Ecological factors influencing small mammal infection by Anaplasma phagocytophilum and Borrelia burgdorferi s.l. in agricultural and forest landscapes

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Ecological factors influencing small mammal infection by *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in agricultural and forest landscapes

Running title:

Tick-borne infection in small mammals

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Significance statement: We investigated intrinsic and extrinsic ecological factors of two tick-borne infections in small mammals. We found that the annual fluctuation and the structure of the small mammal community (i.e. abundance and species composition) together with the difference in small mammal species susceptibility can influence the prevalence of the studied infectious agents. We found no evidence of a relationship between the temporal and spatial variations of the prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in small mammals and the questing *Ixodes ricinus* nymph abundance, the most widespread hard tick vector species in Europe. Our results suggest that endophilic tick species, which are known to be competent vectors for *A. phagocytophilum* and suspected to be competent vectors for *B. burgdorferi* s.l., and more prevalent on the small mammals at nymphal and adult stages, may play a more important role in the maintenance of the enzootic cycle of these bacteria than previously thought.

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Summary

Small mammals are key components of numerous tick-borne disease systems, as hosts for immature ticks and pathogen reservoirs. To study the factors influencing tick-borne infection in small mammals, we trapped small mammals and collected questing ticks in spring and autumn in 2012 and 2013 at 24 sites in a 10x15 km rural landscapes (Brittany, France). Tissue samples were screened by real-time PCR for *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato. Of the two dominant small mammal species captured, bank voles (*Myodes glareolus*) had higher prevalence than wood mice (*Apodemus sylvaticus*) for both infections, presumably because of specific differences in immunological defenses. Prevalence of infections was higher in 2013 than in 2012, likely because small mammals were fivefold less abundant in 2013, favoring tick aggregation. Bacterial prevalence, which was higher in autumn, was not associated to questing *Ixodes ricinus* nymph abundance which was 6 times higher in spring, but rather to the structure of the small mammal community. These findings suggest the involvement of endophilic tick species, *I. trianguliceps* and/or *I. acuminatus*, in bacterial transmission. Our study highlights that the entire community of hosts and vectors, and their interactions, should be considered to fully understand the epidemiology of vector-borne diseases.

Introduction

By their hematophagous feeding behavior, ticks are vectors of a large diversity of infectious agents worldwide (Jongejan and Uilenberg, 2005). Hard ticks only consume three blood meals during their lifecycle. Their first blood meal allows the tick to molt from larva to nymph, the second from nymph to adult and the third allows the adult female tick to produce eggs.

By their abundance and their ubiquity, small mammals are key components of the maintenance of many tick-borne infectious agents transmitted by hard tick species (Ixodidae), including some pathogens of public health and veterinary importance like those responsible for Lyme disease and granulocytic anaplasmosis (Franke *et al.*, 2013; Stuen *et al.*, 2013; Hofmeester *et al.*, 2016). The transmission dynamics of tick-borne infectious agents is therefore partly determined by the propensity of small mammals to acquire them according to intrinsic (individual) and extrinsic (host and vector community structure, landscape context) factors.

Here, we studied two groups of bacteria hosted by small mammals with contrasted life history traits: *Anaplasma phagocytophilum*, the etiological agent of granulocytic anaplasmosis, and *Borrelia burgdorferi* sensu lato (s.l.), the etiological agent of Lyme disease. The strains of *A. phagocytophilum* found in European small mammals are specific of this group of hosts and are considered to be transmitted mainly by *Ixodes trianguliceps* (Bown *et al.*, 2008; Blaňarová *et al.*, 2014; Jahfari *et al.*,

2014), which is an endophilic (i.e. species living in the hosts' burrows) tick species that parasitizes small mammals (Cotton and Watts, 1967; Randolph, 1975; Gilot *et al.*, 1976). The *A. phagocytophilum* infections in small mammals are short-living, lasting only few weeks (Bown *et al.*, 2003). Transovarial transmission (i.e. from adult female tick to larvae) for this tick-borne infectious agent has only been reported in *Dermacentor albipictus*, an American species, and never in the *Ixodes* genus (Baldridge *et al.*, 2009; Stuen, 2013). Thus, European small mammals are thought to acquire *A. phagocytophilum* mainly from the bite of infected nymphal and adult female of *I. trianguliceps* ticks and maybe of other endophilic tick species.

The *B. burgdorferi* s.l. genospecies, which are *Borrelia* species discriminated genetically, found in small mammals in Europe are mainly *B. afzelii* and *B. burgdorferi* sensu stricto (s.s.) (Kurtenbach *et al.*, 2002; Wodecka, 2011; Franke *et al.*, 2013). These genospecies can also be found in medium-sized mammal like hedgehogs, hares and squirrels (Gern *et al.*, 1997; Kjelland *et al.*, 2011; Pisanu *et al.*, 2014). The *B. burgdorferi* s.l. bacteria can be transmitted by *I. ricinus*, the most common host-generalist exophilic (i.e. tick species with active host questing on the vegetation) tick species in Europe. Two endophilic tick species, *I. trianguliceps* and *I. acuminatus*, are known to host these bacteria, and are thus suspected vectors (Doby *et al.*, 1990; Hubbard *et al.*, 1998; Rauter and Hartung, 2005; Szekeres *et al.*, 2015). The infections by *B. burgdorferi* s.l. are long-lasting (Gern *et al.*, 1994; Humair *et al.*, 1999). Transovarial transmission in ticks is thought to be very rare or impossible (Richter *et al.*, 2012; Rollend *et al.*, 2013). As small mammals rarely harbor adult female *I. ricinus*, where they are infected with *B. burgdorferi* s.l. the source is assumed to be *I. ricinus* nymphs.

Tick-borne infection of small mammals relies on their exposure to infected ticks. The small mammals, and sometimes birds, are considered the major hosts for *I. ricinus* larvae, which are virtually free of the two bacteria studied here, but can become infectious as nymphs and adult females (Tälleklint and Jaenson, 1994; Pichon *et al.*, 2003; Hofmeester *et al.*, 2016). The acarological risk for humans and domestic animals is often estimated by the density of questing infected *I. ricinus* nymphs, which is the density of *I. ricinus* nymphs times the infection prevalence. Thus, the exposure of small mammals to infectious ticks is assumed to rely partly on the questing activity of *I. ricinus* nymphs.

Activity of exophilic ticks like *I. ricinus* depends on abiotic conditions, particularly humidity and temperature, resulting in a seasonal activity pattern. Depending on climate and annual meteorological conditions, the activity pattern of *I. ricinus* nymphs is either unimodal (for wet mild summers) with an abundance peak in early summer or bimodal (for hot dry summers, as in our study area) with a major abundance peak in late spring and a minor abundance peak in early autumn (Kurtenbach *et al.*, 2006; Daniel *et al.*, 2015; Alonso-Carné *et al.*, 2016). This second peak has been attributed to the early emergence of larvae fed in early spring (Perret *et al.*, 2004). By contrast, endophilic ticks (e.g. *I. trianguliceps* and *I. acuminatus*) spend all their life in their hosts' burrows, where the abiotic conditions are buffered (Cotton and Watts, 1967) and thus rely less on climatic conditions.

The exposure of small mammals to ticks relies also on their population density because this determines their individual parasitic burden (Renwick and Lambin, 2012). Small mammal population size can fluctuate substantially within and between years (Crespin *et al.*, 2002; Lambin *et al.*, 2006). It is subject to large inter-annual fluctuations whereby a peak in small mammal density can trigger nymphal tick upsurge the following year (Ostfeld *et al.*, 2006; Perez *et al.*, 2016). This temporal variation in small mammal population size can also drive the transmission dynamics of tick-borne infections (Goodwin *et al.*, 2001; Ostfeld *et al.*, 2006; Rosà and Pugliese, 2007). For instance, having many ticks aggregated on a few hosts can increase the transmission rate of infectious agents (Perkins *et al.*, 2003; Ferreri *et al.*, 2014) . In temperate areas, most small mammal species start breeding in spring and their population sizes reach a peak in autumn before decreasing until the following spring (Watts, 1969; Montgomery, 1989; Butet *et al.*, 2006). Thus late spring (peak of *I. ricinus* nymph activity) and autumn (peak of small mammal population size) are the most interesting seasons to investigate interactions between small mammals and ticks.

Exposure to ticks also varies by small mammal species according to differences in body size, habitat use, grooming behaviour (Keesing *et al.*, 2009; Pisanu *et al.*, 2010), and immunological response to tick attachment (Randolph, 1994; Hughes and Randolph, 2001a). Immunological differences also dictate small mammals' competence as reservoir hosts for tick-borne infectious agents (Kurtenbach *et al.*, 1995).

Within each small mammal species, among individual differences may also influence the probability of tick exposure and therefore of conta racting tick-borne infection. Heavier individuals, presumed older, generally experienced higher encounter rates with ticks (Harrison *et al.*, 2010; Mysterud *et al.*, 2015). Home range size, which is linked to body mass, is also frequently associated to tick load in small mammals and thus to the probability to acquire tick-borne infection (Dallas *et al.*, 2012; Devevey and Brisson, 2012; Godsall *et al.*, 2013). For instance, Devevey and Brisson (2012) found a positive relationship of the body mass and the home range size of male white-footed mice (*Peromyscus leucopus*) with their nymphal tick load.

There is also a trade-off between reproduction and survival which is mediated by testosterone level in male rodents. This trade-off results in a weaker immunological response in the more sexually active males, inducing a higher tolerance to tick attachment and prolonged infections (Hughes and Randolph, 2001a, 2001b). Sexual activity in males is also associated to increased home range size, as a way to maximize mating opportunities (Attuquayefio *et al.*, 1986; Godsall *et al.*, 2013). Conversely, female rodents in early breeding season have reduced home range size linked to territoriality and female-female competition (Korn, 1986; Godsall *et al.*, 2013). Body mass may thus interact with sex in determining the exposure risk.

In the present study, we investigated various biotic and abiotic ecological factors of small mammal infection by *A. phagocytophilum* and *B. burgdorferi* s.l.. We hypothesized that, among intrinsic factors: 1) there are species specific differences in the infection probability of small mammals reflecting differences in their immune response and/or exposure to ticks; 2) males are more likely infested by ticks and thus more infected than females because of sexual activity and/or immune response trade-off; 3) the *B. burgdorferi* s.l. infection probability is positively linked to body mass, as body mass is a proxy for age and for home range, and thus for tick exposure, in males; and among extrinsic factors: 4) the highest infection prevalence occurs the year with the lowest small mammal abundance, because a highest tick load per individual is expected when small mammals are less abundant; and 5) infection with *B. burgdorferi* s.l. is more prevalent when the abundance of questing *I. ricinus* nymphs is highest, but this is not true for *A. phagocytophilum* which is dependent on endophilic ticks.

Results

Small mammal community

A total of 608 small mammals were screened for the presence of *A. phagocytophilum*: 452 wood mice (*Apodemus sylvaticus*), 147 bank voles (*Myodes* [*Clethrionomys*] *glareolus*), 4 field voles (*Microtus agrestis*), 2 common pine voles (*Microtus subterraneus*) and 3 crowned shrews (*Sorex coronatus*) (**Table1**). The same individuals were screened for *B. burgdorferi* s.l., with the exception of two wood mice. Only wood mice and bank voles, which constituted 98% of the captures, were considered in the following statistical analyses. Wood mice and bank vole abundance per site were significantly higher in 2012 than in 2013 (wood mice: $V = 1045$, $p < 10^{-7}$, Cohen's $d = 1.174$; bank voles: $V = 656.5$, $p < 10^{-5}$, Cohen's $d = 0.752$). Wood mouse abundance was significantly higher in autumn than in spring ($V = 197.5$, p-value = 0.003, Cohen's $d = 0.447$), but bank vole abundance was not different between seasons ($V = 191.5$, $p = 0.801$).

[**Table 1 here**]

The relationships between our explanatory variables was explored following a model selection procedure of LMMs with body mass as a function of year, season, small mammal species, and sex, with the sampling site as random factor. In the best LMM according to AICc values, body mass differed significantly between years ($p < 10^{-3}$; mean 2012 = 19.1 ± 0.176 g; mean 2013 = 20.3 ± 0.206 g; Cohen's $d = 0.273$), between seasons ($p < 10^{-15}$; mean spring = 20.9 ± 0.252 g;

mean autumn = 18.2 ± 0.206 g; Cohen's d = 0.610), and between sexes ($p < 10^{-5}$; mean female = 18.7 ± 0.252 g; mean male = 19.8 ± 0.257 g; Cohen's d = 0.254). This model explained about one half of the variance in body mass (marginal pseudo-R^2 = 0.380; conditional pseudo-R^2 = 0.526). The average body mass did not differ significantly between wood mice and bank voles (p-ANOVA of the global LMM = 0.639; mean = 19.3 ± 0.182 g).

Tick burden on small mammals

Almost all caught small mammals were examined for their tick burden (**Table 2**). Although all larvae were not identified at the species level, all were belonging to the *Ixodes* genus. Many of the identified larvae were *I. ricinus* or *I. acuminatus*. Wood mice were more frequently infested by larvae than bank voles (Chi^2 test: $p < 10^{-4}$, $Chi^2 = 16.7$). Other tick stages were scarce. Among them, endophilic tick species were more frequent than the exophilic *I. ricinus* ticks (Chi^2 test: $p < 10^{-3}$, $Chi^2 = 11.4$), for which nymphs were present on only 0.7% of the small mammals in autumn 2012 (3 nymphs on two wood mice). The prevalence of nymphal and adult female endophilic ticks on bank voles and on wood mice was not significantly different (Chi^2 test: $p = 0.544$, $Chi^2 = 0.368$), even when considering tick species separately (*I. trianguliceps*: $p = 0.541$, $Chi^2 = 0.375$; *I. acuminatus*: $p = 0.953$, $Chi^2 = 0.004$).

[**Table 2 here**]

To identify the factors affecting the individual tick burden of small mammals, a model selection procedure was undertaken for negative binomial GLMMs of the total tick burden and of the burden of nymphal and adult female ticks (i.e. potentially infectious ticks) as a function of year, season, small mammal species, sex, body mass, interaction between sex and body mass (the effect of body mass can be sex dependent, i.e. particularly more marked in males), and total small mammal abundance. The best GLMM of the total tick burden included the small mammal species ($p < 10^{-3}$), body mass ($p < 10^{-6}$), and the interaction between sex and body mass ($p = 0.024$, pseudo-$R^2 = 0.084$). Wood mice harbored significantly more ticks than bank voles. Body mass was positively associated to the tick burden, with a stronger increase of tick burden with body mass in males (**Figure 1a**). When considering nymphal and adult female ticks only, the best model included the abundance of small mammals, which was negatively associated to the tick burden ($p < 10^{-3}$, pseudo-$R^2 = 0.090$; **Figure 1b**).

[**Figure 1 here**]

When considering wood mice alone, the best model for total tick burden included body mass ($p < 10^{-7}$, pseudo-$R^2 = 0.069$), and the best model for nymphal and adult female tick burden included the abundance of small mammals ($p = 0.009$, pseudo-$R^2 = 0.068$). For bank voles, the best model for the total tick burden included the season (0.005, pseudo-$R^2 = 0.061$), with bank voles more infested in autumn, and the best model for nymphal and adult female tick burden included year ($p < 10^{-15}$, pseudo-$R^2 = 0.201$), with a higher infestation rate in 2013 compared to 2012, and sex ($p < 10^{-15}$), with males being more infested than females. There was no effect of the abundance of one small mammal species on the tick burden of the other.

Intrinsic factors influencing Anaplasma phagocytophilum *and* Borrelia burgdorferi *s.l. infection of wood mice and bank voles*

To identify the factors affecting the *A. phagocytophilum* and *B. burgdorferi* s.l. infection probability of small mammals (how likely a given individual is to be infected), a model selection procedure was undertaken on binomial GLMMs of the infectious status of small mammals (1 = positive, 0 = negative) as a function of year, season, small mammal species, sex, body mass and the interaction between sex and body mass. We found *A. phagocytophilum* in 42 small mammals, corresponding to an average prevalence of 6.92% [95%CI: 5.03-9.24] (**Table 1**). The best model for *A. phagocytophilum* infection included the small mammal species, year, and season. This model explained almost half of the variation in infection rate as expressed by the conditional pseudo-R^2, among which a quarter is due only to the fixed effects only, as expressed by the marginal pseudo-R^2 (**Table 3**). Bank voles were about three times more likely to be infected than wood mice (wood mice: 4.45% [95%CI: 2.74-6.80]; bank voles: 13.6% [95%CI: 8.51-20.2]). Small mammals were significantly less likely to be infected in spring than in autumn (spring: 1.68% [95%CI: 0.460-4.25]; autumn: 10.1% [95%CI: 7.14-13.6]). Similarly, a lower infection rate was observed in 2012 compared to 2013 (2012: 5.69% [95%CI: 3.81-8.12]; 2013: 11.5% [95%CI: 6.11-19.3]). The body mass, sex, and the interaction between body mass and sex were not included in the best model. The analyses were conducted separately for each small mammal species and showed similar results between years and seasons (not detailed).

[**Table 3 here**]

We found *B. burgdorferi* s.l. in 26 individuals, or an average prevalence of 4.28% [95%CI: 2.82-6.21] (**Table 1**). The best model of *B. burgdorferi* s.l. infection probability included the small mammal

species, year and season (**Table 4**). This model explained almost half of the variation in infection probability, where a quarter of the variation is explained by the fixed effects, according to conditional and marginal pseudo-R^2, respectively. There was a statistical significant effect of small mammal species on individual infection probability, bank voles being about two times more likely to be infected than wood mice (wood mice: 3.13% [95%CI: 1.72-5.19]; bank voles: 7.48% [95%CI: 3.79-13.0]). As for *A. phagocytophilum*, a significantly lower rate of *B. burgdorferi* s.l. infection was observed in spring than in autumn (spring: 2.07% [95%CI: 0.674-4.76]; autumn: 5.80% [95%CI: 3.63-8.73]) and a lower infection rate was observed in 2012 compared to 2013 (2012: 1.21% [95%CI: 0.444-2.61]; 2013: 18.7% [95%CI: 11.8-27.4]). Sex, body mass and their interaction were not retained in the best model.

The analyses were also conducted separately for the two small mammal species. For wood mice, infected individuals were only found in autumn 2013. No significant effect of sex or body mass was observed in autumn 2013 ($p > 0.05$). For bank voles, year and body mass had significant effects in single explanatory variable models (year: $p = 0.001$, conditional pseudo-$R^2 = 0.127$, marginal pseudo-$R^2 = 0.127$; body mass: $p = 0.004$, marginal pseudo-$R^2 = 0.165$, conditional pseudo-$R^2 = 0.165$; **Figure 2**). Five individuals were coinfected by *A. phagocytophilum* and *B. burgdorferi* s.l.: four wood mice and one bank vole, all captured in autumn 2013.

[**Table 4 and figure 2 here**]

Influence of small mammal community structure and questing I. ricinus *nymph abundance on* Anaplasma phagocytophilum *and* Borrelia burgdorferi *s.l. prevalence in wood mice and bank voles*

A peak in questing *I. ricinus* nymph abundance was observed in late spring. We found a six fold higher number of *I. ricinus* nymphs per 100 m^2 in May-June than in October (May-June: mean = 38.1 ± 6.26 [SE], median = 30, min = 0, max = 156; and October: mean = 6.06 ± 1.11, median = 3, min = 0, max = 67) (see Perez *et al.*, 2016 for more detailed results).

The relationships of the prevalence of the two tick-borne infectious agents was analysed using GLMs with variables relating to the small mammal community structure (abundance and species composition) and questing *I. ricinus* nymph abundance. Given the dominance of two small mammal species, the structure of the small mammal community was only characterized by the total small mammal abundance and the proportion of these two dominant small mammal species per site , year, and season.

The best GLM for the prevalence of *A. phagocytophilum* in both small mammal species together included year ($p = 0.006$), season ($p < 10^{-6}$), and a negative relationship with the proportion of wood mice ($p < 10^{-5}$; pseudo-$R^2 = 0.273$; **Figure 3a**). Questing *I. ricinus* nymph abundance was not retained in the best model and, when accounting for year and season, was not significantly associated to the infection prevalence in small mammals (p-nymph abundance = 0.330, p-year = 0.091, p-season = 0.004, pseudo-$R^2 = 0.148$). It was even significantly negatively associated to infection prevalence when considered alone ($p = 0.002$, pseudo-$R^2 = 0.064$).

The best GLM for *A. phagocytophilum* infection prevalence in wood mice included the year ($p = 0.011$) and the season ($p = 0.002$, pseudo-$R^2 = 0.207$; **Figure 3b**). The best GLM for *A. phagocytophilum* infection prevalence in bank voles included a positive relationship with total small mammal abundance ($p < 10^{-4}$) and a negative relationship with the proportion of wood mice ($p = 0.003$; pseudo-$R^2 = 0.274$; **Figure 3c**).

For *B. burgdorferi* s.l., the best GLM considering the prevalence of both small mammal species together only included year ($p < 10^{-10}$, pseudo-$R^2 = 0.273$; **Figure 3d**). Questing *I. ricinus* nymph abundance was not retained in the best models and, when considered with year and season, was not significantly associated to the infection prevalence in small mammals (p-nymph abundance = 0.230, p-year $< 10^{-9}$, p-season = 0.082, pseudo-$R^2 = 0.410$), nor when considered alone ($p = 0.967$, pseudo-$R^2 = 0$).

Wood mice infected with *B. burgdorferi* s.l. were only found in autumn 2013. The best GLM of the prevalence of these bacteria in wood mice in this season only included the abundance of small mammals, which displayed a significant negative relationship ($p = 0.006$, pseudo-$R^2 = 0.242$; **Figure 3e**). The best GLM for the prevalence of *B. burgdorferi* s.l. in bank voles included the year ($p < 10^{-3}$) and the proportion of wood mice ($p = 0.008$), which displayed a significant positive relationship, (pseudo-$R^2 = 0.318$; **Figure 3f**).

[**Figure 3 here**]

Discussion

Differences in infection probability among bank voles and wood mice

We investigated by PCR methods the infection status of 5 species and 608 small mammals for *A. phagocytophilum* and 606 small mammals for *B. burgdorferi* s.l.. GLMMs were used to explain the

infection status of the two main small mammal species, wood mice and bank voles, as a function of intrinsic factors, and revealed significant differences in individual infection probability.

The bank voles were about three times more likely than wood mice to be infected with *A. phagocytophilum* and two times more likely for *B. burdorferi* s.l., confirming the hypothesis 1): there is a difference in the infection probability of small mammal species reflecting differences in their immune response and/or exposure to ticks. Actually, this difference in prevalence of *B. burgdorferi* s.l. between the two small mammal species is unlikely to result from differences in *I. ricinus* tick exposure, because these two small mammal species have home ranges of similar size (Kikkawa, 1964) and because bank voles were less heavily infested by this tick species than wood mice (Doby *et al.*, 1992; L'Hostis *et al.*, 1996; Boyard *et al.*, 2008; Perez *et al.*, 2016). The difference in tick burden can be partly explained by the immune acquired resistance to tick attachment in bank voles (Randolph, 1994; Dizij and Kurtenbach, 1995; Perez *et al.*, 2016).

The difference of *A. phagocytophilum* prevalence between wood mice and bank voles had already been partly analysed and discussed elsewhere (Chastagner *et al.*, 2016). The *I. trianguliceps* ticks, the most likely vectors of *A. phagocytophilum* in small mammals, are known to be more prevalent on bank voles than on wood mice, despite the acquired resistance of bank voles to this tick species (Gilot *et al.*, 1976; Randolph, 1994). Thus, Chastagner *et al*. (2016) proposed exposure to *I. trianguliceps* ticks as a possible explanation of the highest prevalence found in bank voles compared to wood mice. However, despite that most *I. trianguliceps* ticks were found on bank voles (six out of nine), this hypothesis is not statistically supported by the findings that nymphal and adult female *I. trianguliceps* were not significantly more prevalent on bank voles than on wood mice.

Yet, our results can be explained, for *A. phagocytophilum*, by a longer infection period in bank voles than in wood mice, and, for *B. burgdorferi* s.l., by a higher bacterial load in bank voles than in wood mice associated to a strongest immune response of wood mice to the spirochetes (Kurtenbach *et al.*, 1994; Bown *et al.*, 2003). Results from a study of the while-footed mouse (*Peromyscus leucopus*) and prairie vole (*Microtus ochrogaster*) which showed that species with higher tick burden were more capable of overcoming bacterial infection, presumably because of an evolutionary trade-off (Rynkiewicz *et al.*, 2013).

Relationship of infection probability with sex and body mass

As expected in hypothesis 2), we found a stronger increase in tick burden with body mass in males than in females wood mice and bank voles together, and a higher nymphal and adult female tick burden in male than in female bank voles. Male-biased tick load has previously been observed by Boyard et al. (2008) in wood mice, and by Siński et al. (2006) in yellow-necked mice (*Apodemus*

flavicollis) and in common voles (*Microtus arvalis*), but not in bank voles. However, contrary to what was expected in hypothesis 2, we did not find that males were significantly more infected with *B. burgdorferi* s.l or *A. phagocytophilum* than females. Perhaps the direct effect of sex on probability of tick-borne infection is not observable at the level of tick exposure experienced by small mammals in our study.

A study by Harrison et al. (2010) suggests that the sex-biased difference in parasite load might be mainly due to sex-driven body mass dimorphism. We observed such a dimorphism and actually found an increase in tick burden with body mass in both small mammal species. However we found a significant positive relationship only between the body mass and the probability of infection by *B. burgdorferi* s.l. in bank voles, but not in wood mice, partially supporting hypothesis 3). The increase of *B. burgdorferi* s.l. infection probability with the body mass in our study is consistent with the study of Tälleklint et al. (1993) who found that bank voles weighing more than 20 g were significantly more infective for *B. burgdorferi* s.l. (i.e. larvae became more infected when feeding on the animals) than those weighing less than 20 g. The positive relationship of body mass with infection probability almost certainly reflects an age effect, because this bacteria display long-lived infections.

The effect of sex and body mass might also be nonlinear in bank voles because of their density dependent acquired resistance to ticks. Kallio *et al*. (2014) found that the probability of *A. phagocytophilum* infection probability in bank voles increases with age of the animal until 2-5 months (estimated with morphological measures) before decreasing. This pattern could be explained by a lower exposure of young individuals of low body mass, which have smaller home ranges, with low tick exposure and thus low infection probability. A better resistance to tick attachment of the older individuals can explain their reduced infection probability. We observed a similar pattern in our data, considering body mass as a proxy for age: the only infected individual weighing more than 25 g was a pregnant female and no infected individual weighed less than 15 g. However, even considering a polynomial relationship, we did not find any significant relationship between infection probability and body mass (not shown). Our results suggest that the interactions of sex with body mass, breeding status, and past exposure could be important determinants of tick-borne infections in small mammals.

Inter and intra-year variations in infection probability

We observed a marked temporal infection pattern similar for *A. phagocytophilum* and *B. burgdorferi* s.l.. The infection probabilities were higher in 2013 compared with 2012, and in autumn compared with spring. Body mass was significantly different between years and between seasons, which could have obscured the effect of those variables. However, small mammals were heavier in spring than in autumn, which does not correspond with the observed difference in infection prevalence between seasons. Small mammals were heavier in 2013, but the difference is not as marked as between

seasons and is therefore not likely to have an important role in explaining the observed difference in prevalence between years.

While the overall *I. ricinus* questing nymph abundance was not significantly different between these two years (Perez *et al.*, 2016), the inter-annual difference can be explained by finding a lower abundance of small mammals in 2013 (n = 110) than in 2012 (n = 502), validating our hypothesis 4). Thus, in 2013, it is likely that more ticks attached on each small mammal, increasing the probability for a given individual to host an infected tick (Rosà *et al.*, 2007; Ferreri *et al.*, 2014). Nevertheless, this explanation is not valid for the intra-year variation: as small mammal abundance was higher in autumn and questing *I. ricinus* nymph abundance was higher in spring (Perez *et al.*, 2016), we thus expected a higher infection probability in spring. Furthermore, this temporal infection pattern was not associated to *I. ricinus* nymph abundance, as the probability of *B. burgdorferi* s.l. infection did not increase with this variable, neither in space nor in time, questioning our hypothesis 5).

Such an intra-annual pattern has already been observed in other studies for *A. phagocytophilum* (Bown *et al.*, 2003) and *B. burgdorferi* s.l. (Humair *et al.*, 1993). For *B. burgdorferi* s.l., a possible explanation is the exposure of small mammals to *I. ricinus* nymphs throughout the summer, because we cannot exclude a persisting activity of ticks at this season (Dobson *et al.*, 2011). Even with the recruitment of new cohorts, the animals caught in autumn could have been exposed to infected ticks during summer (cumulative infection probability)(Humair *et al.*, 1993; Tälleklint *et al.*, 1993). However, despite some support from the positive relationship between body mass and infection probability in bank voles with *B. burgdorferi* s.l., this explanation is not valid for *A. phagocytophilum*, because these bacteria cause short-lived infections.

The similarity of the infection patterns for both infectious agents suggests a common explanation. Small mammals actually rarely host *I. ricinus* nymphs or adults (Bown *et al.*, 2008) while they are the main hosts of all stages of the endophilic tick species *I. trianguliceps* and *I. acuminatus*. Our findings are consistent with the maintenance of transmission cycle in small mammals for at least one *A. phagocytophilum* ecotype without *I. ricinus*, but rather by *I. trianguliceps*, the main vector of *A. phagocytophilum* strains found in small mammals (Bown *et al.*, 2008, 2009; Blaňarová *et al.*, 2014; Jahfari *et al.*, 2014), and/or maybe by *I. acuminatus* (which was abundant on small mammals, while fixed *I. ricinus* nymphs were very scarce and only found on wood mice). Furthermore, the activity of *I. trianguliceps* nymphs peaks in summer and the activity of *I. trianguliceps* adults peaks in spring/early summer and autumn (Randolph, 1975; Cull *et al.*, 2017). This activity pattern is thus compatible with our results for the two groups of infectious agents studied. This hypothesis is also compatible with the concentration of potentially infectious ticks on hosts with a decreasing abundance of small mammals (more nymphal and adult female ticks per hosts), which were mainly *I. trianguliceps* or *I. acuminatus*. This concentration of potentially infectious ticks occurred mainly in the low abundance year, namely 2013 in our study (Katelina *et al.*, 1971).

We thus suspect a role of *I. trianguliceps* and/or *I. acuminatus* in the maintenance of the enzootic transmission of *B. burgdorferi* s.l. in small mammal communities (Doby *et al.*, 1990; Szekeres *et al.*, 2015). Such parallel enzootic transmission cycle of *B. burgdorferi* s.l. by tick species which do not bite humans has recently also been suspected in southern California (Macdonald *et al.*, 2017), and has been suggested for bird-associated *Borrelia* sp. and bird specialized ticks in birds communities in Europe (Heylen *et al.*, 2013).

Influence of the structure of the small mammal community on infection prevalence

The small mammal community structure seems to have a more important role on the transmission of the studied tick-borne infectious agents by influencing the encounter rate with endophilic ticks. Particularly, the proportion of wood mice in the community, which displayed heavier tick burden, seems to have a negative effect on the *A. phagocytophilum* prevalence in bank voles more susceptible to the bacteria, perhaps because they draw away nymphal and adult female ticks away from them. We did not observe a relationship between abundance the wood mice and the tick burden of bank voles, but such an effect of the abundance of one host species on the tick burden of another one have been demonstrated elsewhere (Brunner and Ostfeld, 2008). Thus, wood mice may act as a dilution host species for these bacteria. Conversely, there was a significant positive relationship between the proportion of wood mice and the prevalence of *B. burgdorferi* s.l. in bank voles. This may be an artefact caused by the increase in wood mouse abundance in autumn (while bank vole abundance decreased in autumn 2013), increasing the proportion of wood mice in the small mammal community per site, when the prevalence was higher. An alternative hypothesis is an increase in *I. ricinus* tick abundance in sites where wood mouse abundance is high which flows on into an increase in transmission by this vector tick species.

We did not consider the prevalence of *B. burgdorferi* in questing *I. ricinus* nymphs which may have allowed us to estimate the density of infected nymphs. However, various studies demonstrated that the density of infected *I. ricinus* nymphs for *B. burgdorferi* s.l is more dependent on their total density than to their infection prevalence (Tälleklint and Jaenson, 1996; Jouda *et al.*, 2004). Furthermore it is not relevant to compare prevalence of undetermined genospecies or strains. Indeed, more investigations on the circulating genospecies both in host and vector community are needed.

Conclusion

Small mammals vary in their ability to host ticks and to host infectious agents: bank voles are poor hosts for *I. ricinus* ticks, but good reservoir hosts for *A. phagocytophilum* and *B. burgdorferi* s.l., and the opposite is true for wood mice. This variability creates complex small mammal community effects

on the transmission of tick-borne infectious agent. Even less abundant (and countable), endophilic vector species - like *I. trianguliceps* or *I. acuminatus* - may have an important role in the maintenance of the cycles of zoonotic tick-borne infectious agents, while *I. ricinus* may primarily act as a bridge between the different host components and humans (Heylen *et al.*, 2013). It is thus important to consider all of the host and vector species and their interactions, together with their interactions with the infectious agents (competence), to better understand the dynamics of infectious diseases (Roche *et al.*, 2013). Small mammals are also subject to cyclic inter-annual fluctuations in population size that induce variation in the tick burden, which is a key determinant of the transmission dynamics of infectious agents and coinfection of ticks. The spatial context (i.e. landscape heterogeneity and connectivity), not accounted for here, can also structure the community composition, abundance, and dispersal of hosts and vectors, and thus, indirectly, the transmission rate and the diffusion of infectious agents (Lambin *et al.*, 2010). The fact that about a quarter of the variation in *A. phagocytophilum* infection probability is explained by the sampling site is in favour of such a hypothesis. Further studies to test for parallel maintenance cycle of *B. burgdorferi* s.l. involving different host and tick species are still needed.

Experimental Procedures

Study area and sampling scheme

Small mammals were sampled in the Zone Atelier Armorique (https://osur.univ-rennes1.fr/za-armorique/), which is located in north-eastern Brittany, France (48°30’ N, 1°32’ W). The land use of this agricultural area is mainly dedicated to cropping (wheat and maize) and livestock (cattle pastures) with traditional hedgerow networks between land patches (notably pedunculated oaks, *Quercus robur*, and chestnut trees, *Castanea sativa*). In its south-eastern part, this area also includes an exploited deciduous forest of 979 ha composed mainly of beeches (*Fagus sylvatica*) and pedunculated oaks.

Twenty-four sampling sites located equally in the forest and in the agricultural landscapes were selected. For the 12 “forest” sites, 6 were set in the forest core and six at the forest edges. For the 12 “agricultural” sites, 6 were along woods and 6 along hedgerows. This sampling scheme captured the diversity of the agricultural landscapes concerning woodland cover, meadows-crops covers and hedgerow network density of this area (Perez *et al.*, 2016). As grassland-woodland ecotones are shared by wild and domestic animals and favor interactions between ticks and hosts (Agoulon *et al.*, 2012), all sampling sites, except those in the forest core, were adjacent to pastures. To avoid autocorrelation, all sites were at least 500 m apart.

Small mammal sampling and ethic statement

Small mammals were trapped using 100-m trap lines constituted of 34 INRA live-traps with dormitory boxes spaced every 3 m and baited with a piece of apple, commercial rodent seeds and dry cat food. For each trapping session, traps were checked after 24 h and 48 h. Trapping sessions (n = 4) were carried out in May-June ("spring") and October ("autumn") 2012 and 2013, as we expected the highest interactions between small mammals and ticks at these periods, in accordance with the bimodal activity of *I. ricinus* nymphs (Kurtenbach *et al.*, 2006). Caught animals were euthanized in the field-lab by pentobarbital injection. Animals were identified at the species level, sexed and weighed to 0.5 g precision before dissection. Ticks attached on small mammals were removed for subsequent identification at the species level in the lab using binocular microscope and a determination key (Pérez-Eid, 2007).

The traps that we used are designed not to stress or harm the animals. The animals captured during this experiment were not of protected species and, therefore, no special authorization was needed for trapping, according to French law. All individuals were euthanized by authorized experimenters according to French law and to the European guidelines on the use of animals in science (Close *et al.*, 1997).

Questing Ixodes ricinus *nymphs sampling*

Questing ticks were collected on each site by dragging a 1-m² white flannel blanket on the substrate along a 300-m long transect (Gray and Lohan, 1982; Vassallo *et al.*, 2000). Each transect contained 10 sub-transects (10-m²) spaced every 20 m for a total sampled area of 100 m². At the woodland-ecotone sites, one transect was performed in the woodland (at about 10m² from the edge) and another one in the adjacent grassland. The number of questing *I. ricinus* nymphs from the two transects was then averaged. Ticks were collected in 70% ethanol before identification of tick stage and tick species with binocular microscope in the lab using determination key (Pérez-Eid, 2007). Ticks were sampled when the substrate was reasonably dry, not in dewing condition or after rainfall.

DNA extraction from small mammals and detection of Anaplasma phagocytophilum *and* Borrelia burgdorferi *s.l. in DNA samples*

The DNA from small mammal ear and spleen samples was extracted with NucleoSpin® Tissue Kit (Macherey-Nagel, Düren, Germany). The detection of *A. phagocytophilum* was presented in Chastagner et al. (2016). Briefly, a real-time PCR targeting the msp2 genes from DNA extracts of small mammal was performed according to the protocol of Courtney *et al*. (2004). To detect *B. burgdorferi* s.l. from DNA extracted from ears of small mammal, a real-time PCR in SybrGreen targeting the flaB gene was performed as described in Jacquot *et al*. (2016). We could not obtain

information on *B. burgdorferi* s.l. genospecies in our study. However, assuming similar host and vector ranges of the genospecies found in small mammals in Europe (*B. burgdorferi* s.s., *B. afzelii*, *B. bavariensis*, *B. spielmani*), we do not expect this simplification to bias the results. The considered factors are expected to have the same effects on each genospecies (Kurtenbach *et al.*, 2002; Richter *et al.*, 2004).

Statistical analyses

To assess potential confounding effects between body mass and small mammal species, sex, season or year, body mass was modelled as a function of those factors in Linear Mixed Models (LMMs) with multiple explanatory variables. Because of differences in landscape context, the sampling site was included as a random factor. The models were ranked according to the AICc (Akaike Information Criterion corrected for finite sample size) and the 95% most confident models according to their Akaike weight (lowest AICc-value) were conserved for variable significance evaluation and parameter estimation. The significance of the remaining variables in the 95% confidence set models was evaluated with a type II ANOVA. The first of these models which included only significant variables was considered as the best model. Because small mammal species could respond differently to some variables, the effect of sex, body mass, season and year was also assessed separately for each one.

To test our hypotheses on tick exposure, the total tick burden and the adult female and nymphal tick burden of small mammals was modelled as a function of year, season, small mammal species, sex, body mass, interaction between sex and body mass (the effect of body mass can be sex dependent, i.e. particularly more marked in males), and total small mammal abundance. Because of potential difference in tick abundance in different landscape context (Perez *et al.*, 2016), negative binomial Generalized Linear Mixed Model (GLMMs) with multiple explanatory variables was used with site as random factor. The same model selection procedure was applied as described above. Because each small mammal species could respond differently to some of the variables and to the abundance of the other species, separate models for each one were also built with the above mentioned variables and including a term for the abundance of the other species.

The infection status of small mammals for both infectious agents was modelled using binomial (with a binary response variable: negative = 0; positive = 1) GLMMs with multiple explanatory variables. The infection status of small mammals was modelled as a function of year, season, small mammal species, sex, body mass and the interaction between sex and body mass. We applied the same model selection procedure as described above.

To analyse the effect of small mammal community structure and questing *I. ricinus* nymph abundance, the prevalence of both infectious agents in small mammals per site was modelled using Generalized

Liner Models (GLMs). The explanatory variables were the questing *I. ricinus* nymph abundance, the total small mammal abundance, the proportion of each of the two dominant small mammal species in the community, the year, and the season. Because each species can be influenced differently by these variables, we also built separate models for each species. To assess whether some variables can have effects on the prevalence of the whole community, the prevalence of both species together was also modelled as a function of the same variables. The same model selection procedure as described above was then applied. As the proportions of each species were highly correlated, models containing these two variables at the same time were excluded.

Additionally, coinfection rates were compared using a Chi-square test on the whole data set or by trapping session. Abundance of small mammal species per site between year and between seasons were compared using paired Wilcoxon-Mann-Whitney tests. All statistical analyses were performed with the R 3.1.0 software, with the packages 'effsize', 'car', 'lme4', 'MuMIn', and 'visreg' (R Development Core Team, 2014).

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Competing interests

The authors declare having no competing interest.

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Tables and Figure legends

Table 1: Summary of the detection by real-time PCR of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato per trapping session by small mammal species and by sex.

Table 2: Summary of the tick burden per species by small mammal host species, year and season.

Table 3: Top 95% set of Generalized Linear Mixed Models of *Anaplasma phagocytophilum* infection probability of wood mice and bank voles.

Set of the 95% most supported models according to their AIC weights. Models are ranked based on their AICc values; the best model is the one with the lowest AICc value. Sampling site is included as random factor. The fixed variable coefficients are given with the following baseline factors: "wood mouse" for species; "female" for sex; "2012" for year; "autumn" for season. Significance of the variables in a given model are indicated by "*" for $p < 0.05$, "**" for $p < 0.01$, "***" for $p < 0.001$. "R^2marg": marginal pseudo-R^2; "R^2cond": conditional pseudo-R^2; "df": degree of freedom; "AICc": Akaike Information criteron corrected for finite sample size; "ΔAICc": difference of AICc relative to the most supported model. The conserved model is in bold. The null model is in italic.

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Figure 1: Total tick burden and nymphal and adult female tick burden as a function respectively of body mass and total small mammal abundance for wood mice and bank voles together.

Total tick burden (larvae, nymphs, and adult females) of wood mouse and bank vole as a function of body mass by species and by sex (**a**), and potentially infectious tick (nymphs and adult females only) burden by wood mouse and bank vole as a function of total small mammal abundance per site by year and season (**b**). The curves represent the fitted tick burden in negative binomial Generalized Linear Mixed Models with site as random factor (black; see legend for species and sex in (**a**)), and its 95% confidence interval (grey, averaged 95% confidence interval in (**a**) for readability). Those models correspond to the best models according to our AIC-based model selection procedure; see the text for further details. The gray gradient represents the density of individuals (see legend).

Figure 2: *Borrelia burgdorferi* s.l. infection frequency of bank voles as a function of body mass per one g-class.

Bars represent the cumulated frequency (left scale) of positive (dark grey) and negative (light grey)

bank voles as a function of body mass by one g-class. The curves represent the fitted infection probability (right scale) according to univariate binomial Generalized Linear Mixed Model with site as random factor (solid line; $p = 0.004$; marginal pseudo-$R^2 = 0.165$; conditional pseudo-$R^2 = 0.165$), and its 95% confidence interval (dashed lines).

Figure 3: Prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in wood mice, bank voles, and both species as a function of the variables included in the best GLMs.

Prevalence of *A. phagocytophilum* in both species as a function of the proportion of wood mice in the small mammal community and year (**a**), in wood mice as a function of year and season (**b**), and in bank voles as a function of total abundance of small mammals and the proportion of wood mice in the small mammal community (**c**); prevalence of *B. burgdorferi* s.l. in both species as a function of year (**d**), in wood mice as a function of total small mammal abundance in autumn 2013 (**e**), and in bank voles as a function of the proportion of wood mice in the small mammal community (**f**). The curves (**a**, **e**, and **f**) and the dark gradient (**c**) represent the fitted prevalence in binomial Generalized Linear Models. The area of the circles and/or squares is proportional to the number of tested individuals (see the legend). Those models correspond to the best models according to our AIC-based model selection procedure; see the text for further details.

Table 1: Summary of the detection by real-time PCR of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato per trapping session by sammal mammal species and by sex.

Species / Sex	Infectious status (positive/tested) [a]									
	2012				2013				**Total**	
	Spring		Autumn		Spring		Autumn			
	Aph	Bbs	Aph	Bbs	Aph	Bbs	Aph	Bbs	**Aph**	**Bbs**
Apodemus sylvaticus	**1/158**	**0/158**	**9/207**	**0/206**	**0/16**	**0/16**	**10/71**	**14/70**	**20/452**	**14/450**
Female	1/76	0/76	3/82	0/81	0/9	0/9	4/24	4/24	8/191	4/190
Male	0/81	0/81	6/124	0/124	0/7	0/7	6/47	10/46	12/259	10/258
Unknown	0/1	0/1	0/1	0/1					0/2	0/2
Myodes glareolus	**2/53**	**5/53**	**16//77**	**1/77**	**1/12**	**0/12**	**1/5**	**5/5**	**20/147**	**11/147**
Female	2/41	3/41	12/39	1/39	1/5	0/5	0/2	2/2	15/87	6/87
Male	0/12	2/12	4/38	0/38	0/7	0/7	1/3	3/3	5/60	5/60
Microtus agrestis	**0/2**	**0/2**	**0/2**	**0/2**					**0/4**	**0/4**
Female	0/1	0/1	0/2	0/2					0/3	0/3
Male	0/1	0/1							0/1	0/1
Microtus subterraneus					**0/1**	**0/1**	**0/1**	**1/1**	**0/2**	**1/2**
Female					0/1	0/1	0/1	1/1	0/2	1/2
Sorex coronatus			**1/1**	**0/1**	**0/1**	**0/1**	**1/1**	**0/1**	**2/3**	**0/3**
Female			1/1	0/1	0/1	0/1	1/1	0/1	2/3	0/3
All species	**3/213**	**5/213**	**26/287**	**1/286**	**1/30**	**0/30**	**12/78**	**20/77**	**42/608**	**26/606**
Female	3/118	3/118	16/124	1/123	1/16	0/16	5/28	7/28	25/286	11/285
Male	0/94	2/94	10/162	0/162	0/14	0/14	7/50	13/49	17/320	15/319
Unknown	0/1	0/1	0/1	0/1					0/2	0/2

[a] Aph: *Anaplasma phagocytophilum*; and Bbs: *Borrelia burgdorferi* sensu lato.

Table 2: Summary of the tick burden per species by small mammal host species, year and season.

Host species / Tick species	Number of inspected hosts, infested hosts, and total tick burden per species [a]									
	2012				2013				**Total**	
	Spring		Autumn		Spring		Autumn			
	L/N/F	InfH	L/N/F	InfH	L/N/F	InfH	L/N/F	InfH	**L/N/F**	**InfH**
Apodemus sylvaticus	159	93	206	69	16	14	72	32	**453**	***208***
I. acuminatus	149/0/0	*66/0/0*	45/2/2	*25/2/2*	20/1/0	*9/1/0*	0/0/6	*0/0/5*	**212/3/8**	***100/3/7***
I. ricinus	66/0/0	*38/0/0*	161/3/0	*55/2/0*	24/0/0	*11/0/0*	0	*0*	**251/3/0**	***104/2/0***
I. trianguliceps	0	*0*	0	*0*	0/4/0	*0/2/0*	0	*0*	**0/4/0**	***0/2/0***
Ixodes sp.	77/0/0	*36/0/0*	162/0/0	*39/0/0*	12/0/0	*6/0/0*	163/0/0	*28/0/0*	**414/0/0**	***109/0/0***
Myodes glareolus	54	*6*	75	*20*	12	*5*	6	*2*	**147**	***33***
I. acuminatus	8/0/0	*3/0/0*	12/1/1	*9/1/1*	4/1/0	*2/1/0*	0/1/1	*0/1/1*	**22/3/2**	***14/3/2***
I. ricinus	1/0/0	*1/0/0*	25/0/0	*16/0/0*	1/0/0	*1/0/0*	0	*0*	**27/0/0**	***18/0/0***
I. trianguliceps	0	*0*	1/0/0	*1/0/0*	0/5/0	*0/2/0*	0	*0*	**1/5/0**	***1/2/0***
Ixodes sp.	3/0/0	*3/0/0*	25/0/0	*14/0/0*	0	*0*	5/0/0	*2/0/0*	**33/0/0**	***19/0/0***
Microtus agrestis	2	*1*	2	*0*					**4**	***1***
I. acuminatus	1/0/0	*1/0/0*	0	*0*					**1/0/0**	***1/0/0***
I. ricinus	1/0/0	*1/0/0*	0	*0*					**1/0/0**	***1/0/0***
Microtus subterraneus					1	*0*	1	*0*	**2**	***0***
Sorex coronatus			1	*0*	1	*0*	1	*0*	**3**	***0***
All host species	215	*100*	284	*89*	30	*19*	80	*34*	**609**	***242***
I. acuminatus	158/0/0	*70/0/0*	57/3/3	*34/3/3*	24/2/0	*11/2/0*	0/1/7	*0/1/6*	**239/6/10**	***115/6/9***
I. ricinus	68/0/0	*40/0/0*	186/3/0	*71/2/0*	25/0/0	*12/0/0*	0	*0*	**279/3/0**	***123/2/0***
I. trianguliceps	0	*0*	1/0/0	*1/0/0*	0/9/0	*0/4/0*	0	*0*	**1/9/0**	***1/4/0***
Ixodes sp.	80/0/0	*39/0/0*	187/0/0	*53/0/0*	12/0/0	*6/0/0*	168/0/0	*30/0/0*	**447/0/0**	***128/0/0***
All tick species	**306/0/0**	***100/0/0***	**431/6/3**	***87/5/3***	**61/11/0**	***15/6/0***	**168/1/7**	***30/1/6***	**966/18/10**	***232/12/9***

[a] 'L/N/F': number of larvae, nymphs and adult females, respectively; and 'InfH': the corresponding number of infested hosts.

Table 3: Top 95% set of Generalized Linear Mixed Models of *Anaplasma phagocytophilum* infection probability of wood mice and bank voles .

Intercept	Species	Sex	Mass	Sex:Mass	Year	Season	R²marg	R²cond	df	AICc	ΔAICc
-3.16***	1.30**	-0.629			1.38**	-1.91***	0.255	0.449	6	246.7	0.00
-3.49* **	**1.36*** **				**1.34** **	**-1.83** **	**0.242**	**0.438**	**5**	**247.5**	**0.76**
-2.66**	1.31**	-0.602	-0.031		1.47**	-1.84**	0.261	0.462	7	248.4	1.65
-2.82**	1.37***		-0.040		1.46**	-1.75**	0.248	0.458	6	248.8	2.10
-3.48**	1.31**	1.068	0.012	-0.089	1.47**	-1.85**	0.272	0.482	8	249.4	2.71
*-2.63****								*0.270*	*2*	*186.3*	*35.00*

Set of the 95% most supported models according to their AIC weights. Models are ranked based on their AICc values; the best model is the one with the lowest AICc value. Sampling site is included as random factor. The fixed variable coefficients are given with the following baseline factors: "wood mouse" for species; "female" for sex; "2012" for year; "autumn" for season. Significance of the variables in a given model are indicated by "*" for $p < 0.05$, "**" for $p < 0.01$, "***" for $p < 0.001$. "R²marg": marginal pseudo-R²; "R²cond": conditional pseudo-R²; "df": degree of freedom; "AICc": Akaike Information criteron corrected for finite sample size; "ΔAICc": difference of AICc relative to the most supported model. The conserved model is in bold. The null model is in italic.

Table 4: Top 95% set of Generalized Linear Mixed Models of *Borrelia burgdorferi* sensu lato infection probability of wood mice and bank voles.

Intercept	Species	Sex	Mass	Sex:Mass	Year	Season	R²marg	R²cond	df	AICc	ΔAICc
-5.06* **	**2.11*** **				**3.46*** **	**-1.44***	**0.477**	**0.477**	**5**	**156.9**	**0.00**
-5.33***	2.08***		0.016		3.41***	-1.49*	0.474	0.474	6	158.8	1.91
-5.08***	2.12***	0.036			3.46***	-1.44*	0.477	0.477	6	158.9	2.04
-7.14***	2.17***	2.840	0.100	-0.138	3.39***	-1.48*	0.487	0.499	8	160.4	3.59
-5.35***	2.08**	0.034	0.016		3.41***	-1.49*	0.474	0.474	7	160.8	3.95
-5.06***	2.11**				3.46***		0.477	0.477	4	160.9	4.06
*-3.13****								*0.000*		*211.4*	*54.58*

Set of the 95% most supported models according to their AIC weights. Models are ranked based on their AICc values; the best model is the one with the lowest AICc value. Sampling site is included as random factor. The fixed variable coefficients are given with the following baseline factors: "wood mouse" for species; "female" for sex; "2012" for year; "autumn" for season. Significance of the variables in a given model are indicated by "*" for $p < 0.05$, "**" for $p < 0.01$, "***" for $p < 0.001$. "R²marg": marginal pseudo-R²; "R²cond": conditional pseudo-R²; "df": degree of freedom; "AICc": Akaike Information criteron corrected for finite sample size; "ΔAICc": difference of AICc relative to the most supported model. The conserved model is in bold. The null model is in italic.

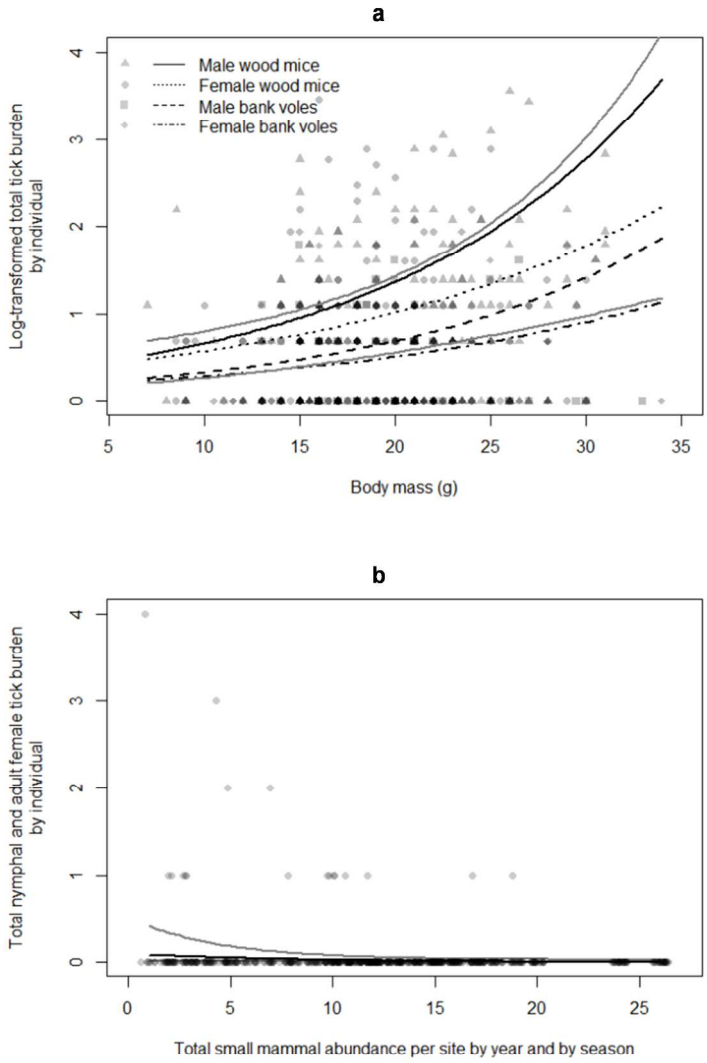

Figure 1

Figure 1 : Total tick burden and nymphal and adult female tick burden as a function respectively of body mass and total small mammal abundance for wood mice and bank voles together.
Total tick burden (larvae, nymphs, and adult females) of wood mouse and bank vole as a function of body mass by species and by sex (a), and potentially infectious tick (nymphs and adult females only) burden by wood mouse and bank vole as a function of the total small mammal abundance per site by year and season (b). The curves represent the fitted tick burden in negative binomial Generalized Linear Mixed Models with site as random factor (black; see legend for species and sex in (a)), and its 95% confidence interval (grey, averaged 95% confidence interval in (a) for readability). Those models correspond to the best models according to our AIC-based model selection procedure; see the text for further details. The gray gradient represents the density of individuals (see legend).

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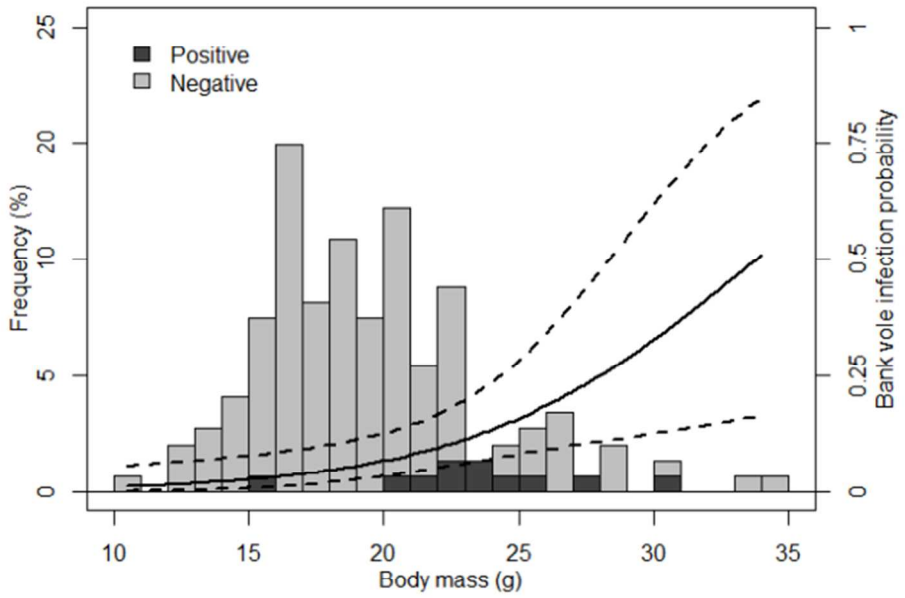

Figure 2

Figure 2 : Borrelia burgdorferi s.l. infection frequency of bank voles as a function of body mass per one g-class.
Bars represent the cumulated frequency (left scale) of positive (dark grey) and negative (light grey) bank voles as a function of their body mass by one g-class. The curves represent the fitted infection probability (right scale) according to univariate binomial Generalized Linear Mixed Model with site as random factor (solid line; p = 0.004; marginal pseudo-R^2 = 0.165; conditional pseudo-R^2 = 0.165), and its 95% confidence interval (dashed lines).

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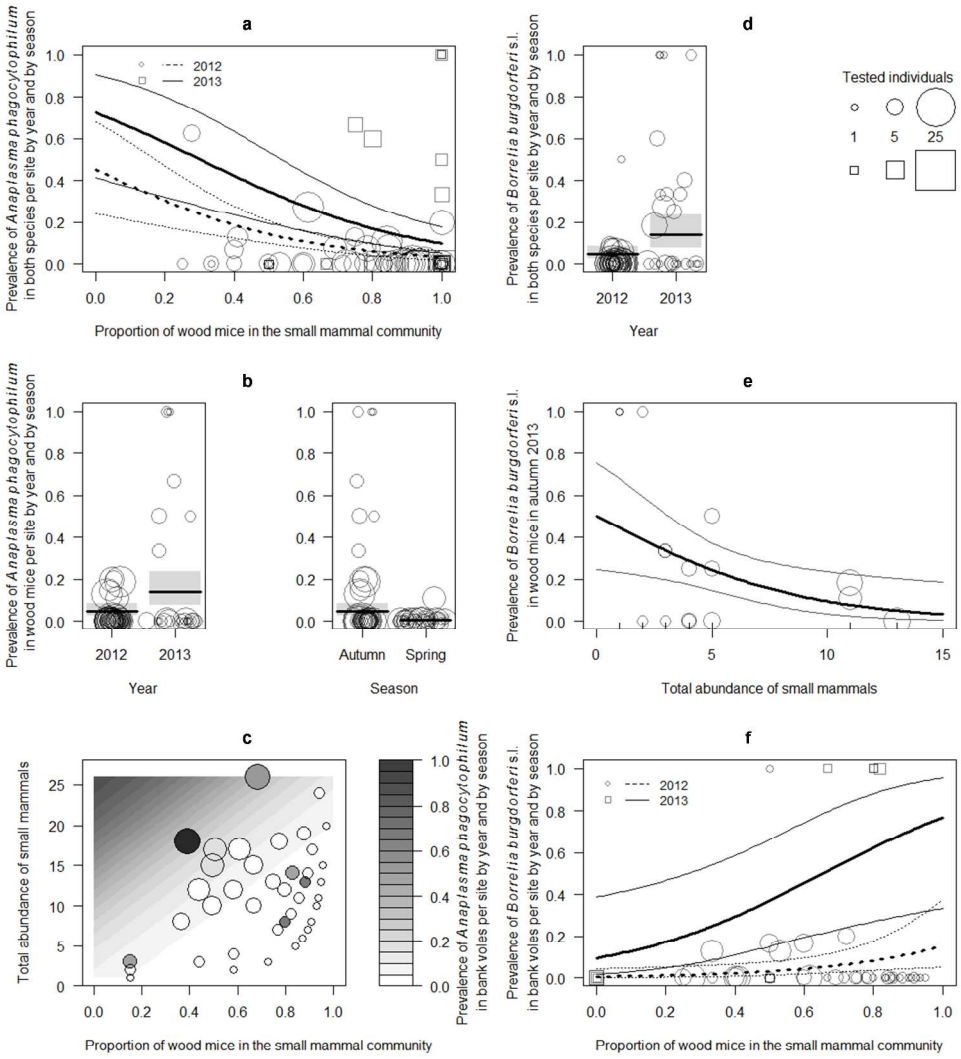

Figure 3

Figure 3: Prevalence of Anaplasma phagocytophilum and Borrelia burgdorferi s.l. in wood mice, bank voles, and both species as a function of the variables included in the best GLMs.
Prevalence of A. phagocytophilum in both species as a function of the proportion of wood mice in the small mammal community and year (a), in wood mice as a function of year and season (b), and in bank voles as a function of total abundance of small mammals and the proportion of wood mice in the small mammal community (c); prevalence of B. burgdorferi s.l. in both species as a function of year (d), in wood mice as a function of total small mammal abundance in autumn 2013 (e), and in bank voles as a function of the proportion of wood mice in the small mammal community (f). The curves (a, e, and f) and the dark gradient (c) represent the fitted prevalence in binomial Generalized Linear Models. The area of the circles and/or squares is proportional to the number of tested individuals (see the legend). Those models correspond to the best models according to our AIC-based model selection procedure; see the text for further details.

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