

SEROEPIDEMIOLOGY OF *TOXOPLASMA GONDII* INFECTION IN PEOPLE APPLYING FOR MEDICAL CERTIFICATES

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Medical certificates are documents that state the health status of a person. This study aimed to determine the seroprevalence and risk factors for *Toxoplasma gondii* infection in applicants of medical certificates and to investigate seroprevalence association with characteristics of these individuals. We examined 404 applicants in a public health center in Durango City, Mexico for the presence of anti-*Toxoplasma* IgG and IgM antibodies using enzyme-linked immunoassays. Of the 404 subjects (mean age of 35.94 ± 13.01) studied, 29 (7.2%) were positive for anti-*T. gondii* IgG antibodies and 9 (31.0%) of them were also positive for anti-*T. gondii* IgM antibodies. IgG and IgM seropositivities were associated with vision impairment ($P = 0.04$) and a history of surgery ($P = 0.03$), respectively. Prevalence of high (>150 IU/ml) IgG antibody levels was associated with hearing impairment ($P = 0.03$), and histories of lymphadenopathy ($P = 0.04$) and miscarriages ($P = 0.03$). Multivariate analysis showed that *T. gondii* seropositivity was associated with being born out of Durango State (odds ratio [OR] = 4.65; 95% confidence interval [CI]: 1.25–17.29; $P = 0.02$) and soil contact (OR = 4.27; 95% CI: 1.71–10.67; $P = 0.002$) and negatively associated with consumption of sheep meat (OR = 0.12; 95% CI: 0.02–0.65; $P = 0.01$). These results could be used for the design of optimal preventive measures against toxoplasmosis and its sequelae.

Keywords: *Toxoplasma gondii*, seroprevalence, medical certificates, cross-sectional study, risk factors

Introduction

Toxoplasma gondii (*T. gondii*) is a coccidian parasite that causes infections worldwide [1]. This parasite is transmitted to humans by a number of routes including ingestion of raw or undercooked meat containing tissue cysts, consumption of food or water contaminated with oocysts shed by cats [2], and vertically [3]. Most infections with *T. gondii* remain asymptomatic; however, some infected individuals develop a severe disease with lymphadenopathy, chorioretinitis, and meningoencephalitis [2, 4, 5]. In addition, infection with *T. gondii* has been associated with

mental illnesses, i.e., schizophrenia [6, 7], mood disorders [8, 9], anxiety [9], and cognitive impairment [10, 11]. Infection with *T. gondii* has also been associated with traffic accidents [12] and work accidents [13]. Therefore, *T. gondii* infection may affect work performance. However, the impact of *T. gondii* infection in workers has been scantily studied. Memory impairment and dizziness or syncope were associated to *T. gondii* infection in migrant agricultural workers [14]. Infection with *T. gondii* was linked to ill status, chronic tonsillitis, and reflexes impairment in workers occupationally exposed to raw fruits and vegetables [15]. Memory impairment associated with

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T. gondii infection in gardeners has also been reported [11]. A recent study of interstate truck drivers showed an association of *T. gondii* seropositivity and hearing impairment [16].

A medical certificate is a document that states the health status of a person. In order to obtain this certificate, applicants usually undergo a medical examination and laboratory tests. People seek for medical certificates usually to fulfill requirements to get a job or to be admitted to a school. The magnitude of *T. gondii* infection and its morbidity in people who apply for a medical certificate is unknown. To the best of our knowledge, the seroepidemiology of *T. gondii* in these people has not been studied. Therefore, we sought to determine the seroprevalence of *T. gondii* exposure in people applying for medical certificates in a public health center in Durango City, Mexico and the seroprevalence association with the sociodemographic, clinical, and behavioral characteristics of these individuals.

Materials and methods

Study design and subjects studied

We performed a cross-sectional study in 404 subjects who applied for a medical certificate in a public health center in Durango City, Mexico. A medical certificate is an important document describing the health status of a person based on a medical examination and laboratory tests. This document is usually required by employers or schools to determine the eligibility of applicants for a job or an academic course. Participants were studied from September to November 2015. Inclusion criteria for enrollment were subjects aged 18 years and older who applied for a medical certificate at the Health Center No. 2 of the Secretary of Health in Durango City, Mexico and who accepted to participate in the study. Gender and socioeconomic status were not restrictive criteria for enrollment. Applicants for medical certificates in the health center are mostly working class people looking for a job at the government or private companies.

Sociodemographic, clinical, behavioral, and housing characteristics of participants

We used a standardized questionnaire to obtain the sociodemographic, clinical and behavioral characteristics, and housing conditions of the subjects studied. Sociodemographic data were age, birthplace, residence, educational level, occupation, and socio-economic status. Clinical data included history of lymphadenopathy, transplant, surgery or blood transfusion, presence of any disease, frequent abdominal pain or headache, dizziness, and impairments in memory, reflexes, hearing, and vision. In women, obstetric history was also obtained. Behavioral items included contact with animals, cleaning cat feces, traveling, type of

meat consumed, frequency of meat consumption, eating raw or undercooked meat, animal brains, dried or cured meat, beef liver, unpasteurized milk or untreated water, and unwashed raw vegetables or fruits. Other behavioral data included frequency of eating away from home (in restaurants or fast food outlets), washing hands before eating, and soil contact. Housing conditions included availability of potable water, form of elimination of excretas, education of the head of the family, crowding, and type of flooring at home.

Laboratory tests

A serum sample was obtained from each participant. Serum samples were stored at -20°C until analyzed. Anti-*T. gondii* IgG antibodies were determined in serum samples 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. Serum samples positive for anti-*T. gondii* IgG antibodies were also analyzed for anti-*T. gondii* IgM antibodies by the commercially available EIA “*Toxoplasma* IgM” kit (Diagnostic Automation Inc., Calabasas, CA, USA). All assays were performed following the instructions of the manufacturer.

Statistical analysis

Results were analyzed with the aid of the software Epi Info 7 and SPSS 15.0 (SPSS Inc. Chicago, Illinois). For calculation of the sample size, a reference seroprevalence of 6.1% [17] as the expected frequency for the factor under study, 5000 as the population size from which the sample was selected, a 2.5% of confidence limits, and a 95% confidence level were considered. The result of the sample size calculation was 329 subjects. We assessed the association of *T. gondii* exposure and the characteristics of participants with the Pearson's χ^2 test or the two-tailed Fisher's exact test (when values were small). As a criterion for selection of variables for multivariate analysis, only variables with a *P* value < 0.05 obtained in the bivariate analysis were selected. Odds ratio (OR) and 95% confidence interval (CI) were obtained using logistic regression with the Enter method. A *P* value of < 0.05 was considered statistically significant.

Ethical aspects

The Ethics Committee of the General Hospital of the Secretary of Health in Durango City, Mexico approved this study. The purpose and procedures of this study were explained to all participants, and a written informed consent was obtained from all of them.

Results

Participants had a mean age of 35.94 ± 13.01 years (range 15–76 years). Of the 404 subjects studied, 29 (7.2%) were positive for anti-*T. gondii* IgG antibodies. Of these 29 IgG seropositive subjects, nine (31.0%) were also positive for anti-*T. gondii* IgM antibodies. Of the 29 anti-*T. gondii* IgG positive participants, 12 (41.4%) had IgG levels higher

than 150 IU/ml, four (13.8%) between 100 and 150 IU/ml, and 13 (44.8%) between 10 and 71 IU/ml. *Table 1* shows a correlation of the sociodemographic characteristics of participants and *T. gondii* seroprevalence. Seroprevalence of *T. gondii* infection did not vary with age, gender, residence, educational level, or socioeconomic status of participants (*Table 1*). In contrast, seroprevalence of *T. gondii* exposure in subjects born out of Durango State was signifi-

Table 1. Socio-demographic characteristics of people seeking medical certificates in Durango, Mexico and prevalence of *T. gondii* infection

Characteristic	No.*	Prevalence of <i>T. gondii</i> infection		P value
		No.	%	
Age groups (years)				
30 or less	178	12	6.7	0.81
31–50	151	12	7.9	
>50	70	4	5.7	
Gender				
Male	128	9	7.0	0.93
Female	276	20	7.2	
Birth place				
Durango State	378	23	6.1	0.001
Other Mexican State	23	6	26.1	
Abroad	1	0	0.0	
Residence place				
Durango State	400	28	7.0	0.07
Other Mexican State	1	1	100.0	
Residence area				
Urban	376	26	6.9	0.26
Suburban	3	0	0.0	
Rural	18	3	16.7	
Educational level				
No education	2	1	50.0	0.05
1 to 6 years	50	5	10.0	
7–12 years	212	17	8.0	
>12 years	136	6	4.4	
Occupation				
Agriculture	4	1	25.0	0.03
Housewife	47	1	2.1	
Business	64	7	10.9	
Employee	188	16	8.5	
Student	11	0	0.0	
Chef	12	3	25.0	
Professional	52	0	0.0	
None	3	0	0.0	
Other [†]	18	1	5.6	

Table 1. (cont'd)

Characteristic	No.*	Prevalence of <i>T. gondii</i> infection		P value
		No.	%	
Socio-economic level				
Low	63	4	6.3	0.06
Medium	336	24	7.1	
High	2	1	50.0	

*Subjects with available data.

†Construction worker, hairdresser, tattooer, taxi driver, butcher, manager, nurse, and photographer.

cantly higher than those born in Durango State or abroad ($P = 0.001$). In addition, seroprevalence varied with occupation ($P = 0.001$), being agricultural worker and chef the occupations with the highest seroprevalences.

Regarding clinical characteristics (Table 2), seroprevalence of *T. gondii* was significantly ($P = 0.04$) higher in participants with vision impairment than in those without this clinical feature. In women, *T. gondii* exposure was not associated with histories of cesarean sections or miscarriages. The frequency of other clinical characteristics of participants including lymphadenopathy, history of transplant, surgery or blood transfusion, presence of any disease, frequent abdominal pain or headache, dizziness, impairments in memory, reflexes, and hearing were similar among *T. gondii* positive and *T. gondii* negative subjects.

Further analysis of the 29 IgG seropositive participants showed that the frequency of IgM seropositivity was significantly ($P = 0.03$) higher in subjects with a history of surgery (6/10: 60%) than in those without this history (3/19: 15.8%). Other clinical characteristics did not show an association with *T. gondii* IgM seropositivity. Prevalence of high IgG antibody levels (>150 IU/ml) was higher in subjects with a history of lymphadenopathy (5/72: 6.9%) than in those without this history (7/330: 2.1%) ($P = 0.04$). In addition, subjects with hearing impairment had a higher prevalence of high antibody levels (5/66: 7.6%) than those without this impairment (7/337: 2.1%) ($P = 0.03$). In women, prevalence of high IgG levels was higher in women with a history of miscarriage (3/45: 6.7%) than in those without this history (1/163: 0.6%) ($P = 0.03$).

Table 2. Bivariate analysis of seropositivity to *T. gondii* and a selection of clinical characteristics in people seeking medical certificates in Durango, Mexico

Characteristic	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		P value
		No.	%	
Clinical status				
Healthy	341	24	7.0	0.78
Ill	60	5	8.3	
Lymphadenopathy ever				
Yes	72	9	12.5	0.05
No	330	20	6.1	
Headache frequently				
Yes	144	15	10.4	0.06
No	259	14	5.4	
Memory impairment				
Yes	79	7	8.9	0.52
No	324	22	6.8	
Dizziness				
Yes	59	7	11.9	0.16
No	343	22	6.4	
Reflexes impairment				
Yes	18	2	11.1	0.37
No	385	27	7.0	

Table 2. (cont'd)

Characteristic	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
Hearing impairment				
Yes	66	8	12.1	0.11
No	337	21	6.2	
Visual impairment				
Yes	114	13	11.4	0.04
No	289	16	5.5	
Surgery ever				
Yes	193	10	5.2	0.13
No	210	19	9.0	
Blood transfusion				
Yes	32	2	6.3	1.00
No	372	27	7.3	
Pregnancies				
Yes	208	12	5.8	0.15
No	60	7	11.7	
Deliveries				
Yes	151	9	6.0	1.00
No	57	3	5.3	
Cesarean sections				
Yes	87	5	5.7	0.99
No	121	7	5.8	
Miscarriages				
Yes	45	4	8.9	0.29
No	163	8	4.9	
Stillbirths				
Yes	12	1	8.3	0.52
No	196	11	5.6	

With respect to behavioral characteristics, a number of variables showed *P* values lower than 0.05 in the bivariate analysis including consumption of meat from sheep (*P* = 0.003), turkey (*P* = 0.03), and armadillo (*P* = 0.02), and

soil contact (*P* = 0.001). A selection of behavioral characteristics of participants and their correlation with *T. gondii* seropositivity is shown in Table 3. Other behavioral factors including contact with animals; cleaning cat feces; trav-

Table 3. Bivariate analysis of selected putative risk factors for infection with *T. gondii* in people seeking medical certificates in Durango, Mexico

Characteristics	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		<i>P</i> value
		No.	%	
Cleaning cat excrement				
Yes	88	4	4.5	0.31
No	314	24	7.6	
National trips				
Yes	299	17	5.7	0.05
No	105	12	11.4	

Table 3. (cont'd)

Characteristics	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		P value
		No.	%	
Pork meat consumption				
Yes	371	26	7.0	0.49
No	32	3	9.4	
Beef consumption				
Yes	383	27	7.0	0.65
No	21	2	9.5	
Goat meat consumption				
Yes	35	0	0.0	0.09
No	367	29	7.9	
Sheep meat consumption				
Yes	124	2	1.6	0.003
No	279	27	9.7	
Turkey meat consumption				
Yes	172	7	4.1	0.03
No	231	22	9.5	
Opossum meat consumption				
Yes	2	1	50.0	0.14
No	398	28	7.0	
Armadillo meat consumption				
Yes	4	2	50.0	0.02
No	397	27	6.8	
Iguana meat consumption				
Yes	7	2	28.6	0.08
No	394	27	6.9	
Frequency of meat consumption				
Never	14	3	21.4	0.08
Up to 3 times a week	284	17	6.0	
4–7 times a week	100	8	8.0	
Sausages or ham consumption				
Yes	346	22	6.4	0.09
No	54	7	13.0	
Animal brain consumption				
Yes	46	5	10.9	0.35
No	354	24	6.8	
Unwashed raw fruits				
Yes	63	6	9.5	0.42
No	339	23	6.8	
Soil contact				
Yes	174	21	12.1	0.001
No	227	8	3.5	
Washing hands before eating				
Yes	379	29	7.7	0.24
No	25	0	0.0	

Table 3. (cont'd)

Characteristics	Subjects tested No.	Prevalence of <i>T. gondii</i> infection		P value
		No.	%	
Availability of potable water				
In the home	368	24	6.5	0.14
In the land	13	0	0.0	
In the street	10	2	20.0	
Crowding at home				
No	139	14	10.1	0.15
Semi-crowded	181	9	5.0	
Overcrowded	62	3	4.8	
Education of the head of family				
7 years or more	234	20	8.5	0.09
4 to 6 years	127	4	3.1	
Up to 3 years	35	4	11.4	

eling; consumption of meat other than those from sheep, turkey, and armadillo; frequency of meat consumption; eating raw or undercooked meat, animal brains, dried or cured meat, beef liver, unpasteurized milk or untreated water, and unwashed raw vegetables or fruits; frequency of eating away from home; and washing hands before eating showed *P* values equal to or higher than 0.05 in the bivariate analysis. None of the housing conditions, i.e., availability of potable water, form of elimination of excretes, education of the head of the family, crowding, and type of flooring at home correlated with *T. gondii* exposure. Multivariate analysis of sociodemographic and behavioral factors with *P* < 0.05 obtained in the bivariate analysis showed that *T. gondii* seropositivity was positively associated with being born out of Durango State (OR = 4.65; 95% CI: 1.25–17.29; *P* = 0.02) and soil contact (OR =

4.27; 95% CI: 1.71–10.67; *P* = 0.002) and negatively associated with consumption of sheep meat (OR = 0.12; 95% CI: 0.02–0.65; *P* = 0.01) (Table 4). No further associations of sociodemographic and behavioral factors were found by multivariate analysis.

Discussion

To the best of our knowledge, there is no report about the seroepidemiology of *T. gondii* in people seeking for medical certificates. Therefore, this work aimed to determine the seroprevalence and correlates of *T. gondii* infection in these applicants attending a primary healthcare center in the northern Mexican city of Durango. People seeking for medical certificates usually ask for this certificate to fulfill

Table 4. Multivariate analysis of selected characteristics of people seeking medical certificates in Durango, Mexico and their association with *T. gondii* infection

Characteristics	Odds ratio	95% confidence interval	P value
Birth place			
Durango State	1		
Other Mexican State	4.65	1.25–17.29	0.02
Occupation			
Agriculture	5.16	0.18–141.4	0.33
Housewife	0.32	0.01–5.87	0.44
Business	2.28	0.23–22.45	0.48
Employee	1.61	0.18–13.96	0.66
Chef	1.07	0.06–18.38	0.96
Other	1		
Sheep meat consumption (yes)	0.12	0.02–0.65	0.01
Turkey meat consumption (yes)	0.57	0.20–1.57	0.28
Armadillo meat consumption (yes)	14.77	0.76–286.8	0.07
Soil contact (yes)	4.27	1.71–10.67	0.002

requirements to get a job or to be admitted to a school. Consultations for medical certificates may detect a disease in applicants that may hamper their work or studies.

In the present study, we found a 7.2% seroprevalence of *T. gondii* infection in applicants of medical certificates. This seroprevalence is comparable with seroprevalences of 6.1% in the general population [17] and 7.4% in blood donors [18] reported in the same Durango City, Mexico. Interestingly, several sociodemographic, clinical, and behavioral characteristics of participants were associated with *T. gondii* exposure in the present study. Most importantly, using multivariate analysis seropositivity to *T. gondii* was positively associated with being born out of Durango State and with soil contact; a negative association was found with consumption of sheep meat. The association of *T. gondii* exposure with being born out of Durango State found in the present study is consistent with previous observations in several population groups in Durango, Mexico; higher seroprevalences of *T. gondii* infection in people born out of Durango State than those born in Durango have been reported in the general population [17], elderly people [19], inmates [20], and in patients with vision and hearing impairments, HIV, cancer, or undergoing hemodialysis [21]. The fact that *T. gondii* exposure was positively associated with soil contact but negatively associated with consumption of sheep meat suggests that infection in participants might have occurred by ingestion of oocysts instead of tissue cysts. Contact with soil is a well-established risk factor for *T. gondii* infection because *T. gondii*-infected cats contaminate soil with oocysts [22]. This is not the first study that found an association of *T. gondii* exposure with contact with soil in the population studied in Durango. We found an association of infection with soil flooring at home in several population groups, for instance, patients suffering from mental disorders due to psychoactive substance use [23] and workers occupationally exposed to unwashed raw fruits and vegetables [24].

Regarding clinical characteristics of participants associated with *T. gondii* exposure, we found that IgG seropositivity was associated with vision impairment. This finding is of epidemiological importance since *T. gondii* infection might be affecting the vision in applicants for medical certificate who intend to work or to study. Infection with *T. gondii* may lead to cyst formation in the retina and cause retinochoroiditis [2, 4]. Vision impairment as a consequence of retinochoroiditis may have important occupational health consequences by reducing the performance at work or school. Furthermore, high IgG levels were associated with hearing impairment in our study. This finding is consistent with previous observations reported in other population groups in Durango: seropositivity to *T. gondii* was linked to hearing impairment in truck drivers [25] and people of the Tepehuanos ethnic group [26] in Durango, Mexico. The association of infection and hearing impairment in applicants of medical certificates found in the present study may also be critical for occupational health since this impairment may hamper the performance in work and study. Both retinochoroiditis and otopathology have been

found in newborns with congenital toxoplasmosis [27]. However, ear disease due to *T. gondii* infection in adults has been poorly studied.

Intriguingly, IgM seropositivity was also associated with a history of surgery. It is not clear why subjects with this history have a higher prevalence of IgM antibodies than those without this history. A previous study of women with stillbirths reported an association of IgG seropositivity with a history of surgery [28]. Further studies to determine the link of surgeries and *T. gondii* infection should be conducted. Prevalence of high IgG antibody levels was also associated with a history of lymphadenopathy and miscarriages. Information about the history of these clinical characteristics should be obtained during consultations because they may help clinicians to suspect of *T. gondii* infection among applicants of medical certificates.

Our results suggest that testing for *T. gondii* antibodies in people seeking for medical certificates may be an innovative and helpful screening tool in occupational health to detect subjects with likely infection with *T. gondii* or toxoplasmosis, i.e., those suffering from vision and hearing impairments.

Conclusions

The seroprevalence of *T. gondii* infection in people seeking medical certificates appears similar to that in the general population. Common risk factors for *T. gondii* infection were identified, and unusual factors need to be further studied. Results point towards environmental contamination with oocysts. These results could be used for the design of optimal preventive measures against toxoplasmosis and its sequelae.

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References

1. Dubey JP: History of the discovery of the life cycle of *Toxoplasma gondii*. Int J Parasitol 39, 877–882 (2009)
2. Montoya JG, Liesenfeld O: Toxoplasmosis. Lancet 363, 1965–1976 (2004)
3. Kravetz J: Congenital toxoplasmosis. BMJ Clin Evid 2013, pii: 0906 (2013)
4. Maenz M, Schlüter D, Liesenfeld O, Schares G, Gross U, Pleyer U: Ocular toxoplasmosis past, present and new aspects of an old disease. Prog Retin Eye Res 39, 77–106 (2014)
5. Tack DM, Holman RC, Folkema AM, Mehal JM, Blanton JD, Sejvar JJ: Trends in encephalitis-associated deaths in the United States, 1999–2008. Neuroepidemiology 43, 1–8 (2014)
6. Sutherland AL, Fond G, Kuin A, Koeter MW, Lutter R, van Gool T, Yolken R, Szoke A, Leboyer M, de Haan L: Beyond

- the association. *Toxoplasma gondii* in schizophrenia, bipolar disorder, and addiction: systematic review and meta-analysis. *Acta Psychiatr Scand* 132, 161–179 (2015)
7. Alvarado-Esquivel C, Urbina-Álvarez JD, Estrada-Martínez S, Torres-Castorena A, Molotla-de-León G, Liesenfeld O, Dubey JP: *Toxoplasma gondii* infection and schizophrenia: a case control study in a low *Toxoplasma* seroprevalence Mexican population. *Parasitol Int* 60, 151–155 (2011)
 8. Duffy AR, Beckie TM, Brenner LA, Beckstead JW, Seyfang A, Postolache TT, Groer MW: Relationship between *Toxoplasma gondii* and mood disturbance in women veterans. *Mil Med* 180, 621–625 (2015)
 9. Groër MW, Yolken RH, Xiao JC, Beckstead JW, Fuchs D, Mohapatra SS, Seyfang A, Postolache TT: Prenatal depression and anxiety in *Toxoplasma gondii*-positive women. *Am J Obstet Gynecol* 204, 433.e1–7 (2011)
 10. Gajewski PD, Falkenstein M, Hengstler JG, Golka K: *Toxoplasma gondii* impairs memory in infected seniors. *Brain Behav Immun* 36, 193–199 (2014)
 11. Alvarado-Esquivel C, Liesenfeld O, Márquez-Conde JA, Estrada-Martínez S, Dubey JP: Seroepidemiology of infection with *Toxoplasma gondii* in workers occupationally exposed to water, sewage, and soil in Durango, Mexico. *J Parasitol* 96, 847–850 (2010)
 12. Flegr J, Havlicek J, Kodym P, Malý M, Smahel Z: Increased risk of traffic accidents in subjects with latent toxoplasmosis: a retrospective case-control study. *BMC Infect Dis* 2, 11 (2002)
 13. Alvarado-Esquivel C, Torres-Castorena A, Liesenfeld O, Estrada-Martínez S, Urbina-Álvarez JD: High seroprevalence of *Toxoplasma gondii* infection in a subset of Mexican patients with work accidents and low socioeconomic status. *Parasit Vectors* 5, 13 (2012)
 14. Alvarado-Esquivel C, Campillo-Ruiz F, Liesenfeld O: Seroepidemiology of infection with *Toxoplasma gondii* in migrant agricultural workers living in poverty in Durango, Mexico. *Parasit Vectors* 6, 113 (2013)
 15. Alvarado-Esquivel C, Estrada-Martínez S, Liesenfeld O: *Toxoplasma gondii* infection in workers occupationally exposed to unwashed raw fruits and vegetables: a case control seroprevalence study. *Parasit Vectors* 4, 235 (2011)
 16. Alvarado-Esquivel C, Pacheco-Vega SJ, Hernández-Tinoco J, Salcedo-Jáquez M, Sánchez-Anguiano LF, Berumen-Segovia LO, Rábago-Sánchez E, Liesenfeld O: *Toxoplasma gondii* infection in interstate truck drivers: a case-control seroprevalence study. *Parasit Vectors* 8, 77 (2015)
 17. Alvarado-Esquivel C, Estrada-Martínez S, Pizarro-Villalobos H, Arce-Quiñones M, Liesenfeld O, Dubey JP: Seroepidemiology of *Toxoplasma gondii* infection in general population in a northern Mexican city. *J Parasitol* 97, 40–43 (2011)
 18. Alvarado-Esquivel C, Mercado-Suarez MF, Rodríguez-Briones A, Fallad-Torres L, Ayala-Ayala JO, Nevarez-Piedra LJ, Duran-Morales E, Estrada-Martínez S, Liesenfeld O, Márquez-Conde JA, Martínez-García SA: Seroepidemiology of infection with *Toxoplasma gondii* in healthy blood donors of Durango, Mexico. *BMC Infect Dis* 7, 75 (2007)
 19. Alvarado-Esquivel C, Liesenfeld O, Burciaga-López BD, Ramos-Nevárez A, Estrada-Martínez S, Cerrillo-Soto SM, Carrete-Ramírez FA, López-Centeno Mde L, Ruiz-Martínez MM: Seroepidemiology of *Toxoplasma gondii* infection in elderly people in a northern Mexican city. *Vector Borne Zoonotic Dis* 12, 568–574 (2012)
 20. Alvarado-Esquivel C, Hernández-Tinoco J, Sánchez-Anguiano LF, Ramos-Nevárez A, Cerrillo-Soto SM, Sáenz-Soto L, Liesenfeld O: High seroprevalence of *Toxoplasma gondii* infection in inmates: a case control study in Durango City, Mexico. *Eur J Microbiol Immunol (Bp)* 4, 76–82 (2014)
 21. Alvarado-Esquivel C, Liesenfeld O, Torres-Castorena A, Estrada-Martínez S, Urbina-Álvarez JD, Ramos-de la Rocha M, Márquez-Conde JA, Dubey JP: Seroepidemiology of *Toxoplasma gondii* infection in patients with vision and hearing impairments, cancer, HIV, or undergoing hemodialysis in Durango, Mexico. *J Parasitol* 96, 505–508 (2010)
 22. Elmore SA, Jones JL, Conrad PA, Patton S, Lindsay DS, Dubey JP: *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. *Trends Parasitol* 26, 190–196 (2010)
 23. Alvarado-Esquivel C, Carrillo-Oropeza D, Pacheco-Vega SJ, Hernández-Tinoco J, Salcedo-Jáquez M, Sánchez-Anguiano LF, Ortiz-Jurado MN, Alarcón-Alvarado Y, Liesenfeld O, Beristain-García I: *Toxoplasma gondii* exposure in patients suffering from mental and behavioral disorders due to psychoactive substance use. *BMC Infect Dis* 15, 172 (2015)
 24. Alvarado-Esquivel C, Estrada-Martínez S, Liesenfeld O: *Toxoplasma gondii* infection in workers occupationally exposed to unwashed raw fruits and vegetables: a case control seroprevalence study. *Parasit Vectors* 4, 235 (2011)
 25. Alvarado-Esquivel C, Pacheco-Vega SJ, Hernández-Tinoco J, Salcedo-Jáquez M, Sánchez-Anguiano LF, Berumen-Segovia LO, Rábago-Sánchez E, Liesenfeld O: *Toxoplasma gondii* infection in interstate truck drivers: a case-control seroprevalence study. *Parasit Vectors* 8, 77 (2015)
 26. Alvarado-Esquivel C, Estrada-Martínez S, García-López CR, Rojas-Rivera A, Sifuentes-Álvarez A, Liesenfeld O: Seroepidemiology of *Toxoplasma gondii* infection in Tepic in Durango, Mexico. *Vector Borne Zoonotic Dis* 12, 138–142 (2012)
 27. Salviz M, Montoya JG, Nadol JB, Santos F: Otopathology in congenital toxoplasmosis. *Otol Neurotol* 34, 1165–1169 (2013)
 28. Alvarado-Esquivel C, Pacheco-Vega SJ, Salcedo-Jáquez M, Sánchez-Anguiano LF, Hernández-Tinoco J, Rábago-Sánchez E, Centeno-Tinoco MM, Flores-García ID, Ramos-Nevárez A, Cerrillo-Soto SM, Guido-Arreola CA, Beristain-García I, Liesenfeld O, Berumen-Segovia LO, Saenz-Soto L, Sifuentes-Álvarez A: Stillbirth history and *Toxoplasma gondii* infection in women attending public health centers in a northern Mexican City. *Eur J Microbiol Immunol (Bp)* 5, 164–171 (2015)