

## STILLBIRTH HISTORY AND *TOXOPLASMA GONDII* INFECTION IN WOMEN ATTENDING PUBLIC HEALTH CENTERS IN A NORTHERN MEXICAN CITY

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Through a cross-sectional study design, 150 women attending public health centers with a history of stillbirths were examined for anti-*Toxoplasma gondii* IgG and IgM antibodies in Durango City, Mexico. Bivariate and multivariate analyses were used to assess the association of *T. gondii* seropositivity with the characteristics of the women with stillbirth history.

Of the 150 women (mean age: 32.09 ± 9.16 years) studied, 14 (9.3%) had anti-*T. gondii* IgG antibodies and six (42.9%) of them were also positive for anti-*T. gondii* IgM antibodies. Multivariate analysis showed that *T. gondii* seropositivity was associated with high frequency (4–7 days a week) of eating meat (OR = 5.52; 95% CI: 1.48–20.59; *P* = 0.01), history of lymphadenopathy (OR = 4.52; 95% CI: 1.14–17.82; *P* = 0.03), and history of surgery (OR = 8.68; 95% CI: 1.04–72.15; *P* = 0.04).

This is the first study on the seroepidemiology of *T. gondii* infection in women with a history of stillbirths in Mexico. The association of *T. gondii* exposure with a history of surgery warrants for further research. Risk factors for *T. gondii* infection found in the present survey may help to design optimal educational programs to avoid *T. gondii* infection.

**Keywords:** *Toxoplasma*, stillbirths, epidemiology, seroprevalence, risk factors, cross-sectional study, Mexico

### Introduction

*Toxoplasma gondii* (*T. gondii*) is a parasite widely distributed around the world [1, 2]. Infections with *T. gondii* usually occur by ingestion of water or food contaminated by oocysts shed by *T. gondii*-infected cats or by ingestion of tissue cysts in meat from *T. gondii*-infected animals [1–3]. The clinical spectrum of *T. gondii* infections varies from asymptomatic to life-threatening disease [1]. Infections with *T. gondii* may lead to lymphadenopathy [2], chorioretinitis [4], meningoencephalitis, congenital disease, and neonatal mortality [2, 5].

Congenital toxoplasmosis can cause fetal death and stillbirths or long-term disabling sequelae [6]. Stillbirths due to infections are more common in developing than in developed countries [7]. There is poor knowledge about the epidemiology of *T. gondii* infection in women with stillbirths. In a study in the Caribbean island of Trinidad, researchers found the highest seroprevalence of *T. gondii* infection in neonates of mothers who had experienced stillbirths [8]. In a previous study of pregnant women suffering from hypertensive disorders in northern Mexico, we found a higher seroprevalence of *T. gondii* infection in women with stillbirth history than those without this his-

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tory [9]. However, very little is known about the characteristics of the women with stillbirths and *T. gondii* infection and about the seroprevalence of *T. gondii* infection in these women in Mexico. Therefore, we sought to determine the seroprevalence of *T. gondii* infection in women with stillbirth history attended in two public health centers and a hospital in Durango City, Mexico, and to determine the association of *T. gondii* seropositivity with the socio-demographic, clinical, and behavioral characteristics of the women with stillbirth history.

## Materials and methods

### *Study design and study population*

We performed a cross-sectional study of 150 women with a stillbirth history who attended two public primary health centers (Clínica de Medicina Familiar, ISSSTE; Centro de Salud con Servicios Ampliados 450, Secretary of Health) and the Mothers and Children's Hospital of the Secretary of Health from August 2013 to January 2015 in Durango City, Mexico. Inclusion criteria for enrollment included: 1) women with a history of stillbirth (fetal death after 20 weeks of pregnancy), 2) aged 16–50 years, and 3) who accepted to participate in the study. Occupation, socio-economic status, and educational level were not restrictive criteria for enrollment.

### *Socio-demographic, clinical, and behavioral characteristics of the women*

We obtained the socio-demographic, clinical, and behavioral characteristics from the women studied with the aid of a standardized questionnaire. Socio-demographic data included age, birthplace, residence, occupation, educational level, and socio-economic status. Clinical items included obstetric history (number of pregnancies, deliveries, cesarean sections, miscarriages, and stillbirths), presence of any underlying disease, presence of frequent headaches and impairments of memory, reflexes, vision, and hearing, and history of blood transfusions or transplants. Behavioral data included animal contacts, 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 from home (in restaurants and fast food outlets), consumption of dried or cured meat (chorizo, ham, sausages, or salami), unwashed raw vegetables or fruits, drinking unpasteurized milk or untreated water, and soil contact (gardening or agriculture).

### *Serology of T. gondii infection*

A blood sample was obtained from each participant. Blood was centrifuged and sera were stored at  $-20^{\circ}\text{C}$  until analyzed. Serum samples were examined for anti-*T. gondii*

IgG antibodies by a commercially available enzyme immunoassay “*Toxoplasma* IgG” kit (Diagnostic Automation Inc., Calabasas, CA, USA). All sera positive for anti-*T. gondii* IgG antibodies were additionally analyzed for anti-*T. gondii* IgM antibodies by a commercially available enzyme immunoassay “*Toxoplasma* IgM” kit (Diagnostic Automation Inc., Calabasas, CA, USA). Both tests were performed following the manufacturer's instructions.

### *Statistical analysis*

Results were analyzed with the Epi Info version 7 and SPSS version 15.0 software. For comparison of the frequencies, the Pearson's chi square and the Fisher exact test (when values were small) were used. We performed bivariate analysis followed by multivariate analysis to assess the association between the characteristics of the women and *T. gondii* infection. All variables with a *P* value equal to or less than 0.10 obtained in the bivariate analysis were included in the multivariate analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression analysis using backward stepwise logistic regression analysis. Goodness of fit of our regression model was assessed with the Hosmer–Lemeshow test. A *P* value  $<0.05$  was considered as statistically significant.

### *Ethics considerations*

This study was approved by the ethical committees of the Mothers and Children's Hospital of the Secretary of Health, Centro de Salud de Servicios Ampliados 450, and Clínica de Medicina Familiar of the Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado in Durango City. The purpose and procedures of the study were explained to all participants, and a written informed consent was obtained from all of them.

## Results

Of the 150 women with stillbirth history studied, 14 (9.3%) had anti-*T. gondii* IgG antibodies and six (42.9%) of them were also positive for anti-*T. gondii* IgM antibodies. Of the 14 anti-*T. gondii* IgG-positive women, 8 (57.1%) had IgG levels higher than 150 IU/ml, 2 (14.3%) between 100 and 150 IU/ml, and 4 (28.6%) between 9 and 99 IU/ml. *Table 1* shows the socio-demographic characteristics of the 150 women and their correlation with *T. gondii* seropositivity. The mean age of the women studied was  $32.09 \pm 9.16$  years (range 16–50 years). The socio-demographic variable “ethnic group” was associated ( $P = 0.01$ ) with seropositivity to *T. gondii* by bivariate analysis. Other socio-demographic variables including age, birthplace, residence, occupation, educational level, and socio-economic status showed *P* values higher than 0.10 by bivariate analysis.

**Table 1.** Socio-demographic characteristics of women with stillbirth history and seroprevalence of *T. gondii* infection

Characteristics	No. of subjects tested	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
Age groups (years)				
30 or less	69	4	5.8	0.26
>30	81	10	12.3	
Ethnic group				
Mexicanero	1	0	0.0	0.01
Tepehuano	3	2	66.7	
White	8	0	0.0	
Mestizo	138	12	8.7	
Birth place				
Durango State	138	12	8.7	0.31
Other Mexican State	12	2	16.7	
Residence place				
Durango State	147	14	9.5	1
Other Mexican State	3	0	0.0	
Residence area				
Urban	127	11	8.7	0.23
Suburban	8	0	0.0	
Rural	15	3	20.0	
Educational level				
No education	3	1	33.3	0.15
1–6 years	21	1	4.8	
7–12 years	105	12	11.4	
13 or more years	21	0	0.0	
Occupation				
Unemployed	120	12	10.0	0.73
Employed	30	2	6.7	
Socio-economic level				
Low	39	4	10.3	0.92
Medium	110	10	9.1	
High	1	0	0.0	

With respect to clinical data, three variables showed *P* values less than 0.10 by bivariate analysis including “lymphadenopathy” ( $P = 0.04$ ), “history of surgery” ( $P = 0.01$ ), and “history of deliveries” ( $P = 0.08$ ). Other clinical characteristics including number of pregnancies, cesarean sections, miscarriages or stillbirths, presence of any underlying disease, presence of frequent headaches and impairments of memory, reflexes, vision and hearing, and history of blood transfusions or transplants showed *P* values higher than 0.10 by bivariate analysis. A correlation of seropositivity to *T. gondii* with a selection of clinical characteristics of the women is shown in Table 2. The frequency of anti-*T. gondii* IgM antibodies was also significantly ( $P =$

0.04) higher in women with surgery history (6/95; 6.3%) than that in women without this history (0/55; 0%). Other clinical characteristics of women were not associated with IgM seropositivity. Stillbirths had occurred from 1 day to 35 years (median 2 years) ago.

Concerning behavioral characteristics, the variable “frequency of eating meat” but no other clinical characteristics including animal contacts, traveling, consumption of raw or undercooked meat, type of meat consumed, eating in restaurants and fast food outlets, consumption of dried or cured meat, unwashed raw vegetables or fruits, drinking unpasteurized milk or untreated water, and soil contact showed *P* values of less than 0.10 by bivariate analysis.

**Table 2.** Bivariate analysis of clinical data and infection with *T. gondii* in women with stillbirth history in Durango City, Mexico

Characteristics	No. of subjects tested	Prevalence of <i>T. gondii</i> infection		P value
		No.	%	
<b>Clinical status</b>				
Healthy	119	9	7.6	0.15
Ill	30	5	16.7	
<b>Lymphadenopathy ever</b>				
Yes	23	5	21.7	0.04
No	127	9	7.1	
<b>Headache frequently</b>				
Yes	73	8	11	0.50
No	77	6	7.8	
<b>Memory impairment</b>				
Yes	42	3	7.1	0.75
No	108	11	10.2	
<b>Dizziness</b>				
Yes	50	7	14	0.23
No	100	7	7	
<b>Reflex impairment</b>				
Yes	12	1	8.3	1.00
No	138	13	9.4	
<b>Hearing impairment</b>				
Yes	20	1	5	0.69
No	130	13	10	
<b>Visual impairment</b>				
Yes	45	4	8.9	1.00
No	105	10	9.5	
<b>Surgery ever</b>				
Yes	95	13	13.7	0.01
No	55	1	1.8	
<b>Blood transfusion</b>				
Yes	27	4	14.8	0.28
No	122	10	8.2	
<b>Pregnancies</b>				
One	21	1	4.8	0.69
More than one	129	13	10.1	
<b>Deliveries</b>				
Zero	54	2	3.7	0.08
One or more	96	12	12.5	
<b>Cesarean sections</b>				
Yes	52	6	11.5	0.55
No	98	8	8.2	

**Table 2.** (cont.)

Characteristics	No. of subjects tested	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
Miscarriages				
Yes	43	2	4.7	0.35
No	107	12	11.2	
Stillbirths				
One	138	12	8.7	0.31
Two to four	12	2	16.7	

A selection of clinical characteristics of women and their association with *T. gondii* seropositivity is shown in Table 3. Multivariate analysis of variables with *P* values equal to or less than 0.10 obtained in the bivariate analysis showed that *T. gondii* seropositivity was associated with high frequency (4–7 days a week) of eating meat (OR = 5.52; 95% CI: 1.48–20.59; *P* = 0.01), history of lymphadenopathy (OR = 4.52; 95% CI: 1.14–17.82; *P* = 0.03), and history of surgery (OR = 8.68; 95% CI: 1.04–72.15; *P* = 0.04). The Hosmer–Lemeshow test showed an acceptable fit of our regression model (*P* = 0.86).

## Discussion

The epidemiology of *T. gondii* infection in women with stillbirth history in Mexico is largely unknown. In the present study, we obtained the seroprevalence and correlates of *T. gondii* infection in women with stillbirth history in the northern Mexican City of Durango. *T. gondii* seropositivity was associated with high frequency of eating meat, history of lymphadenopathy, and history of surgery. Only few reports about the seroprevalence of *T. gondii* infection in women with stillbirths in the world exist. The se-

**Table 3.** Bivariate analysis of selected putative risk factors for infection with *T. gondii* in women with stillbirth history in Durango, Mexico

Characteristics	No. of subjects tested	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
Cleaning cat excrement				
Yes	39	1	2.6	0.11
No	111	13	11.7	
National trips				
Yes	103	12	11.7	0.22
No	47	2	4.3	
Pork meat consumption				
Yes	145	13	9	0.39
No	5	1	20	
Venison consumption				
Yes	56	7	12.5	0.3
No	94	7	7.4	
Squirrel meat consumption				
Yes	17	0	0	0.37
No	132	14	10.6	
Armadillo meat consumption				
Yes	2	1	50	0.17
No	148	13	8.8	

**Table 3.** (cont.)

Characteristics	No. of subjects tested	Prevalence of <i>T. gondii</i> infection		P value
		No.	%	
Frequency of meat consumption				
Up to 3 times a week	118	8	6.8	0.07
4–7 times a week	31	6	19.4	
Degree of meat cooking				
Raw	1	0	0	0.8
Undercooked	3	0	0	
Well done	146	14	9.6	
Raw milk consumption				
Yes	59	3	5.1	0.15
No	91	11	12.1	
Unwashed raw fruits				
Yes	51	3	5.9	0.38
No	99	11	11.1	
Untreated water				
Yes	91	7	7.7	0.39
No	59	7	11.9	
Frequency of eating out of home				
Never	11	1	9.1	0.28
1–10 times a year	61	3	4.9	
>10 times a year	78	10	12.8	
Washing hands before eating				
Yes	140	12	8.6	0.23
No	10	2	20	
Floor at home				
Ceramic or wood	65	9	13.8	0.23
Concrete	73	4	5.5	
Soil	12	1	8.3	

ro-prevalence (9.3%) found in the present study is slightly higher than the 6.1% seroprevalence of *T. gondii* infection reported in the general population [13] and the 6.7% seroprevalence in women with miscarriage history [14] in Durango City, Mexico. However, the seroprevalence is markedly lower than the 21.4%–66.7% seroprevalences reported in women with stillbirths in Iran [10], Indonesia [11], India [12], and the Caribbean island of Trinidad [8]. The difference in seroprevalences might be a reflection of the seroprevalences of *T. gondii* infection in the general populations among the countries studied, v. g., 39.3% in Iran [15] and 30.9% in India [16].

Consumption of meat is a well-known and important risk factor for *T. gondii* infection [1]. In the present study, subjects who consumed meat 4–7 days a week had a significantly higher seroprevalence of *T. gondii* infection than those who consumed meat up to 3 days a week. This find-

ing suggests that an important number of women with stillbirth history might have acquired *T. gondii* infection owing to frequent consumption of meat. In the current study, we studied women who have had stillbirths from 1 day to 35 years (median 2 years) ago. Therefore, factors associated with *T. gondii* infection found in our study should be further investigated in a larger number of women with recent stillbirths. The association of infection with high frequency of meat consumption has epidemiological importance and points toward the need of educational programs in women to avoid *T. gondii* infection with especial attention to pregnant women with high frequency of meat consumption. Health education may help to reduce the risk of congenital toxoplasmosis [17].

With respect to the association of *T. gondii* infection with a history of lymphadenopathy, our finding suggests that women with stillbirth history may have presented

clinical signs of toxoplasmosis albeit this disease was not diagnosed during or before pregnancy. Lymphadenopathy is a characteristic sign of toxoplasmosis while not always observed during pregnancy [1, 2]. It is also true that lymphadenopathy is not exclusive of toxoplasmosis but physicians should think of *T. gondii* infection as a differential diagnosis in any pregnant women presenting with lymph node enlargement. Toxoplasmosis in Mexico is a neglected disease and poor knowledge on the clinical manifestations of toxoplasmosis among physicians in the region has been reported [18]. Therefore, our finding further remarks the need for educational programs to physicians attending pregnant women to increase the detection rate of toxoplasmosis and to provide information for preventing *T. gondii* infections to all their patients.

Intriguingly, we found an association of *T. gondii* infection with a history of surgery. In a previous cross-sectional study of psychiatric patients in a public hospital in Durango City, Mexico, we also found an association of *T. gondii* seropositivity with a history of surgery [19]. We are not aware of further studies that had shown this association. The finding of this association in two independent studies with different population groups should prompt further research into the association of *T. gondii* exposure with a history of surgery. In this regard, it is well known that organ transplantation can be a source for *T. gondii* infection [20, 21]. However, none of the women in our study had undergone surgery for transplantation. In a previous study, history of abdominal hernia repair was linked to *T. gondii* seropositivity, although it was not clear whether this link was due to the surgery or by muscle damage by *T. gondii* [22]. However, none of the women in our study had a history of abdominal hernia repair. The association of *T. gondii* exposure with a history of surgery may be caused by a number of factors. In theory, instruments or materials used in surgeries may be contaminated with *T. gondii* via blood or tissues from other *T. gondii*-infected patients undergoing surgery. Alternatively, surgical suture materials such as “catgut” are made of intestines of animals. It is unclear whether catgut was made or is currently made of cat intestines but it is well known that important stages of the *T. gondii* life cycle occurs in the intestines of cats and other felids [23]. It is also unclear whether solutions used to preserve catgut may also preserve *T. gondii*. Finally, the association observed here and in another study may not be of causal nature.

## Conclusions

This is the first study on the seroepidemiology of *T. gondii* infection in women with history of stillbirths in Mexico. Well-established risk factors for *T. gondii* infection found in the present survey may help to design optimal educational programs to avoid *T. gondii* infection. The association of *T. gondii* exposure with a history of surgery warrants for further research.

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