Low seroprevalence of Toxoplasma gondii IgG and IgM antibodies in pregnant women of northern Durango, Mexico

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Abstract

Toxoplasmosis is caused by intracellular parasite Toxoplasma gondii (T. gondii) a zoonotic disease that infects almost all warm-blooded animals, including humans. In addition, T. gondii infection can result in serious and life-threatening consequences for the developing fetus or newborn, including spontaneous abortion, congenital blindness, hydrocephalus, mental retardation, and even death. The aim of this research was to estimate the frequency of seropositivity to IgM and IgG antibodies against T. gondii in pregnant women attended in the health services of the “Lagunera” region of Durango State in the period of January 2018 - October 2018. A cross-sectional study was conducted on 434 pregnant women who used the health services of Durango State. Serum was analyzed for T. gondii infection by detecting IgG and IgM anti-T. gondii antibodies using commercial kits through the immunological technique of ELISA. In addition, a standardized questionnaire was applied to evaluate risk factors and sociodemographic characteristics of the participants. From 434 participants, 4 were detected with IgG antibodies (0.92%) and none of these had a positive result to IgM antibodies (0%). Dry meat consumption (OR = 13.5; 95% CI 0.94 - 192.58; p= 0.055) and other types of meat consumption (OR = 15.61; 95% CI 1.08 - 224.05; p= 0.043) were associated factors to infection. The results showed a lower seroprevalence of antibodies against T. gondii than any other study reported before. In addition, our results indicate the majority of the population studied is at risk of having a primary T. gondii infection.

Background

Toxoplasma gondii (T. gondii), an obligate intracellular parasite responsible of disease called toxoplasmosis a zoonotic disease which infects almost all warm-blooded animals including humans; up to a third of the world’s population is reported to be seropositive (1,
2). Named *T. gondii* based on its morphology (Toxon: arc, plasma: form) and described for first time by Nicolle and Manceaux on tissues of a North African rodent, the gundis (Ctenodactylus gundi) (3). The infection could be acquired by ingestion of food (raw or uncooked meat from infected animals) or water contaminated with oocysts, direct contact with cat feces or soil contaminated with oocysts, blood transfusions and congenitally from infected mothers to their child (1, 2, 4), geographic location is also related to infection as variations in climate (5). Toxoplasmosis during pregnancy may develop a congenital infection which can lead to abortion, spontaneous stillbirth or clinical manifestations like neurological abnormalities and ocular lesions. Most newborns with congenitally acquired infections with *T. gondii* are asymptomatic; however, clinical manifestations of toxoplasmosis develop later in life.

Mexico is a country with sociodemographical and geographical variations which also contributes to wide seroprevalence of *T. gondii* infection across the country. A national seroprevalence indicates that higher frequency of toxoplasmosis was found in humid/warm environments than in dry or cold places (6). A national *T. gondii* seroprevalence derivate from the National Health Survey 2000 and the National Health and Nutrition Survey 2006 (7) found an increase in the toxoplasmosis distribution in the north region (35%) finding the highest prevalence in coastal regions (67.5%).

There is a lack of knowledge about the seroepidemiology of *T. gondii* infection in pregnant women in Mexico in general, and there is no data available about this infection in the north of Mexico, specifically in the “Lagunera” region of Durango State. There are previous studies of *T. gondii* seroprevalence in Durango City which is 200 km away from the “Lagunera” region; 6.1% in urban and 8.2% in rural pregnant women were found. In the present study, we sought to determine the seroprevalence of *T. gondii* infection in pregnant women attending at public health services in the “Lagunera” region of Durango
State and to determine the association of T. gondii seropositivity with the sociodemographic, clinical, behavioral and housing characteristics of the pregnant women.

Methods

**Population and Detection of anti-T. gondii antibody**

The present cross-sectional study estimated seroprevalence of IgM and IgG antibodies against T. gondii. This study was carried out in the health services that belong to the health jurisdiction No. 2 of the “Lagunera” region of Durango State from January 2018 to October 2018. The majority of the participants in this study belong to the city of Gómez Palacio, which is located in the northwest area of Mexico and northern area of Durango State. The geographic coordinates are 25 33 '00" and 25 32' 27" north latitude and 103 18 '27" and 103 40 '30" west longitude, at an altitude of 1,150 meters above sea level. This city has a semi-arid climate, with an average annual rainfall of 235 mm and an average annual temperature of 20 °C. Inclusion criteria were: pregnant women who were in any trimester of pregnancy, 12 - 45 years of age and pregnant women who signed the informed consent. In pregnant women under the age of 18 years old written informed consent was obtained from each participant and from their parents/guardians. The socioeconomic level, as well as occupation and/or educational level were not part of the criteria of non-inclusion. The participants were recruited consecutively. In total, 434 pregnant women were selected for this study. Sociodemographic, clinical data, risk factors of contracting the disease and associates were taken using a standardized questionnaire. Sociodemographic data, which include age, weight, height, area of residence, educational level, occupation, marital status and housing conditions index, were obtained from all participants. Housing Conditions Index (HCI) was obtained by using the Bronfman's criteria (8). Clinical data included weeks of pregnancy, previous deliveries, previous abortions, previous stillbirths, recent illnesses,
blood transfusions, previous surgeries, vision problems and previously diagnosed diseases. Risk factors and associated factors included overcrowding, pet ownership, cleaning of pets, frequency of consumption of different types of meat, consumption of other animals (deer, turkey, sheep, boar, goat, etc.), consumption of fruits and vegetables, washing of hands before eating, farming practices and farm visits. A serum sample was obtained from each pregnant woman. The serum was stored at -20 °C until further analysis. All samples were analyzed for IgG anti-*T. gondii* antibodies using a commercial kit of ELISA Toxoplasma IgG kit (Diagnostic Automation / Cortez Diagnostics, Woodland Hills, California, USA). Seropositive for IgG anti-*T. gondii* antibodies were then analyzed for IgM anti-*T. gondii* antibodies using a commercial Toxoplasma IgM ELISA kit (Diagnostic Automation / Cortez Diagnostics).

**Statistical analysis and ethics**

The calculation of sample size was determined by the following formula, taking into account the prevalence of toxoplasmosis in pregnant women of 15.6% in the Mexican population (9): 

\[ n = Z^2 p q / i^2 \]

Where: 
- \( Z \): 1.96 = 95% I. C. 
- \( p \): 0.156 
- \( q \): 0.844 
- \( i \): 0.050 
- \( n \): 203

According to the previous calculation, a sample size of 203 patients was obtained. Nevertheless we obtained a sample size of 434 patients this was 2 fold of the sample size calculation. A database with corresponding coding of variables was created and a descriptive analysis was carried out using frequencies of the same. A bivariate analysis was performed with Odds Ratio (OR) calculation between different variables associated with seropositive cases, considering as significant those with a p value less than 0.05 and those that are biologically feasible for inclusion in the subsequent multivariate analysis. A multivariate analysis was performed with calculation of OR between the different variables associated with the seropositive cases with statistical significance derived from the
bivariate analysis. All analyzes were performed using the statistical software IBM SPSS ver. 24.0 and STATA ver. 14.0.

Results

A total of 434 patients were analyzed with an average age of 22.2 years (± 5.95). Patients surveyed belonged to the following localities: Lerdo, Gómez Palacio, Mapimí, Nazas, Cuencamé, San Pedro del Gallo, San Luis del Cordero, General Simón Bolívar, San Juan de Guadalupe and Tlahualilo. Of the 434 patients analyzed, 4 (0.92%) were detected with IgG antibodies against *T. gondii* and none of these had a positive result to IgM antibodies against *T. gondii* (0%). Table 1 shows the sociodemographic and behavioral characteristics of the patients studied, highlighting most frequent age group 16-20 years, representing 46.54% of population which indicates the growing teenage pregnancies in the area of study. Another characteristic to be highlighted is 69.59% of population lived in an urban area and 30.41% in rural area, also representing a heterogeneous urban/rural population; additionally, 3 seropositive participants belonged to an urban area and 1 to a rural area. With respect to HCl, the majority 62.67% had good and only 2.53% had bad HCl; this also indicates that the housing conditions of the patients were adequate. With regards to first time pregnant women, 285 patients that represent 65.67% of the total and the most frequent age group (16-20) with 46.54%. This high percentage reflects teenage pregnancies in the population.

A bivariate analysis showed in Table 2 was carried out according to significant results of the variables of dry meat consumption, consumption of other types of meat, it was also decided to include other variables with biological feasibility in a multivariate analysis (Table 3). It is important to highlight consumption of other types of meat as a risk to have toxoplasmosis (OR 15.61 (95% CI 1.08 - 224.05 p = 0.043), this should be taken into consideration due to the amplitude of the confidence intervals. All this due to the low
frequency of the infection in the study population.

Discussion

The aim of this study was to estimate the frequency of seropositivity to IgM and IgG antibodies in pregnant women against *T. gondii* treated in the health services of the “Lagunera” region of Durango State, since there were no data of the prevalence of toxoplasmosis in the “Lagunera” region of Durango State. The low seroprevalence of this study is possibly due to climatic conditions, type of food consumption and behavioral habits of the population. Climatic conditions affect oocyst survival.

In our study three seropositive pregnant woman belong to urban and one to rural area this difference can’t be considered as important due to the “Lagunera” region of Durango being a cattle and agriculture zone where meat consumption is very frequent and generally local meat is consumed. Rural and urban areas in the “Lagunera” region of Durango are very similar, both are characterized by dust swirls which cover the area, raising and spreading oocysts through, making it difficult to know their main source; this will be considered for a future study.

*T. gondii* infection in general, and in pregnant women in particular, has been previously studied in Mexico. In 1992 a national survey of the general population showed that infection by *T. gondii* is present throughout the country (6). The prevalence varied according to the geographical area, with the northern zone being the lowest in relation to the prevalence. In the present study, a seroprevalence of IgG antibodies against *T. gondii* of 0.91% was determined, a result much lower than that found in 1992 by Velasco et al. (6) in the northern region of the country, where they obtained an 11.5% prevalence for an active infection; specifically in the State of Durango, a prevalence of 9.6% was found with a prevalence of 22.1%, similar only to Tamaulipas State. In 2012 Caballero-Ortega et al. (7) took a serum bank provided for the National Health Survey 2000 and the National
Health and Nutrition Survey 2006 contrasting with the national toxoplasmosis prevalence of 1987, and the climatic changes in Mexico during 1987, 2000 and 2006 but no correlation was found with temperature and precipitation. Nonetheless, a significantly positive correlation with temperature and seroprevalence was found in 21 states, which adjusted prevalence in 2000 and 2006 Durango State had 20-29% and 60-69% respectively. This is particularly interesting since this study’s seroprevalence was 0.94% in a small area of Durango State, differing from the global prevalence in the State in 2006 of 60-69%. This could be due to Durango having a geographical diversity, since in its territory sits west in the plateau region, a sub-humid weather with abundant rains and forests; in the north, a desert zone, where the “Lagunera” region is located; and to the east plains which has a dry semi-warm and semi-dry temperature, where Durango city is located. The different climates could explain the great differences in prevalence among this State.

In 2006 a similar study was carried out in the city of Durango, Durango by Alvarado-Esquivel et al. (10), seroprevalence of antibodies against T. gondii in pregnant women was reported in the general hospital of Durango city; sample size was 343 pregnant women unlike this study with 434 pregnant women. The seroprevalence of IgG and IgM antibodies were 6.1% and 0% respectively. Alvarado-Esquivel et al. argues low prevalence of antibodies IgM and IgG type against T. gondii is due to low socioeconomic level, low consumption of meat and high temperatures of Durango city during the day. However, the risk factors evaluated were living in houses with soil floor, living in rural areas and consuming turkey meat; unlike his study where the majority of the population studied were living in houses with firm floor, belonged to an urban area and consumed dried meat and other types of meat; where consumption of dry meat and consumption of other types of meat turned out to be a risk factor associated with this disease in the studied
population. It should be noted that distance between Gómez Palacio and Durango is approximately 200 kilometers, which results in differences such as habits of the population of Durango that differ from this study in the consumption of turkey meat, number of people who had a soil floor and weather. Durango is in a valley unlike the “Lagunera” region of Durango, which is a semi-desert zone, that could explain the difference in the frequency of IgG antibodies with respect to this study affecting possibly survival of *T. gondii* by the increase in temperature of environment which implies a subsequent investigation to this work.

Recently, in a study carried out in 2016 by Alvarado-Esquivel et al. (11) in the city of Aguascalientes, a seroprevalence of IgG and IgM antibodies against *T. gondii* was found of 6.2% and 4.8% respectively. The sample size of that study was 338, unlike our sample of 434 pregnant women; and the variables studied were very similar, differing from having abdominal pain, memory loss, hearing loss and had suffered from hepatitis. One of the possible explanations that the authors give to the low seroprevalence is due to the climatic conditions of the city of Aguascalientes, which has a semi-arid climate and an annual rainfall of 500 mm; an explanation very consistent with this study since, very similarly, the city of Gómez Palacio has a semi-arid climate but an average annual rainfall of 250 mm, which contributes greatly to the survival of *T. gondii* in the soil. The risk factors evaluated were white ethnicity, low educational level (0 - 6 years), not eating out of home, not washing hands and using latrines. It should be noted that in this study, prevalence was associated with consumption of dry meat and other types of meat, which indicates that toxoplasmosis is acquired by the consumption of tissue cysts, unlike Alvarado-Esquivel et al. 2016 study, where association of seroprevalence of toxoplasmosis was due to habits of population, which suggests that there is an infection directly by land
containing sporulated oocysts; the authors argue that not washing hands before eating (7.1%) and the use of latrines (2.36%) are risk factors that reflect poor hygiene and cleanliness of the studied population, facilitating ingestion of *T. gondii* oocysts; risk factors found in this study represent 2.53% of people who had bad HCl, which means that their homes had a soil floor, use of latrines, public hydrant water and/or overcrowding, as well as 12.9 % of people who do not wash their hands before eating (not showed data), however in this study there was no association to these risk factors with *T. gondii* infection.

In respect to international prevalence of toxoplasmosis in pregnant women, seroprevalence of this study is lower than reported in Zambia by Frimpong et al. in the year 2017 (12) of 5.87% of IgG antibodies and 0% of IgM antibodies. Another study conducted in France by Tourdjman et al. in 2015 (13) reported a prevalence in 2010 of 36.7% higher than reported in this study. In the United States (4) in 2001, the seroprevalence of IgG antibodies against *T. gondii* was reported in women of reproductive age (15 to 44 years) of 15% and a study conducted from 2011-2014 the same author et al. (14) reported a prevalence of 7.5% of IgG antibodies against *T. gondii*. In Norway Findal et al. in 2015 (15) they reported a seroprevalence of IgG antibodies against *T. gondii* of 9.3%. Another study in Korea by Song et al. in the year 2005 (16) showed a prevalence of 0.79%, similar to that reported in this work of 0.92%. In the search for information only the study conducted by Song et al. was the only one who reported a similar prevalence to this study.

Dubey in 1998 examined oocyst survival according to temperature, oocysts were rendered noninfective by storage for 62 days at 35 °C, 28 days at 40 °C, 2 days at 45 °C, 120 min at 50 °C, and 10 min at 55 °C (17). In the “Lagunera” region of Durango, the hot season usually lasts 4.2 months, from April to August, average daily maximum temperature is
more than 33 °C; in 2017 the hottest day had a maximum temperature of 42.6°C. Cool season lasts around 2.5 months, from November to February, and the average daily maximum temperature is less than 24°C. This could be detrimental to oocyst survival in soil and the possible explanation to low seroprevalence. Another possible explanation for the low seroprevalence in this study is due to behavioral characteristics of the population and the possible low prevalence of *T. gondii* in animals for human consumption, combined with the semi-arid climate of the region and the survival of the parasite in the soil, which are crucial factors for low seroprevalence in the area. For example, a study conducted in Colombia by Gomez-Marin et al. in 2011 (18), which aimed to determine incidence of congenital toxoplasmosis in Colombian newborns from 19 hospitals or health services in seven different cities of five natural geographic regions, had as a finding a significant correlation between precipitation average of each city and incidence of markers for congenital infection. Another study in domestic cats conducted in France by Afonso et al. in 2013 (19), where a correlation was found with the highest prevalence and years with coldest and wettest winters. Another study carried out in France by Morin et al. in 2012 (20) calculated seasonal variations of toxoplasmosis infection in pregnant women; it was observed that in rural areas the seasons with significantly less infections happened in the first half of the year but with maximum risks at the end of summer and at the end of autumn.

With regards to consumption of dried meat and other types of meat, there are studies which have associated the consumption of certain types of meat with *T. gondii* infection, such as the one carried out by Cook et al. in the year 2000 (21) where probability of infection in pregnant women was associated with consumption of undercooked lamb meat, beef and consumption of cured or dried meat. In another study, in which the objective was to know the risk factors of toxoplasmosis in the United States by Jones et al. (22), the
adjusted OR's were: eat raw beef 6.67, eat lamb 8.39, eat cured meat, dry or smoked 1.97 and work with meat 3.15. Another study, in 2015 carried out by Guo et al. (23) showed that raw fermented sausage, raw cured meat, meat that is not dried in hot air and fresh processed meat were associated with higher risks of exposure, compared to cooked meat and frozen meat. In this study 3 out of 4 pregnant women with IgG antibodies against *T. gondii* had eaten dried meat, which is consistent with the cited literature that associates the consumption of dried meat with *T. gondii* infection. To the date, there is no data available of toxoplasmosis studies in animals in the Comarca Lagunera of Durango; it will be very important to consider toxoplasmosis seroprevalence studies in animals like birds and mammals in the Comarca Lagunera of Durango as well to explore general population seroprevalence.

The results showed that pregnant women in the “Lagunera” region of Durango State have a very low seroprevalence of *T. gondii* infection, possibly due to the climatic conditions of the region and habits of the population. Due to the low seroprevalence, no risk factors were found conclusively associated with the infection in this population, but this work is useful to know some habits that could be considered as a risk in this population. However, this indicates that living in an area with a very low seroprevalence of toxoplasmosis, this population it has a low risk to acquire the infection but, the majority of the population studied is at risk of having a primary *T. gondii* infection.

**Declarations**

**Ethics approval and consent to participate**

The project was submitted for approval by the Ethics Committee of the Faculty of Chemical Sciences (UJED) with license or registration number 123301538X0201-COFEPRIS in Gómez Palacio, Durango, Mexico. The registry of this project is R-2017-123301538X0201-027. All procedures were communicated to the participants and written
informed consent was obtained from them. In pregnant women under the age of 18 years old written informed consent was obtained from each participant and from their parents/guardians to obtain biological samples. Confidentiality of the participants was respected and their right to leave the study at any time if necessary.

**Availability of data and materials**

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

**Competing interests**

The authors declare no conflicts of interest.

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**Author contributions**

RAFC oversaw fieldwork, instruments and manuscript writing. EHOC conceptualized, designed the study and contributed to manuscript. CAE contributed with technical assistance and contributed to study design and manuscript. EHOC and RAFC performed statistical analysis. All authors read and approved the manuscript.

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Tables

Table 1. - Sociodemographic/behavioral characteristics of pregnant women.

| Characteristic | n  | %  |
|---------------|----|----|
| Age group     |    |    |
| <15           | 24 | 5.53|
| 16-20         | 202| 46.54*|
| 21-25         | 94 | 21.66|
| 26-30         | 72 | 16.59|
| 31-35         | 25 | 5.76|
| >35           | 17 | 3.92|
| Place of residence        |       |       |
|--------------------------|-------|-------|
| Urban                    | 302   | 69.59*|
| Rural                    | 132   | 30.41 |

| Educational level (years)|       |       |
|--------------------------|-------|-------|
| 0-6                      | 42    | 9.68  |
| 7-9                      | 238   | 54.84*|
| 10-12                    | 118   | 27.19 |
| >13                      | 36    | 8.29  |

| Occupation               |       |       |
|--------------------------|-------|-------|
| Housekeeper              | 337   | 77.65*|
| Employee                 | 30    | 6.91  |
| Student                  | 25    | 5.76  |
| Professional             | 6     | 1.38  |
| Others                   | 36    | 8.29  |

| Crowding                 |       |       |
|--------------------------|-------|-------|
| Not overcrowded          | 11    | 2.53  |
| Semi-overcrowded         | 231   | 53.23*|
| Overcrowded              | 192   | 44.24 |

| Housing conditions       |       |       |
|--------------------------|-------|-------|
| Good                     | 272   | 62.67*|
| Regular                  | 151   | 34.79 |
| Bad                      | 11    | 2.53  |

| Gestational age          |       |       |
|--------------------------|-------|-------|
| 0-12                     | 29    | 6.68  |
| 13-26                    | 132   | 30.41 |
| 27-40                    | 273   | 62.90*|

| Miscarriages             |       |       |
|--------------------------|-------|-------|
| No                       | 75    | 17.28 |
| Yes                      | 359   | 82.72*|

p <0.05; Chi² test.

Table 2.- Bivariate analysis of risk factors to have anti-T. gondii IgG antibodies in pregnant women.
| Characteristic                        | Odds ratio | 95% Confidence Interval |
|--------------------------------------|------------|-------------------------|
| Pet ownership                        | 0.48       | 0.06 - 3.49             |
| Pork consumption                     | 1.33       | 0.13 - 12.91            |
| Sausages consumption                 | 0.70       | 0.07 - 6.87             |
| Turkey consumption                   | 0.86       | 0.12 - 6.17             |
| Deer consumption                     | 4.44       | 0.44 - 44.03            |
| Dry meat consumption                 | 16.54      | 1.69 - 161.48           |
| Other meat types consumption         | 7.77       | 1.07 - 56.45            |
| Tap water drinking source            | 1.76       | 0.18 - 17.06            |
| Hand washing before eating           | 0.44       | 0.04 - 4.3              |
| Agriculture or gardening             | 1.25       | 0.12 - 12.25            |
| Blood transfusions                   | 5.17       | 0.52 - 51.54            |
| Recent illness                       | 6.84       | 0.70 - 66.44            |
| Frequent headaches                   | 0.5        | 0.052 - 4.84            |
| Visual impairment                    | 1.77       | 0.18 - 17.31            |

*p < 0.05; Logistic regression

Table 3.- Multivariate analysis of selected characteristics of pregnant women and their association with anti-T. gondii IgG antibodies.

| Characteristic                        | Odds ratio | 95% Confidence Interval |
|--------------------------------------|------------|-------------------------|
| Deer consumption                     | 4.11       | 0.21 - 78.89            |
| Dry meat consumption                 | 13.5       | 0.94 - 192.58           |
| Other meat types consumption         | 15.61      | 1.08 - 224.05           |
| Blood transfusions                   | 2.141      | 0.09 - 49.68            |
| Recent illness                       | 7.31       | 0.66 - 80.09            |

*p <0.05; Logistic regression

Adjusted by: age, school level, occupation, place of residence, housing condition index.