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Seroepidemiology of *Toxoplasma gondii* in pregnant women in Aguascalientes City, Mexico

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ABSTRACT

OBJECTIVES: We determined the seroprevalence and correlates of *T. 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 three public health centers.

PRIMARY AND SECONDARY OUTCOME MEASURES: Women were examined for anti-*Toxoplasma gondii* IgG and IgM antibodies by commercially available enzyme immunoassays, and an avidity test. Bivariate and multivariate 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 anti-*T. gondii* IgG antibodies and one (4.8%) of them was also positive for anti-*T. gondii* IgM antibodies. Avidity of IgG anti-*T. gondii* was high in the IgM positive sample. Multivariate analysis of sociodemographic, behavioral and housing variables showed that *T. gondii* seropositivity was associated with White ethnicity (OR = 149.4; 95% CI: 10.8-2054.1; $P<0.01$), not washing hands before eating (OR = 6.41; 95% CI: 1.73-23.6; $P=0.005$), and use of latrine (OR = 37.6; 95% CI: 4.63-306.31; $P=0.001$).

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*

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exposure found in this study including food hygiene may be useful to determine preventive measures against *T. gondii* infection and its sequelae.

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 seroprevalence of *Toxoplasma gondii* infection was determined in Pregnant women.
- Prevalence association with sociodemographic, clinical, and behavioral characteristics of pregnant women was determined.
- The sample size was small and the seropositivity rate was low to perform a wider analysis of the association of *Toxoplasma gondii* exposure and characteristics of the pregnant women.

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INTRODUCTION

Infection with the parasite *Toxoplasma gondii* (*T. gondii*) is common in humans and animals around the world.[1, 2] This infection is acquired by ingestion of water or food contaminated with oocysts shed by cats or other felids, by ingestion of tissue cysts in meat from mammals and birds,[2, 3] and congenitally.[4, 5] Most infections with *T. gondii* are asymptomatic; however, infection with the parasite can lead to acute toxoplasmosis that presents as lymphadenopathy or chorioretinitis.[5] Immunocompromised individuals may develop a life-threatening disease with meningoencephalitis.[5, 6] Primary infection with *T. gondii* during pregnancy may lead to congenital disease with miscarriages or stillbirths,[5, 7, 8] or disease in eye and central nervous system.[5, 9, 10] Most newborns with congenitally acquired infections with *T. gondii* are asymptomatic; however, clinical manifestations of toxoplasmosis develop later in life.[9] 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.[11, 12]

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 in the central Mexican city of Aguascalientes in particular. In two previous studies in the northern Mexican state of Durango, *T. gondii* seroprevalences of 6.1% in urban,[13] and 8.2% in rural [14] pregnant women were found. In the present study, we sought to determine the seroprevalence of *T. gondii* infection in pregnant women attended in three public health centers in Aguascalientes City, Mexico, and to determine the association of *T. gondii* seropositivity with the socio-demographic, clinical, behavioral, and housing characteristics of the pregnant women.

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MATERIALS AND METHODS

Study design and study population

Through a cross-sectional study design, we examined pregnant women who attended their prenatal care consultations in three public health centers (Instituto de Servicios de Salud del Estado de Aguascalientes) in Aguascalientes City, Mexico, from October 2014 to February 2016. Inclusion criteria were: 1) pregnant women with 1 to 9 months of pregnancy; 2) aged 13-45 years old; and 3) who accepted to participate in the study. Socio-economic status, occupation, or educational level were not restrictive criteria for enrollment. Participants were enrolled consecutively. In total, 338 pregnant women (mean age: 22.95 ± 6.19; range 13-42 years) were included in the study.

Socio-demographic, clinical, behavioral and housing characteristics of the pregnant women

Socio-demographic, clinical, and behavioral characteristics, and housing conditions of the pregnant women were obtained with the aid of a standardized questionnaire. Socio-demographic items included age, ethnic group, birthplace, residence, educational level, occupation and socio-economic 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, cesarean sections, miscarriages and stillbirths). Behavioral items included animal contacts, foreign traveling, consumption of raw or undercooked meat, type of meat consumed (pork, lamb, beef, goat, boar, chicken, turkey, rabbit, deer, squirrel, horse, or other), eating away

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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 unpasteurized 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 women. Sera were stored at -20° C until analyzed. All serum samples were tested for anti-*T. gondii* IgG antibodies by a commercially available enzyme immunoassay "*Toxoplasma* IgG" kit (Diagnostic Automation/Cortez Diagnostics Inc., Woodland Hills, CA, USA). Sera positive for anti-*T. gondii* IgG antibodies were further tested for anti-*T. gondii* IgM antibodies by a commercially available enzyme immunoassay "*Toxoplasma* IgM" kit (Diagnostic Automation/Cortez Diagnostics Inc.). Positive samples for anti-*T. gondii* IgM antibodies 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 (EIA and ELFA) IgM tests were positive. Avidity of IgG anti-*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 version 7 and SPSS version 15.0 software. For calculation of the sample size, we used: a) a reference

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seroprevalence of 6.1% [13] as the expected frequency for the factor under study, b) 15000 as the population size from which the sample was selected, c) a 3.0% of confidence limits, and d) a 95% confidence level. The result of the sample size calculation was 241 subjects. We used the Pearson´s chi square 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, behavioral and housing characteristics of the pregnant women. To avoid bias in the process of data analysis, clinical characteristics were analyzed separated from other characteristics. As a criterion of selection of variables for the multivariate analysis we included only variables with a *P* value ≤ 0.10 obtained in the bivariate analysis. Odd ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression analysis using the Enter method. Statistical significance was set at a *P* value < 0.05.

Ethics aspects

This study was approved by the Ethical Committee of Instituto de Servicios de Salud del Estado de Aguascalientes. The purpose and procedures of this study were explained to all participants, and a written informed consent was obtained from all of them.

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RESULTS

Of the 338 pregnant women studied, 21 (6.2%) had anti-*T. gondii* IgG antibodies and two (9.5%) of them were also positive for anti-*T. gondii* IgM antibodies by 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 to 150 IU/ml, and 14 (66.6%) between 10 to 99 IU/ml. Table 1 shows the socio-demographic characteristics of the pregnant women and their correlation with *T. gondii* IgG seropositivity. The variables "ethnic group" and "educational level" showed *P* values <0.10 by bivariate analysis. Other socio-demographic variables of pregnant women including age, birthplace, residence, occupation, educational level and socio-economic status showed *P* values higher than 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 including health status, presence or history of lymphadenopathy, presence of frequent headaches, impairments of reflexes, vision and hearing, history of blood transfusions, or transplants, and obstetric history (deliveries, cesarean sections, miscarriages and stillbirths) did not show any association with *T. gondii* seropositivity.

With respect to behavioral and housing characteristics, bivariate analysis showed that the variables "frequency of eating meat", "washing hands before eating", and "type of

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toilet facility" showed *P* values ≤ 0.10 (Table 3). Whereas other behavioral and housing variables including animal contacts, foreign traveling, consumption of raw or undercooked meat, type of meat consumed, consumption of dried or cured meat, or animal brains, unwashed raw vegetables or fruits, untreated water or unpasteurized milk, soil contact, type of flooring at home, and crowding showed *P* values higher than 0.10 by bivariate analysis. Multivariate analysis of sociodemographic, behavioral and housing variables with *P* values ≤ 0.10 obtained in the bivariate analysis showed that *T. gondii* seropositivity was associated with White ethnicity (OR = 149.4; 95% CI: 10.8-2054.1; *P*<0.01), no washing hands before eating (OR = 6.41; 95% CI: 1.73-23.6; *P*=0.005), and use of latrine (OR = 37.6; 95% CI: 4.63-306.31; *P*=0.001). Table 4 shows the results of the multivariate analysis.

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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 in three public health centers 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.[15] Results of the present study showed a 6.2% seroprevalence of *T. gondii* infection in pregnant women in Aguascalientes City. This seroprevalence is comparable to the 6.1% seroprevalence of *T. gondii* infection reported in pregnant women in the northern Mexican city of Durango,[13] and the 8.2% seroprevalence reported in pregnant women in rural Durango State, Mexico.[14] 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.[16] Similarly, the 6.2% seroprevalence found in our study is lower than seroprevalences reported in pregnant women in Eastern China (15.2%),[17] in Northern Iran (39.8%),[18] and Northeast Brazil (68.5%).[19] In contrast, the seroprevalence found in our study is comparable to seroprevalences in pregnant women reported in Norway (9.3%),[20] and Korea (3.7%).[21] It is not clear why similar

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seroprevalences among these countries exist. It is possible that behavioral characteristics as 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,[22] and our results confirms previous observations of this association in adults in other groups of population in Mexico including people of Huichol ethnicity,[23] migrant agricultural workers,[24] and gardeners.[25] The association between *T. gondii* infection and abdominal pain has been scantly reported. Gastric toxoplasmosis with abdominal pain was reported in a 22-year-old Haitian woman with acquired immunodeficiency syndrome,[26] and in a 49-year-old man with the same syndrome in the USA.[27] Further research to confirm the association of *T. gondii* exposure and abdominal pain in immunocompetent subjects 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,[28, 29] and in human immunodeficiency virus-infected patients.[30, 31] 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 import role in transmission of *T. gondii* in the women studied.

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We looked for sociodemographic, behavioral 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 U.S. seroprevalence of *T. gondii* infection was reported to be higher among non-Hispanic black persons than among non-Hispanic white persons.[32] Clinical manifestations of toxoplasmosis may vary among ethnic groups. In adults 60 years and older in the USA, latent toxoplasmosis affected immediate memory, particularly in White Americans,[33] 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 favoring infection via oocysts. In a study of children in Iran, researchers found an association of *T. gondii* seropositivity and not washing hands before meals.[34] Similarly, in a study of children in China, hand washing habits was a protective factor against *T. gondii* infection.[35] Washing hands is an important practice to prevent congenital toxoplasmosis.[36]

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

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pregnant women are at risk for a primary infection. The factors associated with *T. gondii* exposure found in this study including poor hygiene may be useful to develop preventive measures against *T. gondii* infection and its sequelae.

Acknowledgements

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Competing interests

The authors declare that no competing interests exist.

Authors' contributions

CAE, MCTS, and FJJ designed the study protocol, and participated in the coordination and management of the study. MDEMT, ROGG, and MERR obtained blood samples, submitted the questionnaires and performed the data analysis. CAE performed the laboratory tests. SEM performed the statistical analysis. CAE, JHT, LFSA, 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 pregnant women and prevalence of *T. gondii* infection.

Characteristic	No.	Prevalence of *T. gondii* infection		*P* value
		No.	%	
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				
0 to 6 years	42	6	14,3	0.03
7-12 years	263	15	5.7	
>12 years	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	
Socio-economic level				
Low	76	7	9.2	0.28
Medium	258	14	5.4	

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

Characteristic	Women tested No.	Prevalence of *T. gondii* infection No.	%	*P* value
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	
Deliveries				
Yes	119	5	4.2	0.30
No	215	15	7.0	

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Cesarean 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	

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Table 3. Bivariate analysis of selected putative risk factors for infection with *T. gondii* in pregnant women.

Characteristic	Women tested No.	Prevalence of *T. gondii* infection No.	%	*P* value
Cats in the neighborhood				
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 to 10 times a year	177	9	5.1	

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>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	

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

Characteristic	Odds ratio	95% confidence interval	*P* value
White ethnicity	149.4	10.8 - 2054.1	0.00
Poor education (0-6 years)	2.91	0.73 - 11.55	0.12
Never eating out of home	0.54	0.07 - 3.73	0.53
No washing hands before eating	6.41	1.73 - 23.6	0.005
Use of latrine	37.6	4.63 - 306.31	0.001

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

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

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		8).
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 (Pages 6-8).**
Bias	9	Describe any efforts to address potential sources of bias **INFORMATION ABOUT EFFORTS TO AVOID BIAS WAS ADDED (Page 8).**
Study size	10	Explain how the study size was arrived at **INFORMATION ABOUT THE CALCULATION OF SAMPLE SIZE WAS INCLUDED (Pages 7-8).**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **INFORMATION ABOUT THE VARIABLES CHOSEN IN THE ANALYSIS WAS INCLUDED (Pages 6-8).**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **A DESCRIPTION OF THE STATISTICAL ANALYSIS WAS INCLUDED (Pages 7-8).**
		(*b*) Describe any methods used to examine subgroups and interactions **METHODS USED TO EXAMINE SUBGROUPS WERE DESCRIBED (Page 8).**
		(*c*) Explain how missing data were addressed **ANALYSIS WAS PERFORMED WITH AVAILABLE 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 (pages 6-8).**
		(*e*) Describe any sensitivity analyses **NOT APPLICABLE.**

Continued on next page

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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 **INFORMATION ABOUT THE ELIGIBILITY OF SUBJECT WAS INCLUDED (Page 6).**
		(b) Give reasons for non-participation at each stage **PARTICIPATION WAS VOLUNTARY (Page 6).**
		(c) Consider use of a flow diagram **THE NUMBER OF PROCEDURES WAS SMALL AND A FLOW DIAGRAM MIGHT BE NOT NECESSARY.**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **CHARACTERISTICS OF THE STUDY PARTICIPANTS WERE INCLUDED (Pages 6-8).**
		(b) Indicate number of participants with missing data for each variable of interest **NUMBER OF PARTICIPANTS WITH MISSING DATA FOR EACH VARIABLE IS SHOWN IN TABLES 1-3.**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures **TABLES WITH SUMMARY OF RESULTS WERE INCLUDED (Pages 22-27).**
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 **INFORMATION ABOUT 95% CONFIDENCE INTERVALS WAS INCLUDED (Page 8).**
		(*b*) Report category boundaries when continuous variables were categorized **INFORMATION ABOUT CATEGORIES AND SUBGROUPS ARE INCLUDED IN TABLES (Pages 22-27).**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NO RELATIVE RISKS WERE ASSESSED.**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

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		RESULTS OF ANALYSIS OF SUBGROUPS WERE SHOWN IN TABLES (Pages 22-27).
Discussion		
Key results	18	Summarise key results with reference to study objectives **KEY RESULTS WITH REFERENCE TO OBJECTIVES WERE DISCUSSED (Pages 11-13).**
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 **THE LIMITATIONS OF THE STUDY WERE INCLUDED (Page 13).**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **AN INTERPRETATION OF RESULTS WAS INCLUDED (Pages 11-13).**
Generalisability	21	Discuss the generalisability (external validity) of the study results **INFORMATION RELATED WITH THE GENERALISABILITY OF THE STUDY RESULTS WAS INCLUDED (Pages 11-13).**
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 **INFORMATION ABOUT FUNDING WAS INCLUDED (Page 14).**

*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.

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Seroepidemiology of Toxoplasma gondii in pregnant women in Aguascalientes City, Mexico: a cross sectional study

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Keywords:	Toxoplasma, pregnant women, Epidemiology < TROPICAL MEDICINE, seroprevalence, risk factors, behavioral characteristics

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

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ABSTRACT

OBJECTIVES: We determined the seroprevalence and correlates of *T. 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 three public health centers.

PRIMARY AND SECONDARY OUTCOME MEASURES: Women were examined for IgG/IgM antibodies to *T. gondii* by 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 one (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, behavioral and housing variables showed that *T. gondii* seropositivity was associated with White ethnicity (OR = 149.4; 95% CI: 10.8-2054.1; $P<0.01$), not washing hands before eating (OR = 6.41; 95% CI: 1.73-23.6; $P=0.005$), and use of latrine (OR = 37.6; 95% CI: 4.63-306.31; $P=0.001$).

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.

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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 allowed us to know the immunological status against infection with *Toxoplasma gondii* in a sample of pregnant women in central Mexico.
- This study provides a number of risk factors for infection with *Toxoplasma 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 *Toxoplasma gondii* exposure and characteristics of the pregnant women.

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INTRODUCTION

Infection with the parasite *Toxoplasma gondii* (*T. gondii*) is common in humans and animals around the world.[1, 2] This infection is acquired by ingestion of water or food contaminated with oocysts shed by cats or other felids, by ingestion of tissue cysts in meat from mammals and birds,[2, 3] and congenitally.[4, 5] Most infections are asymptomatic; however, infection with the parasite can lead to acute toxoplasmosis that presents as lymphadenopathy or chorioretinitis.[5] Immunocompromised individuals may develop a life-threatening disease with meningoencephalitis.[5, 6] Primary infection with *T. gondii* during pregnancy may lead to congenital disease with miscarriages or stillbirths,[5, 7, 8] or disease in eye and central nervous system.[5, 9, 10] Most newborns with congenitally acquired infections with *T. gondii* are asymptomatic; however, clinical manifestations of toxoplasmosis develop later in life.[9] 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.[11, 12]

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 in the central Mexican city of Aguascalientes in particular. In two previous studies in the northern Mexican state of Durango, *T. gondii* seroprevalences of 6.1% in urban,[13] and 8.2% in rural [14] pregnant women were found. In the present study, we sought to determine the seroprevalence of *T. gondii* infection in pregnant women attended in three public health centers in Aguascalientes City, Mexico, and to determine the association of *T. gondii* seropositivity with the socio-demographic, clinical, behavioral, and housing characteristics of the pregnant women.

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MATERIALS AND METHODS

Study design and study population

Through a cross-sectional study design, we examined pregnant women who attended their prenatal care consultations in three public health centers (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 to 9 months of pregnancy; 2) aged 13-45 years old; and 3) who accepted to participate in the study. Socio-economic status, occupation, or educational level were not restrictive criteria for enrollment. Participants were enrolled consecutively. In total, 338 pregnant women (mean age: 22.95 ± 6.19; range 13-42 years) were included in the study.

Socio-demographic, clinical, behavioral and housing characteristics of the pregnant women

Socio-demographic, clinical, and behavioral characteristics, and housing conditions of the pregnant women were obtained with the aid of a standardized questionnaire. Socio-demographic items included age, ethnic group, birthplace, residence place. residence area, educational level, occupation and socio-economic 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, cesarean sections, miscarriages and stillbirths). Behavioral items included presence of cats at home, cats in the neighborhood, raising farm animals, foreign traveling,

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consumption of raw or undercooked meat, type of meat consumed (pork, lamb, beef, goat, boar, chicken, turkey, rabbit, deer, squirrel, horse, or other), 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 unpasteurized 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 women. Sera were stored at -20° C until analyzed. All serum samples were tested for IgG antibodies to *T. gondii* by a commercially available enzyme immunoassay "*Toxoplasma* IgG" kit (Diagnostic Automation/Cortez Diagnostics Inc., Woodland Hills, CA, USA). Sera positive for IgG antibodies to *T. gondii* were further tested for IgM antibodies to *T. gondii* by a commercially available enzyme immunoassay "*Toxoplasma* IgM" kit (Diagnostic Automation/Cortez Diagnostics Inc.). 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 (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.

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Statistical analysis

Statistical analysis was performed with the aid of Epi Info version 7 and SPSS version 15.0 software. For calculation of the sample size, we used: a) a reference seroprevalence of 6.1% [13] as the expected frequency for the factor under study, b) 15000 as the population size from which the sample was selected, c) a 3.0% of confidence limits, and d) a 95% confidence level. The result of the sample size calculation was 241 subjects. We used the Pearson´s chi square 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, behavioral and housing characteristics of the pregnant women. To avoid bias in the process of data analysis, clinical characteristics were analyzed separated from other characteristics. As a criterion of selection of variables for the multivariate analysis we included only variables with a *P* value ≤ 0.10 obtained in the bivariate analysis. Odd ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression analysis using the Enter method. Statistical significance was set at a *P* value < 0.05.

Ethics aspects

This study was approved by the Ethical Committee of Instituto de Servicios de Salud del Estado de Aguascalientes. The purpose and procedures of this study were explained to all participants, and a written informed consent was obtained from all of them.

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RESULTS

Of the 338 pregnant women studied, 21 (6.2%) had IgG antibodies to *T. gondii* and two (9.5%) of them were also positive for IgM antibodies to *T. gondii* by 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 to 150 IU/ml, and 14 (66.6%) between 10 to 99 IU/ml. Table 1 shows the socio-demographic characteristics of the pregnant women and their correlation with *T. gondii* IgG seropositivity. The variables "ethnic group" and "educational level" showed *P* values <0.10 by bivariate analysis. Other socio-demographic variables of pregnant women showed *P* values higher than 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 an history of organ transplantation.

With respect to behavioral 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* values ≤ 0.10. Other behavioral and housing variables showed *P* values higher than 0.10 by bivariate analysis. Results of a selection of behavioral and housing characteristics are shown in Table 3. Multivariate analysis of

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sociodemographic, behavioral and housing variables with *P* values ≤ 0.10 obtained in the bivariate analysis showed that *T. gondii* seropositivity was associated with White ethnicity (OR = 149.4; 95% CI: 10.8-2054.1; *P*<0.01), no washing hands before eating (OR = 6.41; 95% CI: 1.73-23.6; *P*=0.005), and use of latrine (OR = 37.6; 95% CI: 4.63-306.31; *P*=0.001). Table 4 shows the results of the multivariate analysis.

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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 in three public health centers 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.[15] 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,[13] and the 8.2% seroprevalence reported in pregnant women in rural Durango State, Mexico.[14] 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.[16] The low seroprevalence found in pregnant women in Aguascalientes City can be related to the temperate semi-arid 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

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congenital toxoplasmosis in newborns in Colombia, Gómez-Marin *et al* found a significant correlation between a high incidence of markers for congenital toxoplasmosis and higher mean annual rainfall for the city.[17] 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.[18]

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.[19 Similarly, the 6.2% seroprevalence found in our study is lower than seroprevalences reported in pregnant women in Eastern China (15.2%),[20] in Northern Iran (39.8%),[21] and Northeast Brazil (68.5%).[22] In contrast, the seroprevalence found in our study is comparable to seroprevalences in pregnant women reported in Norway (9.3%),[23] and Korea (3.7%).[24] It is not clear why similar seroprevalences among these countries exist. It is possible that behavioral characteristics as 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,[25] and our results confirms previous observations of this association in adults in other groups of population in Mexico including people of Huichol ethnicity,[26] migrant agricultural workers,[27] and gardeners.[28] The association between *T. gondii* infection and abdominal pain has been scantly reported. Gastric toxoplasmosis with abdominal pain was reported in a 22-year-old Haitian woman

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with acquired immunodeficiency syndrome,[29] and in a 49-year-old man with the same syndrome in the USA.[30] Further research to confirm the association of *T. gondii* exposure and abdominal pain in immunocompetent subjects 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,[31, 32] and in human immunodeficiency virus-infected patients.[33, 34] 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 import role in transmission of *T. gondii* in the women studied.

We looked for sociodemographic, behavioral 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 U.S. seroprevalence of *T. gondii* infection was reported to be higher among non-Hispanic black persons than among non-Hispanic white persons.[35] 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,[36] 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 favoring infection via sporulated oocysts. In a study of children in Iran, researchers found an association of *T.*

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gondii seropositivity and not washing hands before meals.[37] Similarly, in a study of children in China, hand washing habits was a protective factor against *T. gondii* infection.[38] Washing hands is an important practice to prevent congenital toxoplasmosis.[39]

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 associated with *T. gondii* exposure found in this study including poor hygiene may be useful to develop preventive measures against *T. gondii* infection and its sequelae.

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Competing interests

The authors declare that no competing interests exist.

Authors' contributions

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CAE, MCTS, and FJJ designed the study protocol, and participated in the coordination and management of the study. MDEMT, ROGG, and MERR obtained blood samples, submitted the questionnaires and performed the data analysis. CAE performed the laboratory tests. SEM performed the statistical analysis. CAE, JHT, LFSA, 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 pregnant women and prevalence of *T. gondii* infection.

Characteristic	No.	Prevalence of *T. gondii* infection		*P* value
		No.	%	
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				
0 to 6 years	42	6	14,3	0.03
7-12 years	263	15	5.7	
>12 years	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	
Socio-economic level				
Low	76	7	9.2	0.28
Medium	258	14	5.4	

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

Characteristic	Women tested No.	Prevalence of *T. gondii* infection No.	%	*P* value
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	
No. of pregnancies				
One	159	12	75	0.35

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Two to nine	176	9	5.1	
Deliveries				
Yes	119	5	4.2	0.30
No	215	15	7.0	
Cesarean 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	

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

Characteristic	Women tested No.	Prevalence of *T. gondii* infection No.	%	*P* value
Cats in the neighborhood				
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 to 10 times a year	177	9	5.1	

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>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	

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

Characteristic	Odds ratio	95% confidence interval	*P* value
White ethnicity	149.4	10.8 - 2054.1	0.00
Poor education (0-6 years)	2.91	0.73 - 11.55	0.12
Never eating out of home	0.54	0.07 - 3.73	0.53
No washing hands before eating	6.41	1.73 - 23.6	0.005
Use of latrine	37.6	4.63 - 306.31	0.001

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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 semi-arid climate, a mean annual rainfall of 500 mm, an altitude of 1700 meters above sea level, and a mean annual temperature of 18.5°C.
173x113mm (300 x 300 DPI)

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

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

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		8).
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 (Pages 6-8).**
Bias	9	Describe any efforts to address potential sources of bias **INFORMATION ABOUT EFFORTS TO AVOID BIAS WAS ADDED (Page 8).**
Study size	10	Explain how the study size was arrived at **INFORMATION ABOUT THE CALCULATION OF SAMPLE SIZE WAS INCLUDED (Pages 7-8).**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **INFORMATION ABOUT THE VARIABLES CHOSEN IN THE ANALYSIS WAS INCLUDED (Pages 6-8).**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **A DESCRIPTION OF THE STATISTICAL ANALYSIS WAS INCLUDED (Pages 7-8).**
		(*b*) Describe any methods used to examine subgroups and interactions **METHODS USED TO EXAMINE SUBGROUPS WERE DESCRIBED (Page 8).**
		(*c*) Explain how missing data were addressed **ANALYSIS WAS PERFORMED WITH AVAILABLE 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 (pages 6-8).**
		(*e*) Describe any sensitivity analyses **NOT APPLICABLE.**

Continued on next page

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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 **INFORMATION ABOUT THE ELIGIBILITY OF SUBJECT WAS INCLUDED (Page 6).**
		(b) Give reasons for non-participation at each stage **PARTICIPATION WAS VOLUNTARY (Page 6).**
		(c) Consider use of a flow diagram **THE NUMBER OF PROCEDURES WAS SMALL AND A FLOW DIAGRAM MIGHT BE NOT NECESSARY.**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **CHARACTERISTICS OF THE STUDY PARTICIPANTS WERE INCLUDED (Pages 6-8).**
		(b) Indicate number of participants with missing data for each variable of interest **NUMBER OF PARTICIPANTS WITH MISSING DATA FOR EACH VARIABLE IS SHOWN IN TABLES 1-3.**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures **TABLES WITH SUMMARY OF RESULTS WERE INCLUDED (Pages 25-30).**
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 **INFORMATION ABOUT 95% CONFIDENCE INTERVALS WAS INCLUDED (Page 8).**
		(*b*) Report category boundaries when continuous variables were categorized **INFORMATION ABOUT CATEGORIES AND SUBGROUPS ARE INCLUDED IN TABLES (Pages 25-30).**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NO RELATIVE RISKS WERE ASSESSED.**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

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		RESULTS OF ANALYSIS OF SUBGROUPS WERE SHOWN IN TABLES (Pages 25-30).
Discussion		
Key results	18	Summarise key results with reference to study objectives **KEY RESULTS WITH REFERENCE TO OBJECTIVES WERE DISCUSSED (Pages 11-14).**
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 **THE LIMITATIONS OF THE STUDY WERE INCLUDED (Page 14).**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **AN INTERPRETATION OF RESULTS WAS INCLUDED (Pages 11-14).**
Generalisability	21	Discuss the generalisability (external validity) of the study results **INFORMATION RELATED WITH THE GENERALISABILITY OF THE STUDY RESULTS WAS INCLUDED (Pages 11-14).**
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 **INFORMATION ABOUT FUNDING WAS INCLUDED (Page 14).**

*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.

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