infection with *T. gondii* might be  
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12 affecting the health of Yoremes. Results may be useful for an optimal design of preventive  
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14 measures against *T. gondii* infection.  
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### 22 **Strengths and limitations of this study**

- 24 • This is the first cross-sectional study of *Toxoplasma gondii* infection in the Mexican  
25 ethnic group of Yoremes (Mayos).  
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- 28 • The seroprevalence of *Toxoplasma gondii* infection was determined in Yoremes.  
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- 30 • Prevalence association with sociodemographic, clinical, and behavioral  
31 characteristics of Yoremes was determined.  
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- 34 • The sample size was small and the seropositivity rate was low to perform a wider  
35 analysis of the association of *Toxoplasma gondii* exposure and characteristics of the  
36 Yoremes.  
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## INTRODUCTION

*Toxoplasma gondii* (*T. gondii*) is a ubiquitous intracellular parasite.[1, 2] This parasite is currently infecting about one third of humanity.[3] Infection with *T. gondii* is usually asymptomatic.[2, 4] However, *T. gondii* disseminates after infection to many organs and may lead to disease in eyes, lymph nodes, and central nervous system.[4-6] Furthermore, primary infection with *T. gondii* in pregnant women is a threat for congenital disease.[4, 7] Infection with *T. gondii* may lead to a life-threatening disease in immunocompromised patients.[4, 8] 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.[2, 4]

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,[9] Tepehuanos,[10] and Huicholes [11] 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. Climate in the Yoremes region is desert or subtropical, and it is unclear whether this climate (or the food habits among Yoremes) may influence the seroprevalence of *T. gondii*. 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 socio-demographic, clinical, and behavioral characteristics of Yoremes.

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## 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) has an altitude of 25 meters above sea level, a desert climate, and a mean annual temperature of 25.4°C. Tierra Blanca has a mean annual rainfall of 266 mm. Inclusion criteria for the study subjects were: 1) Yoremes ethnicity (people who speak the Yoremes language and identify themselves as Yoremes); 2) aged 12 years and older; and 3) that voluntarily accepted to participate.

### Sample size and sampling method

We calculated the sample size using a reference *T. gondii* seroprevalence of 22.4% [9] as expected frequency of the factor under study, 28,063 as the size of population from which the sample was selected, 16.6% as the least acceptable result, and a confidence level of 95%. The result of the calculation was 197 subjects. Sampling of Yoremes was performed by a convenience method. Firstly, authors met Yoremes leaders to provide information about the study. After obtaining permission from the Yoremes leaders, they invited people under their command. Yoremes who accepted to participate in the study were gathered in two public places (a health center and a high school) to provide a blood sample and submit a questionnaire. Since this strategy was not enough to reach the sample size, authors visited houses in the community to enroll participants until the sample size was reached. In total, 200 Yoremes were included in the study.



### **Socio-demographic, clinical, and behavioral data**

Data from the participants was obtained with the aid of a standardized questionnaire. This questionnaire included socio-demographic, clinical and behavioral items. Socio-demographic 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. Behavioral items included contact with animals, food consumed, traveling, 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, unpasteurized 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  $-20^{\circ}$  C until analyzed. Serum samples were tested for anti-*T. gondii* IgG antibodies with the commercially available “*Toxoplasma* IgG” (Diagnostic Automation Inc., Calabasas, CA, USA) enzyme immunoassay (EIA). Anti-*T. gondii* IgG antibody levels were expressed as International Units (IU)/ml, and a value  $\geq 8$  IU/ml was used as a cut-off for seropositivity. Sera positive for anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* IgM antibodies by

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3 the commercially available “*Toxoplasma* IgM” (Diagnostic Automation Inc.) EIA. The cut-  
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5 off for anti-*T. gondii* IgM seropositivity for each assay was obtained by multiplying the  
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7 mean cut-off calibrator optical density by a correction factor ( $f = 0.35-0.40$ ) printed on the  
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9 label of calibrator. All assays were performed following the manufacturer’s instructions,  
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11 and positive and negative controls were included in each run.  
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### 14 15 16 17 **Statistical analysis**

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19 Data was analyzed with the aid of the software Epi Info version 3.5.4 and SPSS  
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21 version 15.0. To avoid bias in the measure of associations, care was taken in obtaining all  
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23 data about the characteristics of participants, and there was no missing data. We used the  
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25 Pearson’s chi-square test and the Fisher exact test (when values were small) for initial  
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27 comparison of the frequencies among groups. Multivariate analysis was used to assess the  
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29 association between the socio-demographic and behavioral characteristics of Yoremes and  
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31 the seropositivity to *T. gondii*. Only variables with a *P* value equal to or less than 0.10  
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33 obtained in the bivariate analysis were included in the multivariate analysis. Odds ratio  
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35 (OR) and 95% confidence interval (CI) were calculated by logistic regression using the  
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37 stepwise backward method. We used the Hosmer-Lemeshow goodness of fit test to assess  
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39 the fitness of the regression model. Statistical significance was set at a *P* value less than  
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41 0.05.  
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### 50 51 **Ethical aspects**

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53 The Institutional Ethical Committee of the University of Sonora, Mexico approved  
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55 this study. The purpose and procedures of the survey were explained to all Yoremes.  
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3 Participation in the study was voluntary. A written informed consent was obtained from all  
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6 participants and from the next of kin of minor participants.  
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## 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 and 19 (73.1%) of them 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 to 45 IU/ml. A correlation of the socio-demographic 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 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 histories of cesarean sections ( $P=0.03$ ) and miscarriages ( $P=0.02$ ). The frequencies of other clinical characteristics including the presence of underlying diseases, suffering from frequent headaches, impairments in reflexes, hearing and vision, and histories of surgery, blood transfusion or transplant were similar among *T. gondii* positive and *T. gondii* negative Yoremes.

Concerning behavioral characteristics, a number of variables showed  $P$  values equal to or lower than 0.10 in the bivariate analysis including consumption of meat from goat ( $P=0.09$ ) and squirrel ( $P=0.01$ ), consumption of raw dried meat ( $P=0.02$ ), and consumption of beef intestines ( $P=0.10$ ) and beef brains ( $P=0.06$ ), and alcoholism ( $P=0.09$ ). Results of a selection of behavioral characteristics of Yoremes and their correlation with *T. gondii*

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3 exposure are shown in Table 3. Other behavioral characteristics of Yoremes including  
4 contact with animals, traveling, consumption of meat other than goat and squirrel,  
5 frequency of meat consumption, degree of meat cooking, consumption of untreated water,  
6 unpasteurized milk, processed meat, unwashed raw vegetables or fruits, frequency of eating  
7 out of home, contact with soil, and type of flooring at home showed *P* values higher than  
8 0.10 in the bivariate analysis. Multivariate analysis of socio-demographic and behavioral  
9 characteristics showed that *T. gondii* exposure was associated only with increasing age  
10 (OR= 1.02; 95% CI: 1.00-1.04; *P*=0.03), and consumption of squirrel meat (OR= 4.99;  
11 95% CI: 1.07-23.31; *P*=0.04). An acceptable fit (*P*=0.37) of our regression model was  
12 obtained in the Hosmer-Lemeshow test.  
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## 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,[10] Mennonites,[9] and Huicholes,[11] 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 behavioral difference. Seroprevalence of *T. gondii* infection may be influenced by environment conditions with a high seroprevalence in humid regions [12] and a low seroprevalence in dry and hot regions.[13] 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.[12] We also analyzed associations with factors other than the environment. Seroprevalence was found to increase with age, consistent with previous reports in rural [14] and urban [15, 16] populations in northern Mexico. The mean age (31.50 years) in Yoremes was similar to that in

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3 Tepehuanos (31.03 years).[10] However, the mean age in Yoremes was lower than the one  
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5 (37.98 years) in Huicholes [11] and that (38.4 years) in Mennonites.[9]  
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10 Multivariate analysis also showed an association of *T. gondii* exposure with  
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12 consumption of squirrel meat. In two previous studies in the general population in rural  
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14 [14] and urban [15] Durango, consumption of squirrel meat was also associated with *T.*  
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16 *gondii* exposure. These findings remark the importance of consumption of squirrel meat in  
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18 the transmission of *T. gondii* infection in the region. Although squirrel meat is usually  
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20 cooked before eating, failure in obtaining a well-done cooking may occur specially for  
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22 thick pieces of meat. Serological evidence of *T. gondii* infection has been demonstrated in  
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24 squirrels.[17] In addition, *T. gondii* has been detected in organs of Korean squirrels (*Tanias*  
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26 *sibericus*) [18] and grey squirrels (*Sciurus carolensis*) [19] with fatal toxoplasmosis. We  
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28 previously investigated the presence of *T. gondii* in animals in Durango but were unable to  
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30 detect anti-*T. gondii* antibodies in 69 squirrels (*Spermophilus variegatus*) collected.[20]  
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33 However, we cannot rule out *T. gondii* infection in squirrels in the region because the  
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35 sample size was small and infection might occur in other squirrel species than the one  
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37 studied. Further research about the epidemiological link of *T. gondii* infection and  
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39 consumption of squirrel meat including the search for *T. gondii* in squirrels should  
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42 therefore be conducted.  
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46 Intriguingly, in the present study we found an association of *T. gondii* exposure with  
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48 abdominal pain, history of lymphadenopathy, cesarean sections and miscarriages. It is well  
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50 known that *T. gondii* infection is a cause of lymph node enlargement and miscarriages.[2,  
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52 4] In contrast, *T. gondii* infection is not typically associated with abdominal pain but  
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54 abdominal pain has been reported in gastric toxoplasmosis in patients with acquired  
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56 immunodeficiency syndrome.[21, 22] We also found an association of *T. gondii* infection  
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3 with a history of cesarean section. It is not clear why women with cesarean sections had a  
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5 higher seroprevalence of *T. gondii* infection than those without this history. Further  
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7 research about the association of *T. gondii* infection and cesarean section and other surgical  
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9 procedures should be conducted.  
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12 In this work, anti-*T. gondii* IgM antibodies were present in a relatively high number  
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14 of anti-*T. gondii* IgG positive Yoremes compared to previous studies. This finding should  
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16 be interpreted with caution because positive results in IgM tests may indicate persistent  
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18 IgM antibodies rather than acute infection.[23]  
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21 The small sample size and the low rate of seropositivity were limitations of the  
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23 study. These factors did not allow us to perform a wider analysis of the association of  
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25 *Toxoplasma gondii* exposure and characteristics of the Yoremes.  
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## 32 **Conclusions**

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

47 This study was financially supported by Juárez University of Durango State,  
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49 Mexico.  
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## 56 **Competing interests**

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3 No competing interests exist.  
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### 8 **Authors' contributions**

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10 CAE, ARC, MAGCM, and MLAM designed the study protocol, and participated  
11 in the coordination and management of the study. ARC, MAGCM, GJAB and ADNA  
12 obtained blood samples, submitted the questionnaires and performed the data analysis.  
13 CAE performed the laboratory tests. CAE, JHT, LFSA, ARC, MAGCM, MLAM, and OL  
14 performed the data analysis, and wrote the manuscript.  
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### 25 **Data sharing statement**

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27 No additional data is available.  
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**Table 1. Socio-demographic characteristics of Yoremes and seroprevalence of *T. gondii* infection.**

Characteristic	Subjects Tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
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	
Birth place				
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				
Laborer <sup>a</sup>	43	8	18.6	0.21
Non-laborer <sup>b</sup>	157	18	11.5	
Socio-economic level				
Low	111	19	17.1	0.15
Medium	88	7	8.0	
High	1	0	0.0	

<sup>a</sup>Laborer: Agriculture, business, construction, livestock raising, professional, other.

<sup>b</sup>Non-laborer: student or housekeeping.

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

Characteristic	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
<b>Clinical status</b>				
Healthy	170	19	11.2	0.08
Ill	30	7	23.3	
<b>Lymphadenopathy ever</b>				
Yes	57	12	21.1	0.03
No	143	14	9.8	
<b>Abdominal pain frequently</b>				
Yes	51	11	21.6	0.03
No	149	15	10.1	
<b>Headache frequently</b>				
Yes	54	10	18.5	0.15
No	146	16	11	
<b>Memory impairment</b>				
Yes	28	5	17.9	0.37
No	172	21	12.2	
<b>Dizziness</b>				
Yes	46	6	13	0.99
No	154	20	13	
<b>Reflexes impairment</b>				
Yes	23	5	21.7	0.19
No	177	21	11.9	
<b>Hearing impairment</b>				
Yes	16	1	6.3	0.70
No	184	25	13.6	
<b>Visual impairment</b>				
Yes	45	8	17.8	0.27
No	155	18	11.6	
<b>Surgery ever</b>				
Yes	55	10	18.2	0.18
No	145	16	11	
<b>Blood transfusion</b>				
Yes	15	4	26.7	0.11
No	185	22	11.9	
<b>Pregnancies</b>				

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3	Yes	71	12	16.9	0.17
4	No	52	4	7.7	
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6	Deliveries				
7	Yes	51	8	15.7	0.45
8	No	72	8	11.1	
9					
10	Cesarean sections				
11	Yes	23	6	26.1	0.03
12	No	100	10	10	
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14	Miscarriages				
15	Yes	16	5	31.3	0.02
16	No	107	11	10.3	
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18	Stillbirths				
19	Yes	6	1	16.7	0.57
20	No	117	15	12.8	
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**Table 3. Bivariate analysis of selected putative risk factors for infection with *Toxoplasma gondii* in Yoremes.**

Characteristic	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
Cats at home				
Yes	93	10	10.8	0.37
No	107	16	15	
National trips				
Yes	51	8	15.7	0.5
No	149	18	12.1	
Goat meat consumption				
Yes	71	13	18.3	0.09
No	129	13	10.1	
Sheep meat consumption				
Yes	67	12	17.9	0.14
No	133	14	10.5	
Turkey meat consumption				
Yes	44	3	6.8	0.16
No	156	23	14.7	
Duck meat consumption				
Yes	9	2	22.2	0.33
No	191	24	12.6	
Quail meat consumption				
Yes	27	6	22.2	0.13
No	173	20	11.6	
Rabbit meat consumption				
Yes	21	5	23.8	0.16
No	179	21	11.7	
Squirrel meat consumption				
Yes	8	4	50	0.01
No	192	22	11.5	
Snake meat consumption				
Yes	6	2	33.3	0.17
No	194	24	12.4	
Raw dried meat				
Yes	43	10	23.3	0.02
No	157	16	10.2	
Chorizo consumption				
Yes	184	22	12	0.13
No	16	4	25	
Beef intestines consumption				
Yes	58	11	19	0.1
No	142	15	10.6	
Consumption of cow's brain				
Yes	18	5	27.8	0.06
No	182	21	11.5	
Frequency of eating out of home				
Never	35	8	22.9	0.15
1 to 10 times a year	103	12	11.7	

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>10 times a year	62	6	9.7	
Alcoholism				
Yes	20	5	25	0.09
No	180	21	11.7	
Soil contact				
Yes	130	20	15.4	0.17
No	70	6	8.6	

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract  <b>THE STUDY DESIGN IS INCLUDED IN THE ABSTRACT.</b> (b) Provide in the abstract an informative and balanced summary of what was done and what was found  <b>AN ABSTRACT WITH IMPORTANT DATA WAS INCLUDED.</b>
<b>Introduction</b>		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported  <b>A BACKGROUND AND RATIONALE FOR THE STUDY WAS INCLUDED.</b>
Objectives	3	State specific objectives, including any prespecified hypotheses  <b>OBJECTIVES WERE INCLUDED.</b>
<b>Methods</b>		
Study design	4	Present key elements of study design early in the paper  <b>ELEMENTS OF THE STUDY DESIGN WERE INCLUDED.</b>
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection  <b>SETTING, LOCATIONS RELEVANT DATES, PERIOD OF RECRUITMENT, AND DATA COLLECTION WERE INCLUDED.</b>
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants  <b>ELIGIBILITY CRITERIA, AND THE SOURCES AND METHOD OF SELECTION OF PARTICIPANTS WERE INCLUDED.</b> (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable  <b>DATA ABOUT VARIABLES AND DIAGNOSIS WAS INCLUDED.</b>
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there

is more than one group

**INFORMATION ABOUT THE VARIABLES, AND METHODS OF ASSESSMENT WAS INCLUDED.**

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Bias	9	Describe any efforts to address potential sources of bias
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**INFORMATION ABOUT EFFORTS TO AVOID BIAS WAS ADDED.**

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Study size	10	Explain how the study size was arrived at
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**INFORMATION ABOUT THE CALCULATION OF SAMPLE SIZE WAS INCLUDED.**

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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
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**INFORMATION ABOUT THE VARIABLES CHOSEN IN THE ANALYSIS WAS INCLUDED.**

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Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding
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**A DESCRIPTION OF THE STATISTICAL ANALYSIS WAS INCLUDED.**

(b) Describe any methods used to examine subgroups and interactions

**METHODS USED TO EXAMINE SUBGROUPS WERE DESCRIBED.**

(c) Explain how missing data were addressed

**THERE WAS NO MISSING DATA.**

(d) *Cohort study*—If applicable, explain how loss to follow-up was addressed

*Case-control study*—If applicable, explain how matching of cases and controls was addressed

*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy

**ANALYTICAL METHODS ARE SHOWN IN THE MATERIALS AND METHODS SECTION.**

(e) Describe any sensitivity analyses

**NOT APPLICABLE.**

Continued on next page

**Results**

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
<b>INFORMATION ABOUT THE ELIGIBILITY OF SUBJECT WAS INCLUDED.</b>		
(b) Give reasons for non-participation at each stage		
<b>INFORMATION ABOUT NON-PARTICIPATION WAS INCLUDED.</b>		
(c) Consider use of a flow diagram		
<b>THE NUMBER OF PROCEDURES WAS SMALL AND A FLOW DIAGRAM MIGHT BE NOT NECESSARY.</b>		
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
<b>CHARACTERISTICS OF THE STUDY PARTICIPANTS WERE INCLUDED.</b>		
(b) Indicate number of participants with missing data for each variable of interest		
<b>THERE WAS NO MISSING DATA.</b>		
(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)		
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time
<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure		
<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures		
<b>TABLES WITH SUMMARY OF RESULTS WERE INCLUDED.</b>		
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
<b>INFORMATION ABOUT 95% CONFIDENCE INTERVALS WAS INCLUDED.</b>		
(b) Report category boundaries when continuous variables were categorized		
<b>INFORMATION ABOUT CATEGORIES AND SUBGROUPS ARE INCLUDED IN TABLES.</b>		
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period		
<b>NO RELATIVE RISKS WERE ASSESSED.</b>		
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
<b>RESULTS OF ANALYSIS OF SUBGROUPS WERE SHOWN IN TABLES.</b>		
<b>Discussion</b>		
Key results	18	Summarise key results with reference to study objectives

**KEY RESULTS WITH REFERENCE TO OBJECTIVES WERE DISCUSSED.**

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
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**THE LIMITATION OF THE STUDY WERE INCLUDED.**

Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
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**AN INTERPRETATION OF RESULTS WAS INCLUDED.**

Generalisability	21	Discuss the generalisability (external validity) of the study results
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**INFORMATION RELATED WITH THE GENERALISABILITY OF THE STUDY RESULTS WAS INCLUDED.****Other information**

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based
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**INFORMATION ABOUT FUNDING WAS INCLUDED.**

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

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

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

Cosme Alvarado-Esquivel,<sup>1\*</sup> Antonio Rascón-Careaga,<sup>2</sup> Jesús Hernández-Tinoco,<sup>3</sup> María Alba Guadalupe Corella-Madueño,<sup>2</sup> Luis Francisco Sánchez-Anguiano,<sup>3</sup> María Lourdes Aldana-Madrid,<sup>4</sup> Gerardo Javier Almada-Balderrama,<sup>2</sup> Alan Daniel Nuñez-Aguirre,<sup>2</sup> Oliver Liesenfeld<sup>5#</sup>

<sup>1</sup>Biomedical Research Laboratory, Faculty of Medicine and Nutrition, Juárez University of Durango State. Avenida Universidad S/N. 34000 Durango, Mexico.

<sup>2</sup>Department of Chemical and Biological Sciences. University of Sonora. Mexico.

<sup>3</sup>Institute for Scientific Research “Dr. Roberto Rivera-Damm”, Juárez University of Durango State. Avenida Universidad S/N. 34000 Durango, Mexico.

<sup>4</sup>Department of Research and Postgraduate in Food. University of Sonora. Mexico.

<sup>5</sup>Institute for Microbiology and Hygiene, Campus Benjamin Franklin, Charité Medical School. Hindenburgdamm 27. D-12203 Berlin, Germany.

#Present address: Roche Molecular Diagnostics, Pleasanton, CA. USA.

\*Corresponding author: Cosme Alvarado-Esquivel. Biomedical Research Laboratory. Faculty of Medicine and Nutrition, Juárez University of Durango State, Avenida Universidad S/N, 34000 Durango, Dgo, Mexico. Tel and Fax: +52-618 8130527; e-mail: alvaradocosme@yahoo.com



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6 **Key words:** *Toxoplasma gondii*, seroprevalence, Yoremes (Mayos), ethnic groups, cross-  
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## ABSTRACT

**OBJECTIVES:** We sought to determine the prevalence of anti-*T. gondii* antibodies in Yoremes and to identify associations of *T. gondii* exposure with socio-demographic, clinical and behavioral characteristics of Yoremes.

**DESIGN:** A cross sectional survey.

**SETTING:** Yoremes were enrolled in the locality of Tierra Blanca in the municipality of Navojoa 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 standardized questionnaire to obtain the characteristics of the Yoremes. The association of *T. gondii* exposure and the Yoremes' characteristics was assessed by bivariate and multivariate analyses.

**RESULTS:** Of the 200 Yoremes studied (mean age:  $31.50 \pm 18.43$  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 socio-demographic and behavioral characteristics showed that *T. gondii* exposure was associated with increasing age (OR= 1.02; 95% CI: 1.00-1.04;  $P=0.03$ ) and consumption of squirrel meat (OR= 4.99; 95% CI: 1.07-23.31;  $P=0.04$ ). Furthermore, seroprevalence of *T. gondii* infection was significantly higher in Yoremes with history of lymphadenopathy ( $P=0.03$ ) and those suffering from frequent abdominal pain ( $P=0.03$ ). In women, *T. gondii*

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3 exposure was associated with history of cesarean sections ( $P=0.03$ ) and miscarriages  
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5 ( $P=0.02$ ).  
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8 **CONCLUSIONS:** We demonstrate for the first time serological evidence of *T. gondii*  
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10 exposure among Yoremes in Mexico. Results suggest that infection with *T. gondii* might be  
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12 affecting the health of Yoremes. Results may be useful for an optimal design of preventive  
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14 measures against *T. gondii* infection.  
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### 22 **Strengths and limitations of this study**

- 24 • This is the first cross-sectional study of *Toxoplasma gondii* infection in the Mexican  
25 ethnic group of Yoremes (Mayos).  
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- 28 • The seroprevalence of *Toxoplasma gondii* infection was determined in Yoremes.  
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- 30 • Prevalence association with sociodemographic, clinical, and behavioral  
31 characteristics of Yoremes was determined.  
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- 34 • The sample size was small and the seropositivity rate was low to perform a wider  
35 analysis of the association of *Toxoplasma gondii* exposure and characteristics of the  
36 Yoremes.  
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## INTRODUCTION

*Toxoplasma gondii* (*T. gondii*) is a ubiquitous intracellular parasite.[1, 2] This parasite is currently infecting about one third of humanity.[3] Infection with *T. gondii* is usually asymptomatic.[2, 4] However, *T. gondii* disseminates after infection to many organs and may lead to disease in eyes, lymph nodes, and central nervous system.[4-6] Furthermore, primary infection with *T. gondii* in pregnant women is a threat for congenital disease.[4, 7] Infection with *T. gondii* may lead to a life-threatening disease in immunocompromised patients.[4, 8] 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.[2, 4]

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,[9] Tepehuanos,[10] and Huicholes [11] 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. Yoremes live in a region with a climate that is different from those in other regions where other populations groups in Mexico were studied for the seroepidemiology of *T. gondii* infection. Climate in the Yoremes' region is desert 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 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

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3 and the association of *T. gondii* prevalence with the socio-demographic, clinical, and  
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5 behavioral characteristics of Yoremes.  
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## 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) has an altitude of 25 meters above sea level, a desert climate, and a mean annual temperature of 25.4°C. Tierra Blanca has a mean annual rainfall of 266 mm. Inclusion criteria for the study subjects were: 1) Yoremes ethnicity (people who speak the Yoremes language and identify themselves as Yoremes); 2) aged 12 years and older; and 3) that 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% [10] in unexposed subjects, and an odds ratio of 2.6. The result of the calculation was 182 subjects. We added a 5% for refusals and the final sample size was 198 subjects. Sampling of Yoremes was performed by a convenience method. Firstly, authors met Yoremes leaders to provide information about the study. After obtaining permission from the Yoremes leaders, they invited the people they lead. Yoremes who accepted to participate in the study were gathered in two public places (a health center and a high school) to provide a blood sample and submit a questionnaire. Since this strategy was not enough to reach the sample size, authors visited houses in the community to enroll participants until the sample size was reached. This new strategy is not likely to influence the results since a minority of

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3 cases was obtain by this type of sampling. In total, 200 Yoremes were included in the  
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### 10 **Socio-demographic, clinical, and behavioral data**

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12 Data from the participants was obtained with the aid of a standardized  
13 questionnaire. This questionnaire included socio-demographic, clinical and behavioral  
14 items. Socio-demographic items were age, sex, birthplace, residence, education,  
15 occupation, and socioeconomic status. Clinical items included current health status, history  
16 of lymphadenopathy, frequent presence of headache and abdominal pain, dizziness,  
17 impairments of memory, reflexes, hearing, and vision, and a history of blood transfusion,  
18 transplants or surgery. In women, obstetric history was also obtained. Behavioral items  
19 included contact with animals, food consumed, traveling, frequency of eating away from  
20 home (in restaurants or fast food outlets), contact with soil (gardening or agriculture) and  
21 type of flooring at home. Data about food were type of meat consumed, frequency of meat  
22 consumption, consumption of raw or undercooked meat, dried or processed meat, and  
23 consumption of unwashed raw vegetables and fruits, unpasteurized milk, or untreated  
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### 46 **Serological tests for anti-*T. gondii* antibodies**

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48 We obtained a blood sample from each participant. Blood samples were centrifuged  
49 and serum samples were obtained. Sera were stored at  $-20^{\circ}$  C until analyzed. Serum  
50 samples were tested for anti-*T. gondii* IgG antibodies with the commercially available  
51 “*Toxoplasma* IgG” (Diagnostic Automation Inc., Calabasas, CA, USA) enzyme  
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3 immunoassay (EIA). Anti-*T. gondii* IgG antibody levels were expressed as International  
4 Units (IU)/ml, and a value  $\geq 8$  IU/ml was used as a cut-off for seropositivity. Sera positive  
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7 for anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* IgM antibodies by  
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10 the commercially available “*Toxoplasma* IgM” (Diagnostic Automation Inc.) EIA. The cut-  
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13 off for anti-*T. gondii* IgM seropositivity for each assay was obtained by multiplying the  
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16 mean cut-off calibrator optical density by a correction factor ( $f = 0.35-0.40$ ) printed on the  
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19 label of calibrator. All assays were performed following the manufacturer’s instructions,  
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22 and positive and negative controls were included in each run.  
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### 24 **Statistical analysis**

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27 Data was analyzed with the aid of the software Epi Info version 3.5.4 and SPSS  
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30 version 15.0. To avoid bias in the measure of associations, care was taken in obtaining all  
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33 data about the characteristics of participants, and there was no missing data. We used the  
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36 Pearson’s chi-square test and the Fisher exact test (when values were small) for initial  
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39 comparison of the frequencies among groups. Multivariate analysis was used to assess the  
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42 association between the socio-demographic and behavioral characteristics of Yoremes and  
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45 the seropositivity to *T. gondii*. Only variables with a *P* value equal to or less than 0.10  
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48 obtained in the bivariate analysis were included in the multivariate analysis. This strategy  
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51 allowed us to reduce substantially the number of variables in the analysis. Odds ratio (OR)  
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54 and 95% confidence interval (CI) were calculated by logistic regression using the stepwise  
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57 backward method. We used the Hosmer-Lemeshow goodness of fit test to assess the fitness  
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60 of the regression model. Statistical significance was set at a *P* value less than 0.05.



### **Ethical aspects**

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. A written informed consent was obtained from all participants and from the next of kin of minor participants.

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## 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 subjects, 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 to 45 IU/ml. A correlation of the socio-demographic 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 histories of cesarean 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 histories of surgery, blood transfusion or transplant were similar among *T. gondii* positive and *T. gondii* negative Yoremes.

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Concerning behavioral characteristics, a number of variables showed  $P$  values equal to or lower than 0.10 in the bivariate analysis including consumption of meat from goat ( $P=0.09$ ) and squirrel ( $P=0.01$ ), consumption of raw dried meat ( $P=0.02$ ), and consumption of beef intestines ( $P=0.10$ ) and beef brains ( $P=0.06$ ), and alcoholism ( $P=0.09$ ). Results of a selection of behavioral characteristics of Yoremes and their correlation with *T. gondii* exposure are shown in Table 3. Other behavioral characteristics of Yoremes including contact with animals, traveling, consumption of meat other than goat and squirrel, frequency of meat consumption, degree of meat cooking, consumption of untreated water, unpasteurized 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 socio-demographic and behavioral characteristics showed that *T. gondii* exposure was associated only with increasing age (OR= 1.02; 95% CI: 1.00-1.04;  $P=0.03$ ), and consumption of squirrel meat (OR= 4.99; 95% CI: 1.07-23.31;  $P=0.04$ ). 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,[10] Mennonites,[9] and Huicholes,[11] 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 behavioral difference. Seroprevalence of *T. gondii* infection may be influenced by environment conditions with a high seroprevalence in humid regions [12] and a low seroprevalence in dry and hot regions.[13] 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.[12] 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 indigenous population in Brazil.[14] Improving of these epidemiological factors may result in the lowering of seroprevalence of *T. gondii* exposure

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3 in younger generations. We did not include minor (younger than 12 years old) participants  
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5 in this study because the frequency of *T. gondii* infection in young people is usually very  
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7 low. We also analyzed associations with factors other than the environment.  
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9 Seroprevalence was found to increase with age, consistent with previous reports in rural  
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11 [15] and urban [16, 17] populations in northern Mexico. The mean age (31.50 years) in  
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13 Yoremes was similar to that in Tepehuanos (31.03 years).[10] However, the mean age in  
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15 Yoremes was lower than the one (37.98 years) in Huicholes [11] and that (38.4 years) in  
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17 Mennonites.[9]  
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22 Multivariate analysis also showed an association of *T. gondii* exposure with  
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24 consumption of squirrel meat. In two previous studies in the general population in rural  
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26 [15] and urban [16] Durango, consumption of squirrel meat was also associated with *T.*  
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28 *gondii* exposure. These findings remark the importance of consumption of squirrel meat in  
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30 the transmission of *T. gondii* infection in the region. Although squirrel meat is usually  
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32 cooked before eating, failure in obtaining a well-done cooking may occur specially for  
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34 thick pieces of meat. Yoremes usually grill the squirrel meat, and this process may result in  
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36 an uneven cooking. In addition, tasting of raw or undercooked meat while grilling might  
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38 occur. Tasting of fresh raw meat was linked to toxoplasmosis in Italy.[18] Serological  
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40 evidence of *T. gondii* infection has been demonstrated in squirrels.[19] In addition, *T.*  
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42 *gondii* has been detected in organs of Korean squirrels (*Tamias sibericus*) [20] and grey  
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44 squirrels (*Sciurus carolensis*) [21] with fatal toxoplasmosis. We previously investigated the  
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46 presence of *T. gondii* in animals in Durango but were unable to detect anti-*T. gondii*  
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48 antibodies in 69 squirrels (*Spermophilus variegatus*) collected.[22] However, we cannot  
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50 rule out *T. gondii* infection in squirrels in the region because the sample size was small and  
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3 infection might occur in other squirrel species than the one studied. Further research about  
4 the epidemiological link of *T. gondii* infection and consumption of squirrel meat including  
5 the search for *T. gondii* in squirrels should therefore be conducted.  
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10 Intriguingly, in the present study we found an association of *T. gondii* exposure with  
11 abdominal pain, history of lymphadenopathy, cesarean sections and miscarriages. It is well  
12 known that *T. gondii* infection is a cause of lymph node enlargement and miscarriages.[2,  
13 4] In contrast, *T. gondii* infection is not typically associated with abdominal pain but  
14 abdominal pain has been reported in gastric toxoplasmosis in patients with acquired  
15 immunodeficiency syndrome.[23, 24] We also found an association of *T. gondii* infection  
16 with a history of cesarean section. It is not clear why women with cesarean sections had a  
17 higher seroprevalence of *T. gondii* infection than those without this history. Interestingly, in  
18 a study of women with stillbirths in Durango, Mexico, *T. gondii* exposure was associated  
19 with a history of surgery.[25] It raises the question whether a specific type of surgery as  
20 cesarean section or a specific population group as women might have a higher risk of *T.*  
21 *gondii* exposure than others. We did not investigate the indications for the cesarean sections  
22 or the health status of the children born by this surgical procedure. Further research about  
23 the association of *T. gondii* infection and cesarean section and other surgical procedures  
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45 In this work, anti-*T. gondii* IgM antibodies were present in a relatively high number  
46 of anti-*T. gondii* IgG positive Yoremes compared to previous studies. This finding should  
47 be interpreted with caution because positive results in IgM tests may indicate persistent  
48 IgM antibodies rather than acute infection.[26] We did not test all participants for anti-*T.*  
49 *gondii* IgM antibodies. Only IgG positive subjects were tested because a high number of  
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3 false positive results for IgM has been reported when using immunoassays.[26] Therefore,  
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5 a positive IgM test with a negative IgG test has a limited usefulness for drawing diagnostic  
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7 and epidemiological conclusions.  
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10 The small sample size and the low rate of seropositivity were limitations of the  
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12 study. These factors did not allow us to perform a wider analysis of the association of *T.*  
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14 *gondii* exposure and the characteristics of the Yoremes. Reaching the sample size of  
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16 Yoremes was challenging. However, the strategy to enroll participants by visiting them at  
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18 their houses allowed us to include participants who were unable to get out of home for  
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20 sampling because of illnesses or other conditions.  
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## 27 **Conclusions**

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

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44 This study was financially supported by Juárez University of Durango State,  
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46 Mexico.  
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## 52 **Competing interests**

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54 No competing interests exist.  
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## Authors' contributions

CAE, ARC, MAGCM, and MLAM designed the study protocol, and participated in the coordination and management of the study. ARC, MAGCM, GJAB and ADNA obtained blood samples, submitted the questionnaires and performed the data analysis. CAE performed the laboratory tests. CAE, JHT, LFSA, ARC, MAGCM, MLAM, and OL performed the data analysis, and wrote the manuscript.

## Data sharing statement

No additional data is available.



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**Table 1. Socio-demographic characteristics of Yoremes and seroprevalence of *T. gondii* infection.**

Characteristic	Subjects Tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
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	
Birth place				
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				
Laborer <sup>a</sup>	43	8	18.6	0.21
Non-laborer <sup>b</sup>	157	18	11.5	
Socio-economic level				
Low	111	19	17.1	0.15
Medium	88	7	8.0	
High	1	0	0.0	

<sup>a</sup>Laborer: Agriculture, business, construction, livestock raising, professional, other.

<sup>b</sup>Non-laborer: student or housekeeping.

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

Characteristic	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
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				

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2					
3	Yes	15	4	26.7	0.11
4	No	185	22	11.9	
5					
6	Pregnancies				
7	Yes	71	12	16.9	0.17
8	No	52	4	7.7	
9					
10	Deliveries				
11	Yes	51	8	15.7	0.45
12	No	72	8	11.1	
13					
14	Cesarean sections				
15	Yes	23	6	26.1	0.03
16	No	100	10	10	
17					
18	Miscarriages				
19	Yes	16	5	31.3	0.02
20	No	107	11	10.3	
21					
22	Stillbirths				
23	Yes	6	1	16.7	0.57
24	No	117	15	12.8	
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**Table 3. Bivariate analysis of selected putative risk factors for infection with *Toxoplasma gondii* in Yoremes.**

Characteristic	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
Cats at home				
Yes	93	10	10.8	0.37
No	107	16	15	
National trips				
Yes	51	8	15.7	0.5
No	149	18	12.1	
Goat meat consumption				
Yes	71	13	18.3	0.09
No	129	13	10.1	
Sheep meat consumption				
Yes	67	12	17.9	0.14
No	133	14	10.5	
Turkey meat consumption				
Yes	44	3	6.8	0.16
No	156	23	14.7	
Duck meat consumption				
Yes	9	2	22.2	0.33
No	191	24	12.6	
Quail meat consumption				
Yes	27	6	22.2	0.13
No	173	20	11.6	
Rabbit meat consumption				
Yes	21	5	23.8	0.16
No	179	21	11.7	
Squirrel meat consumption				
Yes	8	4	50	0.01
No	192	22	11.5	
Snake meat consumption				
Yes	6	2	33.3	0.17
No	194	24	12.4	
Raw dried meat				
Yes	43	10	23.3	0.02
No	157	16	10.2	
Chorizo consumption				
Yes	184	22	12	0.13
No	16	4	25	
Beef intestines consumption				
Yes	58	11	19	0.1
No	142	15	10.6	
Consumption of cow's brain				
Yes	18	5	27.8	0.06

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2					
3	No	182	21	11.5	
4	Frequency of eating out of home				
5	Never	35	8	22.9	0.15
6	1 to 10 times a year	103	12	11.7	
7	>10 times a year	62	6	9.7	
8	Alcoholism				
9	Yes	20	5	25	0.09
10	No	180	21	11.7	
11	Soil contact				
12	Yes	130	20	15.4	0.17
13	No	70	6	8.6	
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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract  <b>THE STUDY DESIGN IS INCLUDED IN THE ABSTRACT (Page 3).</b> (b) Provide in the abstract an informative and balanced summary of what was done and what was found  <b>AN ABSTRACT WITH IMPORTANT DATA WAS INCLUDED (Pages 3-4).</b>
<b>Introduction</b>		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported  <b>A BACKGROUND AND RATIONALE FOR THE STUDY WAS INCLUDED (Page 5).</b>
Objectives	3	State specific objectives, including any prespecified hypotheses  <b>OBJECTIVES WERE INCLUDED (Pages 5-6).</b>
<b>Methods</b>		
Study design	4	Present key elements of study design early in the paper  <b>ELEMENTS OF THE STUDY DESIGN WERE INCLUDED (Page 7).</b>
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection  <b>SETTING, LOCATIONS RELEVANT DATES, PERIOD OF RECRUITMENT, AND DATA COLLECTION WERE INCLUDED (Pages 7-8).</b>
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants  <b>ELIGIBILITY CRITERIA, AND THE SOURCES AND METHOD OF SELECTION OF PARTICIPANTS WERE INCLUDED (Pages 7-8).</b> (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable  <b>DATA ABOUT VARIABLES AND DIAGNOSIS WAS INCLUDED (Pages 8-</b>

		9).
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
<b>INFORMATION ABOUT THE VARIABLES, AND METHODS OF ASSESSMENT WAS INCLUDED (Pages 8-9).</b>		
Bias	9	Describe any efforts to address potential sources of bias
<b>INFORMATION ABOUT EFFORTS TO AVOID BIAS WAS ADDED (Page 9).</b>		
Study size	10	Explain how the study size was arrived at
<b>INFORMATION ABOUT THE CALCULATION OF SAMPLE SIZE WAS INCLUDED (Page 7).</b>		
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
<b>INFORMATION ABOUT THE VARIABLES CHOSEN IN THE ANALYSIS WAS INCLUDED (Pages 8-9).</b>		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding
<b>A DESCRIPTION OF THE STATISTICAL ANALYSIS WAS INCLUDED (Page 9).</b>		
		(b) Describe any methods used to examine subgroups and interactions
<b>METHODS USED TO EXAMINE SUBGROUPS WERE DESCRIBED (Page 9).</b>		
		(c) Explain how missing data were addressed
<b>THERE WAS NO MISSING DATA.</b>		
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed
		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy
<b>ANALYTICAL METHODS ARE SHOWN IN THE MATERIALS AND METHODS SECTION (pages 8-9).</b>		
		(e) Describe any sensitivity analyses
<b>NOT APPLICABLE.</b>		

Continued on next page

**Results**

Participants	13*	<p>(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed</p> <p><b>INFORMATION ABOUT THE ELIGIBILITY OF SUBJECT WAS INCLUDED (Pages 7-8).</b></p> <p>(b) Give reasons for non-participation at each stage</p> <p><b>INFORMATION ABOUT NON-PARTICIPATION WAS INCLUDED (Page 7).</b></p> <p>(c) Consider use of a flow diagram</p> <p><b>THE NUMBER OF PROCEDURES WAS SMALL AND A FLOW DIAGRAM MIGHT BE NOT NECESSARY.</b></p>
Descriptive data	14*	<p>(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders</p> <p><b>CHARACTERISTICS OF THE STUDY PARTICIPANTS WERE INCLUDED (Pages 8-9).</b></p> <p>(b) Indicate number of participants with missing data for each variable of interest</p> <p><b>THERE WAS NO MISSING DATA.</b></p> <p>(c) <i>Cohort study</i>—Summarise follow-up time (eg, average and total amount)</p>
Outcome data	15*	<p><i>Cohort study</i>—Report numbers of outcome events or summary measures over time</p> <p><i>Case-control study</i>—Report numbers in each exposure category, or summary measures of exposure</p> <p><i>Cross-sectional study</i>—Report numbers of outcome events or summary measures</p> <p><b>TABLES WITH SUMMARY OF RESULTS WERE INCLUDED (Pages 23-27).</b></p>
Main results	16	<p>(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included</p> <p><b>INFORMATION ABOUT 95% CONFIDENCE INTERVALS WAS INCLUDED (Page 9).</b></p> <p>(b) Report category boundaries when continuous variables were categorized</p> <p><b>INFORMATION ABOUT CATEGORIES AND SUBGROUPS ARE INCLUDED IN TABLES (Pages 23-27).</b></p> <p>(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</p> <p><b>NO RELATIVE RISKS WERE ASSESSED.</b></p>
Other analyses	17	<p>Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses</p> <p><b>RESULTS OF ANALYSIS OF SUBGROUPS WERE SHOWN IN TABLES (Pages 23-</b></p>

27).

**Discussion**

Key results	18	Summarise key results with reference to study objectives
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**KEY RESULTS WITH REFERENCE TO OBJECTIVES WERE DISCUSSED (Pages 13-16).**

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
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**THE LIMITATION OF THE STUDY WERE INCLUDED (Page 16).**

Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
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**AN INTERPRETATION OF RESULTS WAS INCLUDED (Pages 13-16).**

Generalisability	21	Discuss the generalisability (external validity) of the study results
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**INFORMATION RELATED WITH THE GENERALISABILITY OF THE STUDY RESULTS WAS INCLUDED (Pages 15-16).**

**Other information**

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based
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**INFORMATION ABOUT FUNDING WAS INCLUDED (Page 16).**

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

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

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

Cosme Alvarado-Esquivel,<sup>1\*</sup> Antonio Rascón-Careaga,<sup>2</sup> Jesús Hernández-Tinoco,<sup>3</sup> María Alba Guadalupe Corella-Madueño,<sup>2</sup> Luis Francisco Sánchez-Anguiano,<sup>3</sup> María Lourdes Aldana-Madrid,<sup>4</sup> Gerardo Javier Almada-Balderrama,<sup>2</sup> Alan Daniel Nuñez-Aguirre,<sup>2</sup> Oliver Liesenfeld<sup>5#</sup>

<sup>1</sup>Biomedical Research Laboratory, Faculty of Medicine and Nutrition, Juárez University of Durango State. Avenida Universidad S/N. 34000 Durango, Mexico.

<sup>2</sup>Department of Chemical and Biological Sciences. University of Sonora. Mexico.

<sup>3</sup>Institute for Scientific Research “Dr. Roberto Rivera-Damm”, Juárez University of Durango State. Avenida Universidad S/N. 34000 Durango, Mexico.

<sup>4</sup>Department of Research and Postgraduate in Food. University of Sonora. Mexico.

<sup>5</sup>Institute for Microbiology and Hygiene, Campus Benjamin Franklin, Charité Medical School. Hindenburgdamm 27. D-12203 Berlin, Germany.

#Present address: Roche Molecular Diagnostics, Pleasanton, CA. USA.

\*Corresponding author: Cosme Alvarado-Esquivel. Biomedical Research Laboratory. Faculty of Medicine and Nutrition, Juárez University of Durango State, Avenida Universidad S/N, 34000 Durango, Dgo, Mexico. Tel and Fax: +52-618 8130527; e-mail: alvaradocosme@yahoo.com



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6 **Key words:** *Toxoplasma gondii*, seroprevalence, Yoremes (Mayos), ethnic groups, cross-  
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## ABSTRACT

**OBJECTIVES:** We sought to determine the prevalence of anti-*T. gondii* antibodies in Yoremes and to identify associations of *T. gondii* exposure with socio-demographic, clinical and behavioral characteristics of Yoremes.

**DESIGN:** A cross sectional survey.

**SETTING:** Yoremes were enrolled in the locality of Tierra Blanca in the municipality of Navojoa 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 standardized questionnaire to obtain the characteristics of the Yoremes. The association of *T. gondii* exposure and the Yoremes' characteristics was assessed by bivariate and multivariate analyses.

**RESULTS:** Of the 200 Yoremes studied (mean age:  $31.50 \pm 18.43$  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 socio-demographic and behavioral characteristics showed that *T. gondii* exposure was associated with increasing age (OR= 1.02; 95% CI: 1.00-1.04;  $P=0.03$ ) and consumption of squirrel meat (OR= 4.99; 95% CI: 1.07-23.31;  $P=0.04$ ). Furthermore, seroprevalence of *T. gondii* infection was significantly higher in Yoremes with history of lymphadenopathy ( $P=0.03$ ) and those suffering from frequent abdominal pain ( $P=0.03$ ). In women, *T. gondii*

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3 exposure was associated with history of cesarean sections ( $P=0.03$ ) and miscarriages  
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5 ( $P=0.02$ ).  
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8 **CONCLUSIONS:** We demonstrate for the first time serological evidence of *T. gondii*  
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10 exposure among Yoremes in Mexico. Results suggest that infection with *T. gondii* might be  
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12 affecting the health of Yoremes. Results may be useful for an optimal design of preventive  
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14 measures against *T. gondii* infection.  
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### 22 **Strengths and limitations of this study**

- 24 • This is the first cross-sectional study of *Toxoplasma gondii* infection in the Mexican  
25 ethnic group of Yoremes (Mayos).  
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- 28 • The seroprevalence of *Toxoplasma gondii* infection was determined in Yoremes.  
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- 30 • Prevalence association with sociodemographic, clinical, and behavioral  
31 characteristics of Yoremes was determined.  
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- 34 • The sample size was small and the seropositivity rate was low to perform a wider  
35 analysis of the association of *Toxoplasma gondii* exposure and characteristics of the  
36 Yoremes.  
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## INTRODUCTION

*Toxoplasma gondii* (*T. gondii*) is a ubiquitous intracellular parasite.[1, 2] This parasite is currently infecting about one third of humanity.[3] Infection with *T. gondii* is usually asymptomatic.[2, 4] However, *T. gondii* disseminates after infection to many organs and may lead to disease in eyes, lymph nodes, and central nervous system.[4-6] Furthermore, primary infection with *T. gondii* in pregnant women is a threat for congenital disease.[4, 7] Infection with *T. gondii* may lead to a life-threatening disease in immunocompromised patients.[4, 8] 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.[2, 4]

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,[9] Tepehuanos,[10] and Huicholes [11] 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. Yoremes live in a region with a climate that is different from those in other regions where other populations groups in Mexico were studied for the seroepidemiology of *T. gondii* infection. Climate in the Yoremes' region is desert 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 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

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3 and the association of *T. gondii* prevalence with the socio-demographic, clinical, and  
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5 behavioral characteristics of Yoremes.  
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## 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) has an altitude of 25 meters above sea level, a desert climate, and a mean annual temperature of 25.4°C. Tierra Blanca has a mean annual rainfall of 266 mm. Inclusion criteria for the study subjects were: 1) Yoremes ethnicity (people who speak the Yoremes language and identify themselves as Yoremes); 2) aged 12 years and older; and 3) that 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% [10] in unexposed subjects, and an odds ratio of 2.6. The result of the calculation was 182 subjects. We added a 5% for refusals and the final sample size was 198 subjects. Sampling of Yoremes was performed by a convenience method. Firstly, authors met Yoremes leaders to provide information about the study. After obtaining permission from the Yoremes leaders, they invited the people they lead. Yoremes who accepted to participate in the study were gathered in two public places (a health center and a high school) to provide a blood sample and submit a questionnaire. Since this strategy was not enough to reach the sample size, authors visited houses in the community to enroll participants until the sample size was reached. This new strategy is not likely to influence the results since a minority of

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3 cases was obtain by this type of sampling. In total, 200 Yoremes were included in the  
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5 study.  
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### 10 **Socio-demographic, clinical, and behavioral data**

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12 Data from the participants was obtained with the aid of a standardized  
13 questionnaire. This questionnaire included socio-demographic, clinical and behavioral  
14 items. Socio-demographic items were age, sex, birthplace, residence, education,  
15 occupation, and socioeconomic status. Clinical items included current health status, history  
16 of lymphadenopathy, frequent presence of headache and abdominal pain, dizziness,  
17 impairments of memory, reflexes, hearing, and vision, and a history of blood transfusion,  
18 transplants or surgery. In women, obstetric history was also obtained. Behavioral items  
19 included contact with animals, food consumed, traveling, frequency of eating away from  
20 home (in restaurants or fast food outlets), contact with soil (gardening or agriculture) and  
21 type of flooring at home. Data about food were type of meat consumed, frequency of meat  
22 consumption, consumption of raw or undercooked meat, dried or processed meat, and  
23 consumption of unwashed raw vegetables and fruits, unpasteurized milk, or untreated  
24 water.  
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### 46 **Serological tests for anti-*T. gondii* antibodies**

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48 We obtained a blood sample from each participant. Blood samples were centrifuged  
49 and serum samples were obtained. Sera were stored at  $-20^{\circ}$  C until analyzed. Serum  
50 samples were tested for anti-*T. gondii* IgG antibodies with the commercially available  
51 “*Toxoplasma* IgG” (Diagnostic Automation Inc., Calabasas, CA, USA) enzyme  
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3 immunoassay (EIA). Anti-*T. gondii* IgG antibody levels were expressed as International  
4 Units (IU)/ml, and a value  $\geq 8$  IU/ml was used as a cut-off for seropositivity. Sera positive  
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7 for anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* IgM antibodies by  
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9  
10 the commercially available “*Toxoplasma* IgM” (Diagnostic Automation Inc.) EIA. The cut-  
11  
12 off for anti-*T. gondii* IgM seropositivity for each assay was obtained by multiplying the  
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14 mean cut-off calibrator optical density by a correction factor ( $f = 0.35-0.40$ ) printed on the  
15  
16 label of calibrator. All assays were performed following the manufacturer’s instructions,  
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18 and positive and negative controls were included in each run.  
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### 24 **Statistical analysis**

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27 Data was analyzed with the aid of the software Epi Info version 3.5.4 and SPSS  
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29 version 15.0. To avoid bias in the measure of associations, care was taken in obtaining all  
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31 data about the characteristics of participants, and there was no missing data. We used the  
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33 Pearson’s chi-square test and the Fisher exact test (when values were small) for initial  
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35 comparison of the frequencies among groups. Multivariate analysis was used to assess the  
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37 association between the socio-demographic and behavioral characteristics of Yoremes and  
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39 the seropositivity to *T. gondii*. Only variables with a *P* value equal to or less than 0.10  
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41 obtained in the bivariate analysis were included in the multivariate analysis. This strategy  
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43 allowed us to reduce substantially the number of variables in the analysis. Odds ratio (OR)  
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45 and 95% confidence interval (CI) were calculated by logistic regression using the stepwise  
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47 backward method. We used the Hosmer-Lemeshow goodness of fit test to assess the fitness  
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49 of the regression model. Statistical significance was set at a *P* value less than 0.05.  
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### **Ethical aspects**

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. A written informed consent was obtained from all participants and from the next of kin of minor participants.

For peer review only

## 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 subjects, 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 to 45 IU/ml. A correlation of the socio-demographic 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 histories of cesarean 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 histories of surgery, blood transfusion or transplant were similar among *T. gondii* positive and *T. gondii* negative Yoremes.

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Concerning behavioral characteristics, a number of variables showed  $P$  values equal to or lower than 0.10 in the bivariate analysis including consumption of meat from goat ( $P=0.09$ ) and squirrel ( $P=0.01$ ), consumption of raw dried meat ( $P=0.02$ ), and consumption of beef intestines ( $P=0.10$ ) and beef brains ( $P=0.06$ ), and alcoholism ( $P=0.09$ ). Results of a selection of behavioral characteristics of Yoremes and their correlation with *T. gondii* exposure are shown in Table 3. Other behavioral characteristics of Yoremes including contact with animals, traveling, consumption of meat other than goat and squirrel, frequency of meat consumption, degree of meat cooking, consumption of untreated water, unpasteurized 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 socio-demographic and behavioral characteristics showed that *T. gondii* exposure was associated only with increasing age (OR= 1.02; 95% CI: 1.00-1.04;  $P=0.03$ ), and consumption of squirrel meat (OR= 4.99; 95% CI: 1.07-23.31;  $P=0.04$ ). 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,[10] Mennonites,[9] and Huicholes,[11] 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 behavioral difference. Seroprevalence of *T. gondii* infection may be influenced by environment conditions with a high seroprevalence in humid regions [12] and a low seroprevalence in dry and hot regions.[13] 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.[12] 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 indigenous population in Brazil.[14] Improving of these epidemiological factors may result in the lowering of seroprevalence of *T. gondii* exposure

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3 in younger generations. We did not include minor (younger than 12 years old) participants  
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5 in this study because the frequency of *T. gondii* infection in young people is usually very  
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7 low. We also analyzed associations with factors other than the environment.  
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9 Seroprevalence was found to increase with age, consistent with previous reports in rural  
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11 [15] and urban [16, 17] populations in northern Mexico. The mean age (31.50 years) in  
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13 Yoremes was similar to that in Tepehuanos (31.03 years).[10] However, the mean age in  
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15 Yoremes was lower than the one (37.98 years) in Huicholes [11] and that (38.4 years) in  
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17 Mennonites.[9]  
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23 Multivariate analysis also showed an association of *T. gondii* exposure with  
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25 consumption of squirrel meat. In two previous studies in the general population in rural  
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27 [15] and urban [16] Durango, consumption of squirrel meat was also associated with *T.*  
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29 *gondii* exposure. These findings remark the importance of consumption of squirrel meat in  
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31 the transmission of *T. gondii* infection in the region. Although squirrel meat is usually  
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33 cooked before eating, failure in obtaining a well-done cooking may occur specially for  
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35 thick pieces of meat. Yoremes usually grill the squirrel meat, and this process may result in  
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37 an uneven cooking. In addition, tasting of raw or undercooked meat while grilling might  
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39 occur. Tasting of fresh raw meat was linked to toxoplasmosis in Italy.[18] Serological  
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41 evidence of *T. gondii* infection has been demonstrated in squirrels.[19] In addition, *T.*  
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43 *gondii* has been detected in organs of Korean squirrels (*Tamias sibericus*) [20] and grey  
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45 squirrels (*Sciurus carolensis*) [21] with fatal toxoplasmosis. We previously investigated the  
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47 presence of *T. gondii* in animals in Durango but were unable to detect anti-*T. gondii*  
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49 antibodies in 69 squirrels (*Spermophilus variegatus*) collected.[22] However, we cannot  
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51 rule out *T. gondii* infection in squirrels in the region because the sample size was small and  
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3 infection might occur in other squirrel species than the one studied. Further research about  
4 the epidemiological link of *T. gondii* infection and consumption of squirrel meat including  
5 the search for *T. gondii* in squirrels should therefore be conducted.  
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10 Intriguingly, in the present study we found an association of *T. gondii* exposure with  
11 abdominal pain, history of lymphadenopathy, cesarean sections and miscarriages. It is well  
12 known that *T. gondii* infection is a cause of lymph node enlargement and miscarriages.[2,  
13 4] In contrast, *T. gondii* infection is not typically associated with abdominal pain but  
14 abdominal pain has been reported in gastric toxoplasmosis in patients with acquired  
15 immunodeficiency syndrome.[23, 24] We also found an association of *T. gondii* infection  
16 with a history of cesarean section. It is not clear why women with cesarean sections had a  
17 higher seroprevalence of *T. gondii* infection than those without this history. Interestingly, in  
18 a study of women with stillbirths in Durango, Mexico, *T. gondii* exposure was associated  
19 with a history of surgery.[25] It raises the question whether a specific type of surgery as  
20 cesarean section or a specific population group as women might have a higher risk of *T.*  
21 *gondii* exposure than others. We did not investigate the indications for the cesarean sections  
22 or the health status of the children born by this surgical procedure, and this was a limitation  
23 of the study. Several factors could be considered to explain *T. gondii* infections in women  
24 with cesarean sections. Congenital toxoplasmosis may precipitate not only early delivery or  
25 induction of delivery but also prompt cesarean section.[26-28] In addition, the use of  
26 contaminated surgical instruments or materials during cesarean sections cannot be ruled  
27 out. Blood transfusion is relatively common in surgical patients, and infection with *T.*  
28 *gondii* by blood transfusion may also occur.[29] Further research about the association of *T.*  
29 *gondii* infection and cesarean section and other surgical procedures should be conducted.  
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In this work, anti-*T. gondii* IgM antibodies were present in a relatively high number of anti-*T. gondii* IgG positive Yoremes compared to previous studies. This finding should be interpreted with caution because positive results in IgM tests may indicate persistent IgM antibodies rather than acute infection.[30] We did not test all participants for anti-*T. gondii* IgM antibodies. Only IgG positive subjects were tested because a high number of false positive results for IgM has been reported when using immunoassays.[30] 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 the Yoremes. Reaching the sample size of Yoremes was challenging. However, the strategy to enroll 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.

## Acknowledgements

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3 This study was financially supported by Juárez University of Durango State,  
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5 Mexico.  
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### 10 **Competing interests**

11  
12 No competing interests exist.  
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### 18 **Authors' contributions**

19  
20 CAE, ARC, MAGCM, and MLAM designed the study protocol, and participated  
21 in the coordination and management of the study. ARC, MAGCM, GJAB and ADNA  
22 obtained blood samples, submitted the questionnaires and performed the data analysis.  
23 CAE performed the laboratory tests. CAE, JHT, LFSA, ARC, MAGCM, MLAM, and OL  
24 performed the data analysis, and wrote the manuscript.  
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### 35 **Data sharing statement**

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37 No additional data is available.  
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**Table 1. Socio-demographic characteristics of Yoremes and seroprevalence of *T. gondii* infection.**

Characteristic	Subjects Tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
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	
Birth place				
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				
Laborer <sup>a</sup>	43	8	18.6	0.21
Non-laborer <sup>b</sup>	157	18	11.5	
Socio-economic level				
Low	111	19	17.1	0.15
Medium	88	7	8.0	
High	1	0	0.0	

<sup>a</sup>Laborer: Agriculture, business, construction, livestock raising, professional, other.

<sup>b</sup>Non-laborer: student or housekeeping.

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

Characteristic	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
<b>Clinical status</b>				
Healthy	170	19	11.2	0.08
Ill	30	7	23.3	
<b>Lymphadenopathy ever</b>				
Yes	57	12	21.1	0.03
No	143	14	9.8	
<b>Abdominal pain frequently</b>				
Yes	51	11	21.6	0.03
No	149	15	10.1	
<b>Headache frequently</b>				
Yes	54	10	18.5	0.15
No	146	16	11	
<b>Memory impairment</b>				
Yes	28	5	17.9	0.37
No	172	21	12.2	
<b>Dizziness</b>				
Yes	46	6	13	0.99
No	154	20	13	
<b>Reflexes impairment</b>				
Yes	23	5	21.7	0.19
No	177	21	11.9	
<b>Hearing impairment</b>				
Yes	16	1	6.3	0.70
No	184	25	13.6	
<b>Visual impairment</b>				
Yes	45	8	17.8	0.27
No	155	18	11.6	
<b>Surgery ever</b>				
Yes	55	10	18.2	0.18
No	145	16	11	
<b>Blood transfusion</b>				
Yes	15	4	26.7	0.11

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4	No	185	22	11.9	
5	Pregnancies				
6	Yes	71	12	16.9	0.17
7	No	52	4	7.7	
8					
9	Deliveries				
10	Yes	51	8	15.7	0.45
11	No	72	8	11.1	
12					
13	Cesarean sections				
14	Yes	23	6	26.1	0.03
15	No	100	10	10	
16					
17	Miscarriages				
18	Yes	16	5	31.3	0.02
19	No	107	11	10.3	
20					
21	Stillbirths				
22	Yes	6	1	16.7	0.57
23	No	117	15	12.8	
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**Table 3. Bivariate analysis of selected putative risk factors for infection with *Toxoplasma gondii* in Yoremes.**

Characteristic	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
Cats at home				
Yes	93	10	10.8	0.37
No	107	16	15	
National trips				
Yes	51	8	15.7	0.5
No	149	18	12.1	
Goat meat consumption				
Yes	71	13	18.3	0.09
No	129	13	10.1	
Sheep meat consumption				
Yes	67	12	17.9	0.14
No	133	14	10.5	
Turkey meat consumption				
Yes	44	3	6.8	0.16
No	156	23	14.7	
Duck meat consumption				
Yes	9	2	22.2	0.33
No	191	24	12.6	
Quail meat consumption				
Yes	27	6	22.2	0.13
No	173	20	11.6	
Rabbit meat consumption				
Yes	21	5	23.8	0.16
No	179	21	11.7	
Squirrel meat consumption				
Yes	8	4	50	0.01
No	192	22	11.5	
Snake meat consumption				
Yes	6	2	33.3	0.17
No	194	24	12.4	
Raw dried meat				
Yes	43	10	23.3	0.02
No	157	16	10.2	
Chorizo consumption				
Yes	184	22	12	0.13
No	16	4	25	
Beef intestines consumption				
Yes	58	11	19	0.1
No	142	15	10.6	
Consumption of cow's brain				
Yes	18	5	27.8	0.06

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No	182	21	11.5	
Frequency of eating out of home				
Never	35	8	22.9	0.15
1 to 10 times a year	103	12	11.7	
>10 times a year	62	6	9.7	
Alcoholism				
Yes	20	5	25	0.09
No	180	21	11.7	
Soil contact				
Yes	130	20	15.4	0.17
No	70	6	8.6	

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract  <b>THE STUDY DESIGN IS INCLUDED IN THE ABSTRACT (Page 3).</b> (b) Provide in the abstract an informative and balanced summary of what was done and what was found  <b>AN ABSTRACT WITH IMPORTANT DATA WAS INCLUDED (Pages 3-4).</b>
<b>Introduction</b>		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported  <b>A BACKGROUND AND RATIONALE FOR THE STUDY WAS INCLUDED (Page 5).</b>
Objectives	3	State specific objectives, including any prespecified hypotheses  <b>OBJECTIVES WERE INCLUDED (Pages 5-6).</b>
<b>Methods</b>		
Study design	4	Present key elements of study design early in the paper  <b>ELEMENTS OF THE STUDY DESIGN WERE INCLUDED (Page 7).</b>
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection  <b>SETTING, LOCATIONS RELEVANT DATES, PERIOD OF RECRUITMENT, AND DATA COLLECTION WERE INCLUDED (Pages 7-8).</b>
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants  <b>ELIGIBILITY CRITERIA, AND THE SOURCES AND METHOD OF SELECTION OF PARTICIPANTS WERE INCLUDED (Pages 7-8).</b> (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable  <b>DATA ABOUT VARIABLES AND DIAGNOSIS WAS INCLUDED (Pages 8-</b>

		9).
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
<b>INFORMATION ABOUT THE VARIABLES, AND METHODS OF ASSESSMENT WAS INCLUDED (Pages 8-9).</b>		
Bias	9	Describe any efforts to address potential sources of bias
<b>INFORMATION ABOUT EFFORTS TO AVOID BIAS WAS ADDED (Page 9).</b>		
Study size	10	Explain how the study size was arrived at
<b>INFORMATION ABOUT THE CALCULATION OF SAMPLE SIZE WAS INCLUDED (Page 7).</b>		
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
<b>INFORMATION ABOUT THE VARIABLES CHOSEN IN THE ANALYSIS WAS INCLUDED (Pages 8-9).</b>		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding
<b>A DESCRIPTION OF THE STATISTICAL ANALYSIS WAS INCLUDED (Page 9).</b>		
		(b) Describe any methods used to examine subgroups and interactions
<b>METHODS USED TO EXAMINE SUBGROUPS WERE DESCRIBED (Page 9).</b>		
		(c) Explain how missing data were addressed
<b>THERE WAS NO MISSING DATA.</b>		
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed
		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy
<b>ANALYTICAL METHODS ARE SHOWN IN THE MATERIALS AND METHODS SECTION (pages 8-9).</b>		
		(e) Describe any sensitivity analyses
<b>NOT APPLICABLE.</b>		

Continued on next page

**Results**

Participants	13*	<p>(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed</p> <p><b>INFORMATION ABOUT THE ELIGIBILITY OF SUBJECT WAS INCLUDED (Pages 7-8).</b></p> <p>(b) Give reasons for non-participation at each stage</p> <p><b>INFORMATION ABOUT NON-PARTICIPATION WAS INCLUDED (Page 7).</b></p> <p>(c) Consider use of a flow diagram</p> <p><b>THE NUMBER OF PROCEDURES WAS SMALL AND A FLOW DIAGRAM MIGHT BE NOT NECESSARY.</b></p>
Descriptive data	14*	<p>(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders</p> <p><b>CHARACTERISTICS OF THE STUDY PARTICIPANTS WERE INCLUDED (Pages 8-9).</b></p> <p>(b) Indicate number of participants with missing data for each variable of interest</p> <p><b>THERE WAS NO MISSING DATA.</b></p> <p>(c) <i>Cohort study</i>—Summarise follow-up time (eg, average and total amount)</p>
Outcome data	15*	<p><i>Cohort study</i>—Report numbers of outcome events or summary measures over time</p> <p><i>Case-control study</i>—Report numbers in each exposure category, or summary measures of exposure</p> <p><i>Cross-sectional study</i>—Report numbers of outcome events or summary measures</p> <p><b>TABLES WITH SUMMARY OF RESULTS WERE INCLUDED (Pages 23-27).</b></p>
Main results	16	<p>(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included</p> <p><b>INFORMATION ABOUT 95% CONFIDENCE INTERVALS WAS INCLUDED (Page 9).</b></p> <p>(b) Report category boundaries when continuous variables were categorized</p> <p><b>INFORMATION ABOUT CATEGORIES AND SUBGROUPS ARE INCLUDED IN TABLES (Pages 23-27).</b></p> <p>(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</p> <p><b>NO RELATIVE RISKS WERE ASSESSED.</b></p>
Other analyses	17	<p>Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses</p> <p><b>RESULTS OF ANALYSIS OF SUBGROUPS WERE SHOWN IN TABLES (Pages 23-</b></p>

27).

**Discussion**

Key results	18	Summarise key results with reference to study objectives
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**KEY RESULTS WITH REFERENCE TO OBJECTIVES WERE DISCUSSED (Pages 13-16).**

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
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**THE LIMITATIONS OF THE STUDY WERE INCLUDED (Page 15-16).**

Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
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**AN INTERPRETATION OF RESULTS WAS INCLUDED (Pages 13-16).**

Generalisability	21	Discuss the generalisability (external validity) of the study results
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**INFORMATION RELATED WITH THE GENERALISABILITY OF THE STUDY RESULTS WAS INCLUDED (Pages 15-16).**

**Other information**

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based
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**INFORMATION ABOUT FUNDING WAS INCLUDED (Page 16).**

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

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

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

Cosme Alvarado-Esquivel,<sup>1\*</sup> Antonio Rascón-Careaga,<sup>2</sup> Jesús Hernández-Tinoco,<sup>3</sup> María Alba Guadalupe Corella-Madueño,<sup>2</sup> Luis Francisco Sánchez-Anguiano,<sup>3</sup> María Lourdes Aldana-Madrid,<sup>4</sup> Gerardo Javier Almada-Balderrama,<sup>2</sup> Alan Daniel Nuñez-Aguirre,<sup>2</sup> Oliver Liesenfeld<sup>5#</sup>

<sup>1</sup>Biomedical Research Laboratory, Faculty of Medicine and Nutrition, Juárez University of Durango State. Avenida Universidad S/N. 34000 Durango, Mexico.

<sup>2</sup>Department of Chemical and Biological Sciences. University of Sonora. Mexico.

<sup>3</sup>Institute for Scientific Research “Dr. Roberto Rivera-Damm”, Juárez University of Durango State. Avenida Universidad S/N. 34000 Durango, Mexico.

<sup>4</sup>Department of Research and Postgraduate in Food. University of Sonora. Mexico.

<sup>5</sup>Institute for Microbiology and Hygiene, Campus Benjamin Franklin, Charité Medical School. Hindenburgdamm 27. D-12203 Berlin, Germany.

#Present address: Roche Molecular Diagnostics, Pleasanton, CA. USA.

\*Corresponding author: Cosme Alvarado-Esquivel. Biomedical Research Laboratory. Faculty of Medicine and Nutrition, Juárez University of Durango State, Avenida Universidad S/N, 34000 Durango, Dgo, Mexico. Tel and Fax: +52-618 8130527; e-mail: alvaradocosme@yahoo.com



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6 **Key words:** *Toxoplasma gondii*, seroprevalence, Yoremes (Mayos), ethnic groups, cross-  
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8 sectional study.  
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## ABSTRACT

**OBJECTIVES:** We sought to determine the prevalence of anti-*T. gondii* antibodies in Yoremes and to identify associations of *T. gondii* exposure with socio-demographic, clinical and behavioral characteristics of Yoremes.

**DESIGN:** A cross sectional survey.

**SETTING:** Yoremes were enrolled in the locality of Tierra Blanca in the municipality of Navojoa 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 standardized questionnaire to obtain the characteristics of the Yoremes. The association of *T. gondii* exposure and the Yoremes' characteristics was assessed by bivariate and multivariate analyses.

**RESULTS:** Of the 200 Yoremes studied (mean age:  $31.50 \pm 18.43$  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 socio-demographic and behavioral characteristics showed that *T. gondii* exposure was associated with increasing age (OR= 1.02; 95% CI: 1.00-1.04;  $P=0.03$ ) and consumption of squirrel meat (OR= 4.99; 95% CI: 1.07-23.31;  $P=0.04$ ). Furthermore, seroprevalence of *T. gondii* infection was significantly higher in Yoremes with history of lymphadenopathy ( $P=0.03$ ) and those suffering from frequent abdominal pain ( $P=0.03$ ). In women, *T. gondii*

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3 exposure was associated with history of cesarean sections ( $P=0.03$ ) and miscarriages  
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5 ( $P=0.02$ ).  
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8 **CONCLUSIONS:** We demonstrate for the first time serological evidence of *T. gondii*  
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10 exposure among Yoremes in Mexico. Results suggest that infection with *T. gondii* might be  
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12 affecting the health of Yoremes. Results may be useful for an optimal design of preventive  
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14 measures against *T. gondii* infection.  
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### 22 **Strengths and limitations of this study**

- 24 • This is the first cross-sectional study of *Toxoplasma gondii* infection in the Mexican  
25 ethnic group of Yoremes (Mayos).  
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- 28 • The seroprevalence of *Toxoplasma gondii* infection was determined in Yoremes.  
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- 30 • Prevalence association with sociodemographic, clinical, and behavioral  
31 characteristics of Yoremes was determined.  
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- 34 • The sample size was small and the seropositivity rate was low to perform a wider  
35 analysis of the association of *Toxoplasma gondii* exposure and characteristics of the  
36 Yoremes.  
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## INTRODUCTION

*Toxoplasma gondii* (*T. gondii*) is a ubiquitous intracellular parasite.[1, 2] This parasite is currently infecting about one third of humanity.[3] Infection with *T. gondii* is usually asymptomatic.[2, 4] However, *T. gondii* disseminates after infection to many organs and may lead to disease in eyes, lymph nodes, and central nervous system.[4-6] Furthermore, primary infection with *T. gondii* in pregnant women is a threat for congenital disease.[4, 7] Infection with *T. gondii* may lead to a life-threatening disease in immunocompromised patients.[4, 8] 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.[2, 4]

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,[9] Tepehuanos,[10] and Huicholes [11] 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. Yoremes live in a region with a climate that is different from those in other regions where other populations groups in Mexico were studied for the seroepidemiology of *T. gondii* infection. Climate in the Yoremes' region is desert 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 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

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3 and the association of *T. gondii* prevalence with the socio-demographic, clinical, and  
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5 behavioral characteristics of Yoremes.  
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## 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) has an altitude of 25 meters above sea level, a desert climate, and a mean annual temperature of 25.4°C. Tierra Blanca has a mean annual rainfall of 266 mm. Inclusion criteria for the study subjects were: 1) Yoremes ethnicity (people who speak the Yoremes language and identify themselves as Yoremes); 2) aged 12 years and older; and 3) that 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% [10] in unexposed subjects, and an odds ratio of 2.6. The result of the calculation was 182 subjects. We added a 5% for refusals and the final sample size was 198 subjects. Sampling of Yoremes was performed by a convenience method. Firstly, authors met Yoremes leaders to provide information about the study. After obtaining permission from the Yoremes leaders, they invited the people they lead. Yoremes who accepted to participate in the study were gathered in two public places (a health center and a high school) to provide a blood sample and submit a questionnaire. Since this strategy was not enough to reach the sample size, authors visited houses in the community to enroll participants until the sample size was reached. This new strategy is not likely to influence the results since a minority of

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3 cases was obtain by this type of sampling. In total, 200 Yoremes were included in the  
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5 study.  
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### 10 **Socio-demographic, clinical, and behavioral data**

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12 Data from the participants was obtained with the aid of a standardized  
13 questionnaire. This questionnaire included socio-demographic, clinical and behavioral  
14 items. Socio-demographic items were age, sex, birthplace, residence, education,  
15 occupation, and socioeconomic status. Clinical items included current health status, history  
16 of lymphadenopathy, frequent presence of headache and abdominal pain, dizziness,  
17 impairments of memory, reflexes, hearing, and vision, and a history of blood transfusion,  
18 transplants or surgery. In women, obstetric history was also obtained. Behavioral items  
19 included contact with animals, food consumed, traveling, frequency of eating away from  
20 home (in restaurants or fast food outlets), contact with soil (gardening or agriculture) and  
21 type of flooring at home. Data about food were type of meat consumed, frequency of meat  
22 consumption, consumption of raw or undercooked meat, dried or processed meat, and  
23 consumption of unwashed raw vegetables and fruits, unpasteurized milk, or untreated  
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### 46 **Serological tests for anti-*T. gondii* antibodies**

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48 We obtained a blood sample from each participant. Blood samples were centrifuged  
49 and serum samples were obtained. Sera were stored at  $-20^{\circ}$  C until analyzed. Serum  
50 samples were tested for anti-*T. gondii* IgG antibodies with the commercially available  
51 “*Toxoplasma* IgG” (Diagnostic Automation Inc., Calabasas, CA, USA) enzyme  
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3 immunoassay (EIA). Anti-*T. gondii* IgG antibody levels were expressed as International  
4 Units (IU)/ml, and a value  $\geq 8$  IU/ml was used as a cut-off for seropositivity. Sera positive  
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7 for anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* IgM antibodies by  
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10 the commercially available “*Toxoplasma* IgM” (Diagnostic Automation Inc.) EIA. The cut-  
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13 off for anti-*T. gondii* IgM seropositivity for each assay was obtained by multiplying the  
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16 mean cut-off calibrator optical density by a correction factor ( $f = 0.35-0.40$ ) printed on the  
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19 label of calibrator. All assays were performed following the manufacturer’s instructions,  
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22 and positive and negative controls were included in each run.  
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### 25 **Statistical analysis**

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27 Data was analyzed with the aid of the software Epi Info version 3.5.4 and SPSS  
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30 version 15.0. To avoid bias in the measure of associations, care was taken in obtaining all  
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33 data about the characteristics of participants, and there was no missing data. We used the  
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36 Pearson’s chi-square test and the Fisher exact test (when values were small) for initial  
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39 comparison of the frequencies among groups. Multivariate analysis was used to assess the  
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42 association between the socio-demographic and behavioral characteristics of Yoremes and  
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45 the seropositivity to *T. gondii*. Only variables with a *P* value equal to or less than 0.10  
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48 obtained in the bivariate analysis were included in the multivariate analysis. This strategy  
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51 allowed us to reduce substantially the number of variables in the analysis. Odds ratio (OR)  
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54 and 95% confidence interval (CI) were calculated by logistic regression using the stepwise  
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57 backward method. We used the Hosmer-Lemeshow goodness of fit test to assess the fitness  
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60 of the regression model. Statistical significance was set at a *P* value less than 0.05.



### **Ethical aspects**

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. A written informed consent was obtained from all participants and from the next of kin of minor participants.

For peer review only

## 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 subjects, 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 to 45 IU/ml. A correlation of the socio-demographic 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 histories of cesarean 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 histories of surgery, blood transfusion or transplant were similar among *T. gondii* positive and *T. gondii* negative Yoremes.

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3 Concerning behavioral characteristics, a number of variables showed *P* values equal  
4 to or low than 0.10 in the bivariate analysis including consumption of meat from goat and  
5 squirrel, consumption of raw dried meat, beef intestines and beef brains, and alcoholism.  
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7 Other behavioral characteristics of Yoremes including contact with animals, traveling,  
8 consumption of meat other than goat and squirrel, frequency of meat consumption, degree  
9 of meat cooking, consumption of untreated water, unpasteurized milk, processed meat,  
10 unwashed raw vegetables or fruits, frequency of eating out of home, contact with soil, and  
11 type of flooring at home showed *P* values higher than 0.10 in the bivariate analysis.  
12  
13 Multivariate analysis of socio-demographic and behavioral variables showed that *T. gondii*  
14 exposure was associated only with increasing age (OR= 1.02; 95% CI: 1.00-1.04; *P*=0.03),  
15 and consumption of squirrel meat (OR= 4.99; 95% CI: 1.07-23.31; *P*=0.04). An acceptable  
16 fit (*P*=0.37) of our regression model was obtained in the Hosmer-Lemeshow test.  
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## 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,[10] Mennonites,[9] and Huicholes,[11] 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 behavioral difference. Seroprevalence of *T. gondii* infection may be influenced by environment conditions with a high seroprevalence in humid regions [12] and a low seroprevalence in dry and hot regions.[13] 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.[12] 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 indigenous population in Brazil.[14] Improving of these epidemiological factors may result in the lowering of seroprevalence of *T. gondii* exposure

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3 in younger generations. We did not include minor (younger than 12 years old) participants  
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5 in this study because the frequency of *T. gondii* infection in young people is usually very  
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7 low. We also analyzed associations with factors other than the environment.  
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9 Seroprevalence was found to increase with age, consistent with previous reports in rural  
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11 [15] and urban [16, 17] populations in northern Mexico. The mean age (31.50 years) in  
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13 Yoremes was similar to that in Tepehuanos (31.03 years).[10] However, the mean age in  
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15 Yoremes was lower than the one (37.98 years) in Huicholes [11] and that (38.4 years) in  
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17 Mennonites.[9]  
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23 Multivariate analysis also showed an association of *T. gondii* exposure with  
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25 consumption of squirrel meat. In two previous studies in the general population in rural  
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27 [15] and urban [16] Durango, consumption of squirrel meat was also associated with *T.*  
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29 *gondii* exposure. These findings remark the importance of consumption of squirrel meat in  
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31 the transmission of *T. gondii* infection in the region. Although squirrel meat is usually  
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33 cooked before eating, failure in obtaining a well-done cooking may occur specially for  
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35 thick pieces of meat. Yoremes usually grill the squirrel meat, and this process may result in  
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37 an uneven cooking. In addition, tasting of raw or undercooked meat while grilling might  
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39 occur. Tasting of fresh raw meat was linked to toxoplasmosis in Italy.[18] Serological  
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41 evidence of *T. gondii* infection has been demonstrated in squirrels.[19] In addition, *T.*  
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43 *gondii* has been detected in organs of Korean squirrels (*Tamias sibericus*) [20] and grey  
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45 squirrels (*Sciurus carolensis*) [21] with fatal toxoplasmosis. We previously investigated the  
46  
47 presence of *T. gondii* in animals in Durango but were unable to detect anti-*T. gondii*  
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49 antibodies in 69 squirrels (*Spermophilus variegatus*) collected.[22] However, we cannot  
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51 rule out *T. gondii* infection in squirrels in the region because the sample size was small and  
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3 infection might occur in other squirrel species than the one studied. Further research about  
4 the epidemiological link of *T. gondii* infection and consumption of squirrel meat including  
5 the search for *T. gondii* in squirrels should therefore be conducted.  
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10 Intriguingly, in the present study we found an association of *T. gondii* exposure with  
11 abdominal pain, history of lymphadenopathy, cesarean sections and miscarriages. It is well  
12 known that *T. gondii* infection is a cause of lymph node enlargement and miscarriages.[2,  
13 4] In contrast, *T. gondii* infection is not typically associated with abdominal pain but  
14 abdominal pain has been reported in gastric toxoplasmosis in patients with acquired  
15 immunodeficiency syndrome.[23, 24] We also found an association of *T. gondii* infection  
16 with a history of cesarean section. It is not clear why women with cesarean sections had a  
17 higher seroprevalence of *T. gondii* infection than those without this history. Interestingly, in  
18 a study of women with stillbirths in Durango, Mexico, *T. gondii* exposure was associated  
19 with a history of surgery.[25] It raises the question whether a specific type of surgery as  
20 cesarean section or a specific population group as women might have a higher risk of *T.*  
21 *gondii* exposure than others. We did not investigate the indications for the cesarean sections  
22 or the health status of the children born by this surgical procedure, and this was a limitation  
23 of the study. Several factors could be considered to explain *T. gondii* infections in women  
24 with cesarean sections. Congenital toxoplasmosis may precipitate not only early delivery or  
25 induction of delivery but also prompt cesarean section.[26-28] In addition, the use of  
26 contaminated surgical instruments or materials during cesarean sections cannot be ruled  
27 out. Blood transfusion is relatively common in surgical patients, and infection with *T.*  
28 *gondii* by blood transfusion may also occur.[29] Further research about the association of *T.*  
29 *gondii* infection and cesarean section and other surgical procedures should be conducted.  
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In this work, anti-*T. gondii* IgM antibodies were present in a relatively high number of anti-*T. gondii* IgG positive Yoremes compared to previous studies. This finding should be interpreted with caution because positive results in IgM tests may indicate persistent IgM antibodies rather than acute infection.[30] We did not test all participants for anti-*T. gondii* IgM antibodies. Only IgG positive subjects were tested because a high number of false positive results for IgM has been reported when using immunoassays.[30] 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 the Yoremes. Reaching the sample size of Yoremes was challenging. However, the strategy to enroll 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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3 This study was financially supported by Juárez University of Durango State,  
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5 Mexico.  
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### 10 **Competing interests**

11  
12 No competing interests exist.  
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### 18 **Authors' contributions**

19  
20 CAE, ARC, MAGCM, and MLAM designed the study protocol, and participated  
21 in the coordination and management of the study. ARC, MAGCM, GJAB and ADNA  
22 obtained blood samples, submitted the questionnaires and performed the data analysis.  
23 CAE performed the laboratory tests. CAE, JHT, LFSA, ARC, MAGCM, MLAM, and OL  
24 performed the data analysis, and wrote the manuscript.  
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### 35 **Data sharing statement**

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37 No additional data is available.  
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**Table 1. Socio-demographic characteristics of Yoremes and seroprevalence of *T. gondii* infection.**

Characteristic	Subjects Tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
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	
Birth place				
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				
Laborer <sup>a</sup>	43	8	18.6	0.21
Non-laborer <sup>b</sup>	157	18	11.5	
Socio-economic level				
Low	111	19	17.1	0.15
Medium	88	7	8.0	
High	1	0	0.0	

<sup>a</sup>Laborer: Agriculture, business, construction, livestock raising, professional, other.

<sup>b</sup>Non-laborer: student or housekeeping.

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

Characteristic	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
<b>Clinical status</b>				
Healthy	170	19	11.2	0.08
Ill	30	7	23.3	
<b>Lymphadenopathy ever</b>				
Yes	57	12	21.1	0.03
No	143	14	9.8	
<b>Abdominal pain frequently</b>				
Yes	51	11	21.6	0.03
No	149	15	10.1	
<b>Headache frequently</b>				
Yes	54	10	18.5	0.15
No	146	16	11	
<b>Memory impairment</b>				
Yes	28	5	17.9	0.37
No	172	21	12.2	
<b>Dizziness</b>				
Yes	46	6	13	0.99
No	154	20	13	
<b>Reflexes impairment</b>				
Yes	23	5	21.7	0.19
No	177	21	11.9	
<b>Hearing impairment</b>				
Yes	16	1	6.3	0.70
No	184	25	13.6	
<b>Visual impairment</b>				
Yes	45	8	17.8	0.27
No	155	18	11.6	
<b>Surgery ever</b>				
Yes	55	10	18.2	0.18
No	145	16	11	
<b>Blood transfusion</b>				
Yes	15	4	26.7	0.11

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2					
3	No	185	22	11.9	
4	Pregnancies				
5	Yes	71	12	16.9	0.17
6	No	52	4	7.7	
7	Deliveries				
8	Yes	51	8	15.7	0.45
9	No	72	8	11.1	
10	Cesarean sections				
11	Yes	23	6	26.1	0.03
12	No	100	10	10	
13	Miscarriages				
14	Yes	16	5	31.3	0.02
15	No	107	11	10.3	
16	Stillbirths				
17	Yes	6	1	16.7	0.57
18	No	117	15	12.8	
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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract  <b>THE STUDY DESIGN IS INCLUDED IN THE ABSTRACT (Page 3).</b> (b) Provide in the abstract an informative and balanced summary of what was done and what was found  <b>AN ABSTRACT WITH IMPORTANT DATA WAS INCLUDED (Pages 3-4).</b>
<b>Introduction</b>		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported  <b>A BACKGROUND AND RATIONALE FOR THE STUDY WAS INCLUDED (Page 5).</b>
Objectives	3	State specific objectives, including any prespecified hypotheses  <b>OBJECTIVES WERE INCLUDED (Pages 5-6).</b>
<b>Methods</b>		
Study design	4	Present key elements of study design early in the paper  <b>ELEMENTS OF THE STUDY DESIGN WERE INCLUDED (Page 7).</b>
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection  <b>SETTING, LOCATIONS RELEVANT DATES, PERIOD OF RECRUITMENT, AND DATA COLLECTION WERE INCLUDED (Pages 7-8).</b>
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants  <b>ELIGIBILITY CRITERIA, AND THE SOURCES AND METHOD OF SELECTION OF PARTICIPANTS WERE INCLUDED (Pages 7-8).</b> (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable  <b>DATA ABOUT VARIABLES AND DIAGNOSIS WAS INCLUDED (Pages 8-</b>

		9).
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
		<b>INFORMATION ABOUT THE VARIABLES, AND METHODS OF ASSESSMENT WAS INCLUDED (Pages 8-9).</b>
Bias	9	Describe any efforts to address potential sources of bias
		<b>INFORMATION ABOUT EFFORTS TO AVOID BIAS WAS ADDED (Page 9).</b>
Study size	10	Explain how the study size was arrived at
		<b>INFORMATION ABOUT THE CALCULATION OF SAMPLE SIZE WAS INCLUDED (Page 7).</b>
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
		<b>INFORMATION ABOUT THE VARIABLES CHOSEN IN THE ANALYSIS WAS INCLUDED (Pages 8-9).</b>
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding
		<b>A DESCRIPTION OF THE STATISTICAL ANALYSIS WAS INCLUDED (Page 9).</b>
		(b) Describe any methods used to examine subgroups and interactions
		<b>METHODS USED TO EXAMINE SUBGROUPS WERE DESCRIBED (Page 9).</b>
		(c) Explain how missing data were addressed
		<b>THERE WAS NO MISSING DATA.</b>
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed
		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy
		<b>ANALYTICAL METHODS ARE SHOWN IN THE MATERIALS AND METHODS SECTION (pages 8-9).</b>
		(e) Describe any sensitivity analyses
		<b>NOT APPLICABLE.</b>

Continued on next page

**Results**

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		<b>INFORMATION ABOUT THE ELIGIBILITY OF SUBJECT WAS INCLUDED (Pages 7-8).</b>
		(b) Give reasons for non-participation at each stage
		<b>INFORMATION ABOUT NON-PARTICIPATION WAS INCLUDED (Page 7).</b>
		(c) Consider use of a flow diagram
		<b>THE NUMBER OF PROCEDURES WAS SMALL AND A FLOW DIAGRAM MIGHT BE NOT NECESSARY.</b>
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		<b>CHARACTERISTICS OF THE STUDY PARTICIPANTS WERE INCLUDED (Pages 8-9).</b>
		(b) Indicate number of participants with missing data for each variable of interest
		<b>THERE WAS NO MISSING DATA.</b>
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
		<b>TABLES WITH SUMMARY OF RESULTS WERE INCLUDED (Pages 23-25).</b>
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		<b>INFORMATION ABOUT 95% CONFIDENCE INTERVALS WAS INCLUDED (Page 9).</b>
		(b) Report category boundaries when continuous variables were categorized
		<b>INFORMATION ABOUT CATEGORIES AND SUBGROUPS ARE INCLUDED IN TABLES (Pages 23-25).</b>
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
		<b>NO RELATIVE RISKS WERE ASSESSED.</b>
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
		<b>RESULTS OF ANALYSIS OF SUBGROUPS WERE SHOWN IN TABLES (Pages 23-</b>

25).

**Discussion**

Key results	18	Summarise key results with reference to study objectives
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**KEY RESULTS WITH REFERENCE TO OBJECTIVES WERE DISCUSSED (Pages 13-16).**

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
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**THE LIMITATIONS OF THE STUDY WERE INCLUDED (Page 15-16).**

Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
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**AN INTERPRETATION OF RESULTS WAS INCLUDED (Pages 13-16).**

Generalisability	21	Discuss the generalisability (external validity) of the study results
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**INFORMATION RELATED WITH THE GENERALISABILITY OF THE STUDY RESULTS WAS INCLUDED (Pages 15-16).**

**Other information**

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based
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**INFORMATION ABOUT FUNDING WAS INCLUDED (Page 16).**

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).