Toxoplasma gondii in sympatric domestic and wild ungulates in the Mediterranean ecosystem

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Abstract

Toxoplasma gondii is a zoonotic protozoan of worldwide distribution. The present study provides information on risk factors affecting *T. gondii* infection in domestic and free-ranging wild ungulates sharing habitats in Mediterranean ecosystems in Spain. Serum samples from 482 extensively reared domestic ruminants and 2351 wild ungulates were tested for *T. gondii* antibodies using the modified agglutination test (MAT, cut-off 1:25). *Toxoplasma gondii* seroprevalence was 41.2% of 194 sheep, 18.6% of 199 cattle and 5.6% of 89 goats. The main risk factors associated with infection in livestock were the presence of cats, feeding on the ground and at stubble fields. In wild ungulates, *T. gondii* antibodies were detected in 10.5% of 1063 red deer, 15.6% of 294 fallow deer, 5.6% of 216 European mouflon, 5.6% of 90 Spanish ibex, 13.6% of 22 roe deer and 18.6% of 666 wild boars. The risk factors affecting *T. gondii* infection in wildlife were species, age and hunting season. Significantly higher seroprevalence was found in domestic ruminants, particularly in sheep, compared to the wild species tested. The present study indicates widespread exposure to *T. gondii* among domestic and wild ungulates in Southern Spain, with significant differences among species sharing the same ecosystem. The high seroprevalence observed in domestic ruminants, particularly in sheep, reinforces the need for farm management practices to control the risk factors associated with *T. gondii* infection in extensively reared livestock. Consumption of raw and undercooked food products from domestic and wildlife species may have important implications for public health.

Keywords

Toxoplasma gondii
Domestic ruminants
Wild ungulates
Spain

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Introduction

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Toxoplasma gondii is a zoonotic protozoan with an indirect cycle with domestic and wild felines as definitive hosts (DH), and all warm-blooded species as intermediate hosts including human beings. One third of the human population worldwide is estimated to be infected by this parasite (Montoya and Liesenfeld 2004). *Toxoplasma gondii* is a food-borne pathogen (EFSA 2007). Consumption of raw or undercooked meat products containing tissues cysts is the major risk factor associated with human toxoplasmosis (Cook et al. 2000). Although *T. gondii* infection in human is usually asymptomatic, it can have fatal consequences in immunosuppressed people and pregnant women (Dubey 2010). Toxoplasmosis is also considered an occupational disease for abattoir workers, butchers and hunters who may become infected during evisceration and handling of meat (EFSA 2007).

Toxoplasma gondii has been isolated from different domestic and wild ungulate species worldwide (reviewed by Dubey 2010). Toxoplasmosis generates severe economic losses in livestock, being one of the major causes of abortion, foetal malformations, pre-term deliveries and stillbirths in sheep and goats. Moreover, the European Food Safety Agency identified the meat of large-game species as an important zoonotic source for *T. gondii* infection (EFSA 2007). Spain is the first and third country in Europe in the number of hunted red deer and wild boars per year, respectively (Apollonio et al. 2010). Over 133,000 red deer (*Cervus elaphus*) and 221,000 wild boars (*Sus scrofa*) are hunted annually in this

country (MAPAMA 2016). Game meat and product are not only consumed in this country, but also around 850 tons are exported annually to other European countries (SSCCC 2009). Previous studies have shown widespread distribution of *T. gondii* in domestic and wild ungulates in Spain, reporting a high heterogeneity in the presence of the parasite depending on the geographic area (García-Bocanegra et al. 2013; Gauss et al. 2005, 2006; Calero-Bernal et al. 2016; San Miguel et al. 2016). However, few studies have assessed the presence of this parasite in domestic and wild species that share the same habitat (Panadero et al. 2010), preventing the determination of the factors that influence the parasite epidemiology in the livestock and wildlife interface. The aim of the present study was to determine the risk factors associated with the *T. gondii* infection in sympatric domestic and free-ranging large-game ungulates from Mediterranean ecosystems in Southern Spain destined for human consumption.

Materials and methods

All samples from wild ungulates were collected from legally hunted animals, by authorised hunters with the correct permits and licences and with the permission of landowners. Animals were sampled during the hunting season under Spanish and EU legislation. No animals were specifically hunted for this study and ethical approval by an Institutional Animal Care and Use Committee was not deemed necessary. The collection of blood samples from domestic ruminants was part of the official Animal Health Campaigns of Regional Government of Andalusia, Spain.

A total of 2351 wild ungulates were sampled in 101 hunting estates from Southern Spain (Fig. 1), during the hunting seasons 2011–2012 and 2015–2016. Blood samples were collected from hunted red deer ($n = 1063$), wild boar ($n = 666$), fallow deer (*Dama dama*, $n = 294$), European mouflon (*Ovis aries*, $n = 216$), Spanish ibex (*Capra pyrenaica hispanica*, $n = 90$) and roe deer (*Capreolus capreolus*, $n = 22$) (Table 1). Samples were obtained from the thoracic cavity or by endocranial venous sinuses puncture (Arenas-Montes et al. 2013; Jiménez-Ruiz et al. 2016).

Fig. 1

Geographic distribution of the hunting states and farms tested, and results obtained for the presence of *Toxoplasma gondii* antibodies in Southern Spain. **a** Black and grey spots indicate positive and negative hunting areas for the presence of *T. gondii*, respectively. **b** Black and grey areas indicate positive and negative hunting areas (polygons) and farms (squares) for the presence of *T. gondii*, respectively. The size of squares represents seroprevalence levels in the farms sampled

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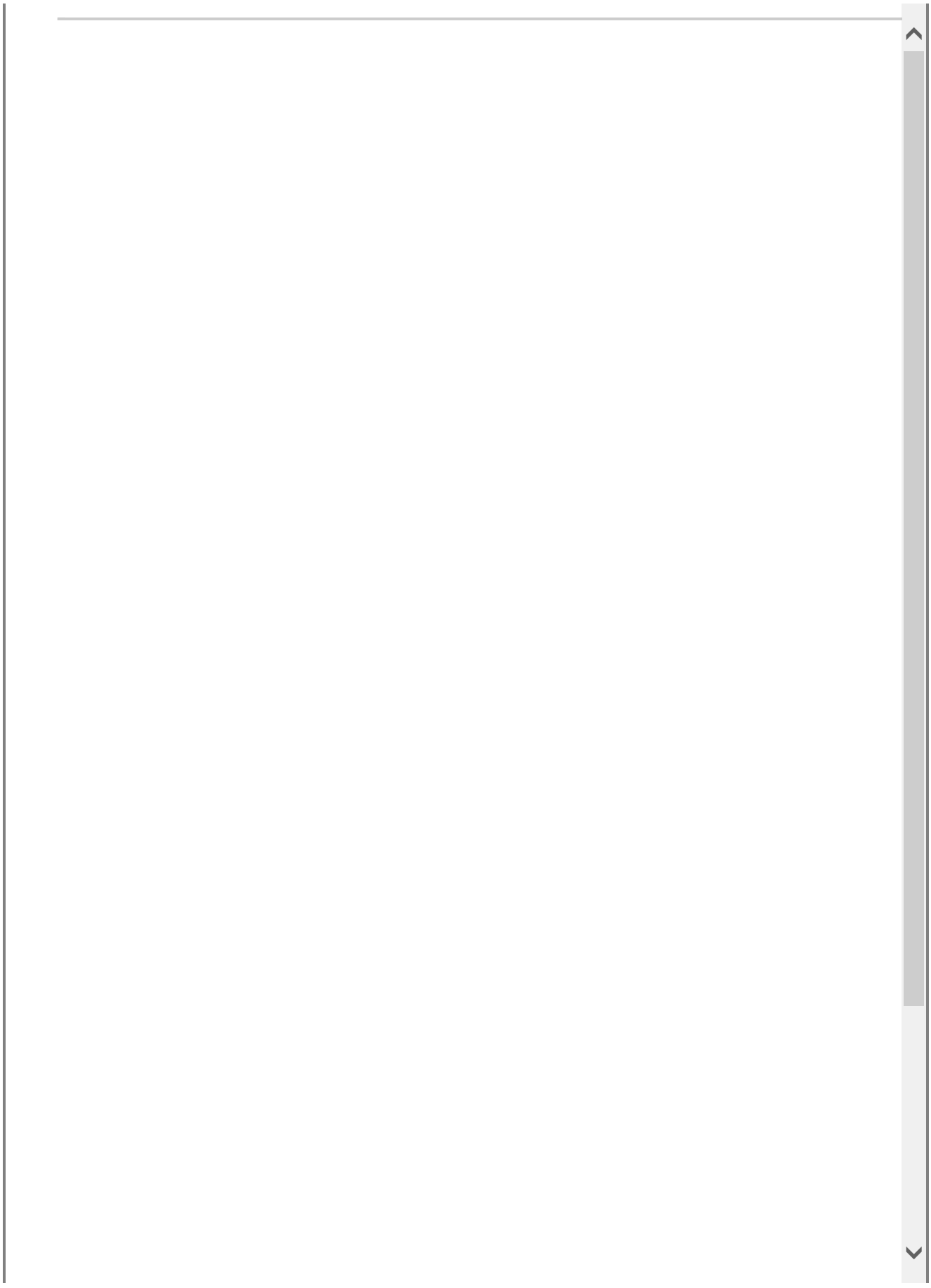

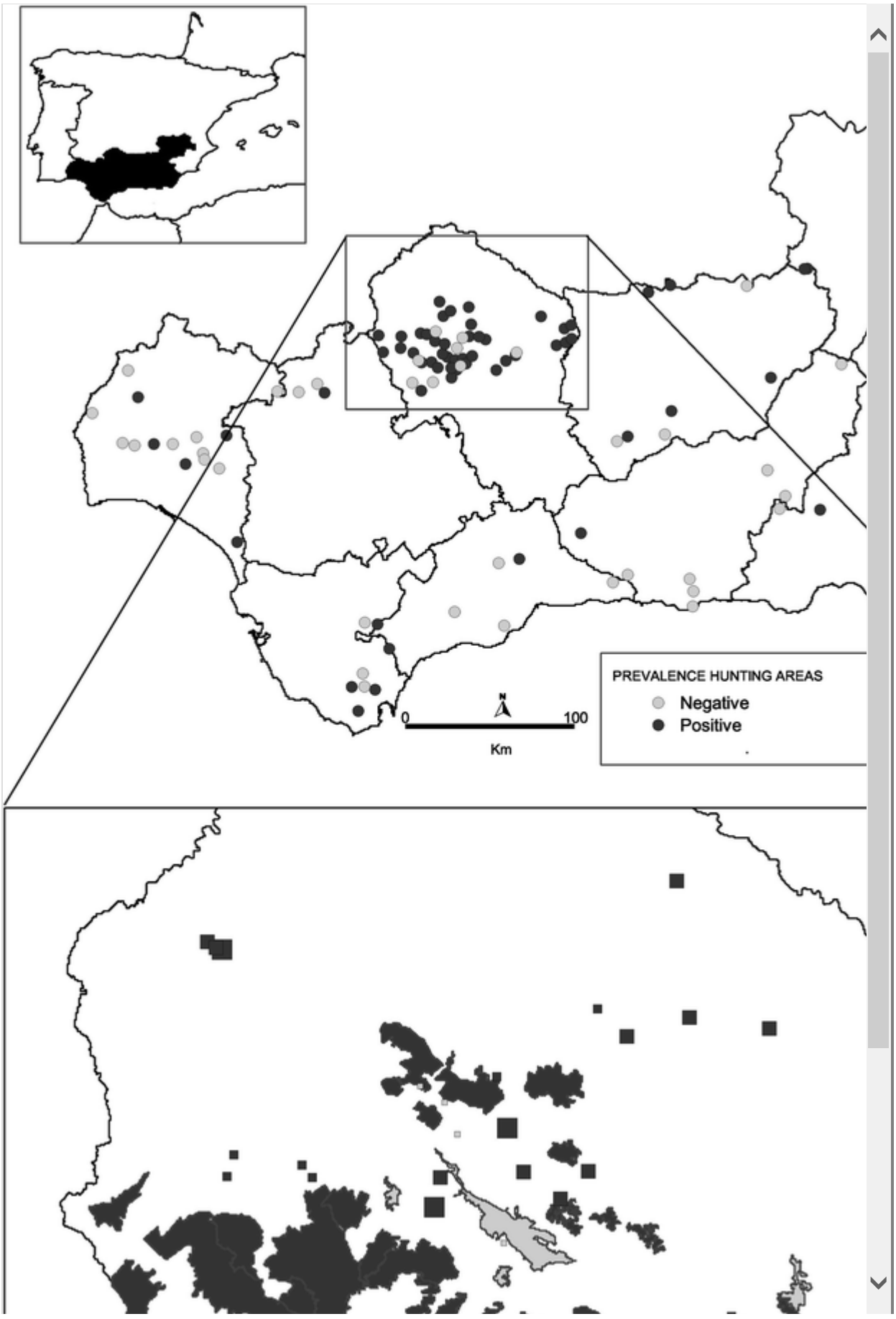
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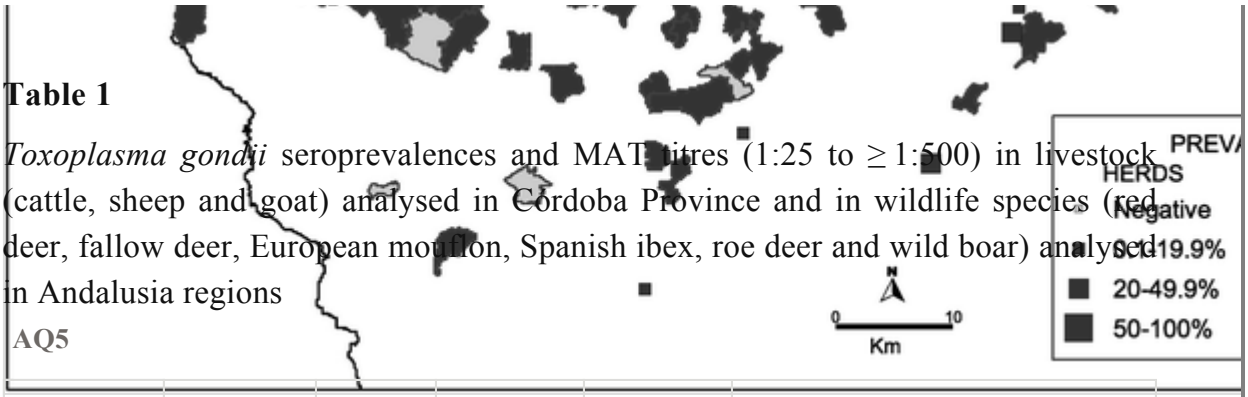

Table 1

Toxoplasma gondii seroprevalences and MAT titres (1:25 to ≥ 1:500) in livestock (cattle, sheep and goat) analysed in Córdoba Province and in wildlife species (red deer, fallow deer, European mouflon, Spanish ibex, roe deer and wild boar) analysed in Andalusia regions

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Host	Area	No. tested	No. positive	% positive	No. of animals with MAT titres			
					1:25	1:50	1:100	≥ 1:500
Cattle	Córdoba	199	37	18.6	27	6	3	1
Sheep	Córdoba	194	80	41.2	8	3	40	29
Goat	Córdoba	89	5	5.6	4			1
Total livestock		482	122	25.3	39	9	43	31
Red deer	Córdoba	676	73	10.8	31	23	15	4
	Other areas	387	39	10.1	16	22	1	
	Total	1063	112	10.5	47	45	16	4
Fallow deer	Córdoba	201	30	14.9	16	10	4	
	Other areas	93	16	17.2	12	2	2	
	Total	294	46	15.6	28	12	6	
Mouflon	Córdoba	178	10	5.6	6		4	
	Other areas	38	2	5.3		2		
	Total	216	12	5.6	6	2	4	
Spanish ibex	Other areas	90	5	5.6	0	5		
Roe deer	Other areas	22	3	13.6	1	2		

Host	Area	No. tested	No. positive	% positive	No. of animals with MAT titres			
					1:25	1:50	1:100	≥ 1:500
Wild boar	Córdoba	604	115	19.4	37	49	25	4
	Other areas	62	9	14.5	4	2	3	
	Total	666	124	18.6	41	51	28	4
Total wildlife		2351	302	12.8	123	117	54	8

The number of domestic ruminant samples collected was based on an estimated prevalence of 50% (which provides the highest sample size in studies with unknown prevalence) and the large population of domestic ruminants in Córdoba Province ($n > 10{,}000$) with precision set at ± 5% and confidence level at 95% (95% CI). These calculations resulted in a total of 385 animals to be sampled. A total of 482 domestic ruminants were finally selected using a convenience sampling according to the proportion of domestic ruminant herds present in the studied hunting areas. Therefore, blood samples from 482 extensively reared domestic ruminants, including 199 cattle, 194 sheep and 89 goats, were collected from 29 farms during 2013, in herds with close proximity or sharing grazing areas to the hunting areas where the wild ungulates were sampled (Fig. 1). The animals were classified into three age groups based on tooth replacement: yearlings (< 1 year old), subadults (1 to 2 years old) and adults (> 2 years old) (Sáenz de Buruaga et al. 2001).

Blood samples were centrifuged at 400×*g* for 15 min and sera were stored at − 20 °C until analysis. Serum samples were tested for antibodies against *T. gondii* by the modified agglutination test (MAT) (Dubey and Desmonts 1987). Sera were tested at 1:25, 1:50, 1:100 and 1:500 dilutions. Sera with titres of 1:25 or higher were considered positive. MAT has been used extensively for the diagnosis of toxoplasmosis in both domestic and wildlife species (reviewed by Dubey 2010).

The prevalence of antibodies against *T. gondii* was estimated from the ratio of positive samples to the total number of samples tested, with the exact binomial confidence intervals of 95% (95% CI). Epidemiological information related to the sampled animals, sampling site habitat, management, biosecurity measures and environmental data were collected by direct interview with farmers and gamekeepers at each sampling site to obtain information on exposure levels to potential risk factors. A risk factors analysis was conducted in the province of Córdoba, the region with the most homogeneous sampling in wild ungulates and where domestic ruminants were sampled. A chi-square and Fisher's exact test were used to test the relevance of the explanatory variables in the risk of an animal being exposed to *T. gondii*. Covariates correlated with a P value < 0.10 in the bivariate analysis variable in each test were included from further analysis. Biologically plausible confounding factors were assessed using Mantel-Haenszel analysis and confounding was considered to be potentially significant if odd ratios (ORs) shifted appreciably (> 30%). Finally, a multiple logistic regression analysis (Hosmer and Lemeshow 2000) was performed including risk factors potentially associated with *T. gondii* exposure (likelihood-ratio Wald's test, $P < 0.05$). The goodness of fit was assessed using the Hosmer-Lemeshow goodness-of-fit test. SPSS 22.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analyses.

Results

Antibodies against *T. gondii* were detected in 41.2% of the sheep (95% CI 34.3–48.2), 18.6% of the cattle (95% CI 13.2–24.0) and 5.6% of the goats (95% CI 0.8–10.4) (Table 1). The overall herd prevalence of antibodies to *T. gondii* was 86.2% and herd seroprevalences for species were 81.8% (9/11) in sheep, 75% (3/4) in goats and 86.7% (13/15) in cattle. Significantly higher seroprevalence was observed in domestic ruminants (25.3% of 482 animals tested) compared to wild ungulates (13.7% of 1659) sampled in Córdoba Province ($P < 0.001$). The seropositivity was also significantly higher in sheep compared to the other species analysed ($P < 0.05$).

Risk factors associated to *T. gondii* infection in domestic ruminants were the species, presence of cats, feeding on ground, and feeding at stubble fields (Table 2). Significantly higher seroprevalence was found in sheep compared to the other domestic ruminant species ($P < 0.05$). Seroprevalence in domestic ruminants from farms with presence of cats was 31.0% (109 of 352), while in the absence of cats, the seroprevalence was 10.0% (13 of 130). Herds in which food was provided directly on the ground showed significantly higher seroprevalence levels than those in which food was not provided on the ground (32.7% of 153 versus 21.9% of 329, respectively). The seroprevalence level in domestic ruminants fed at stubble fields was significantly higher compared to those that did not feed at stubble fields (42.6% of 216 samples versus 11.3% of 266 samples, respectively).

Table 2

Risk factors associated to *Toxoplasma gondii* seropositivity in domestic ruminant species in Southern Spain

Variable	Category	*β*	Sig.	OR	95% CI	
Species	Goat	*	*	*	*	*
	Cattle	1.151	0.022	3.162	1.177	8.498
	Sheep	1.861	< 0.001	6.461	2.417	17.111
Presence of cat	No	*	*	*	*	*
	Yes	1.068	0.002	2.910	1.469	5.763
Feeding on ground	No	*	*	*	*	*
	Yes	0.59	0.018	1.803	1.105	2.943
Feeding at stubble field	No		*	*	*	*
	Yes	1.344	< 0.001	3.836	2.307	6.377
Asterisk indicates reference category						

Antibodies against *T. gondii* were detected in 10.5% of the red deer (95% CI 8.7–12.4%), 15.6% of the fallow deer (95% CI 11.5–19.8%), 5.6% of

the mouflons (95% CI 2.5–8.6%), 5.6% of the Spanish ibexes (95% CI 0.8–10.3%), 13.6% of the roe deer (95% CI 0.0–28.0%) and in 18.6% of the wild boars (95% CI 15.7–21.6%) (Table 1). At least one seropositive animal was detected in 62 (61.4%) of the 101 hunting areas sampled. Risk factors associated with *T. gondii* infection in wildlife in Córdoba Province were species, age and hunting season (Table 3). Significantly higher seroprevalences were observed in red deer, fallow deer and wild boar compared to mouflon. Prevalence of antibodies against *T. gondii* in wild ungulates was significantly higher in adults (16.1% of 883) compared to subadults (10.9% of 495) and yearlings (10.8% of 241). Significant differences in the prevalence of levels of antibodies were observed among hunting seasons, being 2.3 to 2.6 times higher in hunting seasons from 2011 to 2012 and 2013 to 2014, compared to hunting season 2014 to 2015 (Table 3).

Table 3

Risk factors associated to *Toxoplasma gondii* seropositivity in wild ungulates species in Southern Spain

Variable	Category	*β*	Sig.	OR	95% CI	
Species	Mouflon	*	*	*	*	*
	Red deer	0.843	0.018	2.324	1.155	4.675
	Fallow deer	1.335	0.001	3.801	1.760	8.212
	Wild boar	1.559	0.000	4.756	2.388	9.471
Age	Yearlings	*	*	*	*	*
	Subadults	− 0.166	0.521	0.847	0.511	1.405
	Adults	0.516	0.003	1.675	1.187	2.362
Hunting season	2011–2012	0.817	0.000	2.264	1.509	3.397
	2012–2013	0.944	0.002	2.570	1.418	4.659
	2013–2014	0.839	0.000	2.313	1.497	3.575
	2014–2015	*	*	*	*	*

Asterisk indicates reference category

Variable	Category	*β*	Sig.	OR	95% CI	
	2015–2016	0.371	0.121	1.449	0.907	2.317
Asterisk indicates reference category						

Discussion

Toxoplasma gondii is present in many heterogeneous epidemiological scenarios. Although this parasite is distributed worldwide, the factors that determine its presence in animal populations are still poorly understood. The results obtained in the present study indicate that *T. gondii* infection is widespread in the studied area, but significant differences were observed in sheep compared to the other analysed domestic and wild species. Management, ecology and feeding behaviour are potential factors to explain the high *T. gondii* exposure in this species.

The main risk factors associated with *T. gondii* infection in domestic ruminants were the presence of cats, feeding on the ground and feeding at stubble fields. The presence of felines has been shown to be an important risk factor for infection in livestock and wildlife in several studies worldwide, including Spain (Dubey 2010; García-Bocanegra et al. 2010a; García et al. 2012). Only one previous study analysed the seroprevalence of *T. gondii* in domestic cats in the South of Spain (Millán et al. 2009) and reported antibodies in more than 50% of the 53 cats analysed. In regard to wild felines, García-Bocanegra et al. (2010b) reported widespread and continuous presence of the parasite with high seroprevalence (62.8% of 129) in the critically endangered Iberian lynx (*Lynx pardinus*). Interestingly, feeding on the ground and feeding at stubble fields increased the seroprevalence of *T. gondii* in domestic ruminants as indication of environmental contamination and an increased probability of ingestion of *T. gondii* oocysts in the soil and/or even in dry, hard cereal areas. *Toxoplasma gondii* oocysts remain infective in the environment for years (Dubey 2010). The risk factors identified in domestic ruminants in the present study are an indication of contact with oocysts and horizontal

transmission as the main way of *T. gondii* transmission in Mediterranean ecosystems in Southern Spain.

Toxoplasmosis in sheep is a major cause of abortion, foetal malformations, pre-term deliveries and stillbirths and thus can result in severe economic losses (Dubey 2010). In Spain, previous studies showed that *T. gondii* is widely prevalent in sheep (Mainar et al. 1996; García-Bocanegra et al. 2013). Although in the present study seroprevalences in cattle and goats were significantly lower compared to sheep, higher seroprevalence levels were observed in both species in a study in the neighbouring province of Seville, Andalusia (García-Bocanegra et al. 2013). In that study, goats showed the lowest seroprevalence levels compared to sheep and cattle, which is in accordance with our observations in the present study. A low seropositivity was also found in goats (2.8%) in central Spain (Mainar et al. 1996), while a high seroprevalence (48%) was detected in this species in northwestern Spain (Díaz et al. 2016). However, the results from different studies in different locations are not strictly comparable because of different serological tests, cut-off values, and geographical and climatic differences. Furthermore, the seroprevalence obtained in goats in the present study should be interpreted with care because of the limited number of analysed samples.

The possibility of zoonotic diseases transmitted from large-game species to humans is of public health concern. In the present study, the prevalence of antibodies against *T. gondii* in wild ungulates ranged from 5.6 to 18.6%, in similar levels to those observed in domestic ruminants, other than sheep. The highest seroprevalence was found in wild boars. Swine become infected with *T. gondii* through ingestion of food and water contaminated with sporulated oocysts, by ingestion of tissue cysts in infected animal tissues or congenitally (Dubey 2010). The seroprevalence observed in wild boars (18.6% of 666) in the present study is in accordance with that previously detected in Spain (23.8% of 2881) (Calero-Bernal et al. 2016) and France (17.6% of 148) (Richomme et al. 2009). However, other studies performed in this species in the Iberian Peninsula have reported a higher seropositivity to *T. gondii* (38.4% of 507) (Gauss et al. 2005).

The main risk factors associated with *T. gondii* infection in wild ungulates were age and hunting seasons. The higher seroprevalence in older animals reflects a cumulative likelihood for exposure to *T. gondii* and lifelong persistence of antibodies. The results are in accordance with that previously observed in wild and domestic species and suggested that horizontal transmission was the main route of infection in these species (Panadero et al. 2010; García-Bocanegra et al. 2013; Calero-Bernal et al. 2016). Significant decreasing seroprevalence levels were observed in the last sampled hunting seasons, which could be explained by the fact that those years have been the driest of the last decade in the area studied and a humid environment favours the persistence of infective *T. gondii* oocysts (Dubey 2010).

The FAO/WHO reported a global multi-criteria-based ranking of food-borne parasites with *T. gondii* in the top five of importance (FAO/WHO 2014). In addition, the EFSA (2007) recommended *T. gondii* monitoring of game species. In Andalusia, around 56,000 red deer and 43,000 wild boars are hunted every year (MAPAMA 2016). Based on the number of seropositive animals found in the present study, around 66 tons of meat and derived products from red deer and 60 tons of meat and derived products from wild boar could be contaminated by *T. gondii.* Of these, 51 tons of meat and derived products from red deer and wild boar is annually consumed as raw or undercooked food. Further studies are warranted to elucidate the *T. gondii* infection levels in meat and derived products from infected wild ungulates and the risk of transmission of this food-borne zoonotic disease.

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Compliance with ethical standards

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Animals were sampled during the hunting season under Spanish and EU legislation. No animals were specifically hunted for this study and ethical approval by an Institutional Animal Care and Use Committee was not deemed necessary.

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Conflict of interest The authors declare that they have no conflict of interest.

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AQ8

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