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LACK OF ASSOCIATION BETWEEN *TOXOPLASMA GONDII* INFECTION AND OCCUPATIONAL EXPOSURE TO ANIMALS

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The association of infection with *Toxoplasma gondii* and occupational exposure to animals has been scantly determined. We performed a case-control study with 200 subjects from Durango Province, Mexico, occupationally exposed to animals and 200 age- and gender-matched subjects without this occupation. Sera from all participants were analyzed for anti-*T. gondii* IgG and IgM antibodies using enzyme-linked immunoassays. The association of seroprevalence with sociodemographic, work, clinical, and behavioral characteristics in cases was determined.

Cases and controls had similar frequencies of anti-*T. gondii* IgG antibodies (12/200: 6.0% and 11/200: 5.5%, respectively) (OR = 3.0; 95% CI: 0.12–73.64; $P = 1.0$). The frequency of sera with high (>150 IU/ml) levels of anti-*T. gondii* IgG antibodies was comparable among cases and controls ($P = 0.61$). Seroprevalence of anti-*T. gondii* IgM antibodies was similar in cases (4, 2.0%) than in controls (4, 2.0%) ($P = 1.0$). Multivariate analysis showed that seropositivity was associated with eating while working (OR = 7.14; 95% CI: 1.91–26.72; $P = 0.003$) and consumption of duck meat (OR = 5.43; 95% CI: 1.43–20.54; $P = 0.01$).

No association between seropositivity to *T. gondii* and occupational exposure to animals was found. However, risk factors for infection found should be taken into account to reduce the exposure to *T. gondii*.

Keywords: *Toxoplasma gondii,* infection, seroprevalence, occupational exposure, animals, epidemiology, Mexico

Introduction

The parasite *Toxoplasma gondii* is widely spread around the world [1]. Humans and other warm-blooded animals are hosts for *T. gondii* [2, 3], and most of the infected hosts are asymptomatic. However, in humans, infection with *T. gondii* may lead to disease with affection of eyes, lymph nodes, and central nervous system [1, 4]. Immunocompromised individuals may develop a life-threatening toxoplasmosis following reactivation of their latent infection [1, 5]. Furthermore, pregnant women with primary infection with *T. gondii* may transmit the infection to the fetus leading to congenital disease [6–8]. Similarly, animals may develop clinical toxoplasmosis with a variety of outcomes including abortions and a life-threatening disease [2]. Transmission of *T. gondii* may occur by ingesting water or food contaminated with oocysts shed by cats or by ingestion of raw or undercooked meat containing tissue cysts [1, 9].

The epidemiology of *T. gondii* infection in people occupationally exposed to animals has been scantly studied. Only few descriptive studies about the seroprevalence of *T. gondii* infection in people occupationally exposed to

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animals including veterinarians [10, 11] and farmers [12] have been reported. However, to the best of our knowledge, there is no any case-control study that had determined the association between *T. gondii* infection and occupational exposure to live animals. Therefore, we attempted to determine the association of the infection with *T. gondii* and occupational exposure to live animals in the northern Mexican city of Durango. Furthermore, we sought to determine whether any sociodemographic, work, clinical, and behavioral characteristics of the workers occupationally exposed to live animals was associated with *T. gondii* infection.

Materials and methods

Study design and study populations

Through a case-control seroprevalence study, 200 people occupationally exposed to animals (cases) and 200 control subjects without occupational exposure to animals were compared for the prevalence of anti-*T. gondii* IgG and IgM antibodies. This study was performed from August 2013 to July 2014. As a strategy to enroll cases, we visited them at their work place in government facilities (veterinary hospital, veterinary school, animal inspection services, farm, and zoo) and private clinics and animal facilities. Inclusion criteria for the cases were occupational exposure to animals for at least 6 months, aged 18 years and older, any gender, and who accepted to participate in the study. Cases were 18–67 (mean = 31.33 ± 10.35) years old, and included 134 males and 66 females. Controls were subjects without occupational exposure to animals randomly selected from the general population in Durango City. Controls were matched with cases by age (±1 year) and gender. Controls were 18–67 (mean = 31.31 ± 10.35) years old and included 134 males and 66 females. Age was comparable between cases and controls ($P = 0.98$).

Sociodemographic, clinical, work, and behavioral data

We obtained the sociodemographic, clinical, work, and behavioral characteristics of the cases with the aid of a standardized questionnaire. Sociodemographic items were age, gender, birthplace, residence, education, and socioeconomic level. The clinical characteristics in cases included health status, history of lymphadenopathy, blood transfusions, transplantation and surgeries, presence of frequent headache, and impairments in vision, hearing, memory, and reflexes. In female cases, obstetric history was also obtained. Work items were as follows: exposition group (livestock raiser, veterinarian services worker, animal hair dresser), duration in the activity, frequency of animal contact, contact with wild animals, animals contacted, animals most frequently contacted, contact with felids, area of animal contact (urban, suburban, rural), safety practices (wearing gloves, face mask, safety glasses), washing animals, washing animal corrals or pens, contact with animal tissues or fluids, type of animal tissues or fluids contacted, splashes of animal tissues or fluids at face, injury at work, surgical work, history of zoonosis, and eating, smoking, or drinking while working. Behavioral items were contact with cats and their excrement at home, traveling, type of meat consumed (pork, beef, goat, lamb, boar, chicken, turkey, pigeon, duck, rabbit, venison, squirrel, horse, opossum, or other), frequency of meat consumption, consumption of raw or undercooked meat and dried or processed meat (ham, sausages or chorizo), drinking unpasteurized milk or untreated water, consumption of unwashed raw vegetables and fruits, frequency of eating away from home (in restaurants or fast food outlets), contact with soil (gardening or agriculture), and type of floors at home.

Serological detection of T. gondii *antibodies*

Serum samples from all cases and controls were analyzed for anti-*T. gondii* IgG antibodies with the commercially available enzyme immunoassay kit "*Toxoplasma* IgG" (Diagnostic Automation Inc., Calabasas, CA, USA). The cut-off for positivity of this test was 8 IU/ml of specific anti-*T. gondii* IgG antibody. All sera with anti-*T. gondii* IgG antibodies were additionally analyzed for anti-*T. gondii* IgM antibodies by the commercially available enzyme immunoassay "*Toxoplasma* IgM" kit (Diagnostic Automation Inc., Calabasas, CA, USA). Positive and negative controls were included in each assay. All tests were performed according to the manufacturer's instructions.

Statistical analysis

The statistical analyses were performed with the software Microsoft Excel 2010, Epi Info version 7 (Centers for Disease Control and Prevention: http://wwwn.cdc.gov/epiinfo/) and SPSS version 15.0 (SPSS Inc. Chicago, Illinois). For calculation of the sample size, we used a 95% confidence level, a power of 80%, a 1:1 proportion of cases and controls, a reference seroprevalence of 6.1% [13] as the expected frequency of exposure in controls, and an odds ratio of 2.8. The result of the sample size calculation was 195 cases and 195 controls. We used the paired student's *t* test to compare age values among the groups. For comparison of the frequencies of seropositivity to *T. gondii* between cases and controls, the McNemar's test was used. Odds ratio (OR) and 95% confidence interval (CI) were calculated by the Mantel–Haenszel analysis. The Pearson's chi-square test and the Fisher exact test (when values were small) were used to determine the association of *T. gondii* seropositivity with the characteristics of the cases. Multivariate analysis was used to determine the association of *T. gondii* infection with the cases characteristics. As a criterion to include variables in the multivariate analysis, only variables with a P value ≤0.10 obtained in

the bivariate analysis were included. We calculated the odds ratios (OR) and the 95% confidence intervals (CI) by means of the backward stepwise regression method. A *P* value less than 0.05 was considered statistically significant.

Ethics statement

This study was approved by the Ethical Committee 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. In addition, each participant provided a written informed consent.

Results

Anti-*T. gondii* IgG antibodies were present in 12 (6%) of 200 cases and in 11 (5.5%) of 200 controls. There was no difference in the seroprevalence of *T. gondii* infection among case control pairs (OR = 3.0; 95% CI: 0.12–73.64; *P* = 1.0).

Of the 12 anti-*T. gondii* IgG positive cases, seven (3.5%) had anti-*T. gondii* IgG antibody titers higher than 150 IU/ml, and five (2.5%) had titers between 8 to 99 IU/ml. In comparison, of the 11 anti-*T. gondii* IgG positive controls, nine (4.5%) had anti-*T. gondii* IgG antibody levels higher than 150 IU/ml, one (0.5%) between 100 to 150 IU/ml, and one (0.5%) between 8 to 99 IU/ml. High (>150 IU/ml) levels of anti-*T. gondii* IgG antibodies were comparable among cases and controls (*P* = 0.61). The frequency of anti-*T. gondii* IgM antibodies was similar in cases (4, 2.0%) and in controls (4, 2.0%) (*P* = 1.0).

Anti-*T. gondii* IgG antibodies were present in 4 (6.1%) of 66 female cases and in 1 (1.5%) of 66 female controls (*P* = 0.36). Anti-*T. gondii* IgG antibodies were detected in 8 (6.0%) of 134 male cases and in 10 (7.5%) of 134 male controls (*P* = 0.62). The prevalence of high (>150 IU/ml) anti-*T. gondii* IgG antibody titers was similar in male (5/134: 3.7%) and female (2/66: 3.0%) cases (*P* = 1.0).

Of the sociodemographic characteristics in cases, only "being born in Durango State" showed a *P* value ≤0.10 by bivariate analysis *(Table 1)*. Other sociodemographic characteristics in cases including age, gender, residence, education, and socioeconomic status had *P* values >0.10.

Table 1. Sociodemographic characteristics of subjects with occupational exposure to animals and seroprevalence of *T. gondii* infection

Characteristic	Subjects tested	Prevalence of *T. gondii* infection		*P* value
	No.	No.	%	
Age groups (years)				
30 or less	116	5	4.3	0.17
31–50	70	7	10.0	
>50	14	0	0.0	
Sex				
Male	134	8	6	1
Female	66	4	6.1	
Birth place				
Durango State	172	8	4.7	0.06
Other Mexican state or abroad	28	4	14.3	
Residence				
Durango State	197	12	6.1	1
Other Mexican State or abroad	3	0	0.0	
Educational level				
No education	2	0	0.0	0.62
1–6 years	26	3	11.5	
6–12 years	63	3	4.8	
>12 years	109	6	5.5	
Socio-economic level				
Low	25	2	8.0	0.77
Medium	170	10	5.9	
High	5	0	0.0	

With respect to work characteristics in cases *(Table 2)*, only the variable "eating while working" showed a *P* value ≤0.10 by bivariate analysis. Other work characteristics in subjects occupationally exposed to animals including exposition group (livestock raiser, veterinarian services worker, animal hair dresser), duration in the activity, frequency of animal contact, contact with wild animals, type of animals contacted, animals most frequently contacted, contact with felids, area of animal contact (urban, suburban, rural), safety practices (wearing gloves, face mask, safety glasses), washing animals, washing animal corrals or pens, contact with animal tissues or fluids, type of animal tissues or fluids contacted, splashes of animal tissues or fluids at face, injury at work, surgical work, history of zoonosis, and smoking or drinking while working showed *P* values > 0.10. Seroprevalence of *T. gondii* infection was similar in veterinarians (4/86: 4.7%) than in nonveterinarians (8/114: 7.0%) (*P* = 0.56).

Table 2. Bivariate analysis of selected work factors and *T. gondii* seropositivity in subjects with occupational exposure to animals

Characteristic	Subjects tested	Prevalence of *T. gondii* infection		*P* value
	No.	No.	%	
Exposition group				
Livestock raiser	51	2	3.9	0.5
Veterinarian sciences	139	10	7.2	
Animal hair dresser	10	0	0	
Affiliation				
Private	52	5	9.6	0.2
Government	148	7	4.7	
Duration in the activity				
1 to 5 years	89	5	5.6	0.83
More than 5 years	111	7	6.3	
Frequency of animal contact				
4 to 7 days a week	184	11	6	1
Up to 3 days a week	16	1	6.3	
Contact with wild animals				
Yes	76	2	2.6	0.13
No	124	10	8.1	
Felids contact				
Yes	158	11	7	0.46
No	42	1	2.4	
Area of animal contact				
Urban	105	9	8.6	0.22
Suburban	41	2	4.9	
Rural	54	1	1.9	
Wearing gloves				
Yes	69	3	4.3	0.55
No	131	9	6.8	
Wearing mask				
Yes	70	4	5.7	1
No	130	8	6.2	
Wearing safety glasses				
Yes	20	0	0	0.61
No	180	12	6.7	
Washing animals				
Yes	121	8	6.6	0.76
No	79	4	5.1	

Table 2. *(cont'd)*

Characteristic	Subjects tested	Prevalence of *T. gondii* infection		*P* value
	No.	No.	%	
Washing corrals or pens				
Yes	131	9	6.8	0.55
No	69	3	4.3	
Contact with animal tissues or fluids				
Yes	144	7	4.9	0.32
No	56	5	8.9	
Splash of tissues or fluids at face				
Yes	106	7	6.6	0.7
No	94	5	5.3	
Injuries at work				
Yes	121	6	5	0.54
No	79	6	7.6	
Surgical work				
Yes	93	6	6.5	0.8
No	107	6	5.6	
History of zoonosis				
Yes	11	1	9.1	0.5
No	189	11	5.8	
Eating while working				
Yes	45	7	15.6	0.006
No	155	5	3.2	
Smoking while working				
Yes	13	1	7.7	0.56
No	187	11	5.9	
Drinking while working				
Yes	14	2	14.3	0.2
No	186	10	5.4	

With respect to behavioral characteristics in cases *(Table 3)*, the variables consumption of meat from duck, rabbit, horse, opossum and iguana, eating raw or undercooked meat, and eating out of home had *P* values ≤ 0.10 by bivariate analysis. While other behavioral characteristics including contact with cats and their excrement; traveling; consumption of pork, beef, venison, lamb, or meat from goat, boar, chicken, turkey, pigeon, or squirrel; frequency of meat consumption; consumption of dried or processed meat; drinking unpasteurized milk or untreated water; consumption of unwashed raw vegetables and fruits; contact with soil; and type of floors at home showed

Table 3. Bivariate analysis of selected putative risk factors for infection with *Toxoplasma gondii* in subjects occupationally exposed to animals

Characteristic	Subjects tested	Prevalence of *T. gondii* infection		*P* value
	No.	No.	%	
Cats at home				
Yes	112	9	8	0.17
No	87	3	3.4	
Cleaning cat excrement				
Yes	86	7	8.1	0.26
No	114	5	4.4	

Table 3. *(cont'd)*

Characteristic	Subjects tested	Prevalence of *T. gondii* infection		*P* value
	No.	No.	%	
Sheep meat consumption				
Yes	131	10	7.6	0.22
No	69	2	2.9	
Boar meat consumption				
Yes	35	0	0	0.13
No	165	12	7.3	
Pigeon meat consumption				
Yes	25	3	12	0.17
No	175	9	5.1	
Duck meat consumption				
Yes	32	6	18.8	0.005
No	168	6	3.6	
Rabbit meat consumption				
Yes	104	10	9.6	0.03
No	96	2	2.1	
Squirrel meat consumption				
Yes	22	3	13.6	0.13
No	178	9	5.1	
Horse meat consumption				
Yes	31	4	12.9	0.09
No	169	8	4.7	
Opossum meat consumption				
Yes	1	1	100	0.06
No	199	11	5.5	
Armadillo meat consumption				
Yes	4	1	25	0.22
No	196	11	5.6	
Iguana meat consumption				
Yes	8	2	25	0.07
No	192	10	5.2	
Frequency of meat consumption				
Up to 3 times a week	122	5	4.6	0.15
4–7 times a week	78	7	9	
Degree of meat cooking				
Raw or undercooked	18	3	16.7	0.08
Well done	182	9	4.9	
Unwashed raw vegetables				
Yes	69	7	10.1	0.11
No	130	5	3.8	
Untreated water				
Yes	107	9	8.4	0.12
No	93	3	3.2	
Frequency of eating out of home				
Never	9	2	22.2	0.06
1 to 10 times a year	48	1	2.1	

Table 3. *(cont'd)*

Characteristic	Subjects tested	Prevalence of *T. gondii* infection		*P* value
	No.	No.	%	
>10 times a year	143	9	6.3	
Soil contact				
Yes	149	7	4.7	0.18
No	51	5	9.8	

P values > 0.10. Further analysis by logistic regression of sociodemographic, work, and behavioral characteristics of cases that showed *P* values ≤ 0.10 in the bivariate analysis revealed that *T. gondii* seropositivity was associated with eating while working (OR = 7.14; 95% CI: 1.91–26.72; *P* = 0.003) and consumption of duck meat (OR = 5.43; 95% CI: 1.43–20.54; *P* = 0.01).

None of the clinical characteristics in cases including health status, history of lymphadenopathy, blood transfusions, transplantation and surgeries, presence of frequent headache, impairments in vision, hearing, memory and reflexes, and obstetric history in women were associated with *T. gondii* seropositivity.

Discussion

Occupational exposure to live animals is generally considered to be risk factor for toxoplasmosis. However, the association of *T. gondii* infection with occupational exposure to live animals has not previously been studied by age- and gender-matched case-control studies. We therefore performed the present study in order to determine such association in a number of subjects exposed to live animals including workers on veterinarian services, livestock raisers in farms and the city zoo, and animal hairdressers. The frequencies of anti-*T. gondii* IgG and IgM antibodies and anti-*T. gondii* antibody levels were similar in subjects occupationally exposed to animals and control subjects without occupational exposure to animals. Results thus indicate that infection with *T. gondii* is not likely to be acquired by occupational exposure to animals. The 6% seroprevalence of *T. gondii* infection found in subjects with occupational exposure to animals is similar to the 6.1% seroprevalence of *T. gondii* infection reported in the general population in Durango City [13] and lower than the high (21.1%) seroprevalence reported in individuals with occupational exposure to garbage [14] and inmates [15] in the same city. In these three studies, the same immunoassay was used. Therefore, our results do not link occupational exposure to animals with *T. gondii* infection. Interestingly, we found an association of *T. gondii* seropositivity with eating while working. It is likely that workers that have handled infected animals were carrying the parasite from their contaminated hands to their mouths. In a study of 174 workers and residents of swine workers in the USA, Weigel and coworkers [12] attributed an increased risk of *T. gondii* seropositivity in males to less attention paid to cleanliness in food preparation and eating. Workers in contact with animals might be more frequently in contact with *T. gondii* than subjects without contact with animals. It is well known that ingestion of oocysts and animal tissues containing cysts are the main routes of infection with *T. gondii*. Inadequate handling of animals and ingestion of parasites may occur via direct contact with oocysts in excrements of cats or felids or via ingestion of cysts in muscle and central nervous system tissue samples of infected animals. Less likely, blood of infected animals might contain parasite tachyzoites and represent a source of infection.

We investigated a number of work characteristics with likely risk for *T. gondii* infection. Seroprevalence of *T. gondii* did not increase with duration (years) of working with animals. The use of safety practices including wearing gloves, facemask, or safety glasses while handling animals was neither associated with *T. gondii* infection in this study. It is likely that handled animals had low frequency of *T. gondii* infection or a very low number of tissue cysts. In a study of *T. gondii* infection in workers with occupational exposure to raw meat in Durango City, the use of safety practices was not negatively associated with infection neither [16]. The lack of association of *T. gondii* infection with occupational exposure to animals found in the present study is consistent with those found in some descriptive studies. In an American study, researchers did not find a significant risk of *T. gondii* infection in university employees exposed to cats [17]. In a seroprevalence study of veterinarians regularly exposed to cats in Canada, researchers found a low seroprevalence (14.2%) of *T. gondii* infection [10]. In a study about the occurrence of zoonotic infections among Auckland zoo staff in New Zealand, the seroprevalence of antibodies to *T. gondii* reflected that in the Auckland community [18]. In contrast, residents in a district in northeast Tanzania were shown to have a high (52.2%) seroprevalence of *T. gondii* infection in persons who kept livestock [19]. In a recent study in Korea, researchers found an 8% seroprevalence of *T. gondii* infection in veterinarians [11]. They also found that public veterinarians had a higher seroprevalence than those working in veterinary service laboratories (13.4% vs. 5.5%, respectively) [11]. We did not find differences in seroprevalences among affiliation groups. Veterinarians and nonveterinarians had similar seroprevalences of infection. The highest risk for infection with *T. gondii* may be contact with cats

and their excrement and contact with animals with high seroprevalence of *T. gondii* infection. However, in the present study, handling cats or their excrement or animals with high seroprevalence of *T. gondii* infection in our region (cats, dogs, goats, ducks) [20–23] was not associated with *T. gondii* exposure. The lack of such association might be due to the low seroprevalence of *T. gondii* infection found in cases.

Interestingly, we found an association of seropositivity to *T. gondii* and consumption of duck meat. We are not aware of previous reports about an association between *T. gondii* infection and consumption of duck meat. In a previous study in wild birds in Durango, Mexico, two (1 *Anas platyrhynchos*, 1 *Anas diazi*) of four ducks sampled were seropositive for *T. gondii* [23]. The epidemiological link between *T. gondii* infection in humans and ducks is also supported by the observation that handling of ducks was associated with *T. gondii* seropositivity in native communities of James Bay, Canada [24].

The present age- and gender-matched case-control study on the association of occupational exposure to animals with *T. gondii* prevalence is the first of its kind as much as we are informed. We did not observe an association between seropositivity to *T. gondii* and occupational exposure to animals. Results thus indicate that occupational exposure to animals does not represent an important risk for *T. gondii* infection. However, our study has limitations: a) sample number may be too small; b) population may not be the one with highest risk factors; and c) we may not have asked with enough detail. Therefore, further studies are needed to investigate risk factors for infection with *T. gondii* in subjects exposed to animals. Results of such studies can be taken into account to define educational and other measures to reduce the exposure to *T. gondii*.

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