Species or local environment, what determines the infection of rodents by *Toxoplasma gondii*?

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SUMMARY

Toxoplasmosis is largely present in rural areas but its spatial distribution in this environment remains poorly known. In particular, it is unclear if areas of high density of cats, the only hosts excreting *Toxoplasma gondii*, constitute foci of high prevalence. To improve our understanding of the spatial distribution of *T. gondii* in rural areas, we performed a serological survey in rodents from two villages in France. We trapped 710 rodents including commensal rats and meadow or forest voles and mice. The presence of *T. gondii* was examined using PCR, mice inoculation and modified agglutination test for antibodies (MAT). We conducted multivariate and discriminant analyses to identify biological, ecological or spatial variables that could explain *T. gondii* serology in rodents. We then used a logistic regression to assess the relative influence of each explanatory variable. Overall seroprevalence was 4·1%. Commensal-rats were more infected (12·5%) than non-commensal species (3·7%). However, the major determinant of the risk of infection was the distance to the nearest farm (OR = 0·75 for 100 m), which explained the risk in all species or non-commensal species only. We contrast the role of species characteristics and that of the local environment, and discuss the risk of environmental contamination for humans.

Key words: *Toxoplasma gondii*, rodents, ecology, landscape, local scale, rural area, commensal species.

INTRODUCTION

Toxoplasmosis is a worldwide zoonosis caused by the ubiquitous parasite, *Toxoplasma gondii*. Due to the various possible transmission routes of this protozoan, the management and prevention of human infections requires a fine understanding of the dynamics of its life cycle (Gilot-Fromont *et al.* 2012). Felids (domestic cats and their relatives) are the only known definitive hosts for the parasite *T. gondii*. Cats shed unsporulated oocysts in their feces and, once in the environment, oocysts become sporulated and infectious to new hosts. These oocysts constitute both a significant source of infection for humans (Boyer *et al.* 2011) and the main route of infection for intermediate hosts (all warm-blooded animals) (Dubey and Beattie, 1988). The risk of contamination for intermediate hosts and humans by *T. gondii* remains difficult to assess as it depends on both the frequency and spatial distribution of oocysts. It is notably unclear how infection risk varies spatially at a local scale and whether there are specific foci of contamination within, or near, inhabited areas where domestic cats are more abundant.

Rodents, being major prey of felids, are considered to play a key role as intermediate hosts in the maintenance of the *T. gondii* life cycle (Dubey *et al.* 1995; Hejlicek *et al.* 1997). For this reason, rodents as generally considered as relevant markers to assess environmental contamination by toxoplasmosis and other pathogens and to estimate the risk of infection for definitive hosts (Afonso *et al.* 2007*a*; Reperant *et al.* 2009; Antoniou *et al.* 2010).

Before considering toxoplasmosis in rodents as a meaningful indicator to predict environmental contamination by *T. gondii*, it is important to account for potential biological and ecological differences within and between species. For instance, a positive relationship between rodent body mass and their probability to be seropositive for *T. gondii* has been found both within species (due to age) (Reperant *et al.* 2009) and between species (larger species having

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a longer life span) (Afonso *et al*. 2007*b*; Dabritz *et al*. 2008). Species-specific ecological requirements have also been found to influence *T. gondii* prevalence, due to variation in oocyst–rodent contact patterns (Ruiz and Frenkel, 1980; de Thoisy *et al*. 2003). For example, fossorial species that live in burrows (such as *Arvicola terrestris scherman*) are always in contact with soil and eat paratenic hosts of *T. gondii* (such as earthworms); they are therefore thought to be more at risk than other vole species such as *Microtus* species (Afonso *et al*. 2007*b*). In addition to biological and ecological species-level features, the local environment around captured individuals may also influence their probability of infection. In rural areas, *T. gondii* prevalence in mammals and birds is particularly high around farms (Smith *et al*. 1992; Dubey *et al*. 1995; Weigel *et al*. 1995; Meerburg *et al*. 2012). Lehmann *et al*. (2003) found the probability of infection of intermediate hosts (rodents and birds) to be lower at distances further away from pig farms. However, no study has combined an analysis of species-level factors (biology and ecology) with individual-level characteristics (mass and local environment of the captured individual).

This study was designed to examine the effects of these factors. We conducted a serological survey of the local rodent community in two sites in a rural area of Eastern France. Each site corresponded to a village with farms, residential houses, peripheral fields and forests. We expected to capture rodents with different ecological requirements: commensal species (rats), fossorial meadow species (field voles and water voles) and forest species (bank voles, yellow-necked and wood mice). Based on previous findings, we expected the prevalence of *T. gondii* antibodies in rodents to vary between species according to specific ecological requirements, individual biology (mass) and the local environment of each individual (distance to the nearest farm and habitat).

MATERIALS AND METHODS

Study areas and sampling

The study was carried out at two sites located in the French Ardennes (North-Eastern France): Boult-aux-bois (49°25′52″N, 4°50′33″E) and Briquenay (49°24′19″N, 4°52′41″E). Each 1·5 × 1·5 km site was centred on a village; the two village centres were 3·2 km apart. We used aerial views processed in the software QGIS (v. 1.8.0 http://www.qgis.org/) and direct visual assessments to classify the land cover according to the following categories: meadows, crop fields, forests, hedgerows, vegetable gardens and inhabited areas (Fig. 1). The study areas showed equivalent proportions of the different habitat categories: 59–67% meadows, 11–17% crop fields, 10–13% forests, 5–7% hedges, 3–5% inhabited habitats and less than 1% vegetable gardens (Fig. 2). We considered the two study sites as sampling replicates.

Two 10-day trapping sessions were carried out in October 2010 and October 2011. Live traps were set up in the different rodent habitats within each site: hedgerows and forests (for forest species), meadows, pastures and gardens (for fossorial species) and inhabited areas such as areas within and around buildings or along streams running through villages (for commensal species). Trapping efforts were designed to catch at least 30 individuals per species at each site during each field session. Between 19 and 23 lines of live traps were used to catch wood mice and yellow-necked mice, field voles and bank voles during each session. Lines were set up for at least three nights and checked once a day. Sherman traps, placed within galleries under earth mounds, were used to trap the fossorial water vole. Rat traps were mainly used in the villages and around farm buildings. These two types of traps were checked every 2–4 hours or in some case left overnight. All traps were set at a minimum spacing of five metres to target distinct colonies. We recorded the geographical coordinates of each individual trapped using a global positioning system (GPS).

Rodents were euthanased by isoflurane inhalation, weighed, sexed and dissected. Blood samples collected from the clot or heart were centrifuged and the serum separated. Sera were stored in microtubes at −20 °C until analysis. Hearts were placed in sterile plastic collectors containing a suspension of 0·9% (w:v) saline with antibiotics added (120 000 U L^{-1} penicillin-G and 120 mg L^{-1} streptomycin). Species that could not be easily identified using morphological criteria (*Apodemus* species, *Microtus* species and *Myodes glareolus*) were discriminated using a DNA barcoding approach (Galan *et al*. 2012) and specific primer test (Michaux *et al*. 2001).

We used individual GPS coordinates to compute the distance to the nearest farm building containing either domestic animals or food that may attract rodents and hunting cats (Fig. 1).

Serological and parasitological data

Sera were tested for the presence of *T. gondii* antibodies using a modified agglutination test (MAT) for the detection of *T. gondii*-specific IgG antibodies (Dubey and Desmonts, 1987). The antigen was prepared at the Laboratoire de Parasitologie-Mycologie, EA 3800, Reims. Sera were diluted two-fold, starting at 1:3 dilution. Hearts of all seropositive rodents, and of a few seronegative animals, were used for individual bioassays in mice to confirm *T. gondii* infection (Villena *et al*. 2004). Hearts were mixed with a 0·25% trypsin suspension (1 h 30 at 37 °C), the mixture filtered through gauze and centrifuged. Supernatant was suspended in

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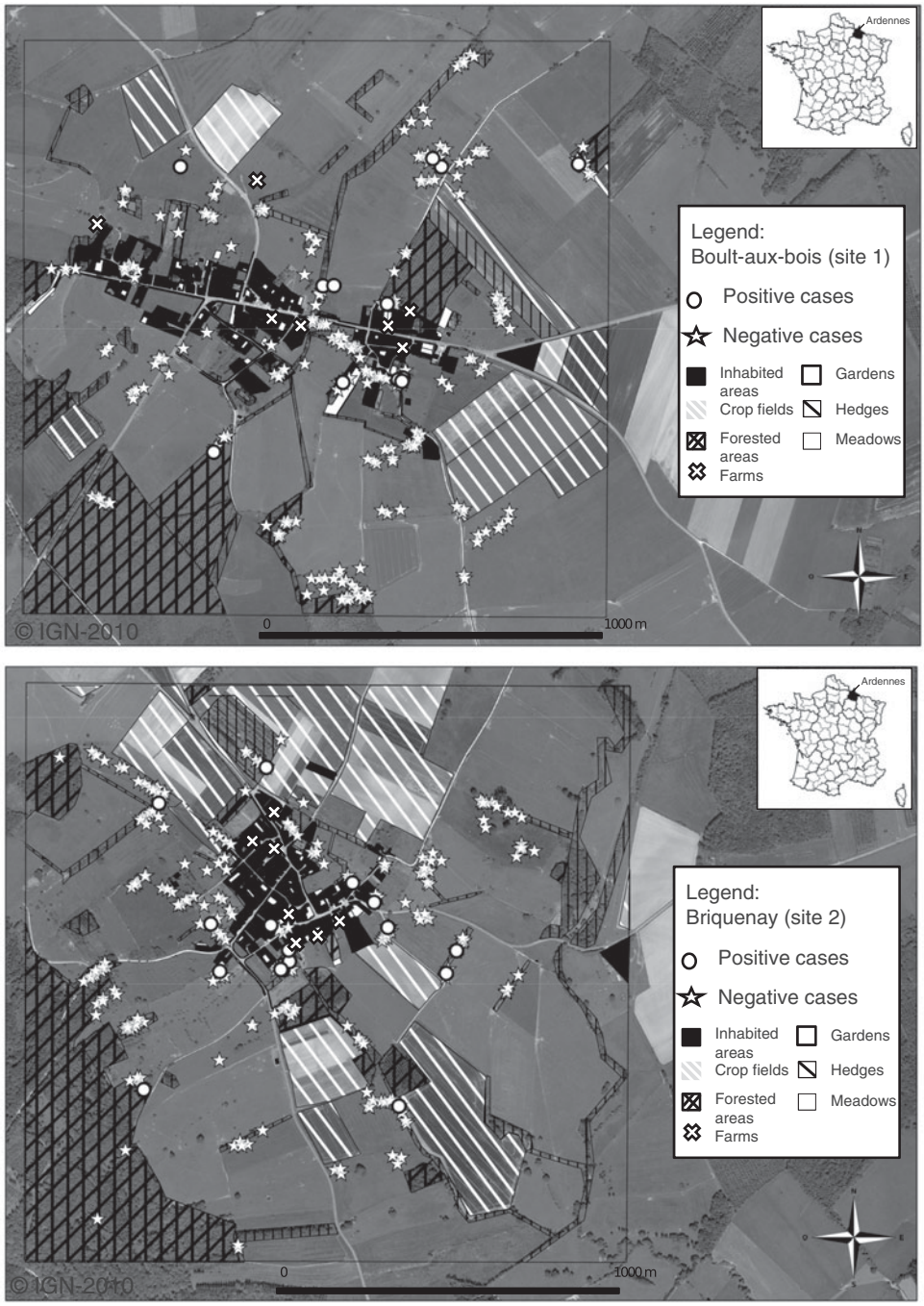

Fig. 1. Distribution of *T. gondii* antibody (seropositive (white circles) and seronegative (white stars)) in samples taken from rodents in two villages of the French Ardennes. The map shows the localization of inhabited areas (black), gardens (white), meadows (grey), crop fields (white-hatched), hedges (fine black hatched) and forested areas (black double-hatched). Farm buildings are identified by white crosses.

saline solution containing penicillin G, streptomycin and amoxicillin. This solution was injected into the peritoneum of two mice. Mice were tested for seroconversion with the MAT test 4 weeks post-inoculation (pi) and euthanased at 60 days pi. Tissue cysts in brains of seropositive mice were detected by microscopic examination. Brain cysts from seropositive mice were isolated by Percoll gradient centrifugation and DNA was extracted using a QIAamp DNA minikit (Qiagen, Courtaboeuf, France). Real-time quantitative PCR (iQ5 instrument, BIORAD) were conducted to detect *T. gondii* DNA by targeting a specific sequence of 529 bp (Reischl *et al.* 2003; Lélu et al. 2011). All laboratory procedures were performed at the Laboratoire de Parasitologie-Mycologie, EA 3800, Reims.

Statistical analyses

We expected a low number of infected rodents and a high number of variables and modalities that could be

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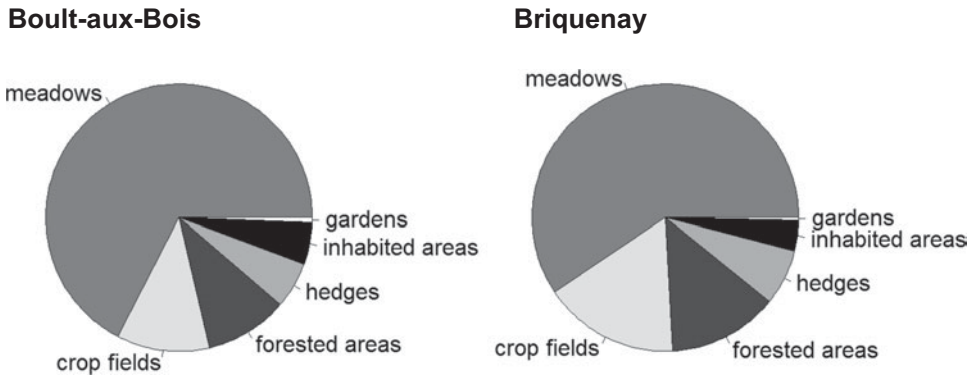

Fig. 2. Distribution of the proportion of each habitat within Boult-aux-bois (left) and Briquenay (right).

considered as explanatory variables of seroprevalence (eight species and six different habitats). We therefore chose not to test the relationships between serology results and all possible variables to minimize the risk of spurious correlations. We then described the dataset to (i) assess which variables were correlated (using correlated variables to explain serology may further lead to confusion) and (ii) identify the factors (variables and modalities) that were most strongly associated with serological status. We used a mixed multivariate descriptive analysis combining quantitative and qualitative variables (Hill and Smith, 1976) which included: species (eight modalities, hereafter called SPECIES), sex (SEX), individual body mass normalized by species and used as a proxy for age (MASS), habitat (six categories: meadows, crop fields, forests, hedges, vegetable gardens and inhabited areas; HABITAT), year (2010 or 2011; YEAR), site (Boult-aux-bois or Briquenay; SITE) and distance to the nearest farm in metres (DISTANCE). This analysis highlighted variables that were correlated and factors (variables and modalities) that were associated with *T. gondii* serological status. We then carried out a discriminant analysis to identify which of these variables and modalities mostly strongly correlated with serological status. The multivariate descriptive analysis was performed in order to limit the probability to find association of serostatus with several correlated factors, and thus to limit type 1 errors. It was done with the R package (*ade4*; Chessel *et al*. 2004).

We performed a logistic regression to analyse the potential explanatory variables of *T. gondii* seroprevalence. Species (here transformed as a dichotomous variable, commensal *vs* non-commensal following the Hill and Smith analysis; thereafter called SPECIES2), habitat (transformed as village *vs* other habitats; HABITAT2), DISTANCE, SEX, MASS, YEAR and SITE were considered. We used an information-theoretic approach for the model selection procedure. We defined *a priori* candidate models and used the corrected Akaike criterion (AICc) to identify the most likely model (Burnham and Anderson, 2002; Hobbs and Hilborn, 2006). Model averaging was performed using R package (*MuMIN*; Bartoń, 2009) for models with $\Delta AICc < 2$. Akaike weights (wi) were used to obtain robust estimates of model parameters (Welch and MacMahon, 2005; Hobbs and Hilborn, 2006; Burnham *et al*. 2011). *A priori* candidate models included between one and three potentially explanatory variables, as well as the interaction between distance and species. This interaction seemed important, as these variables were strongly associated in the Hill and Smith analysis. We therefore checked that the impact of distance on *T. gondii* was the same for commensal rats and for other species. All statistical analyses were performed using R software (R Development Core Team, 2012).

RESULTS

We trapped 710 rodents from eight species. This included forest species (*M. glareolus* ; $n = 203$ and *Apodemus* sp. ; $n = 200$), meadow species (*A. terrestris scherman;* $n = 195$ and *Microtus* sp.; $n = 81$) and one commensal species (*Rattus norvegicus;* $n = 31$) (Table 1). Despite a significant trapping effort in the villages, we did not capture any domestic mice (*Mus musculus domesticus*). The spatial location of all individuals trapped is shown in Fig. 1.

Toxoplasma gondii antibodies were found in 29 of the 710 (4·1%, 95% IC: [2·8; 5·8]) rodents trapped, with titres ranging between 1:6 and 1:6400 (Table 2). We performed bioassays and PCR on 41 individuals (including 12 rodents which were seronegative or had a titre lower than 1:6); isolation was successful in 3 animals with titres of 1:6, 1:400 and 1:3200. Samples taken from 9 individuals, whose titres ranged from 1:3 to 1:6400, gave positive PCR results (Table 2). As *T. gondii* was isolated from 1 individual with titre 1:6, we used this titre as the threshold for the analysis of serological results. Two individuals with serological titres of 1:3 gave positive PCR results and negative inoculation results but, considering inoculation as the gold standard, we retained the titre threshold f 1:6. We checked that using 1:6 or 1:3 as the threshold value did not change the results of the

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Table 1. Presence of *T. gondii* antibodies in rodents trapped in Ardennes, France. The number of positive individuals/total number of individuals trapped are given for each species and year

Species	Boult-aux-bois			Briquenay			Total Overall
	2010	2011	Total (%)	2010	2011	Total (%)	
Apodemus flavicollis	0/7	0/18	0/25 (0·0)	0/2	0/16	0/18 (0·0)	0/43 (0·0)
Apodemus sylvaticus	0/20	1/50	1/70 (1·4)	3/42	0/45	3/87 (3·4)	4/157 (2·5)
Myodes glareolus	4/70	4/38	8/108 (7·4)	2/33	2/62	4/95 (4·2)	12/203 (5·9)
Arvicola scherman	0/43	0/32	0/75 (0·0)	5/77	1/43	6/120 (5·0)	6/195 (3·1)
Microtus agrestis	0/5	0/4	0/9 (0·0)	0/0	0/1	0/1 (0·0)	0/10 (0·0)
Microtus arvalis	2/24	1/29	3/53 (5·7)	0/0	0/13	0/13 (0·0)	3/66 (4·5)
Microtus subterraneus	0/3	0/2	0/5 (0·0)	0/0	0/0	0/0 (0·0)	0/5 (0·0)
Total non-commensal	6/172	6/173	12/345 (3·5)	10/154	3/180	13/334 (3·9)	25/679 (3·7)
Rattus norvegicus (commensal)	0/3	0/7	0/10 (0·0)	1/7	3/14	4/21 (19·0)	4/31 (12·90)
Total	6/175	6/180	12/355 (3·4)	11/161	6/194	17/355 (4·8)	29/710 (4·08)

Table 2. Results of *T. gondii* isolation and PCR from rodents captured in both sites

MAT agglutination titre	Number of samples bioassayed	Number of isolates	Number of PCR positive individuals
0	1	0	0
1:1	4	0	0
1:3	7	0	2
1:6	12	1	0
1:12	9	0	2
1:100	1	0	1
1:400	1	1	1
1:3200	3	1	2
1:6400	3	0	1

model selection procedure or the estimates of the final odds ratios.

The Hill and Smith analysis was performed on 697 individuals (no MASS and SEX information available for 13 rodents). Two main axes were identified (Fig. 3). The first axis accounted for 12·77% of the total variation and indicated that *R. norvegicus* presence was positively associated with inhabited areas and vegetable gardens but negatively associated with the distance to the nearest farm. This result was expected since rat traps were set in the surroundings of inhabited and farm areas. The second axis accounted for 11·93% of the variation. This axis highlighted differences between voles trapped in meadows and forest species, reflecting the specific ecology of different wild rodent species.

Our discriminant analysis revealed three main variables that were strongly related with rodent *T. gondii* serological status: distance to the nearest farm (correlation between variable and canonical score: −0·65), species (*R. norvegicus*, $r = 0{\cdot}58$) and habitat (inhabited areas, $r = 0{\cdot}49$). Based on these results, we converted the variables SPECIES and HABITATS into dichotomous variables. We considered *R. norvegicus vs* non-commensal species (SPECIES2), and habitats located within the village *vs* others (HABITAT2).

We adjusted all models that included three or less of the potential explanatory variables (DISTANCE, SPECIES2, HABITAT2, SEX, SITE, YEAR, MASS and the interaction SPECIES2 * DISTANCE). Models with $\Delta AICc < 2$ included the following variables with decreasing relative importance: DISTANCE, SPECIES2, YEAR, HABITAT2, MASS, SITE, interaction SPECIES2 * DISTANCE and SEX (Table 3). The most highly supported model included the effect of distance only (wi = 0·18). This variable was present in all models with $\Delta AICc < 2$ (Table 4). Parameter estimation confirmed that only the effect of DISTANCE was significant (OR = 0·75, 95% IC: 0·60–0·94, Fig. 4, Table 4). As rats, the most prevalent, were only trapped near farms we checked whether the effect of distance was significant when considering other species only. We thus repeated the analysis without rats (data not shown). The best likely model again included the effect of distance only, with the risk for a rodent to be positive decreasing significantly by 0·75 every 100 m (95% IC: 0·62–0·97). From model parameters, the predicted seroprevalence at distance = 0 (i.e. beside a farm building) was $1/(1 + \exp(-(-2{\cdot}33))) = 0{\cdot}089$.

DISCUSSION

Several studies have described the level of *T. gondii* infection in rodents (reviewed in Afonso *et al.* 2007*b*; Dabritz *et al.* 2008; Mercier *et al.* 2013). However, explanatory factors were most often searched for at the species level, while the local environment of individual rodents was not considered. We present the first study accounting for the potential interplay between biological, ecological and spatial factors when investigating the level and spatial distribution of *T. gondii* infection in rodents. In this study, the overall seroprevalence of the rodent community was

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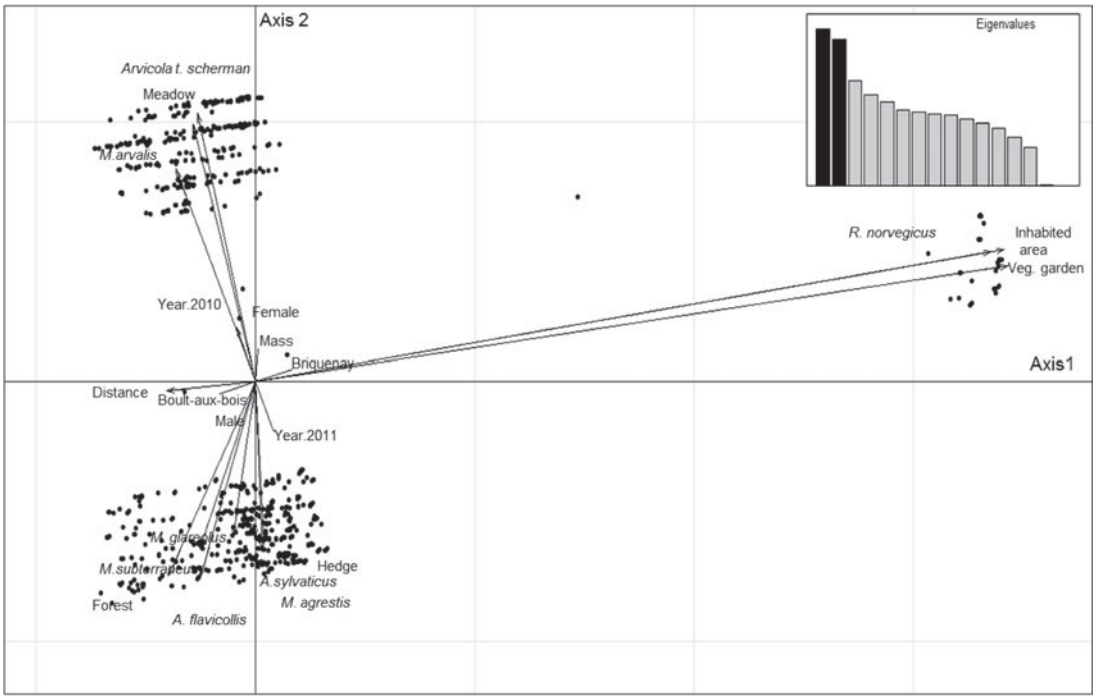

Fig. 3. Factorial map of the Hill and Smith factorial analysis: first (x-axis) and second (y-axis).

low (4%). This value is in line with previous studies (e.g. Jeon and Yong, 2000; Afonso *et al*. 2007*b*; Dabritz *et al*. 2008), and especially with the one conducted in the same area by Afonso *et al*. (2007*a*). The general pattern was stable over two years and at the two sites. Such low prevalence of *T. gondii* and stability of infection patterns are far from being a generality in rodents, and a review of recent works strongly suggests that species and geographic variability is rather a common fact in rodents.

For instance, for small species such as *Apodemus sylvaticus* and *M. glareolus, T. gondii* prevalence has been found to be low (< 5%, e.g. this study, Afonso *et al*. 2007*a*), intermediate (10–20%, e.g. Jackson and Siim, 1986; Kijlstra *et al*. 2008) or high (30–40%, e.g. Fuehrer *et al*. 2010; Thomasson *et al*. 2011) depending on the study. Commensal species (mice and rats) even exhibit the largest range of prevalence, varying from 0 to 60–70% across studies (Kuticic *et al*. 2005; Salibay and Claveria, 2005; Dubey *et al*. 2006; Murphy *et al*. 2008; Vujanic *et al*. 2010; Yin *et al*. 2010; Jittapalapong *et al*. 2011; Thomasson *et al*. 2011; Ahmad *et al*. 2012; Mosallanejad *et al*. 2012). Altogether these results suggest that the interplay between biological, ecological and spatial factors strongly shape the patterns of *T. gondii* prevalence in rodents and that the interpretation of these patterns is not straightforward, as illustrated by the study of Thomasson *et al*. (2011) who found an unexpected high level of *T. gondii* prevalence (41%) in a population of *A. sylvaticus* in an area where the density of cats was very low (< 2·5 individuals km^{-2}).

Numerous factors can play a role in this high variability of prevalence of *T. gondii* in rodents. Between-studies differences may partly result from variation in detection success of *T. gondii*, due to the sensitivity of methods used. Several cases of truly infected mice or rats detected as negative using serological tests have been reported in the literature (Dubey *et al*. 1997; Owen and Trees, 1998; Araujo *et al*. 2010). Araujo *et al*. (2010) isolated *T. gondii* from a rat which was identified as seronegative using MAT test with a low cut-off threshold. This suggests that serological tests, especially when using a high threshold, may underestimate pathogen prevalence. In our study PCR results suggest that we indeed missed some positive individuals by excluding 1:3 dilutions. However, bioassays in 1:3 positive individuals were negative, thus we kept the 1:6 threshold. Direct methods (PCR and bioassay) may also be negative in seropositive individuals and thus could not be used as a gold standard method. Altogether, all these observations suggest that direct and indirect approaches should be combined to detect *T. gondii* and assess prevalence in rodents.

Biological mechanisms such as variability in vertical transmission or susceptibility may also explain the difference of prevalence found between species and/or geographical areas (Thomasson *et al*. 2011; Li *et al*. 2012). High levels of vertical transmission have been documented in *M. domesticus, M. musculus* and *A. sylvaticus* (Owen and Trees, 1998; Marshall *et al*. 2004). Between-species variation in susceptibility to *T. gondii*, which so far has only been observed in commensal species, with rats being more resistant to infection than mice (Li *et al*. 2012), should also be assessed in order to better understand *T. gondii* infection patterns in

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Table 3. Model selection results using Akaike information criterion. Each row represents a candidate model to explain *T. gondii* seroprevalence in rodents as a function of potential explanatory variables. For each model tested, the table indicates the number of degrees of freedom (D.F.), corrected quantitative AICc (QAICc) and corrected AIC (AICc). The model with the minimum AICc (denoted AICmin) was considered as the best model to explain the data; all others models were evaluated based on the difference from this minimum ($\Delta i = AIC_i - AIC_{min}$). All these models have an $\Delta AIC < 2$. The Akaike weight (Wi) represents the relative likelihood for a given model to be the best among all other models. Akaike weight values which range from 0 to 1 can be interpreted as the probability for a model i to be the best model given the data and the numerous repetitions of the model selection exercise. The best model selected using this procedure is indicated in bold

Model	D.F.	QAICc	AICc	Δi	Wi
DISTANCE (to the nearest farm)	2	−115·91	235·85	0·00	0·18
SPECIES2 (commensal *vs* non-commensal) + DISTANCE	3	−115·33	236·69	0·85	0·12
YEAR + DISTANCE	3	−115·38	236·79	0·94	0·11
HABITAT2 (village *vs* other) + DISTANCE	3	−115·49	237·01	1·16	0·10
DISTANCE + MASS	3	−115·53	237·10	1·25	0·10
SPECIES2 + DISTANCE + YEAR	4	−114·59	237·24	1·39	0·09
DISTANCE + SITE	3	−115·71	237·45	1·60	0·08
SPECIES2 * DISTANCE	4	−114·74	237·54	1·69	0·08
HABITAT2 + DISTANCE + YEAR	4	−114·78	237·61	1·76	0·07
DISTANCE + SEX	3	−115·90	237·84	1·99	0·07

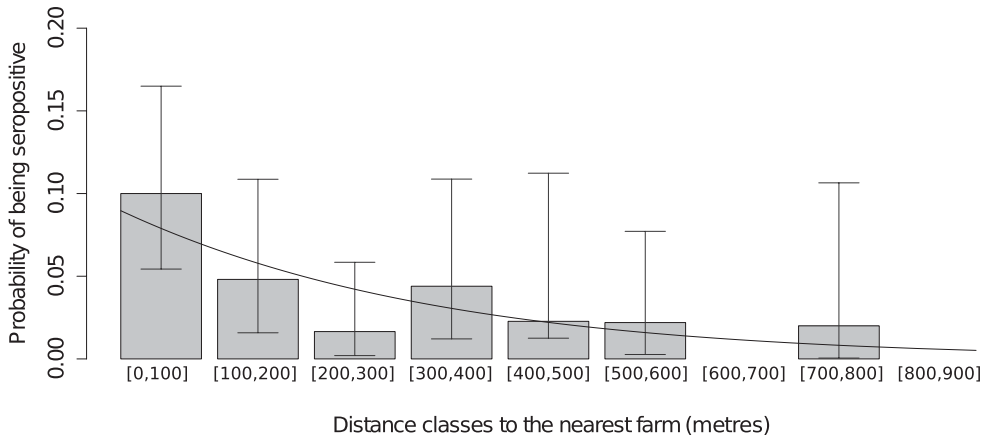

Fig. 4. Proportion of positive rodents observed within each distance class of 100 m with 95% confidence interval calculated following Clopper and Pearson (1934). The probability to be seropositive for rodents, as predicted by the best model, is represented as a function of distance to the nearest farm (black line).

rodent species. Altogether, the results of the studies conducted during the last decade highlight our current lack of understanding of the sources of variability of *T. gondii* prevalence in rodents. The low observed prevalence also limits the use of rodents as indicators to analyse environmental contamination and direct measures should be preferred.

Besides estimating prevalence, we aimed at disentangling the effects of biological, ecological and spatial factors expected to significantly impact the level and spatial distribution of *T. gondii* infection in rodents in rural areas. As the overall value of seroprevalence was low, we were unable to compare all variables and modalities together. For example, species other than rats and habitats other than villages could not be discriminated in our dataset. The preliminary multivariate analysis revealed a potential confounding effect of species (non-commensal *vs* commensal species) and habitat or distance to the nearest farm. These three variables strongly correlated with rodent *T. gondii* serostatus.

Taking these results into account, the logistic regression and selection model procedure highlighted the explanatory power of the distance to the nearest farm but failed to confirm the other effects previously found. Individual body mass or fossorial lifestyle did not influence the probability of *T. gondii* infection, as previously suggested (Afonso *et al.* 2007*a*; Reperant *et al.* 2009). However, these studies did not account for potential confounding effects with the local environment of captured individuals. It is then difficult to make a full conclusion as to any potential interaction between ecology and local environment.

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Table 4. Model-averaged parameter estimate (β) with standard error (S.E.) and the odds ratio with 95% confidence interval (CI) for variables included in models with $\Delta AIC < 2$. The variable 'species' differentiates commensal and non-commensal species

Variable	RVI	β (SE)	Odds ratio	P value
Intercept		−2·33 (0·37)	0·10 (0·05–0·20)	<0·01
DISTANCE to the nearest farm, per 100 m	1·00	−0·29 (0·12)	0·75 (0·60–0·94)	0·01
SPECIES2 = commensal	0·29	1·06 (0·97)	2·89 (0·43–19·39)	0·27
YEAR = 2011	0·11	−0·44 (0·39)	0·64 (0·30–1·39)	0·26
HABITAT2 = village	0·18	0·65 (0·63)	1·91 (0·56–6·52)	0·30
MASS, per 100 g	0·10	0·16 (0·18)	1·18 (0·82–1·68)	0·38
SITE = Briquenay	0·08	0·25 (0·39)	1·28 (0·60–2·75)	0·52
SPECIES2 × DISTANCE	0·08	−2·96 (3·06)	0·05 (0·00–20·90)	0·33
SEX = male	0·07	−0·06 (0·39)	0·94 (0·44–2·00)	0·87

The risk of *T. gondii* infection showed non-significant variations according to the site and year considered. The general pattern was stable over two years and at the two sites, although spatio-temporal variability could be explored further.

Distance to the nearest farm was the variable most clearly related to serological status when considering either all rodents or the subset of non-commensal species. The chance of a rodent testing positive for *T. gondii* decreased by 0·75 with every 100 m increase in distance from the nearest farm (95% IC: 0·60–0·94). This decrease was less pronounced than in a study by Lehmann *et al.* (2003): their estimate of −0·01 per m gave an OR of 0·37 per 100 m. The effect of distance to the nearest farm was confounded with, and probably explained by, the difference between commensal and non-commensal species and the contrast between village areas and other habitats. A similar confounding effect between rodent species and habitat was found in a recent study on *T. gondii* prevalence in a tropical area (Mercier *et al.* 2013). A high presence of cats near and within farms may explain this spatial pattern: Ferreira *et al.* (2011) showed that areas around farms were favoured by cats, probably due to the abundance of resources provided by people. Juveniles and stray cats, that are particularly at risk of toxoplasmosis, are particularly common on farms (Dubey *et al.* 1995; Gauss *et al.* 2003). Lehmann *et al.* (2003) suggested that farms could be considered as a source of *T. gondii* from which the surrounding environment is contaminated.

We detected a species effect which was confounded with distance: *R. norvegicus* exhibited the highest level of infection by *T. gondii* (12·9 *vs* 3·7% on average for other species). This result is also consistent with Lehmann *et al.* (2003) who also found a higher seroprevalence in *R. norvegicus* (50%) than in other wild rodents (0–6%). However, seroprevalence varied between our two sampling sites (0/10 and 4/21 of the trapped rats were infected in Boult-aux-bois and Briquenay, respectively), and the total prevalence did not reach the same level found by Lehmann *et al.* (2003). These results add to the list of inconsistencies found in the literature on both the prevalence of *T. gondii* in *R. norvegicus* and its potential importance in the local maintenance of *T. gondii* (Salibay and Claveria, 2005; Dubey *et al.* 2006; Vujanic *et al.* 2010; Yin *et al.* 2010; Jittapalapong *et al.* 2011; Mosallanejad *et al.* 2012). When making inferences on the role of *R. norvegicus* in the *T. gondii* life cycle, one should keep in mind that its ecology strongly differs from that of other rodents such as voles. *Rattus norvegicus* is a widespread and opportunistic species considered to be a true commensal, as it almost only lives in close proximity to humans where cats are more abundant (Aplin *et al.* 2003; Ferreira *et al.* 2011). *Rattus norvegicus* lives in colonies which are generally established close to food resources and which defend their territory against alien rats (Barnett and Spencer, 1951). Rats are therefore likely to be highly exposed to the oocysts present in anthropized areas where cats are abundant, as was the case in the farms sampled in this study. Moreover, within rat colonies, reproduction is mostly due to a few dominant females (Ziporyn and McClintock, 1991) and females could be more infected by *T. gondii* than males (Yin *et al.* 2010). Other recent works also revealed that *T. gondii* may manipulate rodents by enhancing the sexual attractiveness of infected males and reducing the innate fear of cat odour in infected individuals (Berdoy *et al.* 2000; Lim *et al.* 2013; Vyas, 2013). Altogether, these particularities could generate a strong variability in the level of infection between rat colonies. For instance, vertical transmission could be particularly high within colonies where dominant females are contaminated, since they are the ones mainly involved in reproduction and because their avoidance of infected males may be relaxed though parasitic manipulation. A reduction of innate fear to cat odours could also increase the risk of rats to be predated during their whereabouts within and around farm buildings, further enhancing between-hosts transmission.

CONCLUSION

We presented the first study accounting for the potential interplay between biological, ecological and

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spatial factors in shaping the pattern of *T. gondii* infection in rodents. We found that the proximity of individuals to farm buildings is a major determinant of *T. gondii* rodent infection in rural areas, where farm buildings are located within villages. The key role of farms in *T. gondii* epidemiology needs however to be confirmed in other contexts, such as when farms are located far away from residential areas. Our results suggest that oocyst contamination may decreases with increasing distance from the farms. This parameter could therefore be relevant when estimating the risk of human contamination from the environment. As oocysts are becoming increasingly acknowledged as a source of human infection (Boyer *et al*. 2011), measuring levels of *T. gondii* soil contamination, and the presence of cats in different habitats, could improve our understanding of the spatial dynamics of oocysts. This would enable us to better estimate the risk of human contamination and design preventive measures against toxoplasmosis.

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