Short communication

Seroprevalence of *Toxoplasma gondii* infection in domestic rabbits in Durango State, Mexico

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A B S T R A C T

There is a lack of information concerning the seroprevalence of *Toxoplasma gondii* infection in rabbits in northern Mexico. Through a cross sectional study, antibodies to *T. gondii* were determined in 429 domestic rabbits in Durango State, Mexico using the modified agglutination test. Rabbits were raised in 29 properties in 6 municipalities. Overall, antibodies to *T. gondii* were found in 70 (16.3%) of 429 rabbits, with titers of 1:25 in 42, 1:50 in 19, 1:100 in 5, 1:200 in 3, and 1:800 in 1. Seropositive rabbits were found in 21 (72.4%) of 29 properties, including 16 of 21 homes, 4 of 5 farms, and 1 of 3 pet shops. This is the first study of *T. gondii* infection in rabbits in Durango, Mexico. Results indicate that infected rabbits are a potential source of *T. gondii* infection in humans in Durango State.

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1. Introduction

Toxoplasma gondii is a widely distributed pathogen infecting animals and humans around the world (Dubey, 2010). Infections with *T. gondii* in rabbits may lead to fatal toxoplasmosis (Splendore, 1908; Bergmann et al., 1980; Dubey et al., 1992). In addition, rabbits infected with *T. gondii* are a source of infection for cats that then shed environmentally resistant oocysts (Dubey et al., 2011). Infected rabbits can be a source of infection to humans too. In fact, consumption of rabbit meat was recently associated with *T. gondii* infection in humans in Durango, Mexico (Alvarado-Esquivel et al., 2011a). Very little is known on the seroprevalence and correlates of *T gondii* infection in rabbits in the world. It is not clear what type of environmental or host characteristics may influence the seroprevalence of *T. gondii* infection in rabbits. Efforts to determine the magnitude of infection and its associated epidemiological characteristics should be encouraged since such information may help in planning preventive measures against *T. gondii* infection in rabbits. Therefore, we sought to estimate the seroprevalence and correlates of *T. gondii* infection in rabbits raised in Durango, Mexico.

2. Materials and methods

2.1. Rabbits studied

Through a cross sectional study design, 429 domestic rabbits were sampled from 29 properties in 6 municipalities of Durango State (Table 1) from July to September 2012. Properties included 21 homes where rabbits were raised in the backyards, 5 farms and 3 pet shops. The number of rabbits per property ranged from 1 to 76 (median

Table 1
Seroprevalence of *T. gondii* infection in domestic rabbits in Durango.

Municipality	Meters above sea level	Climate	Properties surveyed	Rabbits tested	Seroprevalence of *T. gondii* infection	
				No.	No.	%
Canatlán	2000	Semi-cold	2	19	11	57.9
Durango	1880	Temperate	18	242	48	19.8
Nombre de Dios	1730	Temperate	3	20	4	20
Poanas	1900	Semi-warm	4	58	5	8.6
San Juan del Rio	1700	Semi-dry	1	76	2	2.6
Vicente Guerrero	1950	Temperate	1	14	0	0
All			29	429	70	16.3

10). All properties were located in the Valley region of Durango State, Mexico. As a strategy for sampling rabbits, an intensive search for rabbits in farms, pet shops, veterinary clinics, animal raising associations, and properties in communities in the valley region was performed. In order to find properties with rabbits, the local veterinary association, community leaders and general population were asked for information, and a search in internet, newspapers, yellow pages, and animal exhibitions was performed. We were able to find a total of 31 properties with rabbits within the region explored during the study period. Of the 31 properties identified, 29 (93.5%) were sampled and 2 (1 farm and 1 home) were excluded because the owners did not cooperate for the sampling. Rabbits aged at least 3 months were included in the study. All rabbits in each property were sampled. We explored the characteristics of the rabbits with the aid of a standardized questionnaire administered to the rabbit owners and included date of sampling, municipality, type of property and management (farm, backyard, pet shop), area (urban, suburban, rural), breed, sex, age, health status, presence of cats in the property, and type of feeding. Several climates exist in the municipalities studied (Table 1) including semi-cold (mean annual (MA) temperature: 15.4 °C, MA rainfall: 550 mm) in 1 municipality, temperate (MA temperature: 16–19 °C, MA rainfall: 500–700.1 mm) in 3 municipalities, semi-warm (MA temperature: 25.9 °C, MA rainfall: 388 mm) in 1 municipality, and semi-dry (MA temperature: 18.0 °C, MA rainfall: 550.5 mm) in 1 municipality. The variables MA temperature and MA rainfall are commonly used for climate classification; however, they may overlap in some climates. Therefore, further characteristics affecting climates including latitude, terrain, and altitude may help for discrimination among climates. Table 1 shows the altitude of the municipalities studied. Other data concerning rabbits are summarized in Table 2.

2.2. Laboratory tests

A few drops of blood were obtained from each rabbit, and blood samples were transported to the laboratory on the day of collection. Sera were collected from whole blood by centrifugation and stored at −20 °C until tested. Rabbit sera were tested for *T. gondii* antibodies using 2-fold serial dilutions from 1:25 to 1:3200 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). There are no reported estimates of sensitivity and

specificity of MAT in rabbits at a given cut-off. However, a titer of 1:25 was used as cut-off for seropositivity in MAT in the present study as was used in previous studies in rabbits (Almería et al., 2004; García-Bocanegra et al., 2010) and other species (Dubey, 2010).

2.3. Statistical analysis

Statistical analysis was performed using Epi Info software version 3.5.4 (Centers for Disease Control and

Table 2
General characteristics of the 429 rabbits studied and seroprevalence of *T. gondii* infection.

Characteristics	Rabbits tested (No.)	Seroprevalence (%)
Age (months)[a]		
0.3–1	31	41.9
2–6	137	13.1
7–12	190	13.7
13–24	63	19
>24	5	20
Gender		
Male	137	12.4
Female	292	18.2
Breed		
Californian	109	15.6
Chinchilla	26	3.8
Mini-lop	15	26.7
New Zealand	122	23
Other pure[b]	12	8.3
Mixed	145	13.1
Area		
Urban	43	16.3
Suburban	82	20.7
Rural	304	15.1
Management		
Farm	145	7.6
Backyard	270	19.6
Shop	14	42.9
Presence of cats		
Yes	305	15.1
No	124	19.4
Health status		
Healthy	423	16.3
Ill	6	16.7
Food		
Concentrated	186	11.3
Mixed[c]	215	19.5
Fruits/vegetables/grains	28	25

[a] Sum does not add up to 429 rabbits due to 3 missing values.
[b] Butterfly Rhin (*n* = 3), Netherland dwarf (*n* = 3), Dwarf Hotot (*n* = 3), Japanese (*n* = 1), and Rex (*n* = 2).
[c] Concentrated and fruits, vegetables, and grains.

Prevention: http://wwwn.cdc.gov/epiinfo/) and SPSS version 15.0 (SPSS Inc., Chicago, IL). We used the Fisher exact test for comparison of the frequencies among groups. To assess the difference of seroprevalences of *T. gondii* infection among different climates, municipalities, and properties the Kruskal–Wallis test was used. A *P*-value of <0.05 was considered statistically significant. To avoid bias, missing values were excluded from the statistical analysis.

3. Results

Antibodies to *T. gondii* were found in 70 (16.3%) of 429 rabbits, with titers of 1:25 in 42, 1:50 in 19, 1:100 in 5, 1:200 in 3, and 1:800 in 1. Seropositive rabbits were found in 21 (72.4%) of 29 properties, including 16 (76.2%) of 21 homes, 4 (80%) of 5 farms, and 1 (33.3%) of 3 pet shops (Tables 1 and 2). Seroprevalence was different among properties and varied from 0 to 100%. Seropositive rabbits were found in 5 of 6 municipalities. There was a statistically significant difference in seroprevalence of *T. gondii* infection in rabbits among the 4 different climates (*P* < 0.001), the 6 municipalities (*P* < 0.001), and 29 properties (*P* < 0.001) studied. Increased seroprevalence was found in rabbits in a municipality with semi-cold climate located at 2000 m above sea level. The highest seroprevalence of *T. gondii* infection (41.9%) was observed in rabbits aged 0.3–1 month old (Table 2). Prevalence of *T. gondii* seropositivity varied also with management (*P* < 0.001) and type of feeding (*P* = 0.03) (Table 2). Increased seroprevalence was found in rabbits raised in backyards, and those for sale in a pet shop. All 6 rabbits of a pet shop in the Canatlán municipality were seropositive for *T. gondii*, they were 0.3 month old, cohabitated with a cat and other animals including chicken and turkeys, and were fed with a mixture of concentrated food and fruits, vegetables and grains. Type of feeding varied among properties with the same management. The highest seroprevalence of *T. gondii* infection (25%) was found in rabbits fed with a mixture of concentrated food and fruits, vegetables and grains. The seroprevalence of *T. gondii* infection was comparable among rabbits regardless their gender, breed, type of living area, presence of cats and health status (*P* > 0.05) (Table 2). Nevertheless, there was a single rabbit with ocular and lower respiratory tract disease that was seropositive (MAT titer 1:50) to *T. gondii*. Such a rabbit was the only one with clinical manifestations compatible with toxoplasmosis.

4. Discussion

Demand for rabbit meat for human consumption is increasing because of the leanness of their meat. Additionally, in Mexico there are no regulations concerning sale and slaughter of these animals. Therefore, infection in rabbits could be of importance in the epidemiology of toxoplasmosis. Additionally, wild rabbits are hunted and consumed by humans. Beverley et al. (1954) found Sabin Feldman dye test antibodies to *T. gondii* in 50.7% of 321 wild rabbits in England, and more importantly rabbit trappers and handlers were at higher risk of *T. gondii* seropositivity than general population. Subsequently, Beverley isolated a strain of *T. gondii* (called Beverley strain

by others and rabbit A strain by him) that was used widely to study the transmission of *T. gondii* (Beverley, 1959). These early studies by them and others (Elias, 1966; Munday, 1972; Cox et al., 1981) indicated that rabbits could be naturally infected. Recently, Almería et al. (2004) and García-Bocanegra et al. (2010) found MAT antibodies (titer 1:25 or higher) in 14.2% of 456 and 11.9% of 85 wild rabbits from Spain, respectively. However, seroprevalence data from domestic rabbits are limited. Fiigueroa-Castillo et al. (2006) detected antibodies in 26.9% of 286 rabbits from 3 rabbit farms in central Mexico (2 farms near Mexico City and 1 in state of Puebla). In the present study, the 16.3% seroprevalence of *T. gondii* infection in rabbits raised in the northern Mexican state of Durango is lower than that reported from rabbits in Central Mexico. However, these results are not directly comparable because of different laboratory methods and types of rabbit managements among the studies were used. Researchers of the other study used a qualitative in-house ELISA and examined rabbits from farms, while we used the MAT and examined rabbits from farms, backyards and pet shops.

T. gondii seropositivity in rabbits in the present study varied in different properties and municipalities in the Durango state but was similar in rabbits from rural, urban and suburban areas. The highest seroprevalence was found in the Canatlán municipality with semi-cold climate and located at the highest altitude (2000 m above sea level). In general, difference in altitudes among the municipalities was only few hundred meters. Climate may be a factor responsible of the differences in seroprevalences. However, further research to evaluate this factor is needed. We are not aware of a previous report of an association of *T. gondii* seropositivity and semi-cold climate. Cats were frequently observed in the properties explored. The lack of association of *T. gondii* infection and presence of cats in the properties in the present study does not exclude parasite contamination since cats enter in a number of properties other than their own. In fact, we have previously documented *T. gondii* infection in cats in Durango City located in the Valley region too. Antibodies to *T. gondii* were found in 21% of 105 (Alvarado-Esquivel et al., 2007) and 9.3% of 150 (Dubey et al., 2009) domestic cats from Durango City. These seropositive cats would have shed oocysts in the environment.

The highest (41.9%) seroprevalence found in the 0.3–1 month old rabbits might be due to colostrally derived antibodies but congenital infection cannot be excluded. Uhlíková and Hübner (1973) in Czech Republic reported asymptomatic congenital *T. gondii* infection in rabbits; 11.5% of newborn rabbits from 9 litters from 3 chronically infected dams were infected. Postnatal exposure to *T. gondii* in the rabbits studied is also a possibility because cats were present in their environment.

In the present work, backyard rabbits and rabbits for sale in a pet shop had the highest seroprevalence of *T. gondii* infection. With respect to backyard rabbits, the increased seroprevalence of *T. gondii* infection may be explained by the type of feeding. Backyard rabbits in Durango are frequently fed with fruits, vegetables and grains. These foods are not usually washed and might be contaminated with *T. gondii*. In fact, *T. gondii* seropositivity in humans in

Durango has been associated with eating unwashed raw fruits (Alvarado-Esquivel et al., 2011b). Further research to evaluate the role of type of feeding and management on the seroprevalence of *T. gondii* infection in rabbits is needed.

There are limitations of the study, including a low number of rabbits and properties sampled in some municipalities. This was due to scarcity of rabbits in some municipalities. This fact prevented for a cluster sampling approach. However, this study has also strengths including that this is the largest study performed in rabbits in Mexico, all rabbits in each property were sampled, and a widely accepted test for detecting anti-*T. gondii* antibodies (MAT) was used. Seropositivity in MAT has been linked to the presence of *T. gondii* in rabbits (Dubey et al., 2011). Although a validation of MAT was not performed in the current study, several studies indicate that a cutoff of 1:25 in rabbits and other animals is suitable for epidemiology studies and is useful for detecting animals with the presence of parasites (Dubey, 2010). Therefore, we used such cutoff in our study.

Results of the present study provide serological evidence of *T. gondii* infection in rabbits raised in the Valley region in Durango. Results are of concern since rabbit meat was recently associated with *T. gondii* infection in humans in Durango, Mexico (Alvarado-Esquivel et al., 2011a).

5. Conclusions

This is the first study that provides serological evidence of, and likely factors associated with *T. gondii* infection in rabbits raised in properties in Durango, Mexico. Results may help in planning of prevention measures against *T. gondii* infection.

Conflict of interest statement

No conflict of interest.

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