Original article

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HIGH SEROPREVALENCE OF *TOXOPLASMA GONDII* INFECTION IN FEMALE SEX WORKERS: A CASE-CONTROL STUDY

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Through an age- and sex-matched case-control study, we sought to determine whether female sex workers have an increased risk of *Toxoplasma gondii* exposure and to determine the sociodemographic, work, clinical, and behavioral characteristics of these workers associated with *T. gondii* exposure. Female workers (n = 136) and controls (n = 272) were examined with enzyme-linked immunoassays (EIA) for the presence of anti-*Toxoplasma* IgG and IgM antibodies. IgM positive sera were additionally tested with enzyme linked-fluorescence immunoassay (ELFA). Anti-*T. gondii* IgG antibodies were found in 21 (15.44%) of 136 cases and in 10 (3.67%) of 272 controls (OR = 4.05; 95% CI: 1.84–8.89; P = 0.0001). Anti-*T. gondii* IgG levels higher than 150 IU/ml were found in 13 (9.6%) of 136 cases and in 8 (2.9%) of 272 controls (P = 0.007). Anti-*T. gondii* IgM antibodies were found in two cases and in six controls by EIA, but all were negative by ELFA. *T. gondii* seropositivity was associated with being born out of Durango State (OR = 10.47; 95% CI: 2.9–36.8; P < 0.01), injuries during sex work (OR = 6.30; 95% CI: 1.1–33.7; P = 0.03), and soil contact (OR = 4.11; 95% CI: 1.2–14.0; P = 0.02). This is the first report of an association of *T. gondii* infection and female sex workers.

Keywords: *Toxoplasma gondii,* seroprevalence, female sex workers, case-control study, risk factors

Introduction

Toxoplasma gondii (T. gondii) is a protozoan parasite with worldwide distribution [1, 2]. Well-known routes for *T. gondii* infection include eating undercooked or raw meat containing *T. gondii* tissue cysts and ingestion of food or water contaminated with *T. gondii* oocysts shed by cats [3]. Most infections with *T. gondii* are asymptomatic; however, some infected individuals may develop clinical manifestations, a disease known as toxoplasmosis. This disease has a number of clinical manifestations including lymphadenopathy, retinochoroiditis, and encephalitis [1, 3, 4]. In addition, evidence is increasing that *T. gondii* infection may be linked to traffic accidents [5, 6], work accidents [7], and mental illnesses including schizophrenia [8, 9] and suicide attempts [10, 11].

It is unclear whether *T. gondii* infection is transmitted by sexual contact. It was recently hypothesized that *T. gondii* can be sexually transmitted from infected men to uninfected women through unprotected sexual contact [12]. Important arguments for such hypothesis were the presence of tachyzoites in seminal fluid and testes, and

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transmission of the infection to females of some animal species through artificial insemination with semen of infected males. In a recent study in Brazil, sexual transmission of *T. gondii* was demonstrated by natural mating in sheep with consequent vertical transmission to their lambs [13]. In an experimental study of dogs, *T. gondii* was detected in testicles and epididymis by immunohistochemistry, sexual transmission of *T. gondii* was demonstrated, and infection was detected in offspring [14]. In an epidemiological study of psychiatric patients in Durango, Mexico, multivariate analysis showed an association of *T. gondii* infection and sexual promiscuity [15]. Furthermore, in two studies of autopsy cases, *T. gondii* was found in testis of patients with acquired immunodeficiency syndrome [16, 17]. To the best of our knowledge, there is no report of *T. gondii* exposure in female sex workers. This group of population has epidemiological importance since sex workers have a high number of sexual contacts and are at risk to acquire and transmit sexually transmitted diseases. Therefore, we sought to determine the association of *T. gondii* exposure with female sex work occupation in Durango City, Mexico. In addition, we determined the sociodemographic, work, clinical, and behavioral characteristics of female sex workers associated with *T. gondii* seropositivity.

Methods

Study design and women studied

We performed a case-control study to determine the association of *T. gondii* infection with the occupation of female sex worker in Durango City, Mexico from May to June 2015.

Female sex workers

One hundred and thirty-six female sex workers registered in the Clinics for Sanitary Inspection of the Municipal Government were enrolled in the study. Inclusion criteria for the cases were current working as female sex workers for at least one year, aged 18 years and older, and who accepted to participate in the study. The mean age of the female sex workers was 34.97 ± 10.46 years (range: 18–67 years).

Control women

Two hundred and seventy-two control subjects with occupations other than sex work matched with female sex workers by age and gender were included in the study. Control women were randomly selected from the general population of Durango City, Mexico. The mean age in controls was 34.87 ± 10.46 (range: 18–67 years) and comparable with that in sex workers ($P = 0.92$).

Sociodemographic, clinical, work, and behavioral data of female sex workers

We obtained the sociodemographic, work, clinical, and behavioral characteristics of the female sex workers through an interviewer-administered standardized questionnaire. Sociodemographic data included age, birthplace, residence, educational level, and socioeconomic status. Work characteristics assessed in female sex workers included duration in the occupation, frequency of condom use, frequency of condom breakage, practice of oral or anal sex, frequency of contact with semen during vaginal, oral or anal sex, mean number of clients per week, approximate number of sexual contacts during life as a sex worker, geographical area (urban, suburban, rural) of work, place of contact with clients, sex work in other Mexican states or abroad, history of injuries during sex work, and history of sexually transmitted diseases. Clinical data in sex workers included presence of any disease, presence or history of lymphadenopathy, frequent abdominal pain or headache, dizziness, impairments in memory, reflexes, hearing and vision, and history of transplant, surgery or blood transfusion, and obstetric data.

Behavioral data included contact with animals or cat feces, traveling, type of meat consumed, frequency of meat consumption, ingestion of raw or undercooked meat, animal brains, unpasteurized milk or untreated water, dried or cured meat, unwashed raw vegetables or fruits, frequency of eating away from home (in restaurants or fast food outlets), consumption of alcohol, tobacco or drug use, washing hands before eating, contact with soil, and type of flooring at home.

Laboratory tests

A blood sample (5 ml) from each participant was drawn and centrifuged. Serum samples were obtained and stored at −20 °C until analyzed. Sera were analyzed by qualitative and quantitative methods for anti-*T. gondii* IgG antibodies with the commercially available enzyme immunoassay (EIA) kit "*Toxoplasma* IgG" (International Immuno-Diagnostics, Foster City, CA, USA). Anti-*T. gondii* IgG antibody levels were expressed as International Units (IU)/ml, and results ≥ 8 IU/ml were considered positive. Furthermore, serum samples positive for anti-*T. gondii* IgG antibodies were additionally analyzed for anti-*T. gondii* IgM antibodies by the commercially available EIA "*Toxoplasma* IgM" kit (Diagnostic Automation Inc., Calabasas, CA, USA). Samples positive for anti-*T. gondii* IgM antibodies by EIA were additionally tested with the commercially available enzyme linked-fluorescence immunoassay (ELFA) kit "VIDAS Toxo IgM" (bioMérieux, Marcy l'Etoile, France). IgM seropositivity was considered when both (EIA and ELFA) IgM tests were positive. All assays were performed following the manufacturer's instructions.

Statistical analysis

Results were analyzed with the software SPSS 15.0 (SPSS Inc., Chicago, Illinois). For calculation of the sample size, we used a 95% confidence level, a power of 80%, a 1:2 proportion of cases and controls, a reference seroprevalence of 6.1% [18] as the expected frequency of exposure in controls, and an odds ratio of 2.7. The result of the sample size calculation was 134 cases and 268 controls. We used the student's *t* test to compare the age among the groups. The association of *T. gondii* seropositivity and the characteristics of the sex workers was determined with the Pearson's χ^2 test and the two-tailed Fisher's exact test (when values were less than 5). Odds ratio (OR) and 95% confidence interval (CI) were obtained by multivariate analysis using logistic regression with the Enter method. Variables were included in the multivariate analysis if they had a *P* value less than 0.05 in the bivariate analysis. Statistical significance was set at a *P* value <0.05.

Ethical aspects

The purpose and procedures of this case control study were explained to all participants, and a written informed consent was obtained from each participant. This study was approved by the Ethical Committee of the General Hospital of the Secretary of Health in Durango City, Mexico.

Results

Anti-*T. gondii* IgG antibodies were found in 21 (15.44%) of 136 female sex workers and in 10 (3.67%) of 272 controls. The difference between the seroprevalences in cases and controls was statistically significant (OR = 4.05; 95% CI: 1.84–8.89; *P* = 0.0001). Anti-*T. gondii* IgG levels higher than 150 IU/ml were found in 13 (9.6%) of the 136 sex workers and in 8 (2.9%) of the 272 controls. Prevalence of high (>150 IU/ml) anti-*T. gondii* IgG levels was significantly higher in cases than in controls (*P* = 0.007). Anti-*T. gondii* IgM antibodies were found in two cases and in six controls by EIA, but all were negative by ELFA.

Of the sociodemographic characteristics of female sex workers, seroprevalence of *T. gondii* was significantly higher in female sex workers born out of Durango State than those born in this Mexican state (*P* < 0.0001). Seroprevalence of *T. gondii* exposure did not vary significantly with age, residence, educational level, or socioeconomic status *(Table 1)*.

With respect to work characteristics, female sex workers had a mean duration in the activity of 17.15 ± 9.43 years (range 3–53 years). Two work characteristics showed a

Table 1. Sociodemographic characteristics of female sex workers and prevalence of *T. gondii* infection

Characteristics	No.	Prevalence of *T. gondii* infection		*P* value
		No.	%	
Age groups (years)				
30 or less	51	8	15.7	0.99
31–50	71	11	15.5	
>50	14	2	14.3	
Birth place				
Durango State	113	9	8.0	<0.0001
Other Mexican State	23	12	52.2	
Residence place				
Durango State	135	20	14.8	0.15
Other Mexican State	1	1	100.0	
Residence area				
Urban	126	21	16.7	0.37
Suburban	9	0	0.0	
Rural	1	0	0.0	
Educational level				
No education	3	0	0.0	0.68
1 to 6 years	39	8	20.5	
7–12 years	87	12	13.8	
>12 years	7	1	14.3	

Table 1. *(cont'd)*

Characteristics	No.	Prevalence of *T. gondii* infection		*P* value
		No.	%	
Socioeconomic level				
Low	28	7	25.0	0.11
Medium	108	14	13.0	

likely association with *T. gondii* seropositivity by bivariate analysis: "mean number of clients per week" ($P = 0.003$) and "injuries during sex work" ($P = 0.03$) *(Table 2)*. Other work characteristics of female sex workers including duration in the occupation, frequency of condom use, frequency of condom breakage, practice of oral or anal sex, frequency of contact with semen during vaginal, oral or anal sex, approximate number of sexual couples during life as a sex worker, geographical area (urban, suburban, rural) of work, place of contact with clients, sex work in other Mexican states or abroad, and history of sexually transmitted diseases showed *P* values >0.05 by bivariate analysis. None of the clinical data in sex workers including health status, lymphadenopathy, frequent abdominal pain or headache, dizziness, impairments in memory, reflexes, hearing and vision, and history of transplant, surgery or

Table 2. Correlation of *T. gondii* infection and work characteristics of female sex workers

Characteristics	No.	Prevalence of *T. gondii* infection		*P* value
		No.	%	
Duration in the activity				
Less than 5 years	13	1	7.7	0.66
5 to 10 years	21	4	19.0	
More than 10 years	102	16	15.7	
Condom use				
Up to 50% of times	33	2	6.1	0.08
More than 50% of times	103	19	18.4	
Condom breakage				
Never	51	11	21.6	0.68
Occasionally (1–5/10 times)	63	9	14.3	
Almost always (6–9/10 times)	1	0	0.0	
Always	1	0	0.0	
Oral sex				
Yes	88	15	17.0	0.48
No	48	6	12.5	
Anal sex				
Yes	12	0	0.0	0.21
No	124	21	16.9	
Mean number of clients a week				
Up to 20	114	13	11.4	0.003
More than 20	22	8	36.4	
Number of clients in life				
Less than 100	68	7	10.3	0.17
100–500	34	6	17.6	
More than 500	33	8	24.2	
Sex work in other Mexican states				
Yes	41	7	17.1	0.72
No	95	14	14.7	

Table 2. *(cont'd)*

Characteristics	No.	Prevalence of *T. gondii* infection		*P* value
		No.	%	
Sex work abroad				
Yes	2	1	50.0	0.28
No	134	20	14.9	
Injuries during sex work				
Yes	9	4	44.4	0.03
No	127	17	13.4	
Sexually transmitted diseases				
Yes	36	9	25.0	0.06
No	99	12	12.0	

blood transfusion, and obstetric data showed *P* values <0.05 by bivariate analysis.

Concerning behavioral characteristics, only two variables showed potential association with *T. gondii* exposure by bivariate analysis: consumption of iguana meat ($P = 0.01$) and soil contact ($P = 0.04$) *(Table 3)*. Other behavioral characteristics assessed including contact with animals or cat feces, traveling, type of meat consumed, frequency of meat consumption, ingestion of raw or undercooked meat, animal brains, unpasteurized milk or untreated water, dried or cured meat, unwashed raw vegetables or fruits, frequency of eating away from home, consumption of alcohol, tobacco or drug use, washing hands before eating, and type of flooring at home showed *P* values >0.05. Multivariate analysis of sociodemographic, work, and behavioral characteristics of female sex workers with

Table 3. Bivariate analysis of selected putative risk factors for infection with *Toxoplasma gondii* in female sex workers

Characteristics	Subjects tested	Prevalence of *T. gondii* infection		*P* value
	No.	No.	%	
Cats at home				
Yes	53	11	20.8	0.17
No	83	10	12.0	
Birds at home				
Yes	40	9	22.5	0.14
No	96	12	12.5	
Pork meat consumption				
Yes	114	20	17.5	0.19
No	22	1	4.5	
Pigeon meat consumption				
Yes	5	2	40.0	0.17
No	131	19	14.5	
Rabbit meat consumption				
Yes	17	5	29.4	0.08
No	119	16	13.4	
Squirrel meat consumption				
Yes	7	3	42.9	0.07
No	129	18	14.0	
Horse meat consumption				
Yes	8	3	37.5	0.10
No	128	18	14.1	

Table 3. *(cont'd)*

Characteristics	Subjects tested	Prevalence of *T. gondii* infection		*P* value
	No.	No.	%	
Opossum meat consumption				
Yes	1	1	100.0	0.15
No	135	20	14.8	
Armadillo meat consumption				
Yes	3	2	66.7	0.06
No	133	19	14.3	
Iguana meat consumption				
Yes	4	3	75.0	0.01
No	132	18	13.6	
Chorizo consumption				
Yes	122	17	13.9	0.23
No	14	4	28.6	
Beef intestines consumption				
Yes	84	9	10.7	0.05
No	52	12	23.1	
Unwashed raw vegetables				
Yes	7	2	28.6	0.29
No	129	19	14.7	
Soil contact				
Yes	34	9	26.5	0.04
No	102	12	11.8	

Table 4. Multivariate analysis of selected characteristics of female sex workers and their association with *T. gondii* infection

Characteristics	Odds ratio	95% Confidence interval	*P* value
Born out of Durango State	10.47	2.9–36.8	<0.01
More than 20 clients per week	2.76	0.6–11.0	0.14
Injuries during sex work	6.30	1.1–33.7	0.03
Iguana meat consumption	7.83	0.2–218.6	0.22
Soil contact	4.11	1.2–14.0	0.02

P value <0.05 obtained in the bivariate analysis showed that *T. gondii* seropositivity was associated with being born out of Durango State (OR = 10.47; 95% CI: 2.9–36.8; *P*<0.01), injuries during sex work (OR = 6.30; 95% CI: 1.1–33.7; *P* = 0.03), and soil contact (OR = 4.11; 95% CI: 1.2–14.0; *P* = 0.02) *(Table 4)*.

Discussion

Very little is known about the transmission of *T. gondii* infection by sexual contact. Available information arises from studies in humans and animals. Janitschke and Nürnberger were the first researchers to study the significance of sexual intercourse for the transmission of *T. gondii* infection [19]. They observed *T. gondii* in testicles and accessory gonads in experimentally infected male rabbits at the 29th day post-infection, and isolated the parasite in one of 50 testicles of sheep for slaughter by mouse inoculation test. However, they did not find the parasite in semen and testis biopsies of men in the mouse inoculation test [19]. In addition, Blewett et al. reported that semen from *T. gondii* infected rams were not infective to mice [20]. In a recent study of mice, *T. gondii* could not be transmitted to female mice and their offspring by mating [21]. In contrast, results of some studies argue in favor of a sexual transmission of *T. gondii*. De Paepe et al. and Jautzke et al. provided microscopic evidence of *T. gondii* in testes in autopsy cases with acquired immunodeficiency syndrome [16, 17]. Furthermore, *T. gondii* infection was associated

with practicing sex without a condom in men having sex with men [22]. These conflicting results about the sexual transmission of *T. gondii* point towards the need for further research to elucidate whether *T. gondii* can be sexually transmitted. The present case control study therefore aimed to determine the association of *T. gondii* infection with the occupation of female sex worker. To the best of our knowledge, this is the first study that assesses such association. We found that female sex workers had a significantly higher seroprevalence of *T. gondii* than age- and gender-matched controls. In addition, female sex workers had a significantly higher prevalence of high (>150 IU/ml) anti-*T. gondii* antibodies than controls. The association of *T. gondii* infection with the occupation of female sex worker found in the present study suggests that female sex workers represent a "new" risk group for *T. gondii* infection. It is not clear why female sex workers had a significantly higher seroprevalence of *T. gondii* infection than controls. The high levels of *T. gondii* IgG antibodies might suggest a frequent contact with the parasite. We searched for contributing factors for infection in female sex workers. Multivariate analysis showed three variables associated with *T. gondii* exposure: being born out of Durango State, injuries during sex work, and soil contact. The characteristic "born out of Durango State" has been associated with *T. gondii* infection in some populations groups in Durango State including inmates [23]; patients with vision and hearing impairment, cancer, HIV, and undergoing dialysis [24]; general urban population [25]; and interstate truck drivers [26]. In these population groups, seroprevalences of *T. gondii* infection in subjects born out of Durango State varied from 13.6% to 40.4%. However, the 52.2% seroprevalence found in female sex workers born out of Durango State is the highest ever found in our studies in different populations groups in Durango. This finding suggests an increasing risk for *T. gondii* exposure in female sex workers. Intriguingly, a history of injuries during sex work was associated with *T. gondii* infection. This finding suggests that *T. gondii* might be transmitted by inoculations through open skin or mucosa. Very little is known on the role of injuries in *T. gondii* infection. In a study in inmates, we found a higher seroprevalence of *T. gondii* infection in subjects with a history of injuries than in those without this history [23]. We are not aware of further studies on the association of *T. gondii* infection with a history of injuries. Further research to elucidate the risk of *T. gondii* infection by injuries should be conducted. On the other hand, the variable contact with soil was also associated with *T. gondii* infection in female sex workers. Soil is a potential source of *T. gondii* [27, 28]. It is therefore likely that female sex workers were not safely handling soil contaminated with cat feces. Serological evidence of *T. gondii* infection has been demonstrated in 9.3% to 21% of cats in Durango City [29, 30]. Therefore, the occurrence of soil contamination with parasite oocysts from infected cats in Durango City is highly likely.

All sera positive for anti-*T. gondii* IgM antibodies by EIA were negative for ELFA. This finding is consistent with a previous observation that EIA for detection of anti-*T. gondii* IgM antibodies may have a high number of false positive results [31].

Conclusions

This is the first report of an association of *T. gondii* infection and the occupation of female sex worker. Results suggest that female sex workers could represent a new risk group for *T. gondii* infection and therefore warrant further research.

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Competing interests

The authors declare that they have no competing interests.

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