

# BMJ Open Seroepidemiology of *Toxoplasma gondii* in pregnant women in Aguascalientes City, Mexico: a cross-sectional study

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**To cite:** Alvarado-Esquivel C, Terrones-Saldívar M del C, Hernández-Tinoco J, *et al*. Seroepidemiology of *Toxoplasma gondii* in pregnant women in Aguascalientes City, Mexico: a cross-sectional study. *BMJ Open* 2016;**6**:e012409. doi:10.1136/bmjopen-2016-012409

► Prepublication history for this paper is available online. To view these files please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2016-012409>).

Received 24 April 2016  
Revised 26 May 2016  
Accepted 7 June 2016



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## ABSTRACT

**Objectives:** We determined the seroprevalence and correlates of *Toxoplasma gondii* infection in pregnant women in Aguascalientes City, Mexico.

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

**Setting:** Pregnant women were enrolled in the central Mexican city of Aguascalientes.

**Participants:** We studied 338 pregnant women who attended prenatal care in 3 public health centres.

### Primary and secondary outcome measures:

Women were examined for IgG/IgM antibodies to *T. gondii* by using commercially available enzyme immunoassays, and an avidity test. Multiple analyses were used to determine the association of *T. gondii* seropositivity with the characteristics of the pregnant women.

**Results:** Of the 338 pregnant women studied, 21 (6.2%) had IgG antibodies to *T. gondii*, and 1 (4.8%) of them was also positive for IgM antibodies to *T. gondii*. Avidity of IgG antibodies to *T. gondii* was high in the IgM-positive sample. Logistic regression analysis of sociodemographic, behavioural and housing variables showed that *T. gondii* seropositivity was associated with white ethnicity (OR=149.4; 95% CI 10.8 to 2054.1;  $p<0.01$ ), not washing hands before eating (OR=6.41; 95% CI 1.73 to 23.6;  $p=0.005$ ) and use of latrine (OR=37.6; 95% CI 4.63 to 306.31;  $p=0.001$ ).

**Conclusions:** Results demonstrate that pregnant women in Aguascalientes City have a low seroprevalence of *T. gondii* infection. However, this low prevalence indicates that most pregnant women are at risk for a primary infection. Factors associated with *T. gondii* exposure found in this study, including food hygiene, may be useful to determine preventive measures against *T. gondii* infection and its sequelae.

## INTRODUCTION

Infection with the parasite *Toxoplasma gondii* (*T. gondii*) is common in humans and animals around the world.<sup>1 2</sup> This infection is acquired by ingestion of water or food contaminated

## Strengths and limitations of this study

- This is the first cross-sectional study of *Toxoplasma gondii* infection in pregnant women in the central Mexican city of Aguascalientes.
- The current study gave us the immunological status against infection with *T. gondii* in a sample of pregnant women in central Mexico.
- This study provides a number of risk factors for infection with *T. gondii* in pregnant women that can be useful to design optimal preventive measures against toxoplasmosis.
- The sample size was small, and the seropositivity rate was low to perform a wider analysis of the association of *T. gondii* exposure and characteristics of the pregnant women.

with oocysts shed by cats or other felids, by ingestion of tissue cysts in meat from mammals and birds,<sup>2 3</sup> and congenitally.<sup>4 5</sup> Most infections are asymptomatic; however, infection with the parasite can lead to acute toxoplasmosis that presents as lymphadenopathy or chorioretinitis.<sup>5</sup> Immunocompromised individuals may develop a life-threatening disease with meningoencephalitis.<sup>5 6</sup> Primary infection with *T. gondii* during pregnancy may lead to congenital disease with miscarriages or stillbirths,<sup>5 7 8</sup> or disease in eye and central nervous system.<sup>5 9 10</sup> Most newborns with congenitally acquired infections with *T. gondii* are asymptomatic; however, clinical manifestations of toxoplasmosis develop later in life.<sup>9</sup> Diagnosis of infection with *T. gondii* during pregnancy is made with the aid of serological tests, particularly the IgG avidity testing that allows for more accurate timing of maternal infection.<sup>11 12</sup>

Very little is known about the seroepidemiology of *T. gondii* infection in pregnant women in Mexico in general, and there is a lack of knowledge about this infection in pregnant women, particularly in the central

Mexican city of Aguascalientes. In two previous studies in the northern Mexican state of Durango, *T. gondii* seroprevalences of 6.1% in urban<sup>13</sup> and 8.2% in rural<sup>14</sup> pregnant women were found. In the present study, we sought to determine the seroprevalence of *T. gondii* infection in pregnant women attending prenatal consultations at three public health centres in Aguascalientes City, Mexico, and to determine the association of *T. gondii* seropositivity with the sociodemographic, clinical, behavioural and housing characteristics of the pregnant women.

## MATERIALS AND METHODS

### Study design and study population

Through a cross-sectional study design, we examined pregnant women who attended the prenatal care consultations at one of the three public health centres (Instituto de Servicios de Salud del Estado de Aguascalientes) in Aguascalientes City, Mexico, from October 2014 to February 2016. Aguascalientes is located in central Mexico; its coordinates and climate conditions are shown in figure 1. Inclusion criteria were: (1) pregnant women with 1–9 months of pregnancy; (2) aged 13–45 years and (3) who accepted to participate in the study. Socioeconomic status, occupation, or educational level were not restrictive criteria for enrolment. Participants were enrolled consecutively. In total, 338 pregnant women (mean age 22.95 ±6.19 years; range 13–42 years) were included in the study.

### Sociodemographic, clinical, behavioural and housing characteristics of the pregnant women

Sociodemographic, clinical and behavioural characteristics, and housing conditions of the pregnant women

were obtained with the aid of a standardised questionnaire. Sociodemographic items included age, ethnic group, birthplace, residence place, residence area, educational level, occupation and socioeconomic status. Clinical data included health status; presence or history of lymphadenopathy; presence of frequent abdominal pain or headaches; impairments of memory, reflexes, vision and hearing; history of surgery; hepatitis; blood transfusions or transplants; and obstetric history (number of pregnancies, deliveries, caesarean sections, miscarriages and stillbirths). Behavioural items included presence of cats at home, cats in the neighbourhood, raising farm animals, foreign travel, consumption of raw or undercooked meat, type of meat consumed (pork, lamb, beef, goat, boar, chicken, turkey, rabbit, deer, squirrel, horse, etc), eating away from home (in restaurants and fast food outlets), consumption of dried or cured meat (chorizo, ham, sausages or salami) or animal brains, unwashed raw vegetables or fruits, untreated water or unpasteurised milk, soil contact (gardening or agriculture), and washing hands before eating. Housing conditions included type of flooring, form of elimination of excretes and crowding.

### Detection of anti-*T. gondii* antibodies

A serum sample was obtained from each pregnant woman. Sera were stored at –20°C until analysed. All serum samples were tested for IgG antibodies to *T. gondii* by a commercially available enzyme immunoassay ‘*Toxoplasma* IgG’ kit (Diagnostic Automation/Cortez Diagnostics, Woodland Hills, California, USA). Sera positive for IgG antibodies to *T. gondii* were further tested for IgM antibodies to *T. gondii* by a commercially



**Figure 1** Geographical location of Aguascalientes State, Mexico. It is located in central Mexico, and the geographical coordinates of its capital (Aguascalientes City) are 21°53'N, 102°18'W. This city has a temperate semiarid climate, a mean annual rainfall of 500 mm, an altitude of 1700 m above sea level and a mean annual temperature of 18.5°C.

available enzyme immunoassay 'Toxoplasma IgM' kit (Diagnostic Automation/Cortez Diagnostics). Positive samples for IgM antibodies to *T. gondii* by enzyme immunoassay were further tested with the commercially available enzyme-linked fluorescence immunoassay (ELFA) kit 'VIDAS Toxo IgM' (bioMérieux, Marcy l'Etoile, France). Seropositivity for IgM antibodies was considered when both (enzyme immunoassay (EIA) and ELFA) IgM tests were positive. Avidity of IgG antibodies to *T. gondii* was assessed in IgM seropositive samples by the VIDAS TOXO IgG Avidity (bioMérieux) assay. All tests were performed following the manufacturer's instructions. Positive and negative controls were included in each run.

### Statistical analysis

Statistical analysis was performed with the aid of Epi Info V.7 and SPSS V.15.0 software. For calculation of the sample size, we used: (1) a reference seroprevalence of 6.1%<sup>13</sup> as the expected frequency for the factor under study, (2) 15 000 as the population size from which the sample was selected, (3) a 3.0% of confidence limits and (4) a 95% confidence level. The result of the sample size calculation was 241 participants. We used the Pearson's  $\chi^2$  test for comparison of the frequencies among groups. Bivariate analysis was followed by multivariate analysis to determine the association between *T. gondii* seropositivity and the sociodemographic, behavioural and housing characteristics of the pregnant women. To avoid bias in the process of data analysis, clinical characteristics were analysed separately from other characteristics. As a criterion of selection of variables for the multivariate analysis, we included only variables with a  $p \leq 0.10$  obtained in the bivariate analysis. ORs and 95% CIs were calculated by logistic regression analysis using the Enter method. Statistical significance was set at a  $p < 0.05$ .

### Ethics aspects

The purpose and procedures of this study were explained to all participants, and a written informed consent was obtained from all of them.

### RESULTS

Of the 338 pregnant women studied, 21 (6.2%) had IgG antibodies to *T. gondii* and 2 (9.5%) women were also positive for IgM antibodies to *T. gondii* by the enzyme immunoassays. Both serum samples positive for IgM by immunoassays were further tested by ELFA and only one resulted positive (4.8%). This IgM-positive sample showed high IgG avidity antibodies. Of the 21 anti-*T. gondii* IgG-positive women, 6 (28.6%) had IgG levels higher than 150 IU/mL, 1 (4.8%) between 100 and 150 IU/mL, and 14 (66.6%) between 10 and 99 IU/mL. **Table 1** shows the sociodemographic characteristics of the pregnant women and their correlation with *T. gondii* IgG seropositivity. The variables 'ethnic group' and 'educational level' showed  $p < 0.10$  by bivariate analysis. Other

**Table 1** Sociodemographic characteristics of pregnant women and prevalence of *Toxoplasma gondii* infection

Characteristic	N	Prevalence of <i>T. gondii</i> infection		p Value
		N	Per cent	
Age groups (years)				
20 or less	141	10	7.1	0.54
21–30	151	10	6.6	
31 or more	41	1	2.4	
Ethnic group				
Mestizo	312	17	5.4	0.001
White	4	3	75.0	
Birth place				
Aguascalientes State	284	18	6.3	0.96
Other Mexican State	51	3	5.9	
Abroad	1	0	0.0	
Residence place				
Aguascalientes State	332	20	6.0	1.00
Other Mexican State	1	0	0.0	
Residence area				
Urban	237	16	6.8	0.88
Suburban	1	0	0.0	
Rural	91	5	5.5	
Educational level (years)				
0–6	42	6	14.3	0.03
7–12	263	15	5.7	
>12	33	0	0.0	
Occupation				
Agriculture	2	0	0.0	0.89
Housewife	273	19	7.0	
Business	11	0	0.0	
Employee	11	0	0.0	
Student	26	2	7.7	
Professional	9	0	0.0	
None	5	0	0.0	
Other	1	0	0.0	
Socioeconomic level				
Low	76	7	9.2	0.28
Medium	258	14	5.4	

sociodemographic variables of pregnant women showed  $p > 0.10$  by bivariate analysis.

Concerning clinical data, bivariate analysis showed that seropositivity to *T. gondii* was positively associated with the variables 'frequent abdominal pain' ( $p = 0.03$ ), 'memory impairment' ( $p = 0.02$ ) and 'history of hepatitis' ( $p = 0.04$ ) and negatively associated with the variable 'history of surgery' ( $p = 0.01$ ; **table 2**). Other clinical variables did not show any association with *T. gondii* seropositivity. None of the women had a history of organ transplantation.

With respect to behavioural and housing characteristics, bivariate analysis showed that the variables 'frequency of eating out of home', 'washing hands before eating' and 'type of toilet facility' showed  $p \leq 0.10$ . Other behavioural and housing variables showed  $p > 0.10$  by bivariate analysis. Results of a selection of behavioural

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

Characteristic	Women tested N	Prevalence of <i>T. gondii</i> infection		p Value
		N	Per cent	
Clinical status				
Healthy	315	21	6.7	1.00
Ill	13	0	0.0	
Lymphadenopathy ever				
Yes	34	2	5.9	1.00
No	291	19	6.5	
Abdominal pain				
Yes	61	8	13.1	0.03
No	271	13	4.8	
Headache frequently				
Yes	97	8	8.2	0.34
No	237	13	5.5	
Memory impairment				
Yes	19	4	21.1	0.02
No	315	17	5.4	
Reflexes impairment				
Yes	9	1	11.1	0.45
No	319	20	6.3	
Hearing impairment				
Yes	27	1	3.7	1.00
No	307	20	6.5	
Visual impairment				
Yes	50	1	2.0	0.33
No	283	20	7.1	
Surgery ever				
Yes	92	1	1.1	0.01
No	240	20	8.3	
Blood transfusion				
Yes	11	0	0	1.00
No	322	21	6.5	
Hepatitis				
Yes	14	3	21.4	0.04
No	315	17	5.4	
Number of pregnancies				
One	159	12	7.5	0.35
Two to nine	176	9	5.1	
Deliveries				
Yes	119	5	4.2	0.30
No	215	15	7.0	
Caesarean sections				
Yes	70	2	2.9	0.26
No	265	19	7.2	
Abortions				
Yes	44	4	9.1	0.49
No	291	17	5.8	
Stillbirths				
Yes	6	0	0.0	1.00
No	329	21	6.4	

**Table 3** Bivariate analysis of a selection of putative risk factors for infection with *Toxoplasma gondii* in pregnant women

Characteristic	Women tested N	Prevalence of <i>T. gondii</i> infection		p Value
		N	Per cent	
Cats in the neighbourhood				
Yes	185	14	7.6	0.28
No	149	7	4.7	
Beef consumption				
Yes	314	18	5.7	0.13
No	21	3	14.3	
Sheep meat consumption				
Yes	167	7	4.2	0.35
No	137	9	6.6	
Chicken meat consumption				
Yes	323	20	6.2	0.41
No	8	1	12.5	
Turkey meat consumption				
Yes	59	2	3.4	0.54
No	270	18	6.7	
Rabbit meat consumption				
Yes	34	3	8.8	0.46
No	297	18	6.1	
Horse meat consumption				
Yes	17	0	0.0	0.61
No	313	21	6.7	
Sausages or ham consumption				
Yes	318	20	6.3	0.48
No	10	1	10.0	
Chorizo consumption				
Yes	298	16	5.4	0.23
No	29	3	10.3	
Unwashed raw fruits				
Yes	49	5	10.2	0.20
No	287	16	5.6	
Untreated water				
Yes	69	5	7.2	0.58
No	262	15	5.7	
Frequency of eating out of home				
Never	38	5	13.2	0.10
1–10 times a year	177	9	5.1	
>10 times a year	116	5	4.3	
Alcohol consumption				
Yes	23	0	0.0	0.38
No	311	21	6.8	
Washing hands before eating				
Yes	309	17	5.5	0.05
No	24	4	16.7	
Toilet facilities				
Sewage pipes	313	18	5.8	0.01
Latrine or another	8	3	37.5	

and housing characteristics are shown in table 3. Multivariate analysis of sociodemographic, behavioural and housing variables with  $p \leq 0.10$  obtained in the bivariate analysis showed that *T. gondii* seropositivity was associated with white ethnicity (OR=149.4; 95% CI

10.8 to 2054.1;  $p < 0.01$ ), no washing of hands before eating (OR=6.41; 95% CI 1.73 to 23.6;  $p = 0.005$ ) and use of latrine (OR=37.6; 95% CI 4.63 to 306.31;  $p = 0.001$ ). Table 4 shows the results of the multivariate analysis.

**Table 4** Multivariate analysis of selected characteristics of pregnant women and their association with *Toxoplasma gondii* infection

Characteristic	OR	95% CI	p Value
White ethnicity	149.4	10.8 to 2054.1	0.00
Poor education (0–6 years)	2.91	0.73 to 11.55	0.12
Never eating out of home	0.54	0.07 to 3.73	0.53
No washing hands before eating	6.41	1.73 to 23.6	0.005
Use of latrine	37.6	4.63 to 306.31	0.001

## DISCUSSION

There is currently no report about the seroepidemiology of *T. gondii* infection in pregnant women in central Mexico. Therefore, this study was aimed to determine the seroprevalence and correlates of *T. gondii* infection in pregnant women attending prenatal consultations at the three public health centres in Aguascalientes City. Testing for *T. gondii* infection during pregnancy is not mandatory in Mexico. Laboratory tests for the serological diagnosis of *T. gondii* infection are not available in many hospitals in this country. In fact, a study of knowledge and practices on toxoplasmosis among physicians attending pregnant women in the northern Mexican city of Durango showed poor knowledge about *T. gondii* laboratory diagnosis; 59% of physicians never requested laboratory tests for detecting *T. gondii* infection, and only few physicians provided recommendations to avoid *T. gondii* infection to pregnant women.<sup>15</sup> Results of the present study showed an overall 6.2% seroprevalence of *T. gondii* infection in pregnant women in Aguascalientes City. Only few studies about the seroepidemiology of *T. gondii* infection in pregnant women in Mexico have been reported. The seroprevalence found in pregnant women in Aguascalientes is comparable to the 6.1% seroprevalence of *T. gondii* infection reported in pregnant women in the northern Mexican city of Durango,<sup>13</sup> and the 8.2% seroprevalence reported in pregnant women in rural Durango State, Mexico.<sup>14</sup> In addition, the seroprevalence found in our study population is lower than the 34.9% seroprevalence reported in women with high-risk pregnancies and habitual abortions in Guadalajara City, Mexico.<sup>16</sup> The low seroprevalence found in pregnant women in Aguascalientes City can be related to the temperate semiarid climate of this city. Prevalence of *T. gondii* infection in humans and animals has been linked to climate. For instance, in a study about the incidence of congenital toxoplasmosis in newborns in Colombia, Gómez-Marín *et al.*<sup>17</sup> found a significant correlation between a high incidence of markers for congenital toxoplasmosis and higher mean annual rainfall for the city. In addition, in a study of cats in France, researchers found the highest seroprevalence of *T. gondii* infection during years with cool and moist winters.<sup>18</sup>

In an international context, the seroprevalence found in pregnant women in Aguascalientes City is lower than the 39.8% seroprevalence of *T. gondii* infection in pregnant women in 10 English-speaking Caribbean countries reported recently.<sup>19</sup> Similarly, the 6.2% seroprevalence found in our study is lower than seroprevalences reported in pregnant women in Eastern China (15.2%),<sup>20</sup> Northern Iran (39.8%)<sup>21</sup> and Northeast Brazil (68.5%).<sup>22</sup> In contrast, the seroprevalence found in our study is comparable to seroprevalences in pregnant women reported in Norway (9.3%)<sup>23</sup> and Korea (3.7%).<sup>24</sup> It is not clear why similar seroprevalences among these countries exist. It is possible that behavioural characteristics like cooking meat or low prevalence of *T. gondii* infection in animals for human consumption in these countries might contribute for the low seroprevalence of *T. gondii* infection in these countries.

In the present study, *T. gondii* infection was significantly higher in pregnant women with memory impairment, frequent abdominal pain and a history of hepatitis than in women without these clinical characteristics. Memory impairment has been associated to *T. gondii* infection in elderly people in Germany,<sup>25</sup> and our results confirm previous observations of this association in adults in other groups of population in Mexico, including people of Huichol ethnicity,<sup>26</sup> migrant agricultural workers<sup>27</sup> and gardeners.<sup>28</sup> The association between *T. gondii* infection and abdominal pain has been scantily reported. Gastric toxoplasmosis with abdominal pain was reported in a 22-year-old Haitian woman with AIDS,<sup>29</sup> and in a 49-year-old man with the same syndrome in the USA.<sup>30</sup> Further research to confirm the association of *T. gondii* exposure and abdominal pain in immunocompetent participants is needed. On the other hand, pregnant women with a history of hepatitis had a significantly higher seroprevalence of *T. gondii* infection than those without this history. Infection with *T. gondii* may lead to liver disease. Toxoplasmic hepatitis has been reported in immunocompetent patients,<sup>31–32</sup> and in HIV-infected patients.<sup>33–34</sup> Additional studies to determine the role of *T. gondii* infection in acute hepatitis should be conducted. In the current study, we also observed that the frequency of *T. gondii* exposure was significantly lower in pregnant women with a history of surgery than in those without this history. This finding suggests that history of surgery did not play an important role in transmission of *T. gondii* in the women studied.

We looked for sociodemographic, behavioural and housing factors associated with *T. gondii* exposure. Multivariate analysis showed that *T. gondii* seropositivity was associated with white ethnicity, not washing hands before eating and use of latrine. In the USA, seroprevalence of *T. gondii* infection was reported to be higher among non-Hispanic black persons than among non-Hispanic white persons.<sup>35</sup> Clinical manifestations of *T. gondii* infection may vary among ethnic groups. In

adults 60 years and older in the USA, latent *T. gondii* infection affected immediate memory, particularly in white Americans.<sup>36</sup> Further research to determine the magnitude of *T. gondii* exposure and the role of *T. gondii* in pathogenicity among ethnic groups is warranted. The association of *T. gondii* exposure and not washing hands before eating and the use of latrine found in the present study reflects poor hygiene and sanitation among the seropositive women, thereby facilitating infection via sporulated oocysts. In a study of children in Iran, researchers found an association of *T. gondii* seropositivity and not washing hands before meals.<sup>37</sup> Similarly, in a study of children in China, hand washing habits was a protective factor against *T. gondii* infection.<sup>38</sup> Washing hands is an important practice to prevent congenital toxoplasmosis.<sup>39</sup>

The present study has limitations. The sample size was small, and the 95% CI of some factors associated with *T. gondii* exposure had wide ranges. Therefore, associations with very wide 95% CI should be interpreted with care.

## CONCLUSIONS

Results demonstrate that pregnant women in Aguascalientes City have a low seroprevalence of *T. gondii* infection. However, this low seroprevalence indicates that most pregnant women are at risk for a primary infection. The factors found to be associated with *T. gondii* exposure in this study, including poor hygiene, may be useful to develop preventive measures against *T. gondii* infection and its sequelae.

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**Acknowledgements** This study was supported by Juárez University of Durango State.

**Contributors** CA-E, MdCT-S and FJ-J designed the study protocol, and participated in the coordination and management of the study. MDEM-T, ROG-G and MER-R obtained blood samples, submitted the questionnaires and performed the data analysis. CA-E performed the laboratory tests. SE-M performed the statistical analysis. CA-E, JH-T, LFS-A and OL performed the data analysis, and wrote the manuscript.

**Funding** This research received financial support from Juárez University of Durango State, Durango, Mexico.

**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** This study was approved by the Instituto de Servicios de Salud del Estado de Aguascalientes.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** No additional data are available.

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