

Seroepidemiology of toxoplasmosis in high-school students in the metropolitan area of Guadalajara, Jalisco, Mexico

Soroepidemiologia da toxoplasmose em estudantes de nível secundário na área metropolitana de Guadalajara, Jalisco, México

Seroepidemiología de la toxoplasmosis en estudiantes de bachillerato en el área metropolitana de Guadalajara, Jalisco, México

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ABSTRACT

Aims: To determine the prevalence of specific antibodies and the associated risk factors for toxoplasmosis in students attending high-school in Zapopan, Jalisco, Mexico. **Methods:** *Toxoplasma gondii*-specific IgG and IgM antibodies were detected by using a home-made indirect immunofluorescence antibodies test. Socio-demographic variables and risk factors were recorded. The correlation was measured by Odds Ratio (95% CI), using Chi-square or Fischer test for statistical significance. **Results:** Blood samples from 174 volunteer students were collected. The age range was 14 to 25 years old, and 109 (63%) were female. Prevalence of *Toxoplasma gondii*-specific IgG and IgM antibodies were 17.8% and 4.6%, respectively. We did not find statistically significant differences due to age or gender. From the risk factors studied, the only significant association was found between *T.gondii*-specific IgG antibodies and the consumption of undercooked meat. Seven out of 11 (63.6%) students who consumed undercooked meat were IgG positive, compared with 22/159 (13.8%) who did not have this habit (OR 10.8, 95%CI 2.9-40.4). Other variables were not statistically significant. **Conclusions:** Prevalence of *Toxoplasma gondii* specific IgG and IgM antibodies were 17.8% and 4.6%, respectively. Students who had consumed undercooked meat had a 10.8 times greater risk of acquiring *Toxoplasma gondii* infection.

Keywords: *Toxoplasma gondii*; TOXOPLASMOSIS/epidemiology; TOXOPLASMOSIS/prevention & control; ANTIBODIES, PROTOZOAN; RISK FACTORS; IMMUNOGLOBULIN G; IMMUNOGLOBULIN M; STUDENTS; MEXICO/epidemiology

RESUMEN

Objetivos: Determinar la prevalencia de anticuerpos anti-*Toxoplasma* y factores de riesgo asociados para toxoplasmosis en estudiantes de bachillerato en Zapopan, Jalisco, México. **Métodos:** Los anticuerpos IgG e IgM anti-*Toxoplasma gondii* fueron determinados usando el método de inmunofluorescencia indirecta realizado en nuestro laboratorio y previamente estandarizado. Variables socio-demográficas y factores de riesgo para toxoplasmosis fueron analizados y correlacionados con la presencia de anticuerpos. La correlación fue realizada mediante razón de momios con un intervalo de confianza del 95%. Fueron utilizados Chi cuadrada ó significancia estadística de Fischer. **Resultados:** Fueron determinados los anticuerpos en 174 estudiantes. El rango de edad fue de 14 a 25 años y 109 (63%) correspondió al género femenino. La prevalencia de anticuerpos anti-*Toxoplasma* fue de 17,8% para IgG y 4,6% para IgM. No se encontraron diferencias estadísticamente significativas con relación a edad y genero. De los factores de riesgo estudiados, la asociación entre anticuerpos anti-*Toxoplasma* clase IgG y el consumo de carne poco cocida fue la única significativa. Siete de cada 11 (63,6%) los estudiantes que consumieron carne mal cocida IgG-positivos, en comparación con 22/159 (13,8%) que no tenían este hábito (odds ratio: 10,8, intervalo de confianza del 95%: 2,9-40,4). Otras variables no fueron estadísticamente significativas. **Conclusiones:** La prevalencia de anticuerpos anti-*Toxoplasma* clase IgG fue de 17,8% y 4,6% a IgM. Los estudiantes que consumían carne mal cocida tuvieron 10,8 veces mayor riesgo para adquirir la infección por *Toxoplasma gondii*.

Palabras clave: *Toxoplasma gondii*; TOXOPLASMOSIS/epidemiología; TOXOPLASMOSIS/prevenición & control; ANTICUERPOS ANTI PROTOZOÁRIOS; FACTORES DE RIESGO; IMUNOGLOBULINA G; IMUNOGLOBULINA M; ESTUDIANTES; MÉXICO/epidemiología

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INTRODUCTION

Toxoplasmosis is an infection caused by *Toxoplasma gondii* (*T.gondii*), an obligated intracellular parasite distributed worldwide, which infects warm-blooded animals, including humans. The cat is the main host of the parasite. Human infection results from ingestion of soil contaminated with cat litter and ingestion of raw or insufficiently cooked meat. Congenital toxoplasmosis results from an acute primary infection acquired by the mother during pregnancy, when the agent is transferred to the fetus through the placenta. Other infection pathways are transfusion, transplantation and direct contamination.¹

Acquired toxoplasmosis is usually asymptomatic, but may be characterized by lymphadenopathy accompanied by reticular cell hyperplasia, pulmonary necrosis, and myocarditis. In congenital disease, there may be central nervous system involvement resulting in hydrocephalus or cerebral calcifications. Retinchoroiditis is caused by congenital or acquired ocular toxoplasmosis. Reactivation of latent infection in immunocompromised patients, such as AIDS patients, is one of the most common causes of central nervous system complications.²

The prevalence rate of the disease in the world is related to different factors such as age, sociocultural and nutritional habits, and contact with domestic cats.³ It is documented that over half a billion of the world's human population has *T. gondii* antibodies.²

The prevalence of *T.gondii* antibodies was between 15% and 50% among the Mexican open population. The highest prevalence was in locations with elevated temperatures, in states located in the Tropic of Cancer or on the coasts, and a prevalence of 24.8% with a dilution of 1 to 16 by **immunofluorescence** antibody test was found in people between 15 and 39 years in the central zone of Mexico.³ High prevalence values have been found in specific groups in Jalisco: 29% in blood donors⁴; 35% and 52% in women with high risk pregnancy alone or those who delivered children with malformations, respectively;^{5,6} 64.4% in pet-cat owners,⁷ and 50% in AIDS patients.⁸ These results support the notion that Jalisco is a highly endemic region of toxoplasmosis.

The prevalence of *T.gondii*-specific antibodies has been found in different countries among high school students. In Korea, 5.4% positivity in males and 5.5% in females were found.⁹ In a study on 947 high school girls aged between 14 to 19 years in Fasa District, Iran, 96 (10%) were seropositive for *T.gondii*-specific IgG antibodies.¹⁰ In Brazil, a high prevalence was found: *T.gondii*-specific IgG antibodies were tested in 133

high school students, residents of São Jerônimo da Serra city, and 67 (50.4%) were seropositive.¹¹ Another report of Fortaleza, Ceará, Brazil, in 584 students between 7 and 18 years old, found 40.8% seropositive subjects.¹² In Rolândia, Paraná, Brazil, a sample of 343 serums was analyzed, and 145 (42.4%) were positive for *T.gondii*-specific antibodies.¹³ However, in Mexico, the prevalence of infection of *T.gondii* in high school students has not been determined. The aim of this work was to determine the prevalence of *T.gondii*-specific antibodies and the associated risk factors in 174 high-school students from the Industrial and Technological High School No. 38 of the Secretariat of Public Education, Jalisco, México.

METHODS

The Guadalajara metropolitan zone is located in the state of Jalisco and officially consists of 8 municipalities, among which six (El Salto, Tlajomulco de Zúñiga, Tlaquepaque, Tonalá and Zapopan) are considered central municipalities. Guadalajara High School No. 38 is located in Zapopan. According to the results of the Second Census of Population and Housing in 2005, the six municipalities of the Guadalajara metropolitan zone had very low levels of marginalization. Housing with some level of crowding and dirty floor is located at El Salto (48.3 and 10.6% respectively), and homes without piped water stands at Tonalá (12.6%). All students had middle socioeconomic level, their homes had drinking potable water, and their houses had tiled floor.

Students attending High School No. 38 in Zapopan, Jalisco, were randomly chosen for blood samples collection. The study protocol and informed-consent form were reviewed and approved by the Committees of Investigation and Ethics of the University of Guadalajara, Mexico. The participants signed an informed-consent form and were screened for *T.gondii*-specific antibodies. A questionnaire was completed by each subject, which included socio-demographic data, age, sex and residence. Hygienic habits were also recorded, such as washing of fruit and vegetables before eating, consumption of undercooked meat and close contact with cats.

Antigen preparations were obtained from tachyzoites of the RH strain of *T.gondii* in the peritoneal exudates of mice infected two days earlier. *T.gondii*-specific antibodies were detected using a home-made indirect immuno-fluorescence test (IFAT). The tachyzoites were incubated in 2% buffered formalin for 30 min at 37°C, washed twice in PBS at 1,000 g for 10 min, and fixed on glass slides. Antihuman IgG (whole

molecule) TRITC conjugate (Sigma T-5903) and anti-human IgM (Sigma Aldrich) were used. The sera were diluted 1:16 to 1:128 for IgG, and 1:8 to 1:32 for IgM, as previously validated in our laboratory.

The average and standard deviation were determined. The association between potential risk factors and each student's positive antibody status was established by the odds ratios (OR) and 95% confidence intervals (CI), using Chi-square or Fischer test for statistical significance.

RESULTS

Blood samples from 174 students were collected. The prevalence of *T.gondii*-specific IgG and IgM antibodies was 17.8% and 4.6%, respectively. The distribution of positive antibodies by IFAT dilutions was 10.34% for IgG 1:16 and 7.46% for IgG 1:128. Eight cases (4.6%) were positive for IgM, of whom five were positive only for IgM and three subjects (1.72%) were positive to both antibodies. In those three, the IgM presented low titers (1:8).

Age range was between 14 and 25 years-old and 63% (109 individuals) were female. The prevalence of IgG *T.gondii*-specific antibodies according to age was higher in 18 years-old or older people. Regarding gender, no statistically significant differences were found, with 20 positive out of 109 (18.3%) in females and 11 out of 65 (16.9%) in males ($p=0.656$) (Table 1).

Regarding prevalence of IgG *T.gondii*-specific antibodies according to residence, there was a trend towards a lower proportion of seropositive students in Guadalajara and Zapopan municipalities and a highest proportion in other municipalities as Tlajomulco de Zuñiga and Tonalá, but the difference was not statistically significant. (Table 2)

Table 1. Prevalence of IgG and IgM *Toxoplasma gondii*-specific antibodies by gender and age in high-school students in the metropolitan area of Guadalajara, Jalisco, Mexico

Gender and age	N	IgG positive N (%)		IgM positive N (%)	
Female	109	20	(18.3)	5	(4.6)
Male	65	11	(16.9)	3	(4.6)
< 16 years	54	6	(11)	5	(9.3)
16	47	8	(17)	2	(4.3)
17	42	9	(21.4)	1	(2.4)
18	13	3	(23.1)	0	(0)
>18	13	3	(23.1)	0	(0)
Unknown age	5	2	(40.0)	0	(0)
Total	174	31	(17.8)	8	(4.6)

Table 2. Prevalence of IgG and IgM *Toxoplasma gondii*-specific antibodies by municipality of residence in high-school students in the metropolitan area of Guadalajara, Jalisco, Mexico

Municipality	N	IgG positive N (%)		IgM positive N (%)	
Guadalajara	51	10	(19.6)	2	(3.9)
Zapopan	105	17	(16.2)	5	(4.7)
Other municipalities	18	4	(22.2)	1	(5.5)
Total	174	31	(17.8)	8	(4.6)
			0.82		0.95

From the risk factors studied, the only significant association was found between *T.gondii*-specific IgG antibodies and the consumption of undercooked meat. Seven out of 11 (63.6%) students that consumed undercooked meat were IgG positive, compared with 22/159 (13.8%) who did not have this habit (OR 10.8, 95% CI 2.9-40.4). Four students did not answer all the questions about risk factors (Table 3).

Table 3. Risk factors for toxoplasmosis in 170 high-school students* in the metropolitan area of Guadalajara, Jalisco, Mexico

FACTOR	N	IgG positive N (%)		IgG negative N (%)		OR (95% CI)	p
Eat undercooked meat							
Yes	11	7	(63.6)	4	(36.4)	10.8 (2.9-40.4)	<0.001
No	159	22	(13.8)	137	(86.2)		
Wash fruit and vegetables						1.70 (0.20-37.0)	0.621
Yes	160	28	(17.5)	132	(82.5)		
No	10	1	(10.0)	9	(90.0)		
Cats at home						1.69 (0.66-4.12)	0.253
Yes	49	11	(22.4)	38	(77.6)		
No	121	18	(15.1)	101	(84.9)		

* Four students did not answer all questions on risk factors.
OR=odds ratio; CI=confidence interval.

One hundred and sixty students washed fruit and vegetables before eating, of whom 28 (17.5%) were positive to IgG *T.gondii*-specific antibodies. Of the 10 students who did not have this habit, only one was IgG positive, but the difference was not statistically significant (OR=1.70, 95%CI=0.20-37.0). Forty-nine students lived with cats and 11 (22.4%) were IgG positive, while among the 121 who had no cats at home, 18 (15.1%) were IgG positive (OR=1.69, 95%CI=0.66-4.12). (Table 3) Association of *T.gondii*-specific IgM antibodies with the studied risk factors had no statistical significance.

DISCUSSION

The prevalence of *T. gondii*-specific antibodies was low (17.8%) and may be due to several factors. All students were healthy, with middle socioeconomic level, and most lived in the municipalities of Guadalajara and Zapopan, which have a higher level of development. The prevalence was lower compared to other studies in students from Fortaleza, Brazil, with a 56.4% prevalence of *T.gondii*-specific IgG antibodies, and from São Jerônimo da Serra, with a prevalence of 50%.^{12,11} This finding could be due either to climate factors or to socio-demographic and habits differences in these populations.

In the age range from 14 to 25 years, the prevalence was 19%, similar to blood donors with less than 20 years of age, using the same technique.⁴ In contrast, our prevalence was higher than the 9% found in blood donors with age range of 18-24 years from Durango City, Mexico.¹⁴ This difference can be due to the technique used and the temperature, which is lowest in the Durango state, located in northern Mexico. These results suggest that the prevalence of *T.gondii*-specific antibodies in young adults less than 20 years is approximately 18% in the metropolitan zone of the municipality of Guadalajara, Jalisco, Mexico. The prevalence of *T.gondii*-specific IgM antibodies was 4.6%, and was higher (8.47%) in students younger than 16 years of age. However, we considered that only five (2.87%) were new infections because of positive IgM antibodies. Three subjects (1.72%) were positive to both antibodies and the IgM presented low titers (1:8) due to its decline.

Regarding gender, no statistical difference was found. These results demonstrate that females and males have the same probability of contracting *T. gondii* infection, which has been demonstrated by other studies from Korea^{9,10} and Brazil.¹¹

Eating undercooked meat was a risk factor for acquiring *T. gondii* infection in other studies. In this study, the frequency of *T.gondii*-specific antibodies

was 10.8 higher in students who ate undercooked meat compared with students that did not have this habit. These results are similar to the higher incidence in women with the habit of eating raw or poorly cooked meat.¹¹ There are various reports about the greater risk of acquiring *T.gondii* infection in people who have cats. In this study, the seroprevalence did not show association with close contact with cats, although in our population a low risk was found. However, in Mexico there are other studies regarding the presence of *T.gondii*-specific antibodies and close contact with cats⁷. In Fortaleza, Brazil, a statistical association was found between *T. gondii* seroprevalence and close contact with cats. These results could be caused by inappropriate management of cat feces and commercial food.¹²

In females, 81.7% had not been in contact with *T. gondii*. This is a very important issue, because these 89 seronegative young women were in fertile age and before this study they were uninformed about the risk of toxoplasmosis transmission during pregnancy. The development of preventive programs against infection by *T.gondii* in young people in schools is urgently needed in our country, where there are two for each 1000 infants born with the congenital infection.¹⁵

Regarding all students with negative antibodies, they should be informed of the risk of transmission and the importance of eating fruit and vegetables that are correctly washed, not eating undercooked or raw meat and about the correct management of cats.

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