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Seroprevalence of *Toxoplasma gondii* in equids from Southern Spain

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ABSTRACT

Antibodies to *Toxoplasma gondii* were determined in serum samples from 616 equids (454 horses, 80 mules and 82 donkeys) in a cross-sectional study of 420 herds in Andalusia (Southern Spain), the region with the highest number of equids in Spain. Antibodies to *T. gondii* were found in 10.8% horses, 15.0% mules and 25.6% donkeys by using the modified agglutination test (MAT) at a cut-off of 1:25. Herd seroprevalence for horses, mules and donkeys was 14.7% (48/327), 23.9% (11/46) and 34.0% (16/47), respectively, and 75 herds (17.8%) had at least one seropositive animal. Significant differences in *T. gondii* seroprevalence were observed among species, with donkeys having the highest seroprevalence and horses the lowest ($P = 0.04$). Seroprevalence was significantly higher in herds with presence of domestic ruminants. This study is the first report of the presence of *T. gondii* antibodies in equine species in Spain and the first reporting *T. gondii* infection in donkeys in Europe. The presence of antibodies is indication of contact with the parasite and therefore, consumption of equine meat could be a potential source of human infection in Spain.

1. Introduction

Toxoplasma gondii is a zoonotic intracellular protozoan parasite of worldwide distribution [1]. Wild and domestic felids are the definitive hosts, excreting oocysts in feces. Humans and virtually all warm-blooded species can be intermediate hosts and can become infected by ingestion of food and water contaminated with sporulated *T. gondii* oocysts, by consumption of tissue cysts in infected animal tissues, or congenitally [1].

Although there are several serologic surveys for *T. gondii* infection in horses worldwide [1,2], information on horses is still scarce and, to our knowledge, nothing is known about *T. gondii* seroprevalence in horses in Spain. The present study reports the prevalence of *T. gondii* antibodies in sera of 616 equids (454 horses, 80 mules and 82 donkeys) from Andalusia (Southern Spain), the region with the highest number of horses in Spain.

2. Materials and methods

2.1. Study design

A cross-sectional survey was designed to analyze the seroprevalence of *T. gondii* in equine herds from Andalusia (Southern Spain; 36° N–38° 60′ N, 1° 75′ W–7° 25′ W). Andalusia is the region of Spain with the highest number of equids. In the last census in Andalusia [3], there were more than 202,000 horses, around 13,200 donkeys and 18,500 mules out of 425,000 equids in Spain. It has been estimated that in Andalusia the horse density is between 1.1 and 3.5 horses/km^2 in the eastern and western regions, respectively [3]. Andalusia also has the second-highest number of equine herds (16.4% of Spanish herds) with almost 20,000 herds [4] of any region in Spain.

In consideration of the number of herds in Andalusia ($n > 10{,}000$), an estimated prevalence of 50% (which provides the highest sample size in studies with unknown prevalence [5]), the desired precision was set at ±5% and confidence level at 95%, resulting in 385 herds to be sampled. A total of 420 herds were finally selected and the sampling was stratified by provinces according to the proportion of herds in each province. Geographical distribution of the sampled herds in each of the 8 provinces that constitute Andalusia (Almería, Granada, Málaga, Cádiz, Huelva, Seville, Córdoba and Jaén) is represented in Fig. 1. Within each

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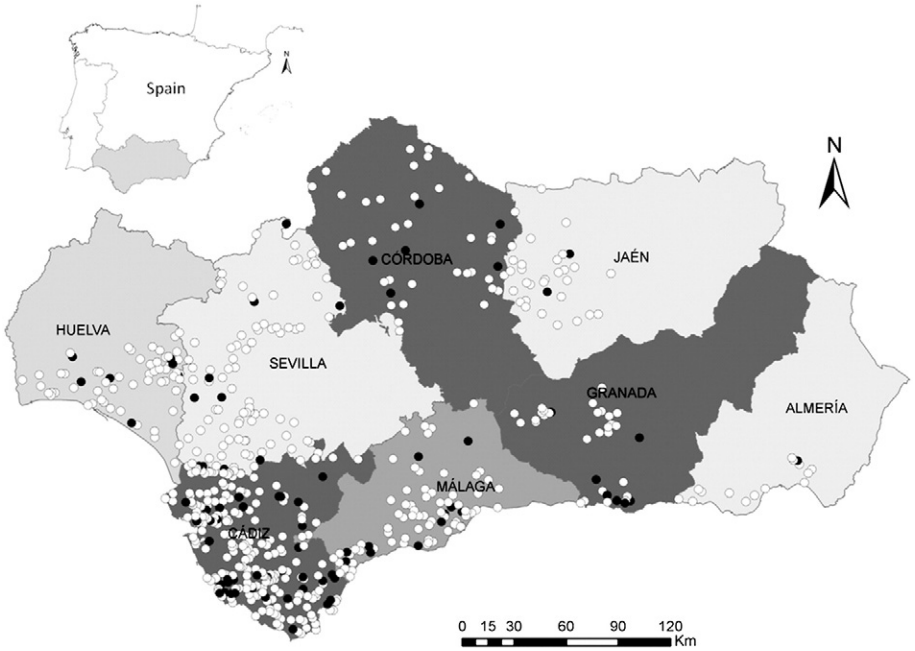

Fig. 1. Map of Andalusia (Southern Spain) showing the location of equids sampled. Black and white dots indicate seropositive and seronegative animals, respectively. The darker gray gradient represents higher seroprevalence against *T. gondii* in the different regions sampled.

herd between 1 and 3 horses were randomly selected. A total of 454 blood samples from horses were included in the study. In addition, blood samples from 80 mules (in 46 herds) and 82 donkeys (in 47 herds) were also collected in Cádiz Province.

2.2. Sample collection and serological analyses

Blood samples were collected by puncture of the jugular vein using a sterile collection system (Vacutainer®, Becton-Dickinson, USA). Samples were transported to the laboratory under refrigeration (4 °C) within 24–48 h of sampling. Blood samples were centrifuged at 400 *g* for 15 min at 4 °C. Sera were separated and stored at −20 °C until assayed for antibodies to *T. gondii* by the modified agglutination test (MAT) as previously described [5]. Sera were diluted to 1:25, 1:50, and 1:500 and those with sera with doubtful results were reexamined. A commercial positive control (Toxotrol-A, Biomerieux, France) diluted from 1:25 to 1:3200 (with a minimum titer of 1:200) was included in each test. Negative controls were also included in all tests. A titer of ≥1:25 was considered indicative of *T. gondii* infection in equids as has been previously considered in these species [6–8].

Epidemiological data were gathered through an on-farm interview with the owners. In total, 23 explanatory variables were included in the analysis. Variables were grouped by a) individual data: species (horse, mules and donkey), age classes (young: <5 years, adult: 5–14 years and geriatric: >14 years), genders (mare, stallion and gelding), and breed (pure bred and crossbreed), b) herd data: location (province), herd size, activity (farming, leisure and work), presence of other domestic animal species (cats, dogs, domestic and wild birds, domestic and wild ruminants and rodents) and type of housing (outside and individual or collective shelter), c) biosecurity: cleaning and disinfection methods and protocols, pest control programs (insects and rodents), water sources and sanitation of water.

2.3. Statistical analysis

The prevalence of antibodies against *T. gondii* was estimated with the exact binomial confidence intervals of 95% [9]. Associations between the serological results and independent variables were analyzed using a Pearson's chi-square test and by Fisher's exact test when observations/category were <6. Differences between categories were analyzed using the Tukey test. Differences were considered statistically significant when $P<0.05$. Statistical analyses were performed using SPSS 15.0 (Statistical Package for Social Sciences (SPSS) Inc.).

3. Results

Antibodies to *T. gondii* were detected in 82 of 616 equids (13.3%; $CI_{95\%}$: 10.6–16.0). Titers of 1:25, 1:50 and ≥1:500 were detected in 55 (67.1%), 22 (26.8%), and 5 (6.1%) animals, respectively.

Individual seroprevalence was 10.8% ($CI_{95\%}$: 7.9–13.7) for horses, 15.0% ($CI_{95\%}$: 7.2–22.8) for mules and 25.6% ($CI_{95\%}$: 16.1–35.1) for donkeys. Statistically significant differences were observed among species ($P=0.04$) (Table 1; Fig. 1). Seroprevalence was significantly higher in donkeys compared to horses ($P=0.001$). No differences were observed between mules and donkeys or horses. Differences among species were also observed, with horses (12.2% of 90 animals) showing significantly lower seroprevalence ($P=0.05$) compared to donkeys when only animals from Cadiz, where the three species were sampled, were considered.

Table 1
Seroprevalence of *T. gondii* in equids from Andalusia, Southern Spain.

Variable	Exposure levels	% MAT positive	Number/ overall[a]	*P*-value
Species*	Horses	10.8	49/454	0.04
	Mules	15.0	12/80	
	Donkeys	25.6	21/82	
Sex	Females	15.5	38/245	0.12
	Males	11.9	44/371	
Age	Foals	11.9	14/118	0.72
	Adults	13.7	48/350	
	Geriatrics	13.5	20/148	
Presence of cats	Yes	14.1	53/375	0.10
	No	10.2	23/225	
Shelter (indoors, outdoors)	Yes	11.5	45/390	0.06
	No	16.4	37/226	
Domestic ruminants presence*	Yes	20.1	27/134	0.01
	No	11.4	44/386	

[a] Missing values omitted.
* *P-value*<0.05.