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SEROEPIDEMIOLOGY OF *TOXOPLASMA GONDII* INFECTION IN HUMAN ADULTS FROM THREE RURAL COMMUNITIES IN DURANGO STATE, MEXICO

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ABSTRACT: There is scarce information concerning the epidemiology of *Toxoplasma gondii* infection in people of rural Mexico. Anti-*T. gondii* immunoglobulin (Ig)G and IgM antibodies were sought in 462 adult inhabitants from 3 rural communities of Durango, Mexico, using enzyme-linked immunoassays. In total, 110 (23.8%) of 463 persons had IgG anti-*T. gondii* antibodies. Ten (2.2%) of them also had IgM anti-*T. gondii* antibodies. Prevalences of *T. gondii* IgG antibodies in the 3 communities varied from 14.8 to 35.8%. The highest prevalence of infection was observed in participants older than 70 yr and in those with good housing conditions. *Toxoplasma gondii* infection was significantly associated with consumption of squirrel (adjusted odds ratio [OR] = 4.22; 95% confidence interval [CI] = 1.11–16.05) and turkey meat (adjusted OR = 4.58; 95% CI = 1.14–18.44). This is the first epidemiological study of *T. gondii* prevalence in rural Mexico.

Toxoplasma gondii antibodies have been found in one third of the world's population (Dubey and Beattie, 1988; Tenter et al., 2000). Although *T. gondii* infections are generally asymptomatic, toxoplasmosis can be a serious illness in humans (Montoya and Liesenfeld, 2004). Cats are the definitive hosts for *T. gondii*; therefore, a major route of parasite transmission in humans and animals is via ingestion of food or water contaminated with oocysts shed by cats. In addition, the parasite is transmitted by consumption of undercooked, or raw, meat containing tissue cysts. Rural communities in developing countries have a major epidemiological importance because these communities usually have deficient hygiene and sanitary practices that may favor transmission of the parasite. In addition, rural communities lack full coverage of health care services and seldom have diagnostic facilities for infectious diseases. Thus, adequate preventive and treatment measures for infectious diseases in these communities are limited. Although there are several reports of *T. gondii* infections in animals (Roch and Varela, 1966; Galván-Ramírez et al., 1999; Dubey et al., 2004; Suzán and Ceballos, 2005; Figueroa-Castillo et al., 2006; Alvarado-Esquivel, Liesenfeld et al., 2007; Dubey et al., 2007), and in humans (Roch and Varela, 1966; Goldsmith et al., 1991; Velasco-Castrejón et al., 1992; Alvarado-Esquivel et al., 2006; Alvarado-Esquivel, Mercado-Suárez et al., 2007) in Mexico, little is known of the epidemiology of *T. gondii* infection in Mexico, especially in rural areas. Therefore, we performed a cross-sectional study to determine the prevalence of *T. gondii* infection in persons living in rural areas of Durango state, Mexico, and to identify characteristics of these persons associated with seropositivity.

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MATERIALS AND METHODS

Study design and study population

An observational prospective survey from December 2006 to August 2007 was conducted in adult populations of 3 rural communities of Durango state, Mexico (Fig. 1). Community 1 (San Dimas) is located at the mountains region in the western part of Durango state at 2,650 m above sea level and has a subhumid climate. Community 2 (Villa Montemorelos) is located in the valley region in the central part of Durango state at 1,870 m above sea level and has a temperate climate. Community 3 (Santa Clara) is located in the semidesert region in the eastern part of Durango state at 2,050 m above sea level and has a dry climate. In total, 463 persons (155 [community 1], 121 [community 2], and 187 [community 3]) voluntarily participated in the study; all were older than 17 yr. The purpose and procedures of the study were explained to all participants, and a written informed consent was obtained from all of them. This study was approved by the Institutional Ethical Committee.

Sociodemographic and behavioral data

Sociodemographic data including age, birthplace, location of residence, occupation, educational level, and housing condition index were acquired from all participants Table I. A housing condition index was obtained using the criteria of Bronfman et al. (1988). Briefly, 5 variables evaluated were: the number of persons in the house, number of rooms, and the type of flooring material in the house, plus the availability of drinkable water, and the presence/absence of facilities for the disposal of human excreta. Contributing and confounding risk factors of behavioral data from all inhabitants included blood transfusion or transplant history; animal contacts; contact with cat feces; foreign travel; kind of meat consumed (pork, lamb, beef, goat, boar, chicken, turkey, rabbit, deer, squirrel, horse, fish, rat, opossum, and snake); consumption of raw, or undercooked, meat; unpasteurized milk or milk products; untreated water; dried or cured meat (chorizo, ham, sausage, or salami); unwashed raw vegetables or fruit, contact with soil (gardening or agriculture); and eating outside of the home (at least once a year).

Serological examination for *T. gondii* antibodies

Blood samples were centrifuged and sera were stored at −20 C at the Laboratory of Microbiology, Faculty of Medicine, Juárez University of Durango State, Mexico, until examined. Sera were first tested for *T. gondii* IgG antibodies; IgG-positive sera were checked for IgM antibodies by commercially available enzyme immunoassay, i.e., *Toxoplasma* IgG and *Toxoplasma* IgM kits (International Immuno-Diagnostics,

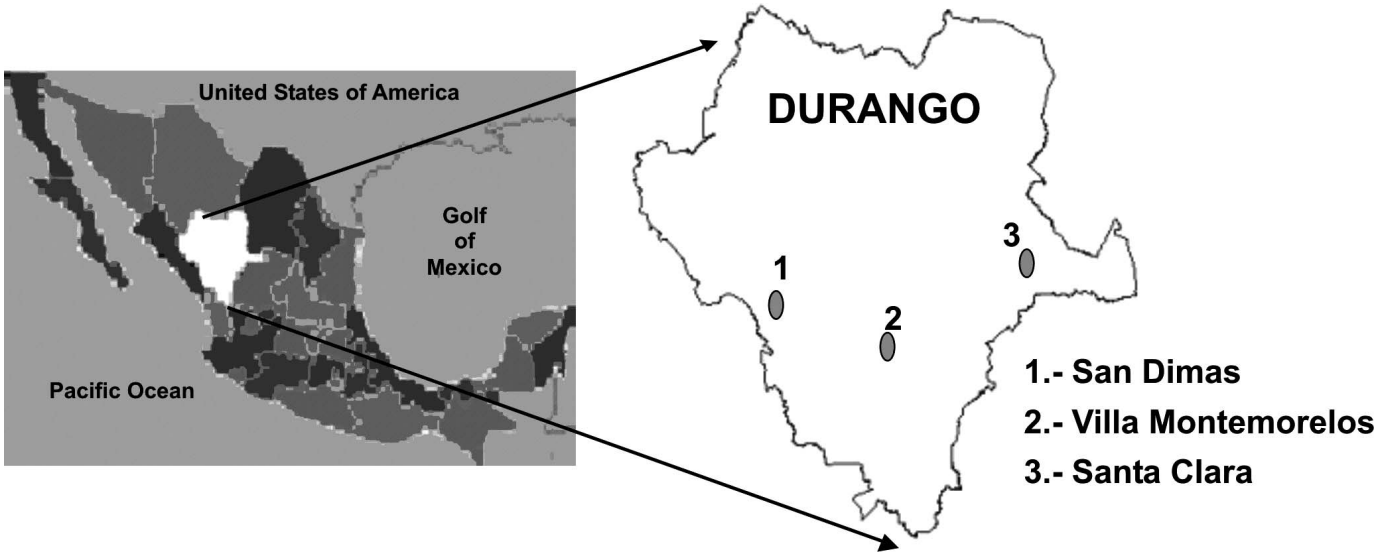

FIGURE 1. Geographical locations of the 3 rural communities explored in Durango state, Mexico. The geographical coordinates of the communities are as follows: community 1 (San Dimas) 24°06′N, 105°56′W; community 2 (Villa Montemorelos) between 22°40′ and 26°50′N, and 102°25′55′′ and 107°08′50′′W; and community 3 (Santa Clara) 24°28′N, 103°21′W.

TABLE I. Sociodemographic characteristics of inhabitants in 3 rural communities of Durango, Mexico.

	Community 1*			Community 2*			Community 3*			All*		
	No. of subjects	Prevalence of *T. gondii* infection		No. of subjects	Prevalence of *T. gondii* infection		No. of subjects	Prevalence of *T. gondii* infection		No. of subjects	Prevalence of *T. gondii* infection	
Characteristic	tested	No.	%	tested	No.	%	tested	No.	%	tested	No.	%
Gender												
Male	53	7	(13.2)	18	2	(11.1)	42	16	(38.1)	113	25	(22.1)
Female	102	16	(15.7)	103	18	(17.5)	145	51	(35.2)	350	85	(24.3)
Age group (yr)												
≤30	61	8	(13.1)	35	7	(20)	34	8	(23.5)	130	23	(17.7)
31–50	61	8	(13.1)	44	7	(15.9)	86	27	(31.4)	191	42	(22)
51–70	25	6	(24)	26	1	(3.8)	41	20	(48.8)	92	27	(29.3)
>70	7	1	(14.3)	12	4	(33.3)	25	12	(48)	44	17	(38.6)§
Educational level												
No education	10	1	(10)	12	3	(25)	25	10	(40)	47	14	(29.8)
Up to 12 yr	143	22	(15.4)	104	17	(16.3)	157	53	(33.8)	404	92	(22.8)
13–19 yr	1	0	(0)	2	0	(0)	4	3	(75)	7	3	(42.9)
>19 yr	1	0	(0)	1	0	(0)	0			2	0	(0)
Occupation												
No laborer†	105	17	(16.2)	102	17	(16.7)	143	53	(37.1)	350	87	(24.9)
Laborer‡	50	6	(12)	18	3	(16.7)	43	14	(32.6)	111	23	(20.9)
Housing conditions index												
Good	2	0	(0)	17	1	(5.9)	23	13	(56.5)	42	14	(33.3)‖
Regular	75	12	(16)	81	17	(21)	146	50	(34.2)	302	79	(26.2)
Bad	71	11	(15.5)	0			4	0	(0)	75	11	(14.7)

* Inhabitants with available data.
† Nonlaborer = no occupation, student or housewife.
‡ Laborer = employee, professional, business, agriculture, cattle rising, factory worker, construction worker, or other.
§ Statistically significant difference among the youngest and oldest age groups: $P = 0.008$.
‖ Statistically significant difference among the good and bad housing conditions: $P = 0.03$.

Foster City, California). Both tests were performed following the instructions of the manufacturer. For both IgG and IgM tests, we used the Toxo G index and Toxo M index, respectively. These indexes were calculated for each determination by dividing the mean value of each sample by the cut-off calibrator mean value. A sample was considered positive for IgG or IgM when a Toxo G index or a Toxo M index was equal to, or greater than, 1.0. In the Toxo IgG test, an index of 1.0 was equivalent to 8 international units/ml. A positive IgG test with a negative IgM test in a person was interpreted as a chronic infection. A positive IgM test in a person with a positive IgG test was interpreted as probability of a recent infection.

Statistical analysis

The statistical analysis was performed with the aid of Epi Info version 3.3.2 and SPSS version 7.5 (SPSS Inc., Chicago, Illinois). For calculation of the sample size, we used a reference seroprevalence of 7.4% (Alvarado-Esquivel, Mercado-Suárez et al., 2007) as expected frequency of the factor under study, with 30,000 as the size of the population from which the sample was selected; the worst acceptable result was 10%, with a confidence level of 95%. The result of the calculation was 384 subjects. Descriptive statistics were used for numerical (mean and median) and categorical (frequency or percentage) variables. We used the Yates corrected test, and the Fisher exact test (when values were <5), for comparison of the frequencies among groups. Bivariate and multivariate analyses were used to assess the association between the characteristics of the subjects and *T. gondii* seropositivity. As a criterion for inclusion of variables in the multivariate analysis, we considered variables with $P \leq 0.25$ obtained in the bivariate analysis to allow potential confounding. An adjusted odds ratio (OR) and a 95% confidence interval (CI) were calculated by multivariate analysis using multiple, unconditional logistic regression. $P < 0.05$ was considered statistically significant.

RESULTS

Overall, 110 (23.8%) of 463 persons had *T. gondii* IgG antibodies and 10 (2.2%) were also positive for *T. gondii* IgM antibodies. Seroprevalence of *T. gondii* IgG antibodies in communities 1, 2, and 3 were 14.8 (23 of 155), 16.5 (20 of 121), and 35.8% (67 of 187), respectively. A significantly higher prevalence of *T. gondii* IgG antibodies was found in community 3 than in communities 1 and 2 ($P < 0.0001$ and $P < 0.001$, respectively). Anti-*T. gondii* IgM antibodies were found in 3 (13%) of the 23 IgG-positive inhabitants from community 1, in 3 (15%) of the 20 IgG-positive inhabitants from community 2, and in 4 (6%) of the 67 IgG-positive persons from community 3.

General sociodemographic characteristics of the 463 persons studied are shown in Table I. All participants were born in Mexico, and their mean age was 42.5 ± 17.6 yr (range 18–91 yr). Most of them were females, attended school up to 12 yr, and lived in regular housing conditions. Overall, the frequency of infection increased with age; 70 yr or older persons (38.6%) had a significantly higher ($P = 0.008$) frequency of infection than those aged 30 or less (17.7%). This trend was observed in communities 2 and 3, but not in community 1. Similarly, the frequency of infection was significantly higher in inhabitants with a good housing condition index (33.3%) than those with a bad housing condition index (14.7%) ($P = 0.03$). Again, housing conditions were worse in participants from community 1 than in those from communities 2 and 3.

In the bivariate analysis, clear differences in type of variables associated with infection among the communities were found. In community 1, squirrel meat consumption was associated with *T. gondii* infection ($P = 0.01$). In community 2, turkey meat consumption and contact with soil (gardening or agriculture) were associated with infection ($P = 0.03$ and $P = 0.01$, respectively). In community 3, no overcrowding at home was associated with infection ($P = 0.008$). When the 3 communities were evaluated together, *T. gondii* infection was associated with goat meat consumption ($P = 0.003$), sheep meat consumption ($P = 0.03$), drainage at home ($P = 0.002$), and no overcrowding ($P = 0.01$). Other sociodemographic and behavioral characteristics of the rural inhabitants did not have any association with *T. gondii* infection by bivariate analysis. Table II shows the results of the bivariate analysis of selected variables and the results of *T. gondii* seropositivity. Further analysis using a logistic regression showed that only 2 variables were significantly associated with *T. gondii* infection, i.e., squirrel meat consumption in community 1 (adjusted OR = 4.22; 95% CI = 1.11–16.05), and turkey meat consumption in community 2 (adjusted OR = 4.58; 95% CI, 1.14–18.44) (Table III).

DISCUSSION

The 23.8% seroprevalence of *T. gondii* infection in the adult population of rural areas in Durango state, Mexico, in the present study is higher than that reported in healthy urban populations in the capital city (Durango City) of Durango state. In 2 previous studies, 7.4% of 432 healthy blood donors of 2 blood banks (Alvarado-Esquivel, Mercado-Suárez et al., 2007) and 6.1% of 343 healthy pregnant women seeking prenatal care in a public hospital in Durango City were seropositive (Alvarado-Esquivel et al., 2006). Differences in sanitation and different IgG and IgM kits used in these investigations could, however, have contributed to differences in the seroprevalences in rural and urban populations. Certainly, rural communities in Durango state had poorer hygiene than in Durango City. Altitude and climate had no apparent effect on seroprevalences in 3 communities. In addition, our seroprevalence is higher than the 3.8% seroprevalence reported in rural populations in the southern Mexican state of Oaxaca (Goldsmith et al., 1991). With respect to the demographic characteristics of the inhabitants, we observed that the frequency of seropositivity in general increased with age, as observed in blood donors from Durango City (Alvarado-Esquivel, Mercado-Suárez et al., 2007). However, this was not the case in community 1, where most inhabitants live in regular or poor housing conditions. We found that seropositivity to *T. gondii* in general increased as the housing condition index increased ($P = 0.03$). Good housing conditions are a reflection of better socioeconomic circumstances. Thus, inhabitants with good housing conditions were more likely to consume meat than those at a lower socioeconomic level. Indeed, paucity of meat consumption was frequently observed in low-income participants. This variable was particularly important in community 3. In contrast, communities 1 and 2 had different variables associated with infection. This fact clearly shows the variability in the routes of infection among the communities examined. In community 1, squirrel meat consumption was associated with infection, whereas in community 2, turkey meat consumption was associated with infection. The squirrel species eaten in community 1 include *Sciurus nayaritensis* and *Sciurus allen*, *Spermophilus madrensis*, and *Spermophilus variegatus* (Servin et al., 1996, 1997). Suzán and Ceballos (2005) found complement-fixing antibodies in 2 of 2 rock squirrels (*Spermophilus variegatus*) captured in a nature reserve outside

TABLE II. Bivariate analysis of putative risk factors for infection with *Toxoplasma gondii* in inhabitants from rural areas of Durango, Mexico.

	Community 1*			Community 2*			Community 3*			All*		
Characteristic	Inhabitants No.	Prevalence of *T. gondii* infection No. (%)	*P*	Inhabitants No.	Prevalence of *T. gondii* infection No. (%)	*P*	Inhabitants No.	Prevalence of *T. gondii* infection No. (%)	*P*	Inhabitants No.	Prevalence of *T. gondii* infection No. (%)	*P*
Pork meat consumption												
Yes	154	22 (14.3)	0.14	101	18 (17.8)	0.31	149	51 (34.2)	0.23	404	91 (22.5)	0.1
No	1	1 (100)		20	2 (10)		38	16 (42.1)		59	19 (32.2)	
Goat meat consumption												
Yes	11	3 (27.3)	0.21	1	0	0.83	102	36 (35.3)	0.44	114	39 (34.2)	0.003
No	142	20 (14.1)		120	20 (16.7)		85	31 (365)		347	71 (20.5)	
Sheep meat consumption												
Yes	32	4 (12.5)	0.44	15	4 (26.7)	0.21	92	34 (37)	0.43	139	42 (30.2)	0.03
No	121	19 (15.7)		106	16 (15.1)		95	33 (34.7)		322	68 (21.1)	
Turkey meat consumption												
Yes	82	14 (17.1)	0.29	26	8 (30.8)	0.03	26	12 (46.2)	0.16	134	34 (25.4)	0.59
No	71	9 (12.7)		95	12 (12.6)		159	54 (34)		325	75 (23.1)	
Deer meat consumption												
Yes	126	20 (15.9)	0.38	4	2 (50)	0.12	32	12 (37.5)	0.49	162	34 (21)	0.27
No	27	3 (11.1)		117	18 (15.4)		154	55 (35.7)		298	76 (25.5)	
Squirrel meat consumption												
Yes	101	20 (19.8)	0.01	3	0	0.57	3	1 (33.3)	0.7	107	21 (19.6)	0.23
No	51	3 (5.9)		118	20 (16.9)		183	66 (36.1)		352	89 (25.3)	
Degree of meat cooking												
Undercooked	30	4 (13.3)	0.52	3	0	0.57	3	2 (66.7)	0.2	36	6 (16.7)	0.4
Well done	125	19 (15.2)		118	20 (16.9)		184	65 (35.3)		427	104 (24.4)	
Gardening or agriculture												
Yes	128	22 (17.2)	0.07	109	15 (13.8)	0.01	165	59 (35.8)	0.56	402	96 (23.9)	0.96
No	25	1 (4)		11	5 (45.5)		22	8 (36.4)		58	14 (24.1)	
Blood transfusion												
Yes	23	3 (13)	0.53	18	5 (27.8)	0.15	19	5 (26.3)	0.25	60	13 (21.7)	0.66
No	130	20 (15.4)		102	15 (14.7)		168	62 (36.9)		400	97 (24.3)	
Drainage at home												
Yes	5	0	0.43	44	4 (9.1)	0.05	152	58 (38.2)	0.06	201	62 (30.8)	0.002
No	145	23 (15.9)		61	14 (23)		28	6 (21.4)		234	43 (18.4)	
Overcrowding at home												
High	69	8 (11.6)	0.55	12	3 (25)	0.38	46	8 (17.4)	0.008	127	19 (15)	0.01
Moderate	56	10 (17.9)		54	11 (20.4)		96	38 (39.6)		206	59 (28.6)	
No	28	5 (17.9)		37	4 (10.8)		45	21 (46.7)		110	30 (27.3)	
Cleaning cat feces												
Yes	46	5 (10.9)	0.25	36	8 (22.2)	0.19	82	33 (40.2)	0.15	164	46 (28)	0.1
No	108	18 (16.7)		79	11 (13.9)		100	32 (32)		287	61 (21.3)	

* Inhabitants with available data.

Mexico City, but nothing is known of *T. gondii* infection in feral squirrels from Durango state.

There was also an association between *T. gondii* seroprevalence and eating turkey meat. Natural and experimental infections by *T. gondii* in turkeys have been reported in the United States (Dubey et al., 1993; Quist et al., 1995), but nothing is known of *T. gondii* infection in turkeys in Mexico. It is of interest that this is the second study where we found an association between turkey meat consumption and *T. gondii* infection in Durango state. In our previous study, we found this association in pregnant women during visitation interviews at attending a public hospital in Durango City (Alvarado-Esquivel et al., 2006). Goat and sheep meat consumption was also associated with *T. gondii* infection by bivariate analysis in our study. This association declined when included in a multivariate analysis. Although *T. gondii* is widely prevalent in sheep and goats worldwide (Dubey and Beattie, 1988), we are not aware of a report of *T. gondii* infection in these animals in Mexico.

TABLE III. Multivariate analysis of selected characteristics of inhabitants in rural areas and their association with *Toxoplasma gondii* infection.

	Community 1			Community 2			Community 3			All		
Characteristic*	Adjusted OR†	95% CI	*P*	Adjusted OR†	95% CI	*P*	Adjusted OR†	95% CI	*P*	Adjusted OR†	95% CI	*P*
Pork meat consumption	—	—	—	—	—	—	0.75	0.32–1.75	0.52	0.99	0.49–2.0	0.99
Goat meat consumption	1.96	0.41–9.24	0.39	—	—	—	0.96	0.37–2.47	0.94	1.49	0.77–2.88	0.23
Sheep meat consumption	0.55	0.15–2.05	0.37	2.29	0.47–10.9	0.29	1.07	0.42–2.71	0.88	0.97	0.52–1.82	0.93
Turkey meat consumption	1.24	0.45–3.41	0.66	4.58	1.14–18.44	0.03	1.55	0.60–3.94	0.35	1.26	0.72–2.21	0.4
Deer meat consumption	1.1	0.26–4.68	0.88	—	—	—	1.03	0.40–2.67	0.93	0.92	0.48–1.79	0.82
Squirrel meat consumption	4.22	1.11–16.05	0.03	—	—	—	—	—	—	1.29	0.60–2.75	0.5
Degree of meat cooking‡	0.52	0.14–1.85	0.31	—	—	—	3.92	0.30–50.52	0.29	0.73	0.27–1.96	0.53
Gardening or agriculture	3.33	0.38–28.5	0.27	0.05	0.008–0.30	<0.01	0.89	0.32–2.41	0.82	0.79	0.39–1.58	0.5
Blood transfusion	0.5	0.11–2.20	0.36	1.28	0.27–5.90	0.74	0.57	0.16–2.01	0.38	0.65	0.30–1.38	0.26
Drainage at home	—	—	—	2.57	0.67–9.82	0.16	0.41	0.15–1.16	0.09	0.6	0.34–1.07	0.08
Cleaning cat feces	0.66	0.21–2.03	0.46	0.65	0.18–2.37	0.52	1.47	0.74–2.88	0.26	1.24	0.77–2.00	0.37

* The variables included were those with a $P < 0.25$ obtained in the bivariate analysis.
† Adjusted by age and the rest of the characteristics included in this table.
‡ Undercooked versus well done.

Contacts with cats and soil, or poor hygiene in food or water, were not positively associated with infection in this study, which may be related to low prevalence of *T. gondii* infection in cats. In a survey of cats using the modified agglutination test, antibodies to *T. gondii* were found in 21% of 105 cats from Durango city, Mexico (Alvarado-Esquivel, Liesenfeld et al., 2007) and 51.5% of 101 dogs (Dubey et al., 2007). It would have been of interest to examine the prevalence of *T. gondii* in cats and other animals in rural areas surveyed in the present study.

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