MISCARRIAGE HISTORY AND *TOXOPLASMA GONDII* INFECTION: A CROSS-SECTIONAL STUDY IN WOMEN IN DURANGO CITY, MEXICO

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Through a cross-sectional study design, 326 women with a history of miscarriage were examined for anti-*Toxoplasma gondii* IgG and IgM antibodies in Durango City, Mexico. Prevalence association with sociodemographic, clinical, and behavioral characteristics in women with miscarriage was also investigated.

Twenty-two (6.7%) of the 326 women studied had anti-*T. gondii* IgG antibodies and two (0.6%) were also positive for anti-*T. gondii* IgM antibodies. Seroprevalence of *T. gondii* infection was not influenced by age, birth place, occupation, educational level, or socioeconomic status. In contrast, logistic regression showed that *T. gondii* exposure was associated with consumption of raw or undercooked meat (OR = 6.84; 95% CI: 1.04–44.95; $P = 0.04$) and consumption of chicken brains (OR = 18.48; 95% CI: 1.26–269.43; $P = 0.03$).

This is the first study on the seroepidemiology of *T. gondii* infection in women with a history of miscarriage in Northern Mexico. Of interest, we also observed an association of *T. gondii* exposure with consumption of chicken brains. Contributing factors for *T. gondii* exposure found in the present study should be taken into consideration for public health measures to avoid infection with *T. gondii* and its sequelae.

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

Introduction

The parasite *Toxoplasma gondii* (*T. gondii*) causes infections in humans worldwide [1, 2]. Infections with *T. gondii* are commonly asymptomatic. However, some infections with *T. gondii* may lead to disease including cervical lymphadenopathy or chorioretinitis [1]. In addition, primary infections with *T. gondii* in pregnant women may lead to congenital disease [1, 3]. Damage to the fetus is characterized by severe necrotic changes to the central nervous system, i.e., the brain and eyes [4, 5]. These commonly occur when infection with *T. gondii* is acquired during the first trimester of pregnancy [1, 3].

Very little is known about the epidemiology of *T. gondii* infection in women with abortions. Seroprevalences of infection with *T. gondii* varying from 17% to 43.8%

in women with abortions have been reported in Pakistan [6], Egypt [7], and India [8]. However, very poor knowledge exists about the characteristics of the women with miscarriages and *T. gondii* infection. In a previous survey, we found an association of *T. gondii* seropositivity with abortion in rural pregnant women in Durango State, Mexico, especially in communities with high seroprevalence of *T. gondii* infection [9]. However, there is no information about the seroprevalence and risks associated with of *T. gondii* infection in urban women with a history of miscarriage in Northern Mexico. Therefore, we performed a survey to determine the prevalence of *T. gondii*, the concentrations of anti-*T. gondii* IgG, and the sociodemographic, clinical, and behavioral characteristics associated to *T. gondii* seropositivity in women with a history of miscarriage.

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Materials and methods

Study populations and study design

Through a cross-sectional study design, we studied 326 women with a history of miscarriage in a public primary health care center (Centro de Salud con Servicios Ampliados 450) and the General Hospital of the Secretary of Health from August to December 2013 in Durango City, Mexico. Inclusion criteria for enrollment in the study were: 1) women with a history of miscarriage (spontaneous fetal loss during the first 20 weeks of pregnancy); 2) residing in Durango City; 3) aged 16 years and older; and 4) who accepted to participate in the study.

Sociodemographic, clinical, and behavioral data

We used a standardized questionnaire to obtain sociodemographic, clinical, and behavioral characteristics from the women studied. Sociodemographic items included age, birth place, residence, occupation, educational, and socioeconomic status. Clinical data included obstetric history (number of pregnancies, deliveries, cesarean section, and miscarriages); presence of any underlying disease, frequent headaches, and impairments of memory, reflexes, vision, and hearing; and history of blood transfusions or transplants. Behavioral items obtained were animal con-

tacts; foreign travel; consumption of raw or undercooked meat (pork, lamb, beef, goat, boar, chicken, turkey, rabbit, deer, squirrel, horse, or other); eating away of home (in restaurants and fast food outlets); consumption of dried or cured meat (chorizo, ham, sausages, or salami), unpasteurized milk, untreated water, and unwashed raw vegetables or fruits; and contact with soil (gardening or agriculture).

Laboratory tests

Sera of the women studied were examined for anti-*T. gondii* IgG antibodies by a commercially available enzyme immunoassay “*Toxoplasma* IgG” kit (Diagnostic Automation Inc., Calabasas, CA, USA). Sera positive for anti-*T. gondii* IgG antibodies were further 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 instructions of the manufacturers.

Statistical analysis

The statistical analysis was performed with the aid of the software: Epi Info version 3.5.4 and SPSS version 15.0. We used the Pearson’s χ^2 and the Fisher exact test (when

Table 1. Sociodemographic characteristics of women with miscarriage history and seroprevalence of *Toxoplasma gondii* infection

Characteristic	No. of women tested	Prevalence of <i>T. gondii</i> infection		Odds ratio	95% Confidence interval	<i>P</i> value
		No.	%			
Age groups (years)						
30 or less	124	6	4.8	1.0		
31–50	160	11	6.9	1.5	0.48–4.56	0.47
>50	42	5	11.9	2.7	0.66–10.61	0.14
Birth place						
Durango State	300	21	7.0	1.9	0.25–39.08	1
Other Mexican State	26	1	3.8	1.0		
Educational level						
No education	8	1	12.5	4.6	0.00–196.79	0.35
1–6 years	79	9	11.4	4.1	0.49–91.34	0.27
7–12 years	206	11	5.3	1.8	0.23–38.65	1
13 or more years	33	1	3.0	1.0		
Occupation						
Unemployed	249	17	6.8	1.1	0.35–3.40	0.91
Employed	77	5	6.5	1.0		
Socioeconomic status						
Low	105	7	6.7	1.0		
Medium	221	15	6.8	1.0	0.37–2.86	0.96

Table 2. Bivariate analysis of selected putative risk factors for infection with *Toxoplasma gondii* in women with miscarriage history in Durango, Mexico

Characteristic	Women tested*	Prevalence of <i>T. gondii</i> infection		P value
	No.	No.	%	
Cleaning cat excrement				
Yes	92	10	10.9	0.06
No	233	12	5.2	
Birds at home				
Yes	122	11	9	0.2
No	204	11	5.4	
Raising farm animals				
Yes	125	14	11.2	0.01
No	200	8	4	
Traveled abroad				
Yes	69	9	13	0.03
No	254	13	5.1	
Chicken meat consumption				
Yes	324	21	6.5	0.13
No	2	1	50	
Pigeon meat consumption				
Yes	31	4	12.9	0.14
No	294	18	6.1	
Duck meat consumption				
Yes	41	5	12.2	0.17
No	285	17	6	
Horse meat consumption				
Yes	22	4	18.2	0.05
No	304	18	5.9	
Degree of meat cooking				
Raw	1	0	0	0.03
Undercooked	6	2	33.3	
Well done	319	20	6.3	
Consumption of chicken's brain				
Yes	3	2	66.7	0.01
No	323	20	6.2	
Unwashed raw vegetables				
Yes	84	8	9.5	0.23
No	242	14	5.8	
Unwashed raw fruits				
Yes	116	12	10.3	0.05
No	210	10	4.8	
Untreated water				
Yes	207	16	7.7	0.35
No	119	6	5	
Soil contact				
Yes	214	16	7.5	0.46
No	112	6	5.4	
Floor at home				
Ceramic or wood	121	5	4.1	0.34
Concrete	167	14	8.4	
Soil	38	3	7.9	

Table 3. Results of the multivariate analysis

Characteristic	<i>P</i> value	Odds ratio	95% Confidence interval
Raising farm animals	0.075	2.36	0.91–6.10
Traveled abroad	0.057	2.59	0.97–6.90
Consumption of:			
Horse meat	0.057	3.65	0.96–13.91
Raw or undercooked meat	0.045	6.84	1.04–44.95
Chicken brains	0.033	18.48	1.26–269.43

values were less than 5) for comparison of frequencies among groups. Bivariate and multivariate analyses were used to evaluate the association between the characteristics of the women and *T. gondii* seropositivity. 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 (ORs) and 95% confidence intervals (CIs) were calculated by multivariate analysis using backward stepwise logistic regression analysis. Statistical significance was set at a *P* value less than 0.05.

Ethics statement

This study was approved by the ethical committees of the Centro de Salud de Servicios Ampliados 450 and the General Hospital of the Secretary of Health in Durango City. The purpose and procedures of the study were explained to all women, and a written informed consent was obtained from all participants.

Results

Of 326 women with miscarriage history, 22 (6.7%) had anti-*T. gondii* IgG antibodies and two (0.6%) were also positive for anti-*T. gondii* IgM antibodies. Of 22 anti-*T. gondii* IgG positive participants, 12 (54.5%) had IgG levels higher than 150 IU/ml, one (4.5%) between 100 to 150 IU/ml, and nine (41%) between 9 to 99 IU/ml. General sociodemographic characteristics of the 326 women and their relation with *T. gondii* seropositivity are shown in Table 1. Most women were born in Durango State, Mexico, unemployed (housewives, students, or no occupation), and of medium socioeconomic status. The mean age of the women was 35.57 ± 12.43 years (range 15–71 years). Seroprevalence of *T. gondii* infection was not significantly influenced by age, birth place, occupation, educational level, or socioeconomic status of women.

Concerning clinical data, seroprevalence of *T. gondii* infection was not associated with characteristics including obstetric history (number of pregnancies, deliveries, cesarean section, and miscarriages); presence of underlying diseases, frequent headaches, and impairments of memory, reflexes, vision, and hearing; and history of blood transfusions or transplants.

With respect to behavioral characteristics (Table 2), the bivariate analysis showed a number of variables with a *P* value equal to or less than 0.10 including cleaning cat excrement (*P* = 0.06); raising farm animals (*P* = 0.01); having traveled abroad (*P* = 0.03); and consumption of horse meat (*P* = 0.05), raw or undercooked meat (*P* = 0.03), chicken brains (*P* = 0.01), and unwashed raw fruit (*P* = 0.05). Other behavioral variables including presence of cats at home; consumption of untreated water, unwashed raw vegetables, unpasteurized milk, dried or cure meat; eating away of home; or soil contact showed *P* values higher than 0.10 in the bivariate analysis. Further analysis by using logistic regression (Table 3) showed that *T. gondii* exposure was positively associated with consumption of raw or undercooked meat (OR = 6.84; 95% CI: 1.04–44.95; *P* = 0.04) and consumption of chicken brains (OR = 18.48; 95% CI: 1.26–269.43; *P* = 0.03).

Discussion

The present study allows us to obtain new epidemiological knowledge of *T. gondii* infection in women with a history of miscarriage. Studying the epidemiology of infection with *T. gondii* is important to understand the magnitude of the infection as a public health problem and to find transmission routes of the infection. Such information is useful for planning optimal measures against infection. In the international context, only few epidemiology studies in women with a history of miscarriage exist. The seroprevalence of infection with *T. gondii* in women with abortion has been reported high (17% to 43.8%) in some African [7] and Asian countries [6, 8]. We found a low (6.7%) seroprevalence of *T. gondii* infection in women with miscarriage history in Durango City, Mexico. The seroprevalence found is comparable with a 6.1% seroprevalence reported in pregnant women [10] and the general population in Durango City [11]. The fact that women with a history of miscarriage in Durango City have a similar seroprevalence of infection than other populations in the same city might suggest that *T. gondii* is not associated with miscarriages in urban women. However, this was not the case in rural women in Durango State; a previous study in pregnant women living in rural areas in Durango State showed that *T. gondii* infection was associated with abortion [9]. In studies of women with spontaneous abortion in

the Southern Mexican state of Yucatan, researchers found high (47%–55%) seroprevalences of *T. gondii* infection [12, 13]. Similarly, in Guadalajara City in central Mexico, researchers found a 44.9% seroprevalence in women with habitual abortions [14]. The seroprevalence found in women in Durango is much lower than those reported in the studies in Guadalajara City and Yucatan State. However, difference of seroprevalences can be explained by a lower seroprevalence in populations in northern than in central and southern Mexican states [15].

Previous studies in Durango State have shown a number of sociodemographic and housing factors associated with *T. gondii* infection including age [11, 16], low education [16], low socioeconomic conditions [9], and soil flooring at home [10]. However, in the present study, we did not find any sociodemographic or housing characteristic associated with *T. gondii* infection. Similarly, we did not find an association of *T. gondii* exposure with clinical characteristics of the women. This lack of association might be due to the presence of mostly chronic *T. gondii* infections. In fact, only two women had anti-*T. gondii* IgM antibodies suggesting recent infections.

Of the behavioral characteristics, multivariate analysis showed that *T. gondii* exposure was associated with consumption of raw or undercooked meat. Consumption of meat from *T. gondii*-infected animals is a major route for *T. gondii* infection in humans [1]. The association of *T. gondii* exposure with consumption of raw or undercooked meat in our study suggests that miscarriage in our setting might be more related with ingestion of meat containing parasite cysts than ingestion of contaminated water or food containing oocysts shed by cats. Multivariate analysis did not allow us to identify any meat from specific animals involved in *T. gondii* exposure. Therefore, meat from any animal could be involved. In fact, serological evidence of *T. gondii* infection has been reported in a number of edible animals in our setting including chickens [17], goats [18], sheep [19], and pigs [20]. Remarkably, we found an association of *T. gondii* seropositivity with consumption of chicken brains. Although chicken brains are usually eaten cooked, it is likely that chicken brains remain undercooked because of thermic protection of the chicken skull. To the best of our knowledge, this is the first report of an association of consumption of chicken brains with *T. gondii* seropositivity in humans. Interpretation of this finding should be taken with care because a limited number of subjects were studied and a wide 95% confidence interval of the odd ratio was obtained. However, serological and molecular evidence of *T. gondii* infection has been reported in chickens in Mexico [17, 21]. Chickens studied in Mexico have been found infected with *T. gondii* of the clonal type III lineage [21]. It is known that *T. gondii* may infect chicken brains [22], and such tissue is frequently selected to search for *T. gondii* cysts [23, 24]. Nested polymerase chain reaction (PCR) has demonstrated *T. gondii* DNA in brains of chickens [25]. Therefore, results of the present study suggest that consumption of chicken brains represents a new risk factor for *T. gondii*

infection, and such risk might be higher than consumption of chicken flesh.

This is the first study on the seroepidemiology of *T. gondii* infection in women with a history of miscarriage in Northern Mexico and of an association of *T. gondii* exposure with consumption of chicken brains. Contributing factors for *T. gondii* exposure found in the present study should be taken into consideration for avoiding *T. gondii* infection.

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