

Short communication

Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain

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Abstract

Roe deer (*Capreolus capreolus*) is an important game animal in Spain. Sera from 278 roe deer from eight areas in mainland Spain were assayed for antibodies to *Toxoplasma gondii* by modified agglutination test (MAT). Titers of 1:25 or higher were found in 109 (39.2%) of 278 deer. No significant differences in antibody prevalence were found between sex or age categories. In contrast, significant differences in seroprevalence between locations were evident. Roe deer from the Northern coastal habitats (high humidity and roe deer density) had the highest prevalence, compared with low prevalence in Central Spain (arid areas and low roe deer density). There was a positive correlation between antibody prevalence and mean annual rainfall ($r_s = 0.85$, $n = 8$, $P < 0.01$). These findings have environmental and/or public health implications because venison can be an important meat source of *T. gondii* infections for humans and feral cats.

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Keywords: *Toxoplasma gondii*; Roe deer; *Capreolus capreolus*; Seroprevalence; Spain

1. Introduction

Virtually all warm-blooded hosts can be intermediate hosts of *Toxoplasma gondii* (Dubey and Beattie, 1988). Humans, especially hunters, may get infected by consumption of uncooked venison (Sacks et al., 1983; Ross et al., 2001), and contacting the agent while dressing game (Dubey, 1994).

The roe deer (*Capreolus capreolus*) is a small sized cervid (subfamily Odocoileinae) abundant throughout

Europe. In Spain, roe deer is an important game animal and its population is increasing (Acevedo et al., 2005; García-Sánchez et al., 2007; García-Sanmartín et al., 2007). In the present study we determined seroprevalence of *T. gondii* infection in roe deer from Spain and provided epidemiologically important information on risk factors including effect of sex, age, geographic origin and climate (rainfall) on the host parasite relationship.

2. Materials and methods

Sera from 278 roe deer from eight localities in mainland Spain (Fig. 1) were used for the present study. Sampling sites in Valdés, Portal and Cantabric

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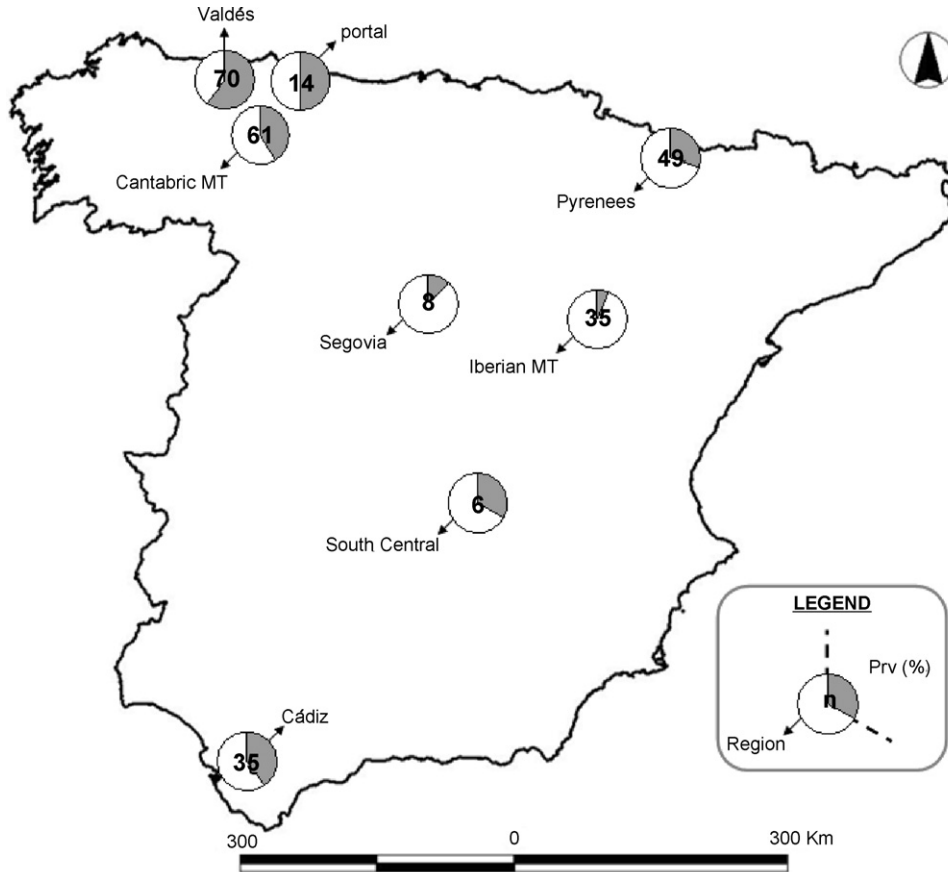


Fig. 1. Map of peninsular Spain showing sampling areas, sample size (n) and prevalence of *T. gondii* roe deer in each area.

Mountains, located in Asturias (Northern Spain) are characterized by a coastal Atlantic climate with high mean annual rainfall, spread human population and high roe deer density (Ruiz-Fons et al., 2008). Sampling sites in the Pyrenees have moderate rainfall and a more continental climate, low roe deer density and sparse human population (Gortazar et al., 2000). Sampling sites from Central Spain (Segovia and Iberian Mountains) and South-Central Spain are characterized by Mediterranean climate with low annual rainfall, low human density and very low roe deer density (Acevedo et al., 2005). Sampling sites in Cádiz (Southern Spain) have a Mediterranean climate with moderate annual rainfall, intermediate human density, and abundant roe deer.

Blood samples were collected from the heart or chest cavity of dead animals, centrifuged, and the sera obtained were stored at -20°C until assayed. Age was determined based on tooth eruption, and each individual was classified either as juvenile (0–2 years) or adult (>2 years), based on the descriptions by Sáenz De Buruaga et al. (1991).

Antibodies to *T. gondii* were determined by the modified agglutination test (MAT) as described previously (Dubey and Desmonts, 1987; Sobrino et al., 2007). Each serum sample was tested at dilutions of 1:25, 1:50, 1:100, and 1:500. Positive and negative controls were included in each run (Sobrino et al., 2007). Sera with a titer of 1:25 or higher were considered positive and those with doubtful results were re-tested. Although the specificity and sensitivity of MAT have not been evaluated for the diagnosis of toxoplasmosis in roe deer, it is the most evaluated and specific test for the diagnosis of toxoplasmosis in animals, particularly pigs (Dubey et al., 1995; Dubey, 1997).

Seroprevalence was statistically analyzed considering the variables geographical area, rainfall, sex and age of the animals. Data about roe deer density were not available from some of the sampled areas and different methods were used in some of the other areas, therefore density could not be included as a variable in the statistical analysis. The statistical data analysis was performed using the SPSS 14.0 Statistical program. We

Table 1
Prevalence of *T. gondii* antibodies in roe deer from Spain by sampling areas

Sampling sites	No. examined	No. positive	Prevalence (%)	S.E. 95%CI
Valdés	70	42	60	48–71
Cantabric Mountains	61	25	41	29–54
Portal	14	7	50	23–76
Pyrenees	49	16	32.7	20–46
Iberian Mountains	35	2	5.7	1.03–20
Segovia	8	1	12.5	0.64–50
South-Central	6	2	33.3	6.3–73
Cádiz	35	14	40	25–57

used nonparametric statistics with 95% confidence level and a P -value <0.05 was considered significant.

3. Results

Antibodies against *T. gondii* were found in 109 (39.2%) of 278 roe deer (Table 1). No significant differences in antibody prevalence were found between sex (females 46.3%, males 38.1%; $\text{Chi}^2 = 1.4$, 1 d.f., $P > 0.05$) or age categories (juveniles 48%, adults 38%; $\text{Chi}^2 = 2.0$, 1 d.f., $P > 0.05$). In contrast, significant differences were evident between locations (Table 1, Fig. 1). Prevalence ranged from 6% (Iberian Mountains, an arid region with low roe deer density) to 60% (Valdés, Asturias, a humid Atlantic climate locality with high roe deer density) (Kruskal–Wallis test, 7 d.f., $\text{Chi}^2 = 33.0$, $P < 0.001$). There was a significant correlation between mean local antibody prevalence and mean annual rainfall ($r_s = 0.85$, $n = 8$, $P < 0.01$; Fig. 2).

4. Discussion

The results of the present study supplement the data on seroprevalence of *T. gondii* in roe deer in Europe

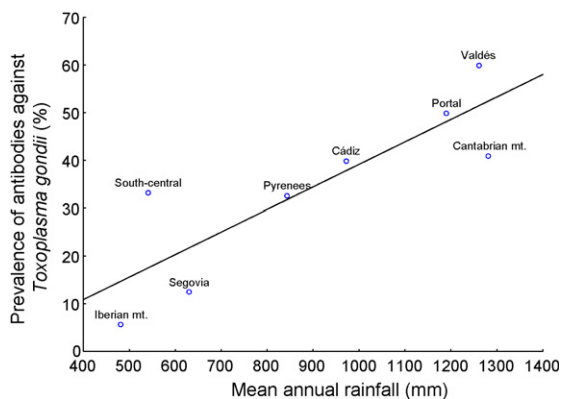


Fig. 2. Linear regression plot of mean annual rainfall (mm) and prevalence of *T. gondii* roe deer in each area.

(Table 2). In addition, we correlated seroprevalence to epidemiological factors. It is noteworthy that the seroprevalence of *T. gondii* in roe deer from Norway (Vikoren et al., 2004) is similar to the present study although these countries are geographically distinct; in both studies similar serological tests were used. The DAT and MAT are essentially identical tests.

No significant effects of sex or age were observed in the seroprevalence levels of *T. gondii* in Spanish roe deer, in contrast with some studies that have found age-related differences in *T. gondii* antibody prevalence in Odocoileinae (e.g. Vanek et al., 1996; Vikoren et al., 2004). These results suggest vertical or congenital transmission as a main route of *T. gondii* infection in our area. Recently, *T. gondii* was isolated from naturally infected white-tailed deer fetuses from the USA (Dubey et al., in press). On the other hand, marked differences in the prevalence of antibodies against *T. gondii* were observed among locations. Roe deer from the Northern coastal habitats had the highest prevalence recorded in European roe deer, while populations from Central Spain had a low prevalence of infection. Location has also been found to be a significant factor for *T. gondii* seroprevalence in wild rabbits (Almeria et al., 2004) and red deer (Gauss et al., 2006) in Spain.

Our results indicated that environment rather than the host are important in the probability of *T. gondii* infection in roe deer in Spain. This fact could be due to different reasons. Firstly, humidity and moderate temperatures may favour oocyst survival and sporulation in the environment, facilitating parasite spread and maintenance (Dubey and Beattie, 1988; Smith and Frenkel, 1995). In fact, a positive and significant correlation of antibody prevalence of *T. gondii* among locations and rainfall was observed in the present study. Secondly, density populations of roe deer from the Atlantic regions (three of the study areas: Valdés, Cantabric Mountains and Portal) are higher than those from dry Mediterranean habitats, and density is a well-known risk factor for many diseases (Acevedo et al.,

Table 2
Prevalence of *T. gondii* antibodies in roe deer from Europe

Country	Source	No. tested	Prevalence (%)	Serologic test ^a (cut-off)	Reference
Czech Republic	Zoo	4	50.0	IFAT (1:40)	Sedláčková and Bartoňová (2006)
	Wild	95	13.0	DT (1:4)	Hejlíček et al. (1997)
Italy	Wild	207	27.0	LAT (1:32)	Gaffuri et al. (2006)
Norway	Wild	760	33.9	DAT (1:40)	Vikoren et al. (2004)
Norway and Sweden	Wild	8	63.0	DT (1:8)	Kapperud (1978)
Spain	Wild	33	21.8	MAT (1:25)	Gauss et al. (2006)
	Wild	278	33.9	MAT (1:25)	Present study

^a DT, dye test, IFAT; indirect fluorescent antibody test; DAT, direct agglutination test.

2007), and it was the main factor affecting seroprevalence of *T. gondii* infection in wild boars in our country (Gauss et al., 2005). However, data concerning roe deer density were not available from some of the sampled areas or using uniform methodology in others and, therefore, this factor could not be analyzed in the present study. Scrubs are the dominant diet of roe deer, and although the plant species consumed differ between geographical regions, it is not expected that the proportion of browsing differs much among locations (Fandos et al., 1987). Therefore, most probably differences in diet were not the main factor for the observed differences among regions. Another possibility for the observed results could be due to man-related factors such as presence of domestic cats and human population dispersion, which can also affect the epidemiology of *T. gondii* (Hejlíček et al., 1997). Human settlements are spread out in forest areas and villages throughout the Cantabric region and domestic cats are abundant in those rural areas. Hence, contact of roe deer with cats in the surroundings farms and villages is more probable in this region than in Mediterranean areas of Spain, where wildcats and stray cats are sparse (Millán et al., 2002). Further studies about these latter factors could clarify their role in the epidemiology of *T. gondii* in roe deer in the analyzed areas.

The present report indicates a widespread and high exposure to *T. gondii* in Spanish roe deer. This fact should be taken into account since consumption of raw or inadequately cooked meat, as well as handling carcasses of roe deer should be regarded as a potential source of infection for humans. Roe deer may also be a good indicator species for environmental contamination with *T. gondii* in Spain.

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