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ORIGINAL ARTICLE

Toxoplasmosis in Natural Populations of Ungulates in France: Prevalence and Spatiotemporal Variations

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Abstract

Toxoplasmosis is characterized by a complex epidemiology. The risk of infection for humans depends on their contact with infective oocysts in a contaminated environment and on the amount of tissue cysts located within consumed meat. Unfortunately, the prevalence of tissue cysts is largely unknown for game species. Although herbivorous game species are a source of infection for humans, the level of infection found in wildlife can also be used to estimate environmental contamination. The aim of this study was to estimate the prevalence of *Toxoplasma gondii* infection and analyze its temporal dynamics in one population of chamois (*Rupicapra rupicapra*), one of mouflon (*Ovis gmelini musimon*), and two of roe deer (*Capreolus capreolus*) in France, surveyed during a period of 6 to 28 years. Taking into account individual risk factors, we specifically analyzed the relationship between *T. gondii* prevalence and meteorological conditions that may influence oocyst survival. Serum samples from 101 chamois, 143 mouflons, and 1155 roe deer were tested for antibodies against *T. gondii* using the modified agglutination test (MAT), an enzyme-linked immunosorbent assay (ELISA) assay, or both. Using MAT with a threshold of 1:6, seroprevalence was 14.7% in mouflon, 16.8% in chamois, and 43.7% in roe deer. In mouflon and roe deer, seroprevalence was positively correlated with age and/or body mass, in accordance with the hypothesis that antibodies have long-term persistence. In roe deer, seropositivity differed between the two populations and changed linearly over time between 1983 and 2010, increasing by a factor 1.75 every 10 years. Moreover, in this species, the highest prevalences were found during dry and cold years or during warm and moist years, depending on the population. Our results suggest that the risk for people to acquire infection through game meat increases over time, but with high variability according to the population of origin and meteorological conditions of the year.

Key Words: *Toxoplasma gondii*—Ungulate—Spatial heterogeneity—Seroprevalence—Modified agglutination test—ELISA.

Introduction

Parasites that may infect several host species raise specific management issues, especially when resistant forms contaminate the environment. Multiple and concurrent sources of infection, including the environment and intermediate hosts, lead to a complex pattern of infection. The control of such diseases requires a multidisciplinary approach combining environmental, educational, and behavioral changes (Chomel 2008).

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The complex life-cycle protozoan *Toxoplasma gondii* is among the most ubiquitous parasites (Tenter et al. 2000). Felids are the only definitive hosts; they excrete environmentally resistant oocysts, which may in turn infect any mammal or bird. Intermediate hosts for *T. gondii*, including humans, are infected by ingesting food or water contaminated with oocysts, by ingesting tissue cysts from other intermediate hosts (carnivorism), or through vertical transmission (Dubey 2010). Because of these multiple routes, the prevention of *T. gondii* infection in humans makes it essential to identify and quantify the risk from all potential sources (Kijlstra and Jongert 2008). The consumption of undercooked meat is generally considered a major source of infection (Cook et al. 2000). However, the level of meat contamination has received unequal attention. Data from domesticated species clearly indicate a decrease over time during recent decades (Kapperud et al. 1996, Gilot-Fromont et al. 2009, Dubey 2010, Halos et al. 2010), whereas the spatiotemporal variations in the level of infection of game species have rarely been assessed. On the other hand, the level of contamination of soil and water has been poorly evaluated (Dumètre and Dardé 2003), and its variations are unknown.

Game species represent an interesting source of information in this context. The consumption of game is considered an emerging risk of toxoplasmosis, due to ingestion, evisceration, and handling (Dubey 1994, Ross et al. 2001, Kijlstra and Jongert 2008). Moreover, herbivorous species are essentially infected through environmental contamination, thus their level of infection reflects environmental contamination by oocysts.

Several studies have reported spatial heterogeneity in seroprevalence in ungulate game species. In geographic areas within the Arctic Circle (north of 66°33′39), prevalences range from 0.4% to 8.6% in moose (*Alces alces*), black-tailed deer (*Odocoileus hemionus*), dall sheep (*Ovis dalli*), caribou (*Rangifer tarandus*), and bison (*Bison bison*) (Zarnke et al. 2000, Jokelainen et al. 2010, Stieve et al. 2010, Malmsten et al. 2011). Low prevalences in northern territories have been attributed to the low density of domestic cats where human population is very scarce. Geographical variations in *T. gondii* prevalence have also been reported under temperate climates. In Spain, prevalence in red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) ranged from 5.7% to 75% depending on the presence of cats and climate (Gauss et al. 2006, Gamarra et al. 2008). However, long-term monitoring data to assess whether prevalence varies over time in wild animals are lacking.

The aim of this study was to estimate the prevalence of *T. gondii* infection and assess its temporal dynamics in chamois (*Rupicapra rupicapra*), mouflon (*Ovis gmelini musimon*), and roe deer (two populations). This work is based on demographic and epidemiological monitoring of French populations during a period of 6 to 28 years. The level of infection of ungulates was expected to vary with time, following both variations in meteorological conditions and long-term changes due to climate, environment, or population, and also to differ according to the species, age, and population considered. For this reason, each population was analyzed separately, and the effect of individual factors was taken into account before analyzing the year-to-year variability. We also estimated which part of the temporal variability was related to a linear trend over years, to annual meteorological variations influencing oocyst survival (rainfall and temperature), or to other nonelucidated effects of year.

Materials and Methods

Sampling sites

All study areas are reserves monitored by the French National Game and Wildlife Agency (Office National de la Chasse et de la Faune Sauvage ONCFS) (Fig. 1). They differ in their climatic conditions, size, and presence of felid species that may contaminate the environment (Table 1). Three felid species are potentially present in these areas—domestic cats (*Felis catus*), European wildcat (*Felis silvestris*), and the European lynx (*Lynx lynx*). The presence of European wildcats and lynxes has been confirmed in the sites where they were potentially present (Table 1). The presence of domestic cats cannot be ruled out in any of the reserves. On the basis of reports of incidental observations conveyed by observers in each reserve, we qualitatively classified the presence of domestic cats as rare (when less than approximately one record per year could be reported), occasional (when sightings have been reported at least once a year), or not confirmed (when we could not obtain any confirmation of an observation in the reserve). The potential of soil contamination of the three species is related to their density and home range size. When they are present, European wildcats and lynxes are sparse: 0.1–0.6 wildcats/km^2 (Sarmento et al. 1996, Anile et al. 2012) and 1.0–2.1 lynxes/100 km^2 (Pesenti et al. 2013). However, their mean annual home range may be large: 122–404 ha for European wildcats (Germain et al. 2008) and 31,900–62,500 ha for lynxes (Herfindal et al. 2005), versus 2–220 ha for domestic cats (Germain et al. 2008). Each infected individual may thus potentially contaminate a large area.

Chamois were trapped in the Bauges Reserve in the northern French Alps (45°40′N, 6°13′E). A population of 2000 chamois occupies this unfenced reserve of 5205 ha, located in a calcareous massif (Pioz et al. 2008). The mountain climate in Bauges is characterized by cold, snowy, and long winters and warm temperate climate with enough rainfall in the summer months. European wildcats, lynx (*L. lynx*) and rare domestic cats have been observed in this area (Table 1; J.M. Jullien, personal communication).

Mouflons were caught in the reserve of Caroux in southern France (43°38′N, 2°58′E) that covers 17,072 ha in a mountainous area at the interface between Atlantic and Mediterranean influences (Garel et al. 2004). Wild felids are not present in this study area, but domestic cats have already been observed in the area closest to the nearest village (M. Garel, personal communication).

Roe deer were trapped from two study sites, Chizé and Trois Fontaines, which differ markedly in environmental characteristics and population dynamics (Gaillard et al. 2003, Morellet et al. 2007). The Reserve of Chizé is located in western France (46°05′N, 0°25′W) and is an enclosed forested reserve of 2614 ha. This site has an oceanic climate with Mediterranean influences, characterized by mild winters and hot, dry summers (Gaillard et al. 2003).

European wildcats are not present in this part of France (Say et al. 2011), and few domestic cats are observed in this

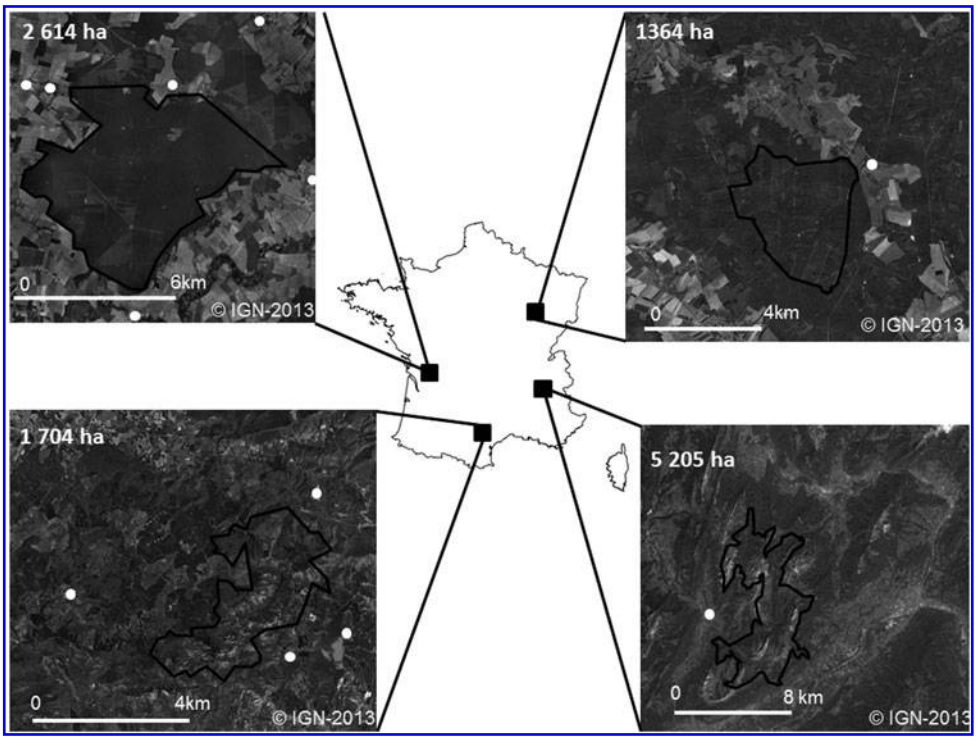

FIG. 1. Location of each study sites (reserves). Clockwise: Chizé, Trois-Fontaines, Caroux and Bauges. White dots represent nearest villages.

closed area (N. Guyon, personal communication). In contrast, the Territoire d'Etude et d'Expérimentation of Trois Fontaines l'abbaye is located in northeastern France (48°43′N, 4°55′E) and comprises 1364 ha of enclosed forested area. The climate is continental with cold winters and hot summers (Gaillard et al. 1993, 2003). This study site is located within the range of the European wildcat (Say et al. 2011), and the presence of wildcats has been confirmed. On the contrary, the presence of domestic cats has not been confirmed but cannot be ruled out despite the presence of a fence along the reserve.

TABLE 1. MAIN FEATURES OF EACH SITE STUDIED FOR THE PREVALENCE OF *TOXOPLASMA GONDII* IN WILD UNGULATES

Study sites	*Reserve*	*Study species*	*Climate*	*Presence of domestic cats Felis catus*	*Presence of other felid species*
Bauges	Unfenced reserve 5205 ha	Chamois	Mountain climate: cold, snowy, and long winter (+1.4°C); warm summer with rainfall (17.8°C)	Confirmed, rare	European wildcats (*Felis silvestris silvestris*) Lynx (*Lynx lynx*) Confirmed
Caroux	Unfenced reserve 17,072 ha	Mouflons	Atlantic and mediterranean influences: cold winter (+2.2°C); warm, dry summer (17.3°C)	Confirmed, occasional	None
Chizé	Enclosed reserve 2614 ha	Roe deer	Oceanic climate: mild winter (+5.5°C); hot and dry summer (+20.4°C)	Confirmed, occasional	None
Trois Fontaines	Enclosed reserve 1364 ha	Roe deer	Continental climate: cold winter (+1.9°C); hot but not dry summer (+20.0°C)	Not confirmed	European wildcats confirmed

For each site, the table gives the type of reserve, the species studied, the climatic conditions, with the mean temperature of the coldest (in winter) and hottest (in summer) months in °C, the estimated level of presence of domestic cats, and the presence of other felid species.

Neither the Chizé nor Trois Fontaines sites experienced major changes in landscape or population management during the study period. The forest cover has been maintained, and population size has been kept stable except during the period between 2003 and 2005 when an experiment was performed in Trois Fontaines. During this period, population size was no longer restrained and reached 450 individuals, against 200–300 during other periods. However, since roe deer become infected through oocysts or vertical transmission, we did not expect *T. gondii* risk to change with roe deer density. In contrast, the meteorological conditions evolved during the period. In Trois Fontaines, mean temperatures increased by $+1.19$°C over the 28 years of study ($R^2 = 0.30$, $p = 0.007$), whereas during the same period, the level of annual rainfall decreased by 200 mm ($R^2 = 0.21$, $p = 0.028$). In Chizé, no change in temperature was observed during the 2000–2010 period ($R^2 = 0.09$, $p = 0.41$), whereas annual rainfall also decreased by 400 mm ($R^2 = 0.45$, $p = 0.047$).

Sampling and diagnostic design

Sera were collected from a total of 101 chamois captured from 2001 to 2006, 143 mouflons captured from 2001 to 2006 (no data in 2002), 843 roe deer from Trois Fontaines (1983–2010), and 312 roe deer from Chizé (2000–2010, no data in 2001, 2003 and 2009). At each capture, age (in years), sex, and body mass were recorded and a blood sample was collected. Sera were centrifuged and stored at -20°C until assay. Each year, a part of the sera were analyzed using the enzyme-linked immunosorbent assay (ELISA) method with the commercial kit Chekit-Toxotest-Anti-IgG-Ruminant® (bioMérieux, France, now called Toxotest Antibody Test Kit, IDEXX) according to the manufacturer's instructions. Positive and negative control samples were provided in the kit. Diagnosis was performed by comparing the optical densities (OD) obtained for samples and controls. Percentage of inhibition (%I) was obtained by the formula: $\%I = 100 - [(\text{sample OD} \times 100)/\text{mean negative control OD}]$. If %I was at least 30%, the sample was considered positive according to the manufacturer's instructions. Since 2007, the same sera were also assayed using the modified agglutination test (MAT; Dubey and Desmonts 1987). Moreover, sera remaining in the serum bank from previous years were tested with the MAT to compare with ELISA results. The MAT assay was performed using successive two-fold dilutions starting at 1:6, and results were expressed as the highest positive dilution.

The first step of analysis was to estimate the concordance between both tests, using the Kappa coefficient (Dohoo et al. 2003). Kappa varies between -1 and 1, and a value of 0 or below means random concordance, whereas kappa = 1 indicates a complete concordance. The value of kappa was computed using the thresholds 1:24, 1:12, and 1:6 for MAT. Risk factor analyses were also performed for the same three thresholds to check whether similar patterns were obtained with different dilutions.

Meteorological data

Oocysts are expected to survive best when the local environment is moist and temperate (Dumètre and Dardé 2003). We used the levels of rainfall (millimeters of water) and temperature (°C) during the 12 months preceding each capture as proxy for the local conditions that may influence oocyst survival and thus seroprevalence in roe deer. Daily sums of rainfall and average temperature were recorded from the weather station located in Lescheraine, Caroux, Beauvoir, and Saint-Dizier, which are located between 5–10 km of each study site, respectively, in the Bauges reserve, Caroux reserve, Chizé, and Trois-Fontaines. Concerning temperatures, we used the average of daily mean temperatures over 12 months before capture. For precipitation amounts, we used the amount of rainfall during the last 12 months that preceded the capture.

We also considered the North Atlantic Oscillation (NAO) winter index, which represents a measure of the difference in pressure level between Iceland and the Azores and provides a single variable to sum up, in a large geographic area, interannual differences in a number of weather variables, such as temperature, wind speed and direction, and precipitation (Hurrell 1995). We used the NAO winter index covering the period from December to March. NAO values for the winter preceding each capture (year N) were extracted from www.cgd.ucar.edu/$\sim$jhurrell/nao.html. We also tested the NAO value for year N $-$ 1 to analyze the influence of past climate variations. We expected the risk of toxoplasmosis to be the highest either during cool years or during warm and moist years (Frenkel et al. 1975, Dumètre and Dardé 2003, Lélu et al. 2012).

Statistical analysis

We analyzed the relationship between potential explanatory variables and antibody carriage, considered as a binary outcome (presence/absence) in three separate datasets corresponding to the three species. We first considered the individual characteristics of age, sex, body mass (unavailable for mouflons), and differentiated populations for roe deer. Age was categorized in four classes: Juvenile (<1 year old), yearling (1–2 years old), prime-aged adults (3–5 years old), and old individuals ($\geq$6 years old). The duration of antibody persistence has not been measured in the species studied; however, they are phylogenetically close to domestic ungulates. Antibodies persist several years in sheep (Dubey 2009), but probably not as long in cattle (Dubey and Thulliez 1993, Gilot-Fromont et al. 2009). In mouflon, which is the species most closely related to sheep, we thus expected seroprevalence to increase with age, whereas the prediction was not clear in other species. After selecting the model with appropriate individual variables using the method described below, and for roe deer populations only, we tested the effect of environmental variables.

In this aim, we used a generalized linear mixed model (GLMM). In addition to the annual meteorological conditions measured through weather stations and NAO, we also considered a random effect of year. This random variable allowed accounting for all unmeasured factors that may vary among year and influence disease risk, and to study other effects after taking into account all the interannual variability. To identify significant variables, the best-fitted model was selected using the Akaike information criterion (AIC; Burnham and Anderson 1992). Only models with $\Delta AIC < 2$ compared to the model with the lowest AIC value were selected. When several models had a $\Delta AIC < 2$, we selected the most parsimonious, *i.e.*, with the fewest parameters. At each

step (individual variables, environmental factors for roe deer), all possible models were tested.

To quantify the association between each variable and serological status, we computed odds ratios (ORs) and their 95% confidence intervals (CIs) for parameter estimates and their standard errors (SE). The significance of each effect was tested using Wald tests, which test the effect of a null value for parameter by comparing the ratio (estimated parameter/SE) to a normal distribution (Dohoo et al. 2003). All statistical analyses were performed using R version 2.13.1 software (R Development Core Team 2010).

Results

For chamois and roe deer, the highest agreement between MAT and ELISA test was obtained using a 1:6 threshold (Table 2). We considered the 1:6 threshold for all species for homogeneity, but we also provided prevalences at other thresholds (Table 3). At the threshold 1:6, prevalence ranged from 14.7% in mouflon to 50.5% in roe deer from Trois Fontaines. Crude prevalence also varied according to sex and age (Table 4). A clear increase with age was observed in Trois Fontaines, where sample sizes are the largest, whereas other populations showed more complex patterns. Moreover, the results of model selection for each species are given in Table 5. Analyses considering 1:24, 1:12, and 1:6 thresholds selected similar models (not shown).

Only models with $\Delta AIC < 2$ or those interesting for interpretation are provided in Table 5. Models retained to explain seroprevalence with individual factors included body mass for chamois and age for mouflon and roe deer. For chamois, every additional kilogram increased the probability of being seropositive by 1.11 (95% CI 1.00–1.22). In mouflons, the probability of being positive increased with age and was the highest in animals in age class 3 (Table 6). In roe deer, the best model for individual variables included age, population, and their interaction (Table 4), and the final model with spatiotemporal variables included the interaction between rainfall and temperature. The relationship between age and prevalence differed between Chizé and Trois Fontaines (Table 6). In Chizé, all OR values comparing each age class to juveniles were higher than 1, but the only significant one was the comparison between yearlings and juveniles (OR = 3.79 [1.42–10.14]. In Trois Fontaines, the seroprevalence was generally higher to the one found in Chizé (OR = 3.67 [1.13–11.89]), and the contrast between juveniles and old adults was significantly more pronounced than in Chizé (OR = 1.80 [1.54–16.21]).

The analysis of temporal variations first showed a linear increase with time, OR = 1.05 per year (95% CI 1.01–1.09; Table 6). The selected model retained the effect of an interaction between the levels of rain and temperature (Tables 5 and 6, Fig. 2): the predicted prevalence was maximal when weather conditions were either warm and moist (above 14°C and 1000 mm rain), which happened only in Chizé, or cool and dry (below 10°C and 750 mm rain), this condition being observed only in Trois Fontaines. Figure 3 represents the between-year variations of seroprevalence, using annual intercepts of the mixed models, before and after considering the linear increase with time and meteorological variables. Even after considering the linear temporal trend and meteorological effects, a strong unexplained temporal variability remained.

Table 2. Agreement Between Modified Agglutination Test and ELISA for *T. gondii* Antibodies for Chamois, Mouflons, and Roe Deer

A. MAT 1:6 /ELISA

	+/+	+/−	−/+	−/−	n	*Kappa*
Chamois	3	14	6	7	97	0.13
Mouflon	5	14	4	112	135	0.31
Roe deer	23	1	5	32	61	0.80

B. MAT 1:12 /ELISA

	+/+	+/−	−/+	−/−	n	*Kappa*
Chamois	2	7	7	81	97	0.18
Mouflon	0	9	9	117	135	−0.08
Roe deer	21	0	7	33	61	0.77

C. MAT 1:24/ELISA

	+/+	+/−	−/+	−/−	n	*Kappa*
Chamois	0	2	9	86	97	0
Mouflon	0	2	9	124	135	0.17
Roe deer	13	0	15	33	61	0.48

Three thresholds of MAT, 1:6 (A), 1:12 (B), and 1:24 (C), were compared to ELISA results.

n, number tested; ELISA, enzyme-linked immunosorbent assay; MAT, modified agglutination test.

Discussion

In this study, we present the first analysis on the presence of *T. gondii* in wild ungulates in a spatiotemporal framework. We bring new elements to explain temporal and spatial variability of *T. gondii* infection risk by investigating the relative effects of individual characteristics and environmental conditions in roe deer populations.

The MAT test does not require specific reagents and thus is particularly appropriate to study seroprevalence in wild species (Vikoren et al. 2004, Gauss et al. 2006, Aubert et al. 2010). Here, for all species studied, the highest values of concordance between MAT and ELISA tests were obtained with a 1:6 threshold for the MAT test; this threshold is lower than 1:12 and 1:24 thresholds often used. However, *T. gondii* was isolated previously from red deer with a titer of 1:6 (Aubert et al. 2010).

Prevalence values were consistent with other studies conducted in Europe, although different thresholds were used. In roe deer, using a 1:25 threshold, Gauss et al. (2006) found antibodies in 21.3% of 33 roe deer, and Aubert et al. (2010) obtained 40% seroprevalence from 60 animals. However, considering the contrast we observed between our two populations, these results are not comparable because of different populations sampled, and without information on the local climate or presence of felids. Interestingly, in a comparison between populations conducted in Spain by Gamarra et al. (2008), seroprevalence varied from less than 10% to 75%, with a strong positive correlation between seroprevalence and the local level of annual rainfall. In a survey of sheep meat in France, Halos et al. (2010) also detected a high variability among French regions, which was

Table 3. Seroprevalence of *T. gondii* in Several Populations of Ungulates in France

		MAT agglutination titer			*ELISA*
Species study area		*1:24*	*1:12*	*1:6*	*ELISA positive*
Chamois, Bauges	*n* positive/total	2/101	9/101	17/101	9/97
	Prevalence (%); 95% CI	1.90 [0.24–6.90]	8.91 [4.15–16.24]	16.83 [10.12–25.57]	9.27 [4.33–16.88]
Mouflons, Caroux	*n* positive/total	2/143	9/143	21/143	6/135
	Prevalence (%);95% CI	1.40 [0.17–4.96]	6.30 [2.92–11.61]	14.68 [9.33–21.57]	4.44 [1.65–9.42]
Roe deer, Trois Fontaines	*n* positive/total	300/843	375/843	426/843	28/61
	Prevalence (%); 95% CI	35.60 [32.35–38.92]	44.48 [41.09–47.91]	50.53 [47.10–53.96]	45.90 [33.06–59.15]
Roe deer, Chizé	*n* positive/total	37/312	49/312	79/312	NA
	Prevalence (%); 95% CI	11.86 [8.49–15.97]	15.70 [11.85–20.22]	25.32 [20.58–30.53]	NA
Roe deer, Total	*n* positive/total	337/1155	424/1155	505/1155	28/61
	Prevalence (%); 95% CI	29.18 [26.57–31.89]	36.70 [33.92–39.56]	43.72 [40.83–46.63]	45.90 [33.06–59.15]

For each population and threshold, the numbers of positive/number tested are indicated, as well as seroprevalence in % and its 95% confidence interval.
MAT, modified agglutination test; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval; NA, not available.

Table 4. Seroprevalence of *Toxoplasma gondii* in Chamois, Mouflon, and Roe Deer, According to Age and Sex

		Chamois		*Mouflon*		*Roe deer Trois Fontaines*		*Roe deer Chizé*	
Age	*Sex*	n *positive/ total*	*Prevalence (%); 95% CI*	n *positive/ total*	*Prevalence (%); 95% CI*	n *positive/ total*	*Prevalence (%); 95% CI*	n *positive/ total*	*Prevalence (%); 95% CI*
Juvenile	Female	2/22	9.10 [1.12–29.16]	2/25	0.08 [0.98–26.03]	25/114	21.93 [14.72– 30.65]	5/25	20.0 [6.83– 40.70]
	Male	3/22	13.64 [2.90–34.91]	2/33	6.06 [0.74– 20.23]	26/111	23.42 [15.91– 32.41]	1/24	4.17 [0.10– 21.12]
Yearling	Female	2/13	15.38 [1.92–45.45]	0/6	0.00 [0.00– 45.92]	75/145	51.72 [43.28– 60.09]	13/44	29.54 [16.76–45.20]
	Male	1/5	20.0 [0.50–71.64]	2/18	11.11 [1.37–34.71]	39/90	43.33 [32.92– 54.20]	19/44	43.18 [28.35– 58.97]
Prime-aged adults	Female	7/24	29.20 [12.61–51.09]	12/34	35.29 [19.74–53.51]	86/133	64.66 [55.90– 72.75]	13/57	22.81 [12.74–35.84]
	Male	1/2	50.0 [1.26– 98.74]	2/18	11.11 [1.37– 34.71]	66/110	60.00 [50.22–69.22]	12/48	25.00 [13.64– 39.59]
Old adults	Female	1/13	7.69 [0.19– 36.02]	1/6	16.67 [0.42– 64.12]	68/88	77.27 [67.10– 85.53]	13/49	26.53 [14.95–41.08]
	Male	0/0	0	0/3	0.00 [0.00–70.76]	41/52	78.85 [65.30–88.94]	3/21	14.28 [3.04–36.34]

CI, confidence interval.

Table 5. Summary of Model Selection for the Seroprevalence of Toxoplasmosis in Three Species, Using AIC

Model	*Deviance*	*AIC*	*ΔAIC*
		Chamois* n = *101	
Mass + (1|year)	**86.1**	**92.1**	**0**
Age * Mass + (1|Year)	74.7	92.7	0.6
Age + Mass + (1|Year)	80.8	92.8	0.7
Age + Sex + Mass + (1|Year)	80.6	94.6	2.5
1 + (1|Year)	91.5	95.5	3.4
Age + (1|Year)	86.5	96.5	4.4
Sex + (1|Year)	91.5	97.5	5.4
		Mouflon *n* = 143	
Age + (1|Year)	**107.8**	**117.8**	**0**
Age + Sex + (1|Year)	106.2	118.2	0.4
Sex + (1|Year)	112.3	118.3	0.5
1 + (1|Year)	115	119	1.2
Age * Sex + (1|Year)	102	120	2.2
		Roe deer *n* = 1 155	
		Individual-level variables	
Age * Pop + (1|Year)	**1 312**	**1 330**	**0**
Age * Pop + Sex + (1|Year)	1 311	1 331	1
Age * Pop + Mass + (1|Year)	1 312	1 332	2
Age + (1|Year)	1 463	1 473	143
1 + (1|Year)	1 551	1 555	225
		Environmental variables	
Rain * Temp + Age * Pop + Year + (1|Year)	**1 289**	**1 315**	**0**
Rain * Temp + NAO + Age * Pop + Year + (1|Year)	1 289	1 317	2
Rain * Temp + NAO_{-1} + Age * Pop + Year + (1|Year)	1 289	1 317	2
NAO + Age * Pop + Year + (1|Year)	1 297	1 319	4
Rain * Temp + Age * Pop + (1|Year)	1 297	1 321	6
Rain + Age * Pop + Year + (1|Year)	1 300	1 322	7
Temp + Age * Pop + Year + (1|Year)	1 300	1 322	7
NAO_{-1} + Age * Pop + Year + (1|Year)	1 300	1 322	7
Rain * Temp + NAO_{-1} + Age * Pop + (1|Year)	1 296	1 322	7
Rain * Temp + NAO + Age * Pop + (1|Year)	1 297	1 323	8
Rain + Temp + Age * Pop + Year + (1|Year)	1 300	1 324	9
Temp + Age * Pop + (1|Year)	1 310	1 330	15
NAO_{-1} + Age * Pop + (1|Year)	1 311	1 331	16
Rain + Age * Pop + (1|Year)	1 312	1 332	17
NAO + Age * Pop + (1|Year)	1 312	1 332	17
Rain + Temp + Age * Pop + (1|Year)	1 310	1 332	17

Models take into account the effects of age (four classes), sex, mass, population (Pop, 2 populations of roe deer), average temperatures (Temp), and annual rainfall (Rain).
AIC, Akaike information criterion.

interpreted as resulting from climatic and environmental differences. In roe deer, seroprevalence are thus highly variable and have to be interpreted in the light of information regarding the population of origin. On the contrary, in mouflon and chamois, previous seroprevalence values were relatively homogeneous, ranging from 14.8% to 20.6% (Martinez-Carrasco et al. 2005, Gauss et al. 2006, Aubert et al. 2010), and we also obtained values in the same range. A possible interpretation is that environmental conditions in the areas where populations live (*i.e.*, mountainous areas) are relatively homogeneous regarding *Toxoplasma* contamination.

The relationship between prevalence and age differed among species. A clear increase with age in both sexes was observed only in Trois Fontaines, the population with the largest sample size (Table 4). This result favors the hypothesis of antibody persistence over time in roe deer, although the pattern is less clear in Chizé. In mouflons and chamois, seroprevalence increased with age except in old adults, where low values were observed. This result may be due to small sample size in this age class or to the sampling being unbalanced among years, which would contribute to confuse the age-prevalence pattern observed. A hypothesis that would specifically explain the low values in the oldest animals is that toxoplasmosis-infected individuals have a lower life expectancy than others, but we found no record of such phenomenon in the literature. The level of infection in juveniles may also be explained by vertical transmission, as has been shown in sheep (Williams et al. 2005), although the low seroprevalence in juveniles does not suggest vertical transmission as a major route here.

TABLE 6. COEFFICIENTS OF THE GENERALIZED LINEAR MIXED MODELS SELECTED TO EXPLAIN *TOXOPLASMA GONDII* SEROPOSITIVITY IN THREE UNGULATE SPECIES

Factor	*Parameter estimate (SE)*	P *value*	*OR*	*95% CI*
Chamois intercept	−4.29 (1.37)	<0.001	0.01	0.00–0.20
body-mass	0.10 (0.05)	0.03	1.11	1.00–1.22
Mouflons intercept	−2.75 (0.68)	<0.001	0.06	0.02–0.24
Age yearling	0.42 (0.95)	0.65	1.52	0.24–9.80
Age = prime-age adult	1.46 (0.62)	0.02	4.31	1.28–14.52
Age = old adult	0.13 (1.24)	0.91	1.14	0.10–12.94
Roe deer	−3.35 (0.58)	<0.001	0.03	0.01–0.11
Intercept = Age juvenile				
Age = yearling	1.33 (0.50)	0.008	3.79	1.42–10.14
Age = prime-age adult	0.80 (0.50)	0.110	2.22	0.83–5.96
Age = old adult	1.03 (0.54)	0.053	2.80	0.97–8.07
Population = Trois Fontaines	1.30 (0.60)	0.032	3.67	1.13–11.89
Age = yearling, Trois Fontaines	−0.27 (0.55)	0.614	0.37	0.26–2.24
Age = prime-age adult, Trois Fontaines	0.94 (0.55)	0.088	2.55	0.87–7.52
Age = old adult, Trois Fontaines	1.61 (0.60)	0.008	1.80	1.54–16.21
Year (linear)	0.05 (0.02)	<0.002	1.05	1.01–1.09
Temp (per degree Celsius)	−0.16 (0.21)	0.460	0.85	0.56–1.29
Rain (per 100 mm)	−0.06 (0.09)	0.470	0.94	0.79–1.12
Temp × Rain	0.27 (0.08)	<0.001	1.30	1.12–1.53

For each modality, parameter estimate with its standard error (SE), *P* value of the Wald test, and adjusted odds ratio (OR) with 95% confidence interval (CI).

Finally, the observed pattern results from the balance between exposure to *Toxoplasma* and the duration of the antibody response. The duration of antibody persistence is a major determinant of the epidemiological pattern observed; however, it may vary even between closely related species, as suggested by the difference between sheep and cattle. The loss of antibodies is particularly probable if the infection doses are low, as can be expected in remote (mountain and fenced) areas. Our results support the hypothesis of a long-term persistence of antibodies only in Trois Fontaines. Because this population is also the most infected, the observed pattern may also be related to successive reinfections of the same animals. Our results do not clearly support our predictions that roe deer in general or mouflons maintain a long-term immunity. This point could be investigated, for example, along the longitudinal follow-up of marked individuals.

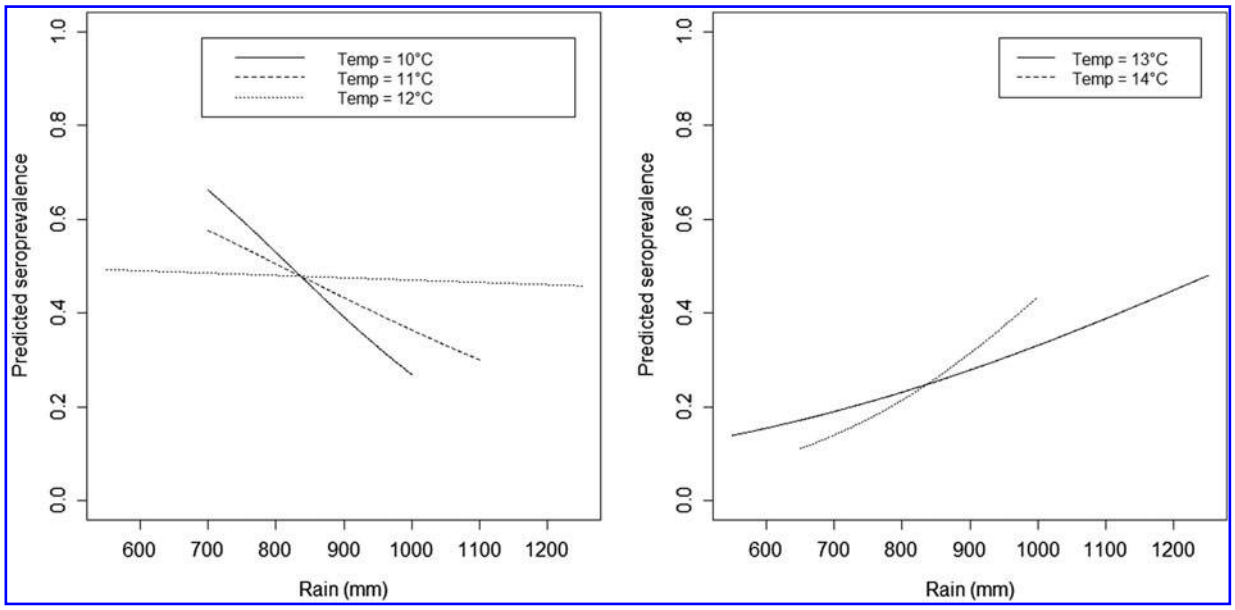

FIG. 2. Influence of maximal temperature (Temp) and rain on the seroprevalence of roe deer for *Toxoplasma gondii* in Trois Fontaines (*left*) and in Chizé (*right*). The curves display the predictions of the model for observed values of temperature and rain for yearlings (1–2 years old) during the year 2000. Each curve represents the predictions of the model according to rain for a value of temperature (legend on the right).

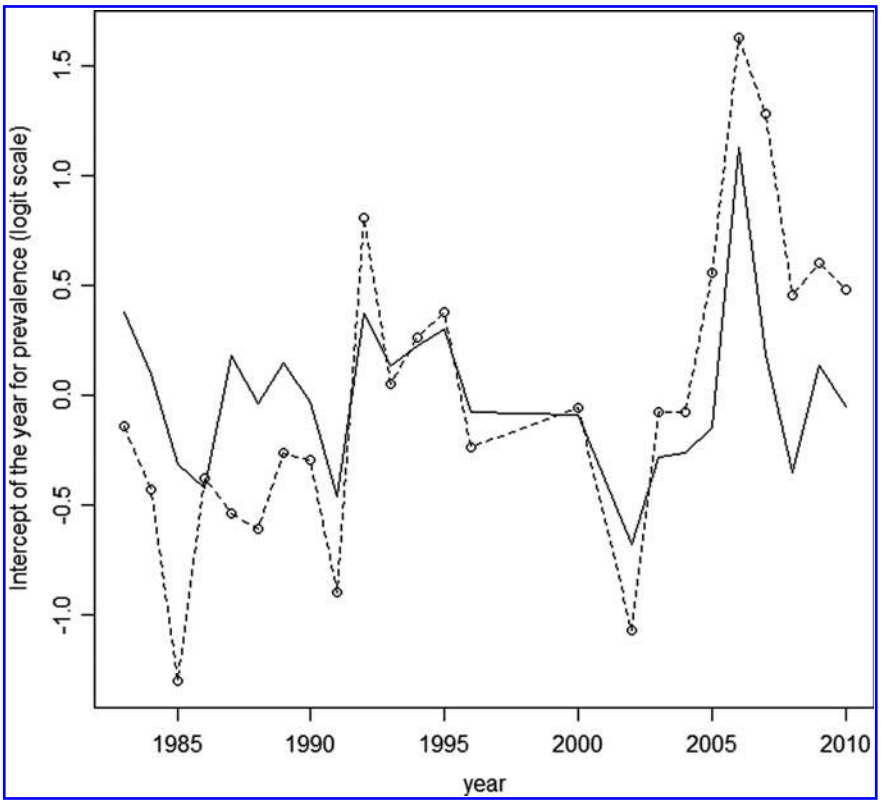

FIG. 3. Yearly variations of seroprevalence. The lines show yearly intercepts on the logit scale for the model with individual variables (dotted line), and for the model including temperature, rain, and linear effect of year (full line).

As previously reported, seroprevalence differed between populations and was twice as high in Trois Fontaines (50.5%) than in Chizé (25.3%). This difference is probably linked to the area of presence of the European wildcat, which does not cover the site of Chizé (Say et al. 2011). However, the seroprevalence in Chizé is remarkably high given the absence of European wildcats and few observations of domestic cats in the area. Several mechanisms may contribute to explain this high level. First, as previously discussed, the possible lifelong persistence of antibodies contributes to these high values: even roe deer infected several years before sampling would be seropositive. As mentioned earlier, some individuals may also acquire infection through vertical transmission, as has been shown in sheep (Williams et al. 2005, Dubey 2009). Finally, a relatively high level of infection also reflects a significant environmental contamination. One may notice that only a few cats may be responsible for this situation—after infection, one cat sheds millions of oocysts in 1 or 2 weeks (Dubey 2010). Oocysts may be disseminated away from the cat home range and can survive for months, especially in moist and shaded conditions that are encountered in forested areas (Gauss et al. 2006, Lélu et al. 2012). In contrast, mountain ungulates occupy open habitats where the survival of oocysts is less probable.

A major result of this study is the temporal variations of toxoplasmosis over the years. To our knowledge, our study presents the longest period of time surveying *T. gondii* prevalence in an ungulate species. In both roe deer populations, seroprevalence significantly increased during the 1983–2010 period. This trend should be interpreted with caution, as it may be due to any factor changing linearly over years. However, neither the general landscape nor population management changed over the study period. Figure 3 confirms that the period of artificial high roe deer density (2003–2005) did not correspond to specific values of *T. gondii* seroprevalence. Because cats are the only source of oocysts in the environment, we propose that meteorological conditions driving the survival of oocysts and cat densities may have changed the risk of infection in ungulates. The meteorological conditions tended to change toward warmer (in Trois Fontaines) and drier (in both populations) conditions over the study period. Predicting the effect of these large-scale changes is not straightforward; however, one may expect oocyst survival to be lowered under drier conditions. Thus, the increasing dryness over time in our study sites cannot explain the linear increase in prevalence. The density of European wildcats has not been studied in the area of Trois Fontaines; however, it may have increased progressively following the complete protection of this species in 1976. To our knowledge, no estimate is available concerning the risk attributable to contact with game carcasses or meat. However, because of this increasing trend in seroprevalence of roe deer, we hypothesize that the risk for people to acquire infection from contact with roe deer meat or carcasses has increased over time.

The spatiotemporal variability correlated with the interaction of rainfall and temperature was observed by Afonso et al. (2006) in urban populations of domestic cats. Here, in Chizé, where temperatures reach high values in summer, the highest predicted prevalences were reached after moist and warm years, which can be interpreted as a result of high oocyst survival under such conditions. This finding is consistent with the clear relationship between rainfall and seroprevalence

found in Spain (Gamarra et al. 2008). Under warm climates with hot periods in the summer, the survival of oocysts should strongly depend on moisture during summer, avoiding oocyst desiccation. In contrast, in Trois Fontaines where mean temperatures are lower than in Chizé, the model predicts high prevalences after cold and dry years. This unexpected result may be related to other processes than oocyst survival. In particular, meteorological conditions may also influence the population dynamics of intermediate and definitive hosts of toxoplasmosis, resulting in variations in the risk.

Meteorological conditions are known to affect the survival of small rodents (Howard 1951). A possible mechanism is that a high survival of rodent prey would favor both felid predator population and the transmission of toxoplasmosis, which should result in a high risk of the maintenance of the *T. gondii* cycle and thus a high risk of environment contamination. Finally, after taking into account the linear increase over years and the link with meteorological variations, a strong variability among years remained unexplained (Fig. 3). Besides the lack of data on in the presence of cats, a possible explanation lies in the scale at which explanatory variables have been measured: Meteorological data obtained at the scale of the site may be too coarse to correctly predict the survival of oocysts in the microenvironment (Beral et al. 2012).

Overall, the infection of the three species of ungulates studied should be considered in a general risk assessment of toxoplasmosis in humans. As the frequency of infection tends to decrease in domestic animals, the relative risk due to wildlife is expected to increase with time. Moreover, the risk due to ungulate meat depends on age, localization, and meteorological conditions during the period preceding the capture. A better knowledge of the level of contamination in the various sources of risk (game meat, other meat products, and environment) is a basis for risk assessment and for adjusting prevention measures in people.

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Author Disclosure Statement

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References

Afonso E, Thulliez P, Gilot-Fromont E. Transmission of *Toxoplasma gondii* in an urban population of domestic cats (*Felis catus*). Int J Parasitol 2006; 36:1373–1382.

Anile S, Amico C, Ragni B. Population density estimation of the European Wildcat (*Felis silvestris silvestris*) in Sicily using camera trapping. Wildl Biol Pract 2012; 8:1–12.

Aubert D, Ajzenberg D, Richomme C, Gilot-Fromont E, et al. Molecular and biological characteristics of *Toxoplasma gondii* isolates from wildlife in France. Vet Parasitol 2010; 171:346–349.

Beral M, Rossi S, Aubert D, Gasqui P, et al. Environmental factors associated with the seroprevalence of *Toxoplasma gondii* in wild boar *Sus scrofa*, France. EcoHealth 2012; 9:303–309.

Burnham KP, Anderson DR. Data-based selection of an appropriate model: The key to modern data analysis. In: McCullough DR, Barrett RH, eds. *Wildlife 2001, Populations.* London: Elsevier Applied Science, 1992:16–30.

Chomel B B. Control and prevention of emerging parasitic zoonoses. Int J Parasitol 2008; 38:1211–1217.

Cook AJ, Gilbert RE, Buffolano W, Zufferey J, et al. Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. Br Med J 2000; 321:142–147.

Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research. Charlottetown, Canada: AVC Inc., 2003:91.

Dubey JP. Toxoplasmosis. J Am Vet Med Assoc 1994; 205: 1593–1598.

Dubey JP. Toxoplasmosis in sheep—The last 20 years. Vet Parasitol 2009; 163:1–14.

Dubey JP. *Toxoplasmosis of Animals and Humans,* 2nd ed. Boca Raton, FL: CRC Press Inc., 2010:1–313.

Dubey JP, Desmonts G. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet J 1987; 19:337–339.

Dubey JP, Thulliez P. Persistence of tissue cysts in edible tissues of cattle fed *Toxoplasma gondii* oocysts. Am J Vet Res 1993;54:270–273.

Dumètre A, Dardé ML. How to detect *Toxoplasma gondii* oocysts in environmental samples? FEMS Microbiol Rev 2003; 796:1–11.

Frenkel JK, Ruiz A, Chinchilla M. Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. Am J Trop Med Hyg 1975; 24:439–443.

Gaillard JM, Delorme D, Boutin JM, Van Laere G, et al. Roe deer survival patterns: A comparative analysis of contrasting populations. J Anim Ecol 1993; 62:778–791.

Gaillard JM, Duncan P, Delorme D, Van Laere G, et al. Effects of hurricane Lothar on the population dynamics of European roe deer. J Wildl Manage 2003; 67:767–773.

Gamarra JA, Cabezon O, Pabon M, Arnal MC, et al. Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain. Vet Parasitol 2008; 153:152–156.

Garel M, Loison A, Gaillard JM, Cugnasse JM, et al. The effects of a severe drought on mouflon lamb survival. Proc R Soc Lond 2004; B 271:S471–S473.

Gauss CB, Dubey JP, Vidal D, Cabezon O, et al. Prevalence of *Toxoplasma gondii* antibodies in red deer (*Cervus elaphus*) and other wild ruminants from Spain. Vet Parasitol 2006; 136:193–200.

Germain E, Benhamou S, Poulle ML. Spatio-temporal sharing between the European wildcat, the domestic cat and their hybrids. J Zool 2008; 276:195–203.

Gilot-Fromont E, Aubert D, Belkilani S, Hermitte P, et al. Landscape, herd management and within-herd seroprevalence of *Toxoplasma gondii* in beef cattle herds from Champagne-Ardenne, France. Vet Parasitol 2009; 161:36–40.

Halos L, Thébault A, Aubert D, Thomas M, et al. An innovative survey underlining the significant level of contamination by *Toxoplasma gondii* of ovine meat consumed in France. Int J Parasitol 2010; 40:193–200.

Herfindal I, Linnell JDC, Odden J, Nilsen EB, Andersen R. Prey density, environmental productivity and home-range size in the Eurasian lynx (*Lynx lynx*). J Zool 2005; 265:63–71.

Howard WE. Relation between low temperature and available food to survival of small rodents. J Mammal 1951; 32:300–312.
Hurrell JW. Decadal trends in the North Atlantic Oscillation: Regional temperatures and precipitation. Science 1995; 269:676–679.
Jokelainen P, Nareaho A, Knaapi S, Oksanen A, et al. *Toxoplasma gondii* in wild cervids and sheep in Finland: North-south gradient in seroprevalence. Vet Parasitol 2010; 171:331–336.
Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, et al. Risk factors for *Toxoplasma gondii* infection in pregnancy. Results of a prospective case-control study in Norway. Am J Epidemiol 1996; 144:405–412.
Kijlstra A, Jongert E. Control of the risk of human toxoplasmosis transmitted by meat. Int J Parasitol 2008; 38:1359–1370.
Lélu M, Villena I, Darde ML, Aubert D, et al. Quantitative estimation of the viability of *Toxoplasma gondii* oocysts in soil. Appl Environ Microbiol 2012; 78:5127–5132.
Malmsten J, Jakubek EB, Bjorkman C. Prevalence of antibodies against *Toxoplasma gondii* and *Neospora caninum* in moose (*Alces alces*) and roe deer (*Capreolus capreolus*) in Sweden. Vet Parasitol 2011; 177:275–280.
Martinez-Carrasco C, Romero MJ, Martin P, Ruiz de Ybanez R, et al. Prevalence of antibodies to *Toxoplasma gondii* in mouflons (*Ovis aries*) in the Sierras de Cazorla, Segura y Las Villas natural park (Jaen, Espana). BIPAS 2005; 25:136–138.
Morellet N, Gaillard JM, Hewison AJM, Ballon P, et al. Indicators of ecological change: New tools for managing populations of large herbivores. J Appl Ecol 2007; 44:634–643.
Pesenti E, Zimmermann F. Density estimations of the Eurasian lynx (*Lynx lynx*) in the Swiss Alps. J Mammal 2013; 94: 73–81.
Pioz M, Loison A, Gauthier D, Gibert P, et al. Diseases and reproductive success in a wild mammal: Example in the alpine chamois. Oecologia 2008; 155:691–704.
R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing 2010. Vienna, Austria, www.R-project.org.
Ross RD, Stec LA, Werner JC, et al. Presumed acquired ocular toxoplasmosis in deer hunters. Retina 2001; 21:226–229.
Sarmento P. Feeding ecology of the European wildcat *Felis silvestris* in Portugal. Acta Theriol 1996; 41:409–414.
Say L, Devillard S, Léger F, et al. Distribution and spatial genetic structure of European wildcat in France. Anim Conserv 2011; doi: 10.1111/j.1469-1795.2011.00478.x.
Stieve E, Beckmen K, Kania SA, et al. *Neospora caninum* and *Toxoplasma gondii* antibody prevalence in Alaska wildlife. J Wildl Dis 2010; 46:348–355.
Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: From animals to humans. Int J Parasitol 2000; 30:1217–1258.
Vikoren T, Tharaldsen J, Fredriksen B, Handeland K. Prevalence of *Toxoplasma gondii* antibodies in wild red deer, roe deer, moose, and reindeer from Norway. Vet Parasitol 2004; 120:159–169.
Williams RH, Morley EK., Hughes JM, Duncanson P, Terry RS, Smith JE, Hide G. High levels of congenital transmission of *Toxoplasma gondii* in longitudinal and cross-sectional studies on sheep farms provides evidence of vertical transmission in ovine hosts. Parasitol 2005; 130:301–307.
Zarnke RL, Dubey JP, Kwok OC, Ver Hoef JM. Serologic survey for *Toxoplasma gondii* in selected wildlife species from Alaska. J Wildl Dis 2000; 36:219–224.

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