EcoHealth 9, 303–309, 2012
DOI: 10.1007/s10393-012-0786-2

EcoHealth

Short Communication

Environmental Factors Associated with the Seroprevalence of *Toxoplasma gondii* in Wild Boars (*Sus scrofa*), France

Marina Beral,[1] Sophie Rossi,[2] Dominique Aubert,[3] Patrick Gasqui,[1] Marie-Eve Terrier,[4] Francois Klein,[3] Isabelle Villena,[3] David Abrial,[1] Emmanuelle Gilot-Fromont,[6,7] Céline Richomme,[4] Jean Hars,[8] and Elsa Jourdain[1]

[1]*INRA, UR346, 63122 Saint-Genes-Champanelle, France*
[2]*Direction des Etudes et de la Recherche, Office National de la Chasse et de la Faune Sauvage, Unité Sanitaire de la Faune, 05000 Gap, France*
[3]*Laboratoire de Parasitologie-Mycologie, Université de Reims Champagne-Ardenne, EA 3800, SFR CAP-SANTE, 51 rue Cognacq-Jay, 51096 Reims, France*
[4]*French Agency for Food, Environmental and Occupationnal Health Safety (ANSES), Nancy Laboratory for Rabies and Wildlife, Technopole Agricole et Vétérinaire, 54220 Malzéville, France*
[5]*Centre National d'Etude et de Recherche Appliquées sur les Cervidés-Sangliers, Office National de la Chasse et de la Faune Sauvage, 1 Place Exelmans, 55000 Bar-le-Duc, France*
[6] *Laboratoire de Biométrie et Biologie Evolutive, Bâtiment Mendel, CNRS, UMR5558, Université Lyon 1, 43 Bd du 11 novembre 1918, 69622 Villeurbanne, France*
[7]*VetAgro-Sup Campus Vétérinaire, 1 Avenue Bourgelat, 69280 Marcy l'Etoile, France*
[8]*Direction des Etudes et de la Recherche, Office National de la Chasse et de la Faune Sauvage, Unité Sanitaire de la Faune, 38610 Gieres, France*

Abstract: *Toxoplasma gondii* is a protozoan parasite infecting humans and animals. Wild boars *Sus scrofa* are a potential source of human infection and an appropriate biological model for analyzing *T. gondii* dynamics in the environment. Here, we aimed to identify environmental factors explaining the seroprevalence of toxoplasmosis in French wild boar populations. Considering 938 individuals sampled from 377 'communes', overall seroprevalence was 23% (95% confidence interval: [22–24]). Using a Poisson regression, we found that the number of seropositive wild boars detected per 'commune' was positively associated with the presence of European wildcats (*Felis silvestris*) and moderate winter temperatures.

Keywords: risk factor, *Toxoplasma gondii*, *Sus scrofa*, seroprevalence, environment, France, zoonosis, wildlife, modified agglutination test

Introduction

Toxoplasma gondii is a protozoan parasite with a complex life cycle. Felids are the definitive hosts, while many animal species are intermediate hosts. In humans, infection and persistence of antibodies are usually lifelong (AFSSA 2005). In France, about 50% of the human population is infected and 200,000–300,000 new infections are believed to occur each year (AFSSA 2005) with strong variations among regions (Berger et al. 2008). Human infection usually occurs via the oral route, by ingestion of oocysts excreted by cats and present in the soil or, more frequently, bradyzoites contained in raw or undercooked meat from intermediate hosts.

Among intermediate hosts, wild boars (*Sus scrofa*) are commonly infected with *T. gondii* (Bengis et al. 2004).

Published online: July 21, 2012

Correspondence to: Elsa Jourdain, e-mail: elsa.jourdain@clermont.inra.fr

Unlike domestic species, for which contamination by imported food supplies is possible (Halos et al. 2010), wild boars are exposed to toxoplasmosis through contact with their local environment. Therefore, they appear as ideal indicators for understanding geographical variations associated with the prevalence of toxoplasmosis.

Our aim was to identify risk factors that may explain toxoplasmosis infection in wild boars in France. Since previous studies showed that the detection of *T. gondii* antibodies in wild boars is positively correlated with the presence of bradyzoites in their muscles (Bártová et al. 2006; Richomme et al. 2009), we used seroprevalence data. Our assumptions were that wild boars become infected by ingesting either soil contaminated by oocysts or bradyzoite-infected intermediate hosts, themselves infected after ingesting oocysts present in the environment. Seroprevalence was therefore expected to vary with (1) oocyst density, which depends on the presence and density of definitive hosts (domestic cats *Felis catus* and European wildcats *Felis silvestris*), and (2) oocyst survival in the environment, which depends on the physical environment (temperature and moisture). Environmental variables possibly determining oocyst density or survival were selected and included in a multivariate model to select variables best explaining wild boar seropositivity in each 'commune'.

Material and Methods

Seroprevalence Data

Blood samples were collected from 938 wild boars shot during the 2003–2004 hunting season in 377 'communes' (Figs. 1, 2). The 'communes' (municipalities) belonged to 21 French 'départements' and their neighbor contiguity was poor, with 141 out of 377 (34%) 'communes' isolated from the others. Blood samples were centrifuged and the resulting serum was stored at −20°C. *T. gondii* antibodies were detected using a modified agglutination test (MAT) considering 1:25 as a positivity threshold (Dubey et al. 2002; Gauss et al. 2005). Seroprevalence was estimated in each 'commune' and 95% confidence intervals (CI) were calculated. Maps were built using Quantum GIS 1.6.0 and ArcGIS 9.1.

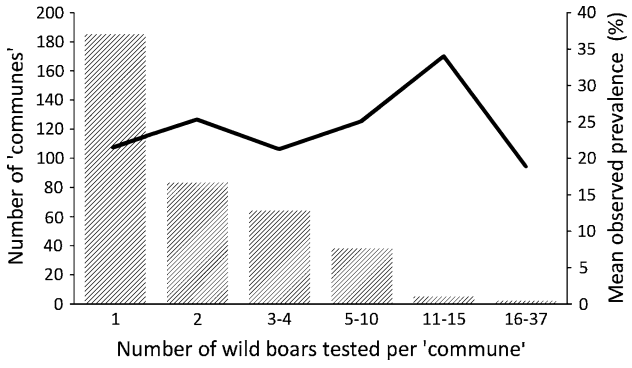

Figure 1. Histogram of wild boar sampling size per 'commune' and plot of mean observed seroprevalence per 'commune'.

Environmental Data

Five variables possibly explaining oocyst density or survival, and measured at the 'commune' scale, were selected (Table 1).

Two variables were used to estimate the presence or density of definitive hosts in each 'commune': (1) "Farm density", as an estimator of domestic cat density, as previously done in Corsica (Richomme et al. 2010); (2) "presence of wildcats", based on a presence map of European wildcats in France (Say et al. 2012).

Because experimental (Dumètre and Dardé 2003) and epidemiological (Afonso et al. 2010) studies suggested that oocyst survival depends on moisture and temperature, three meteorological variables were used: (1) "rainfall", to predict moisture in the environment of oocysts; (2) "maximum temperatures" during summer, to infer periods of hot temperature and drought, which are deleterious for the survival of oocysts (Frenkel et al. 1975); (3) "number of cold periods", i.e., number of 10-day periods with an average minimal temperature below −6°C during the winter preceding the sampling period: this temperature threshold was chosen because temperatures below −6°C prevent the sporulation of oocysts whatever the moisture conditions (Dumètre and Dardé 2003).

To detect linear as well as nonlinear effects, all continuous variables were transformed into variables with three categories and secondarily, when appropriate, into dichotomous variables. We expected that low farm density, absence of wildcat, low rainfall levels, high temperatures, and a high number of cold periods would be associated with low seroprevalence in wild boars.

Generalized Linear Model

The number of seropositive wild boars per 'commune' was modeled with a Poisson generalized linear model (GLM), i.e., a model of counts of infected animals per 'commune'.

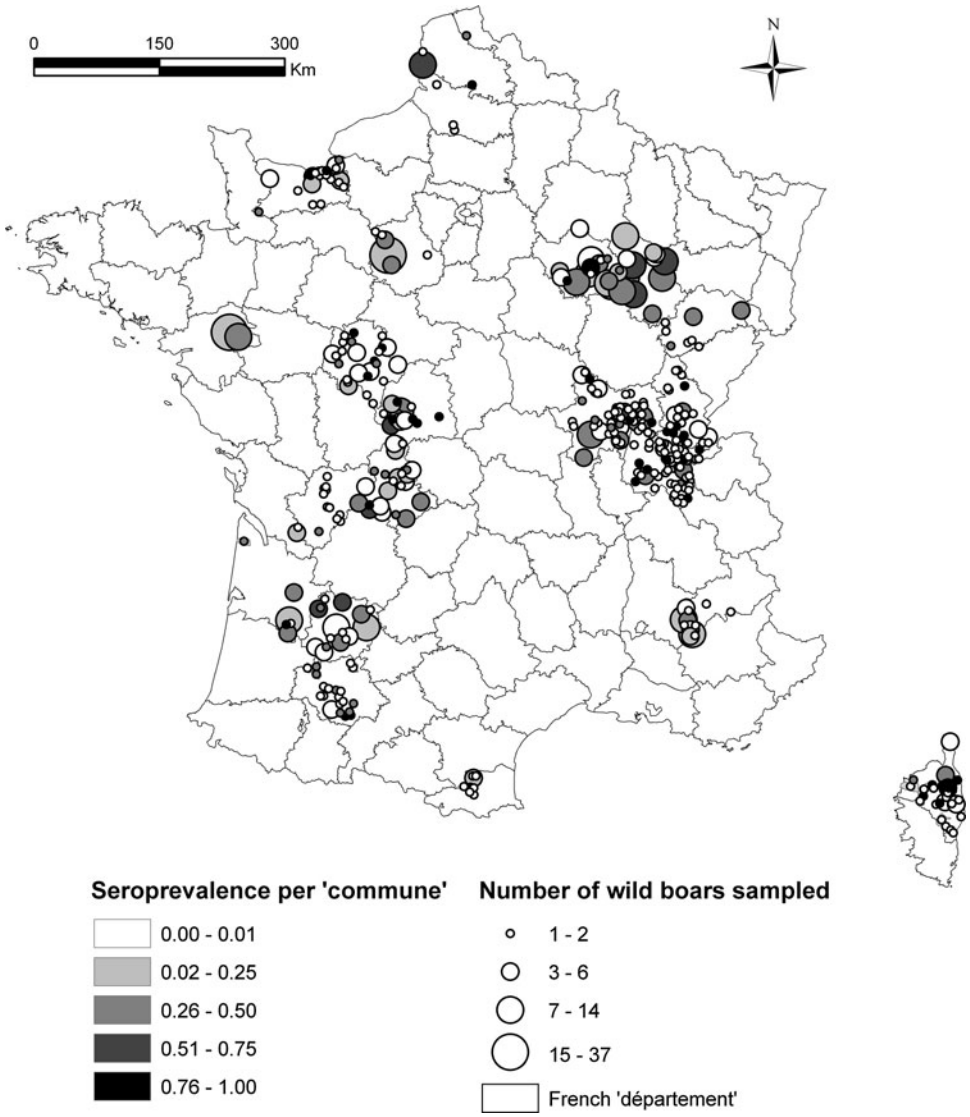

Figure 2. Map showing, for each 'commune' included in the study, the number of wild boars sampled and the observed seroprevalence.

Although these data may also be analyzed by considering the binomial distribution of infected versus non-infected wild boars in each commune (logistic regression), instead of considering the number of infected per commune (Poisson regression), we chose the second option. Indeed, exploratory analyses revealed that the binomial distribution did not correctly fit our data (underdispersion). Poisson regression enabled adjusting prevalence per 'commune' on sample size, by using the total number of wild boars tested by 'commune' as an offset variable (Dohoo et al. 2009), which allowed reducing significantly underdispersion. Because this parsimonious model satisfyingly explained our dataset, we did not consider using a random-effect binomial model.

The full model was defined to include the effects all variables, i.e., ''farm density'', ''presence of wildcats'', ''maximum temperature'', ''rainfall'' and ''number of cold periods''. The full model did not allow for interactions between covariates because there was no biological reason to suspect the existence of such effects. The fit of the Poisson model was assessed using a Kolmogorov–Smirnov test and a dispersion parameter φ was estimated as the ratio of the residual deviance of the full model on its degree of freedom. The full model was simplified by backward elimination using likelihood ratio tests to eliminate factors that did not add any significant information about the number of infected animals, and therefore better estimate the significance of the selected explanatory variables. All statistical procedures were performed using R (Development Core Team 2008, version 2.15.0).

Table 1. Environmental variables included in the full model

Variable	Definition and categories	Source
Farm density (number/km^2)	Number of farms per km^2 Categories: [0.00; 0.54], (0.54; 1.00], (1.00; 5.15]	Ministry of Agriculture and Fisheries, AGRESTE data, www.agreste.agriculture.gouv.fr
Wildcats	Presence of European wildcats (*Felis silvestris*) in the 'commune': "present" ('commune' in a département where wildcats have been observed); "possibly present" ('commune' in a département at the border of the range); "absent" (elsewhere)	Say et al. (2012)
Rainfall (mm)	Cumulative rainfall during the 12 months preceding the sampling period (September 2002 to August 2003) Categories: [28.80; 62.10], (62.10; 74.80], (74.80; 151.00]	Original data from MeteoFrance®, barycentric interpolation of three to four nearest meteorological stations
Maximum temperature (°C)	Maximum temperature of the warmest 10-day period during the summer preceding the sampling period (June 1st, 2003 to September 1st, 2003). Categories: [34.70; 38.90], (38.90; 40.30], (40.30; 42.80]	
Number of cold 10-day periods	Number of 10-day periods with an average minimum temperature below −6°C during the winter preceding the hunting season (November 2002 to February 2003) Categories: [0.00; 3.00], (3.00; 7.32]	

Results

Seroprevalence

The overall seroprevalence was 23% (22–24) and seroprevalence per 'commune' varied between 0 and 100% as shown in Figs. 1 and 2. Seroprevalence was similar in juveniles (21% [16–26]) and adults (25% [22–29]) ($P = 0.840$).

Explanatory Environmental Factors

The assumption of Poisson distribution was supported by the Kolmogorov–Smirnov test ($P = 0.264$) and the dispersion parameter estimate, which was close to 1 ($\varphi = 0.83$). The best fitting model included two environmental variables, "presence of wildcats" and "number of cold periods" (Table 2; Fig. 3). According to this best fitting model, seroprevalence was predicted to be high in 'communes' where wildcats are present compared to 'communes' where they are absent (odds ratio OR = 1.79, $P = 0.005$). Seroprevalence was predicted to be low in 'communes' with more than three cold 10-day periods (OR = 0.63, $P = 0.018$). We checked that the same model was obtained when considering 1:10 instead of 1:25 as a positive MAT threshold.

Discussion

The influence of environmental conditions on the transmission dynamics of diseases is gaining interest in recent studies, especially concerning vector-borne diseases and pathogens with complex life cycles (Junglen et al. 2009). However, this approach has rarely been applied to *T. gondii* (Fredebaugh et al. 2011). Here we show that the infection of a wild-living species may be partly explained by considering potential environmental risk factors at a country scale.

The seroprevalence (23% [22–24]) measured here was consistent with results reported by studies considering the same 1:25 MAT cut-off titer in the USA (18–37%, Diderrich et al. 1996; Dubey et al. 1997), Spain (38%, Gauss et al. 2005), and the French region of Champagne-Ardennes (20%, Richomme et al. 2009). Because infective cysts have been found in wild boars with MAT titers as low as 1:6 (Richomme et al. 2009), we checked that the model was robust using a lower positivity threshold. Because seropositive wild boars are known to frequently carry

Table 2. Coefficients of the best fitting multivariable Poisson regression model selected to explain *T. gondii* seropositivity in French wild boars

Parameter	Estimate	*P*	OR (95% confidence interval)
Offset			
Wildcats: possibly present	0.228	0.288	1.26 (0.81; 1.89)
Wildcats: present	0.583	0.005	1.79 (1.20; 2.73)
Number of cold 10-day periods >3	−0.461	0.018	0.63 (0.43; 0.92)

infective cysts (Bártová et al. 2006; Richomme et al. 2009), our results suggest that French wild boars are frequently infected with *T. gondii*. Since consuming undercooked meat, eviscerating and handling infected wild game have been identified as a source of *T. gondii* infection in humans (Dubey 1994; Ross et al. 2001; Bengis et al. 2004), French wild boars represent a potential risk for the zoonotic transmission of toxoplasmosis, particularly in the current context of increased consumption of undercooked game meat (Bultel and Derouin 2006).

Seroprevalence was particularly high in 'communes' where wildcats are present and low in 'communes' exposed to particularly cold winter temperatures. Both selected environmental variables were consistent with our predictions that (1) the presence of wildcats is determinant for the life cycle of the parasite and (2) harsh winter conditions limit the

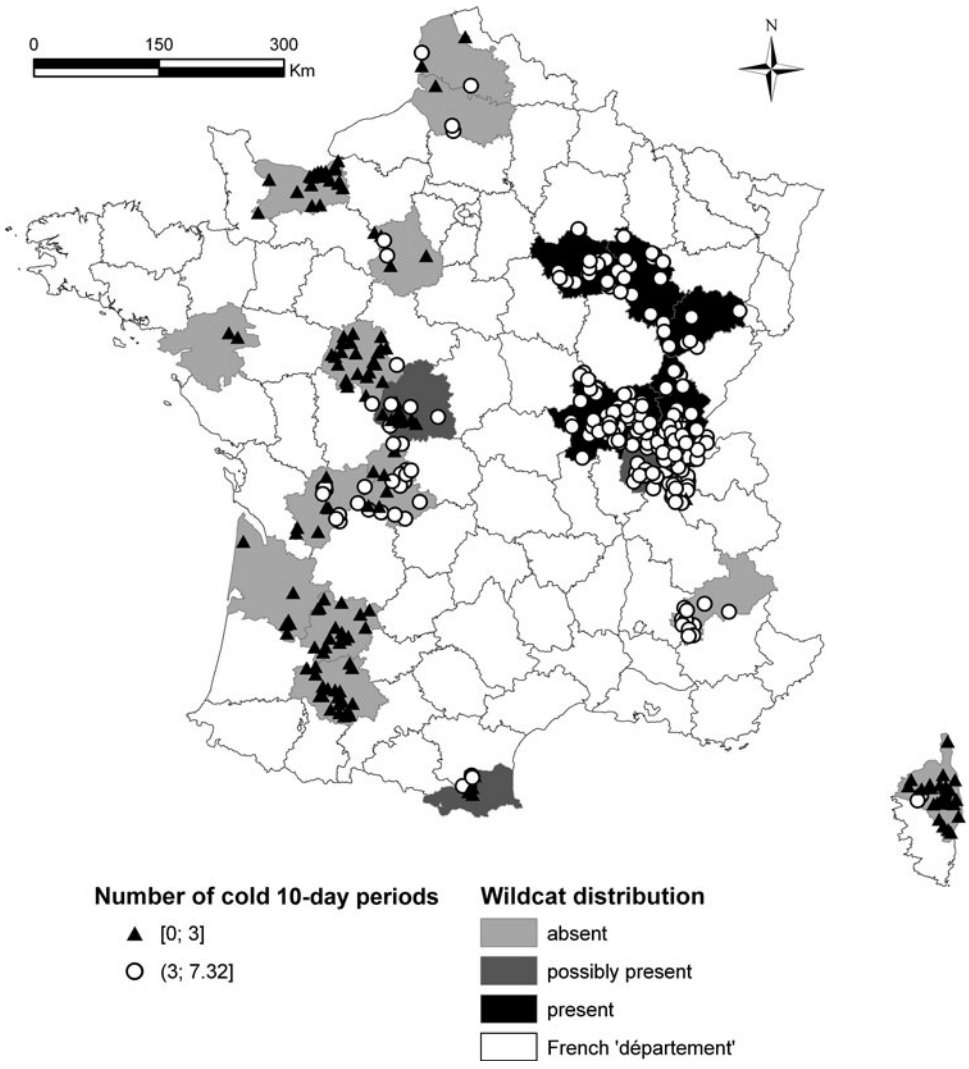

Figure 3. Map showing, for each 'commune' included in the study, the location of 'communes' with ≤3 or >3 cold 10-day periods (i.e., 10-day periods with an average minimum temperature below 6°C) during the winter 2002–2003 and the spatial distribution of wildcats in the corresponding 'départements'.

risk of exposure to *T. gondii*, possibly by preventing oocysts deposited in the environment from sporulating (Dumètre and Dardé 2003). In order to further explore the shape of the relationship between seroprevalence and temperature or wildcat density as continuous variables, data collected from more 'communes' would be needed.

On the contrary, low farm density, low rainfall levels and high temperatures were not found to be significantly associated with low seroprevalence. Beside the relatively low power of this study, our explanatory variables may have been measured at an inappropriate scale. Farm density may not be a relevant estimator of the domestic cat population at the scale of France. Meteorological data were obtained at the 'commune' scale whereas oocyst survival depends on the conditions experienced by oocysts in their microenvironment, and the home range of wild boars (500–3,000 ha) likely overlaps several 'communes' (Fischer et al. 2004; Keuling et al. 2008). Therefore, our ''rainfall'' and ''maximum temperature'' variables were probably too crude to correctly predict local exposure risk. Obtaining data at the appropriate scale would help assessing more accurately environmental factors and improving our model. The data presented here will be useful to identify particular communes in which locally focused follow-up studies may be performed to explore these associations in more detail.

Conclusion

Understanding the dynamics of zoonotic diseases in their natural environment provides keys to improving the management of between-host transmission, and especially the prevention of zoonoses (Lloyd-Smith et al. 2009). Concerning *T. gondii*, these and previous results suggest that physical and meteorological conditions acting on oocyst survival, as well as host species densities and factors determining them (e.g., altitude, forest cover), all influence the natural life cycle of the parasite (Almeria et al. 2004; Afonso et al. 2010; Richomme et al. 2010). These results may contribute to explain the inter-regional variations observed in many species including humans and to better identify sources for human infection.

Acknowledgements

The authors thank the agents of the Office National de la Chasse et de la Faune Sauvage for their help in the field, R. Geers for laboratory analyses, and B. Persyn and S. Ruette for their help in collecting complementary data. The work was financially supported by the French Agency for Food, Environmental and Occupationnal Health Safety.

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