Few vertebrate species dominate the *Borrelia burgdorferi* s.l. life cycle
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Keywords: deer, Lyme borreliosis, small mammals, thrushes, transmission maintenance, *Ixodes ricinus*

Supplementary material for this article is available online

Abstract

**Background.** In the northern hemisphere, ticks of the Ixodidae family are vectors of diseases such as Lyme borreliosis, Rocky Mountain spotted fever and tick-borne encephalitis. Most of these ticks are generalists and have a three-host life cycle for which they are dependent on three different hosts for their blood meal. Finding out which host species contribute most in maintaining ticks and the pathogens they transmit, is imperative in understanding the drivers behind the dynamics of a disease.

**Methods.** We performed a systematic review to identify the most important vertebrate host species for *Ixodes ricinus* and *Borrelia burgdorferi* s.l. as a well-studied model system for tick-borne diseases. We analyzed data from 66 publications and quantified the relative contribution for 15 host species.

**Review results.** We found a positive correlation between host body mass and tick burdens for the different stages of *I. ricinus*. We show that nymphal burdens of host species are positively correlated with infection prevalence with *B. burgdorferi* s.l., which is again positively correlated with the realized reservoir competence of a host species for *B. burgdorferi* s.l. Our quantification method suggests that only a few host species, which are amongst the most widespread species in the environment (rodents, thrushes and deer), feed the majority of *I. ricinus* individuals and that rodents infect the majority of *I. ricinus* larvae with *B. burgdorferi* s.l.

**Discussion.** We argue that small mammal-transmitted *Borrelia* spp. are maintained due to the high density of their reservoir hosts, while bird-transmitted *Borrelia* spp. are maintained due to the high infection prevalence of their reservoir hosts. Our findings suggest that *Ixodes ricinus* and *Borrelia burgdorferi* s.l. populations are maintained by a few widespread host species. The increase in distribution and abundance of these species, could be the cause for the increase in Lyme borreliosis incidence in Europe in recent decades.

1. Background

Zoonotic vector-borne diseases pose an increasing threat to human health, as one-third of the emerging infectious diseases in the last decades was vector-borne (Jones et al 2008). In the northern hemisphere, ticks of the Ixodidae family are vectors for human diseases such as Lyme borreliosis, Rocky Mountain spotted fever and tick-borne encephalitis (Jongejan and Uilenberg 2004). From these, the spirochaete complex *Borrelia burgdorferi* sensu lato (Baranton et al 1992; from here on referred to as *B. burgdorferi*), the causative agent of Lyme borreliosis and vectored by ticks of the *Ixodes ricinus* complex (Xu et al 2003), causes the majority of human disease cases (Dantas-Torres et al 2012). Both in Europe and in North
America, *I. ricinus* and *I. scapularis* populations have spread and increased in density in recent decades, most probably due to a multitude of man-made changes to the environment, which has resulted in an increase in Lyme disease incidence (Kurtenbach et al 2006, Medlock et al 2013).

Lyme disease risk is determined by multiple biological, environmental and societal factors (Randolph 2004, Vanwambeke et al 2010, Mannelli et al 2012). These can be split into two distinct groups, (1) factors determining the number of questing Ixodes ticks infected with *B. burgdorferi*, and (2) factors determining the level of human exposure to ticks (Sprong et al 2012). In this review, we will focus on the first, with in particular the factors that determine the number of questing Ixodes ticks, and their infection with *B. burgdorferi*.

Both *I. ricinus* and *B. burgdorferi* are considered generalist parasites, as they utilize a multitude of host species (Jaenson et al 1994, Margos et al 2011). These host species differ considerably in the numbers of ticks they feed, which differs between the different life stages of the tick (Tälleklint and Jaenson 1994, Gray 1998). *Ixodes ricinus* has three life stages, larva, nymph and adult, which need a blood meal from a vertebrate host during each stage to moult to the next stage or to lay eggs (Gray 1998). Host species differ in their ability to infect *I. ricinus* larvae with different genospecies of *B. burgdorferi*. For example, *B. afzelii* is mainly transmitted by small mammals, while *B. garinii* is mainly transmitted by birds (Hanincova et al 2003a, 2003b), and even within genospecies, different host species differ in their ability to transmit *B. burgdorferi* (Kurtenbach et al 1994). Both the number of ticks a host can feed and the transmission of *B. burgdorferi* could be linked to general host characteristics, such as host body mass, which is related to both immunological and behavioral responses (Carbone et al 2005, Lee 2006, Previtali et al 2012), and could therefore influence both tick burden and reservoir competence for *B. burgdorferi* (Marsot et al 2012, Huang et al 2013, Barbour et al 2015).

The success of transmission and maintenance of *B. burgdorferi* in enzootic cycles depends, therefore, on the density and abundance of the various vertebrate host species. As the transmission of *B. burgdorferi* from one host to another is mediated by ticks, the distribution of the various genospecies is also dependent on the behavior and feeding preference of the vector ticks. Thus, the resulting *B. burgdorferi* distribution in the questing ticks is a function of the densities of different host species, their capacity to feed ticks and their capacity to transmit the bacteria to those ticks. Therefore, it is important to summarize data on the distribution of ticks of different stages over different vertebrate host species and use these data to find patterns that could be related to the increase in disease risk due to indirect effects by human induced changes to the environment.

Although there have been several studies carried out on particular sites (e.g. Matuschka et al 1991) or on various classes of vertebrates (e.g. Matuschka et al 1990), and some descriptive reviews have been published (e.g. Mannelli et al 2012, Franke et al 2013, Pfäffle et al 2013), there is no quantitative review that integrates data on a host assemblage comprising a wide range of vertebrate species. Here, we used a data driven approach to quantify the contribution of various vertebrate host species to feeding *I. ricinus* ticks, and transmitting *B. burgdorferi* to feeding larvae, to infer a mechanism that could support the apparent increase in Lyme borreliosis incidence in Europe. Furthermore, pinpointing the host species groups that are most important in feeding *I. ricinus* might aid in selecting host species to target intervention strategies (Perkins et al 2003).

We compiled data on interactions between vertebrate hosts, *I. ricinus* and *B. burgdorferi* using a systematic review approach. For the species for which data were available that fulfilled our selection criteria, we looked for correlations between: (1) body mass and *I. ricinus* burden for the different stages, (2) nymphal burden and infection prevalence with *B. burgdorferi*, and (3) infection prevalence and realized reservoir competence for *B. burgdorferi*. We hypothesize that host species body mass is positively correlated to *I. ricinus* burden, as host species of greater body mass have a greater day range (Carbone et al 2005) and are therefore more likely to encounter ticks in the vegetation. Furthermore, we hypothesize that the *I. ricinus* burden of a host species is positively correlated with the infection prevalence with *B. burgdorferi* as hosts that feed a large number of ticks are more likely to feed an infected tick and become infected. Lastly, we hypothesize that the average infection prevalence of a host species is positively correlated with the realized reservoir competence of a host species, as hosts that are more often infected are more likely to transmit the disease to a large number of larvae.

Next to these general patterns, we aimed to quantify the relative contribution of different host species in the maintenance of *I. ricinus* and *B. burgdorferi*. For this, we modified the framework proposed by Mather et al (1989) to quantify the importance of different vertebrate species based on differences in density, *I. ricinus* burden and realized reservoir competence for *B. burgdorferi*. The original framework (Mather et al 1989) was created to quantify the relative importance of different host species in infecting *I. scapularis* larvae with *B. burgdorferi* in three study sites in North America. We modified the equations to quantify the relative importance of host species in the feeding of *I. ricinus* as well as the relative importance in infecting larvae with *B. burgdorferi*. As these equations need a vertebrate assemblage for their calculation, we used data from the literature search to create an assemblage including the most widespread vertebrate species occurring in most European forests.
2. Methods

We performed a literature search using PubMed, Web of Science and Scopus to review the parasitism of Ixodes ricinus on vertebrate host species, and the occurrence of Borrelia burgdorferi in vertebrate hosts and in the I. ricinus parasitizing them. We only considered European host species. Our most extensive search was done in PubMed, were we searched for publications in English and German. The last literature search was carried out in January 2015 and concerned the years 1945–2014. The search string used, and part of the selection procedure are given in the supplementary material. An additional screening of relevance concentrated on the type of data the publications included: field-derived or laboratory data. As we used the data for a quantification framework resembling a situation in the field, we chose only publications that contained field-derived data. Finally, we selected for papers including data on: (1) measurements of the tick burden on the vertebrate hosts, (2) measurements of host infection prevalence with B. burgdorferi, or (3) measurements of infection prevalence with B. burgdorferi in feeding ticks. Publications with incomplete or previously published data were excluded. All publications were reviewed by two different contributors (TRH and ECC) and the data extracted from each paper were checked twice.

2.1. Data acquisition

For each of the selected publications the following variables were extracted: location, number of hosts examined, number of hosts infested, number of hosts infected, numbers of I. ricinus of each stage per host, method of B. burgdorferi detection, type of tissue tested, number of samples (ticks or tissue) tested, number of samples (ticks or tissue) positive and genospecies of B. burgdorferi detected. These variables were the primary variables in our database, and were used for subsequent calculations. Difficulties in extracting data resulted from reported data that accounted for total number of ticks only (no stage mentioned) or combined observations of multiple host species. These papers were stored in the database, but not used for further analysis. To fill the database with values for the desired analyses, the following steps were carried out. If the number of infested or infected hosts or ticks in the study was not given, we calculated, if possible, based on the number of samples examined and the reported infestation or infection prevalence. Similarly, if the number of ticks infesting the host animals was not given, then we would calculate it from the number of animals examined and their mean infestation. Most of the time, however, a total number of ticks from a specific stage collected from a total number of hosts was given, so we could calculate the average tick burden per stage. Only about one third of the publications (56/162) contained standard deviations, standard errors or confidence intervals for the parameters we were interested in, so we decided to calculate merely an average value and no other descriptive statistical parameters.

Publications were divided into separate records if the investigators examined (1) different host species, (2) hosts collected in different locations (if specified), (3) different tick stages (4) different Borrelia genospecies, or (5) hosts tested also by xenodiagnosis. From publications in which the same ticks were examined with different methods for B. burgdorferi detection, leading to different results, only data obtained by PCR or sequencing were included. Records containing sequencing data were considered to have tested for the presence of all the B. burgdorferi genospecies described in the paper. Combining these records resulted in a database on tick burdens, host infection prevalence, and infection prevalence in feeding ticks per host species with data from 162 publications (supplementary data).

2.2. Summarizing the data

As most studies presented only total numbers of animals and ticks studied, we calculated the average tick burden for each stage of tick for each host species by using equation (1)

\[ B_i = \frac{\sum_{s=1}^{3} L_{is} \times H_{is}}{\sum_{s=1}^{3} H_{is}} \]

where \( B_i \) is the mean per-capita larval burden of species \( i \), \( L_{is} \) is the total number of larvae counted on host species \( i \) in study \( s \), and \( H_{is} \) is the total number of individuals of host species \( i \) studied in study \( s \). \( L_{is} \) can be substituted by the total number of nymphs counted on species \( i \) in study \( s \) (\( N_{is} \)) or by the total number of adults counted on species \( i \) in study \( s \) (\( A_{is} \)) in order to calculate the mean per-capita nymphal burden (\( B_{n} \)) or the mean per-capita adult burden (\( B_{a} \)), respectively. Our term tick burden is equal to the mean density of ticks as defined by Kahl et al. (2002).

Infection with B. burgdorferi was calculated as the sum of infections with the various genospecies and with untyped B. burgdorferi, counting the mixed infections only once. We only considered B. burgdorferi and not the individual genospecies to be able to use data produced before widespread use of molecular techniques, and to facilitate comparison between different host taxa.

We calculated two different measures of infection. For each host species we calculated the infection prevalence (\( IP_{0h} \)) of that species with B. burgdorferi by using equation (2). Our term infection prevalence is equal to the ratio of pathogen-exposed hosts versus non-exposed hosts (Kahl et al. 2002) and indicates the fraction of animals within a species that has been infected by B. burgdorferi.

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\[
\text{IP}_{bb_i} = \frac{\sum_{s=1}^{n_{\text{ci}}} \text{BBIH}_{i,s}}{\sum_{s=1}^{n_{i}}} H_{i,s} \tag{2}
\]

\(\text{IP}_{bb_i}\) is the infection prevalence of species \(i\) with any genospecies of \(B.\) burgdorferi, \(\text{BBIH}_{i,s}\) is the total number of \(B.\) burgdorferi infected host individuals of species \(i\) in study \(s\) and \(H_{i,s}\) is the total number of host individuals of species \(i\) sampled in study \(s\). Infection can be determined by either testing tissue samples from a host, or by testing \(I.\) ricinus for presence of \(B.\) burgdorferi after they were collected from a host in the field. As these can, potentially, result in very different values, both were calculated separately and were named 'tissue-derived' infection prevalence and 'tick-derived' infection prevalence, respectively.

Next to that, we calculated the realized reservoir competence (\(\text{RC}_{bb_i}\)), i.e., the proportion of blood fed larvae that become infected with \(B.\) burgdorferi (LoGiudice et al 2003), by using equation (3), which is comparable to the specific host infectivity as defined by Kahl et al (2002).

\[
\text{RC}_{bb_i} = \frac{\sum_{s=1}^{n_{\text{ci}}} \text{BBIL}_{i,s}}{\sum_{s=1}^{n_{i}}} L_{i,s} \tag{3}
\]

\(\text{RC}_{bb_i}\) is the realized reservoir competence of species \(i\) for any genospecies of \(B.\) burgdorferi, \(\text{BBIL}_{i,s}\) is the total number of \(B.\) burgdorferi infected larvae sampled from host species \(i\) in study \(s\), and \(L_{i,s}\) is the total number of larvae tested from species \(i\) in study \(s\).

2.3. Selection criteria

In order to improve the data quality in our analysis, we selected data from our database using the following criteria: (1) a minimum of 20 individuals from a species at a location was studied, (2) a minimum of 50 larvae was tested to determine the realized reservoir competence, (3) the study was conducted within the activity period of \(I.\) ricinus, for which we excluded studies performed in December–February, and studies that were performed all year round without specifying numbers for the separate seasons and (4) the study was conducted within habitats in which \(I.\) ricinus normally resides, namely forest, forest ecotone and woodland. Lastly, we excluded studies that only considered migratory birds. This resulted in a dataset with data for 44 species from 66 publications.

2.4. Quantifying the role of species as hosts for \(I.\) ricinus and \(B.\) burgdorferi

We used this dataset to quantify the role of fifteen species as hosts for \(I.\) ricinus and \(B.\) burgdorferi, using modifications of the framework proposed by Mather et al (1989). The original formula was used to quantify the relative importance of host species in infecting larval \(I.\) scapularis with \(B.\) burgdorferi. We modified the original equation to be used to calculate the relative importance of host species in feeding the different stages of \(I.\) ricinus (equation (4)), as well as the relative importance of host species in infecting \(I.\) ricinus larvae with \(B.\) burgdorferi (equation (5)). For equation (3), we separated the number of infected vectors produced by the host species (\(N_i\) in the original model) into two different parameters, \(B_i\) and \(\text{RC}_{bb_i}\). We did this to clarify the similarity between the two equations we used.

\[
\text{RI}_{i} = \frac{B_i D_i}{\sum_{j=1}^{n} B_j D_j} \tag{4}
\]

\[
\text{RI}_{bb_i} = \frac{B_i D_i \text{RC}_{bb_i}}{\sum_{j=1}^{n} B_j D_j \text{RC}_{bb_j}} \tag{5}
\]

\(\text{RI}_{i}\) is the relative importance of host species \(i\) in feeding the larval stage of \(I.\) ricinus, \(B_i\) is the mean per-capita larval burden of species \(i\), and \(D_i\) is the density (\(\text{km}^{-2}\)) in which species \(i\) occurs. The \(\sum_{j=1}^{n} B_j D_j\) gives the total number of larvae fed by all species in the assemblage, as determined by their mean larval burden and their densities. \(B_i\) can be substituted by \(B_n\) or \(B_a\) to calculate the relative importance of a host species in feeding the nymphal (\(\text{RI}_{na}\)) and adult (\(\text{RI}_{ab}\)) stages, respectively.

\(\text{RI}_{bb_i}\) is the relative importance of species \(i\) in infecting larvae with any genospecies of \(B.\) burgdorferi and is equivalent to the relative reservoir capacity used by Kahl et al (2002) or the reservoir potential of Mather et al (1989). This parameter could also be seen as the relative contribution of a host species to the pool of infected nymphs. We did not calculate the relative importance of host species for the separate \(B.\) burgdorferi genospecies, because there were not enough data available for multiple host species in our selection.

As the quantification of the relative importance of a species is dependent on all species in an assemblage, we needed to select a number of host species to perform our calculations. In principle the equations can be used for any specific area where local densities and tick burdens are known. To present the idea behind

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Host taxonomic group</th>
<th>Density ((\text{km}^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apodemus sylvaticus</td>
<td>Rodent</td>
<td>1200</td>
</tr>
<tr>
<td>Capreolus capreolus</td>
<td>Ungulate</td>
<td>11</td>
</tr>
<tr>
<td>Cyanistes caerules</td>
<td>Small bird</td>
<td>200</td>
</tr>
<tr>
<td>Erinaceus europaeus</td>
<td>Medium-sized mammal</td>
<td>1</td>
</tr>
<tr>
<td>Erithacus rubecula</td>
<td>Small bird</td>
<td>80</td>
</tr>
<tr>
<td>Fringilla coelebs</td>
<td>Small bird</td>
<td>100</td>
</tr>
<tr>
<td>Microtus agrestis</td>
<td>Rodent</td>
<td>1000</td>
</tr>
<tr>
<td>Myodes glareolus</td>
<td>Rodent</td>
<td>1200</td>
</tr>
<tr>
<td>Parus major</td>
<td>Small bird</td>
<td>100</td>
</tr>
<tr>
<td>Phylloscopus collybita</td>
<td>Small bird</td>
<td>100</td>
</tr>
<tr>
<td>Prunella modularis</td>
<td>Small bird</td>
<td>200</td>
</tr>
<tr>
<td>Sylvia atricapilla</td>
<td>Small bird</td>
<td>40</td>
</tr>
<tr>
<td>Turdus merula</td>
<td>Thrush</td>
<td>200</td>
</tr>
<tr>
<td>Turdus philomelos</td>
<td>Thrush</td>
<td>80</td>
</tr>
<tr>
<td>Vulpes vulpes</td>
<td>Medium-sized mammal</td>
<td>1</td>
</tr>
</tbody>
</table>
the framework, and to show some overall trends that we think might be true for any area, we selected a hypothetical assemblage of species. We chose a relatively diverse European forest vertebrate assemblage consisting of six mammalian and nine avian species (table 1). All of these species occur regularly in northwestern European forests or forest ecotones. Species were selected based on their area of distribution throughout Europe as described in published handbooks (Niethammer and Krapp 1978, Cramp and Perrins 1994) and on the number of individuals (minimum of 100 individuals) that was studied in the publications used for data acquisition. Densities of the species were collected from the same published handbooks used for determining the area of distribution. Although the relative importance is calculated per host species, we divided the host species into different species groups based on size and taxonomy (table 1). We present only these broad group contributions, to show general patterns regardless of the contribution of individual species.

To test for the sensitivity of the framework to errors in the mean per-capita tick burden, we calculated the relative importance of the host groups for additional scenarios, in which the species composition of the host assemblage remained unaltered but in which the contribution of rodents, birds or ungulates varied by either doubling or halving the mean per-capita tick burden of these groups compared to the initial values, while all other parameters were kept constant.

2.5. Statistical analysis
To test for correlations between body mass, I. ricinus burden, IPbh and RCbh, of the different host species, we performed a stepwise analysis. First we tested for correlations between body mass and I. ricinus burden, secondly, we tested for correlations between I. ricinus burden and IPbh, and thirdly, we tested for correlations between IPbh and RCbh. For the species that were considered in our vertebrate assemblage for the quantification of the importance of different host species, we also tested for correlations between density and body mass. Statistical analyses were performed using R 3.2.2 (R Core Team 2015). All analyses were performed for each host taxa (birds, mammals and reptiles) separately.

We used log–log regressions to test for correlations between host body mass and I. ricinus burden for the three life stages using average body mass of the host species obtained from published handbooks (Niethammer and Krapp 1978, Cramp and Perrins 1994). This was done because the average tick burdens per life stage did not yield integers, which refrained us from performing generalized linear models using Poisson or negative binomial distributions. Because of the presence of zeros, we added the lowest non-zero burden to the tick burdens (0.04 for larvae, and 0.01 for nymphs and adults) in order to calculate the log10. Due to the large variation in body size, we also log10 transformed host body mass. To give more weight to species that were studied more intensively, we weighted the log–log regression by sample size. We also used log–log regressions for testing the correlations between density and body mass of host species.

For both the infection prevalence and the realized reservoir competence we used a generalized linear model with a binomial distribution and a logit link. For the infection prevalence we used the number of host individuals found infected and the number of host individuals found uninfected, using the tissue-derived data, to test for a correlation between infection prevalence and log10 nymphal burden. We supplemented this dataset with tick-derived data for species for which tissue-derived data were missing. For the realized reservoir competence we used, per host species, the total number of larvae found infected and the total number of larvae found uninfected as reported in the selected papers, to test for a correlation between realized reservoir competence and logit infection prevalence. By using the binomial infected-non-infected data, we weighted the correlations by sample size. We tested if the models for realized reservoir competence could be improved by adding log10 body mass to the model, and compared the models using AICc values (Burnham and Anderson 2004) using the package MuMIn (Barton 2014).

To further analyze the impact of species averages taken from few studies with low sample sizes, we did a post-hoc analysis of leverage to check for the importance of single species in determining the regression coefficients. We calculated Cook’s distance for all parameters in all analyses (Cook 1977). If a Cook’s distance was larger than 0.5, we checked the number of studies and the number of individuals on which the estimate was based.

3. Review results
3.1. Tick burdens, infection prevalence and realized reservoir competence of hosts
The 44 host species in our dataset differed ten to thousandfold in I. ricinus burden, infection prevalence with B. burgdorferi and realized reservoir competence for B. burgdorferi (table 2). Because we only had data on three reptile species, we performed analyses on mammals and birds only. Larval I. ricinus burden increased with host body mass for birds (F1,18 = 12.1, p = 0.02, β = 0.97) but not for mammals (F1,15 = 0.9, p = 0.37). Nymphal I. ricinus burden was positively correlated to host body mass both for birds (F1,18 = 30.5, p < 0.001, β = 1.81; figure 1(A)) and for mammals (F1,15 = 26.1, p < 0.001, β = 0.79; figure 1(D)). Adult I. ricinus burden also increased
Table 2. Host species, their taxonomic class, body mass, average *I. ricinus* burden for the different stages, infection prevalence with *B. burgdorferi* and realized reservoir competence for *B. burgdorferi*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Class</th>
<th>Body mass (g)</th>
<th>Average tick burden (larvae/nymphs/adults)</th>
<th>Infection prevalence with <em>B. burgdorferi</em> (tissue-derived/tick-derived)</th>
<th>Realized reservoir competence for <em>B. burgdorferi</em></th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrocephalus scirpaceus</td>
<td>B</td>
<td>11</td>
<td>2.3/0.6/0</td>
<td>—/0.10</td>
<td>—</td>
<td>(Kaiser et al 2002)</td>
</tr>
<tr>
<td>Aegithalos caudatus</td>
<td>B</td>
<td>8</td>
<td>0/0/0</td>
<td>—/—</td>
<td>—</td>
<td>(Dubska et al 2009)</td>
</tr>
<tr>
<td>Apodemus agrarius</td>
<td>M</td>
<td>15</td>
<td>1.6/0/0</td>
<td>—/—</td>
<td>0.27</td>
<td>(Michalik et al 2003, Radziejewskaja et al 2013)</td>
</tr>
<tr>
<td>Carduus carduus</td>
<td>B</td>
<td>16</td>
<td>0/0/0</td>
<td>—/—</td>
<td>—</td>
<td>(Estrada-Peña et al 2005)</td>
</tr>
<tr>
<td>Carduus chloris</td>
<td>B</td>
<td>27</td>
<td>0.4/0.1/0</td>
<td>—/—</td>
<td>—</td>
<td>(James et al 2011)</td>
</tr>
<tr>
<td>Carduus spinus</td>
<td>B</td>
<td>13</td>
<td>0.4/0/0</td>
<td>—/—/0.06</td>
<td>—</td>
<td>(James et al 2011)</td>
</tr>
<tr>
<td>Cervus elaphus</td>
<td>M</td>
<td>130000</td>
<td>8/16/42.8</td>
<td>—/—</td>
<td>—</td>
<td>(Pacilly et al 2014)</td>
</tr>
<tr>
<td>Coccothraustes</td>
<td>B</td>
<td>55</td>
<td>1.4/2/0</td>
<td>—/—</td>
<td>—</td>
<td>(Taragelová et al 2008)</td>
</tr>
<tr>
<td>Cynisteres caeruleus</td>
<td>B</td>
<td>11</td>
<td>0.1/0/0</td>
<td>—/—/0.15</td>
<td>—</td>
<td>(Kipp et al 2006, Dubska et al 2011, James et al 2011, Heylen et al 2013)</td>
</tr>
<tr>
<td>Eliomys quercinus</td>
<td>M</td>
<td>80</td>
<td>—</td>
<td>—/0.89</td>
<td>0.79</td>
<td>(Richter et al 2004)</td>
</tr>
<tr>
<td>Erinaceus europaeus</td>
<td>M</td>
<td>1100</td>
<td>119.9/58.7/10.5</td>
<td>—/—</td>
<td>—</td>
<td>(Pfäffle et al 2011)</td>
</tr>
<tr>
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<td>(Fietz et al 2014)</td>
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<td>Class</td>
<td>Body mass (g)</td>
<td>Average tick burden (larvae/nymphs/adults)</td>
<td>Infection prevalence with B. burgdorferi (tissue-derived/tick-derived)</td>
<td>Realized reservoir competence for B. burgdorferi</td>
<td>References</td>
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<tr>
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<td>1.7/7.3/0</td>
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<td>—</td>
<td>(Václav et al 2011)</td>
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<tr>
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<td>—</td>
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<td>—/—</td>
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<td>180</td>
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<td>0.29/*</td>
<td>—</td>
<td>(Kaiser et al 2002, Dubzska et al 2009, 2011)</td>
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<td>(Brinck et al 1967, Humair et al 1993a, Tälleklint and Jaenson 1994, Hellgren et al 2011)</td>
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<td>-</td>
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Table 2. (Continued.)

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<th>Species</th>
<th>Class</th>
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<th>Infection prevalence with B. burgdorferi (tissue-derived/tick-derived)</th>
<th>Realized reservoir competence for B. burgdorferi</th>
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<td>0.01</td>
<td>(Humair et al 1993b, Kipp et al 2006, Taragel’ova et al 2008, Dubska et al 2009)</td>
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<td>0/1.3/4.2</td>
<td>0.07/—</td>
<td>—</td>
<td>(Heidrich 1999, 2000)</td>
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*a B = birds (Aves), M = mammals (Mammalia) and R = reptiles (Reptilia).

*b — represents missing data.
with host body mass both for birds \((F_{1,18} = 74.4, p < 0.001, \beta = 0.53)\) and for mammals \((F_{1,15} = 73.9, p < 0.001, \beta = 1.15)\).

The 25 host species for which we had data on infection prevalence with \(B. \text{burgdorferi}\) differed ten-fold in infection prevalence (table 2). Infection prevalence increased with nymphal \(I. \text{ricinus}\) burden both for birds (deviance difference = 199.1, \(p < 0.001, \beta = 1.76\); figure 1(B)) and for mammals (deviance difference = 24.6, \(p < 0.001, \beta = 0.34\); figure 1(E)). Of the 17 host species for which we had data on the realized reservoir competence for \(B. \text{burgdorferi}\), 14 also had data on infection prevalence (table 2). In these species, realized reservoir competence for \(B. \text{burgdorferi}\) increased with infection prevalence both for birds (deviance difference = 1048.2, \(p < 0.001, \beta = 1.29\); figure 1(C)) and for mammals (deviance difference = 903.7, \(p < 0.001, \beta = 0.72\); figure 1(F)). For both groups, the model improved significantly by adding \(\log_{10}\) body mass, which was positively correlated to realized reservoir competence in birds \((\Delta \text{AICc} = 59.7, p < 0.001, \beta_{\text{body mass}} = 2.88)\), and negatively correlated to realized reservoir competence in mammals \((\Delta \text{AICc} = 149.6, p < 0.001, \beta_{\text{body mass}} = -3.13)\).

Post-hoc analyses of leverage indicated that for most analyses there were one or two species with a Cook’s distance >0.5. However, most of the time these were the estimates which we gave a higher weight based on high sample size. In the few instances that species with a low sample size (less than 100 individuals) and low number of studies (less than three studies) had a high Cook’s distance, omitting these species in the analysis only increased the fit. The only exception was the analysis of realized reservoir competence for mammals, for which omitting the data for \(Eliomys \text{quercinus}\) and \(Sorex \text{araneus}\) decreased the fit of the model including only infection prevalence with \(B. \text{burgdorferi}\) (deviance difference = 0.25, \(p = 0.62\)). However, excluding these two species from the model including both infection prevalence with \(B. \text{burgdorferi}\) and \(\log_{10}\) body mass resulted in a similar result as for all species, albeit with slightly different parameter estimates (deviance difference = 96.91, \(p < 0.001, \beta_{\text{IP}} = 0.76, \beta_{\text{body mass}} = -3.80\)).

3.2. Relative importance of host groups for \(I. \text{ricinus}\) and \(B. \text{burgdorferi}\)

The quantification of the relative importance of host species feeding \(I. \text{ricinus}\) indicated that rodents
contributed most (89%) to feeding larval *I. ricinus* (figure 2). Although the absolute value changes with different scenarios (range: 80%–94%), rodents were the most important host group feeding *I. ricinus* larvae in all our scenarios (figure S1). Thrushes were the second most important group by feeding 5% of the larvae (range: 3%–9%), followed by smaller birds (4%; 2%–8%).

The relative importance of host groups for nymphs differed most strongly between scenarios. Thrushes had the highest contribution to feeding nympha1 *I. ricinus* (40%; 29%–49%), while rodents (28%; 16%–43%), small birds (23%; 16%–28%) and ungulates (8%; 4%–14%) were also important, depending on the scenario. Ungulates contributed most (92%) to feeding adult *I. ricinus* (figure 2). The absolute value of the importance of ungulates feeding adults changed per scenario (range: 85%–96%), but in all scenarios the majority of adult *I. ricinus* is fed by ungulates. The second most important group were medium-sized mammals (5%; 3%–9%).

The relative importance of host species for *B. burgdorferi* was calculated using only a subset of the host species in the dataset for which realized reservoir competence estimates were available (9 of the 15 species; table 2). In all scenarios, rodents were the most important host group infecting larval *I. ricinus* with *B. burgdorferi* (89%; 80%–94%), followed by thrushes (10%; 5%–18%). However, it has to be noted that no data were available on realized reservoir competence for any of the medium-sized mammals and ungulates. For the fifteen species in our calculations, density decreased with body mass for mammals ($F_{1,4} = 10.05, p = 0.03, \beta = -0.99$) but not for birds ($F_{1,7} = 0.12, p = 0.74$).

### 4. Discussion

#### 4.1. Importance of host species in maintaining *I. ricinus*

Although *I. ricinus* is found to parasitize a large number of host species (Anderson and Magnarelli 1993), we found that the different stages of *I. ricinus* mainly feed on a few host species from different taxonomic groups. For birds, we found that species with higher body mass feed most *I. ricinus* of all stages. As host body mass is not correlated with host density in birds, it is the same large species of bird that contribute most to tick feeding by birds. In our analysis these were two thrushes, Turdus merula and Turdus philomelos, two abundant and widespread species in many European countries (Cramp and Perrins 1994). These species mostly forage on the ground, shifting through the litter layer looking for food (Cramp and Perrins 1994), which might be the main reason for the relatively high number of *I. ricinus* found on these species.

For mammals, which occur in the highest densities, we found a negative correlation between density and host mass, but no relationship between larval burden and body mass. Therefore, small mammals, occurring in high densities and having relatively large larval burdens, emerged from our analysis as the host group that was most important for feeding larval *I. ricinus*. This suggests that larval *I. ricinus* do not actively select for a host species, but rather feed on the hosts that are most abundant low in the vegetation where they quest (Mejlon and Jaenson 1997). The main studied small mammal host species were Apodemus flavicollis, Apodemus sylvaticus, Microtus agrestis, and Myodes glareolus, all widespread and abundant species in many European countries (Niethammer and Krapp 1978).
When looking at nymphs and adults, however, there was a strong correlation between host body mass of mammals and *I. ricinus* burden. This was reflected in our analysis by the higher relative importance of medium-sized mammals and ungulates for these stages, although small mammals were still the most important mammalian host group for nymphs, due to their high densities. Ungulates were the most important host group feeding adult *I. ricinus*, which suggests that adult *I. ricinus* actively select large mammals as hosts, regardless of their relatively low densities. This might be why adult ticks quest higher in the vegetation compared to nymphs and larvae (Mejløn and Jønsson 1997). In our analysis of host importance the only ungulate species included was *Capreolus capreolus*, the most widespread ungulate species in Europe (Niethammer and Krapp 1978). In the absence of roe deer, other ungulate species can serve as important hosts for adults as well.

Tick distributions on hosts are often highly over-dispersed (Randolph 2004) and summarizing this distribution by a mean value might not result in the best parameter. Nevertheless, most papers used in our analysis did not specify any other parameters, which resulted in our use of a mean per-capita tick burden per species. We do not think that this has greatly influenced our results. The differences in mean per-capita tick burden between species are much larger than differences between studies for the same species, and most values used in our analysis are based on large sample sizes. Furthermore, the different scenarios we used in our framework showed the same general patterns, showing that these patterns are not very sensitive to changes in mean tick burden (figure S1). We do, however, strongly urge for a standardized reporting system for summarizing the numbers of ticks found on hosts, for which the reporting of the number of hosts, the prevalence of infestation, the median intensity of infestation, including confidence intervals, and the exponent k of the negative binomial distribution could be used (Rózsa et al 2000).

We show that all stages of *I. ricinus* can be maintained by only a few host species that are widespread throughout Europe. This, together with a large distribution in suitable habitat and climatic conditions, explains why *I. ricinus* has such a wide distribution, and why it occurs in high densities in many areas with a vertebrate assemblage existing of widespread species. It also supports the hypothesis that the increase in Lyme borreliosis may be due to an increase in *I. ricinus* distribution and abundance (Sprong et al 2012, Medlock et al 2013), following increases in range and abundance of widespread host species such as *C. capreolus*, *M. glareolus* and *T. merula* (Gregory et al 2007, Apollonio et al 2010, van Strien et al 2015).

### 4.2. Infection prevalence of host species with *B. burgdorferi*

We found that for small to medium-sized mammals and birds, the infection prevalence of host species with *B. burgdorferi* increased with their nymphal burden, with a stronger pattern for birds compared to mammals (figure 1). We did not have data on infection prevalence for the largest mammals in our analysis: *C. capreolus* and *Cervus elaphus*. Studies that were not incorporated in our selection, for reasons outlined above, show that roe deer have high levels of antibody in their blood, and low infection prevalence in tissues (Pińcha et al 2000, Hulinska et al 2002, Pato et al 2013). These findings support the hypothesis that *B. burgdorferi* is unable to circumvent host complement from deer (Kjelland et al 2011, Pacilly et al 2014), which could also explain the incapability of deer to transmit *Borrelia* spirochaetes (Kurtenbach et al 2002). This shows that the relationship between number of nymphs feeding on a species and infection prevalence might not be linear, signifying the need for more data on infection prevalence in ungulates, other large mammals, and large bird species.

We estimated the infection prevalence of host species for *B. burgdorferi* using tissue-derived data as this is the best method to determine infection prevalence of animals, as not all infected animals carry ticks that can be tested (Hanincová et al 2003a). For our analysis, we complemented the dataset with tick-derived data only for species for which tissue-derived infection prevalence was not available. The difference between estimates of both methods within species are much smaller than the differences between species (table 2). Therefore, we conclude that using a combination of methods did not strongly affect our results, although our results might be underestimates because not all infected animals carry infected ticks (Matuschka et al 1993), and not all tissues from infected animals test positive (Kurtenbach et al 1998).

We recommend future studies to test a combination of multiple tissues and engorged ticks to get the best possible estimate of infection prevalence of hosts with *B. burgdorferi*. For species that are able to transmit *B. burgdorferi*, xenodiagnosis using *I. ricinus* larvae will further increase the accuracy of infection prevalence estimates (De Boer et al 1993).

### 4.3. Realized reservoir competence for *B. burgdorferi*

The realized reservoir competence for *B. burgdorferi* of mammals and birds <100 g increases with *B. burgdorferi* infection prevalence of the species (figure 1). For small mammals we show a negative correlation between body mass and realized reservoir competence when we correct for differences in infection prevalence. It is hypothesized that smaller, short lived, species invest less in their immune system than larger, longer lived, species (Lee 2006). However, this hypothesis is debated for differences at a lower taxonomic
level (Martin et al. 2007), so it might not be the most likely explanation for the correlation we found for rodents and shrews. We think it is more likely that B. burgdorferi has adapted to the species it most often encounters. This idea was supported by a positive correlation between density and realized reservoir competence of mammalian hosts for several tick-borne pathogens in North America (Ostfeld et al. 2014). We found a negative correlation between host body mass and density for mammals in our analysis, so the found correlation of realized reservoir competence with body mass could actually be the result of differences in densities of the different species.

For small songbirds we found a positive correlation between body mass and realized reservoir competence when we correct for differences in infection prevalence. This correlation could be caused by the two largest species (Turdus merula and T. philomelos) which have a far higher realized reservoir competence than the others. These species also have the highest nymphal burden and infection prevalence, showing that these are the bird species that B. burgdorferi most often encounters. Therefore, the adaptation of B. burgdorferi to the species it most often encounters could explain the different correlations with body mass we found for small mammals and birds.

4.4. Importance of host species in infecting larvae with B. burgdorferi

In our analysis, rodents, which occur in high densities and have relatively large larval burdens, but relatively low realized reservoir competence, had the highest relative importance for infecting larvae with B. burgdorferi. Thrushes were the second most important group, having intermediate densities and larval burdens, but a very high realized reservoir competence. This indicates that the number of larvae feeding on a host species and its density are more important than the realized reservoir competence of that host species in determining the contribution of a host species to infect larvae. Furthermore, it suggests that the prevalence of different B. burgdorferi genospecies in questing ticks is mainly dependent on the distribution of larvae over rodents and thrushes.

The feeding pattern of ticks could explain why, in most areas in Europe, B. afzelii is the most common genospecies found in questing nymphs (Rauter and Hartung 2005). We found that 89% of the infected larvae in our analysis had fed on rodents. This should result in a large percentage of B. afzelii-infected nymphs as B. afzelii is transmitted by small mammals (Hanincova et al. 2003a). Thrushes fed 10% of the infected larvae, which could explain the relatively low percentages of B. garinii and B. valaisiana in field-derived nymphs (Gassner et al. 2011).

4.5. Borrelia spp. transmission maintenance

The large difference in infection prevalence between small mammals and birds together with their large differences in relative importance for B. burgdorferi suggest that there are two distinct mechanisms behind the maintenance of small mammal-transmitted Borrelia spp. and bird-transmitted Borrelia spp. (Kurtenbach et al. 2002). Because small mammals have low nymphal burdens, their infection prevalence with B. burgdorferi is relatively low (table 2). However, because they feed such a large proportion of the larvae, even a small infection prevalence of the host species can result in a high density of infected nymphs with small mammal-transmitted Borrelia spp. like B. afzelii. This high density of nymphs infected with small mammal-transmitted Borrelia spp. results in a sufficiently-large number of infected nymphs to, in turn, infect small mammals in spite of their low nymphal burdens.

Bird-transmitted Borrelia spp., like B. garinii and B. valaisiana, on the other hand, seem to be dependent on high infection prevalence of their host species due to relatively high nymphal and adult burdens (table 2). Therefore, even with a low larval burden and intermediate host density, sufficient numbers of infected nymphs are produced to infect birds, which completes the maintenance cycle for bird-transmitted Borrelia spp. However, this strategy is probably not only restricted to bird-transmitted Borrelia spp. Borrelia spielmanii is a candidate for a similar maintenance strategy in mammals as it is often found with low prevalence in questing ticks, but with high prevalence in one of its principal hosts, E. quercinus (Richter et al. 2004).

These differences in maintenance strategies could indicate that less common Borrelia spp., or other tick-borne pathogens with low infection prevalence in questing nymphs, might be maintained by host species with high nymphal or adult burdens (Ostfeld et al. 2014). Also it shows that B. burgdorferi can specialize either on host species that occur in high densities, or on host species that feed large numbers of ticks, with the exception of larger bodied mammalian species such as deer.

4.6. Host species diversity

Ostfeld and Keesing (2000) proposed a dilution effect of host species diversity on Lyme borreliosis risk. This hypothesis has been highly debated, especially in the context of ticks from the I. ricinus complex and Lyme borreliosis (Randolph and Dobson 2012, Wood and Lafferty 2013). Although our analysis did not examine the effect of differences in species richness, our methods could be used to quantify the relative contributions of different species in different assemblages, as long as differences in tick burden and density are accounted for. Our results suggest that few, but widespread vertebrate species feed most of the ticks in
European forests. Therefore, community related factors influencing either the densities or tick burdens of these species can have an effect on the outcomes of the calculations. For example, the presence of predators could have effects on the densities or tick burdens of roids, which may affect the number of B. burgdorferi infected ticks in the vegetation (Ostfeld and Holt 2004).

4.7. Limitations of the data
There is little information available on the infection prevalence with and realized reservoir competence for the different genospecies of B. burgdorferi such as B. afzelii, B. bavariensis, B. burgdorferi s.s., B. garinii, B. lusitaniae, B. spielmani and B. valaisiana (supplementary data). For some widespread host species such as European hedgehog (Erinaceus europaeus), European hare (Lepus europaeus), and field vole (Microtus agrestis) there were no data available at the genospecies level that would satisfy our selection criteria. Furthermore, for some widespread host species, such as Eurasian badger (Meles meles), Eurasian jay (Garrulus glandarius), Eurasian pygmy shrew (Sorex minutus), Eurasian red squirrel (Sciurus vulgaris), European pine marten (Martes martes), great spotted woodpecker (Dendrocopos major), wild boar (Sus scrofa) and wood pigeon (Columba palumbus), either very little, or no data at all were available (supplementary data). For example, the two studies (Humair and Gern 1998, Pisanu et al 2014) that lead the current opinion that red squirrels are important hosts in transmitting and maintaining B. burgdorferi s.s. in Europe had either a very low sample size (Humair and Gern 1998), or the animals were collected throughout the year in different habitat types, without specifying infection prevalence per season/habitat type (Pisanu et al 2014). Therefore, we stress that data on tick stages and genospecies-specific infection should be collected from these host species during the active period of I. ricinus, in natural habitats, in order to be able to analyse a more complete host assemblage. This would also enable the analysis of the relationships between host body mass, density, tick burdens, infection prevalence and realized reservoir competence for the different genospecies.

5. Conclusion
Our analysis suggests that a few vertebrate species that are widespread in Europe are the most important host species feeding I. ricinus and transmitting B. burgdorferi. We demonstrate that vertebrate species with a higher body mass have a higher I. ricinus burden, that host species with higher tick burdens are more likely to be infected with B. burgdorferi and that species that are more often infected with B. burgdorferi also transmit the infection more often to larval I. ricinus. These patterns suggest that B. burgdorferi adapts to the species it most often encounters.

To our knowledge, this review is the first to quantify the relative importance of host species for the different stages of I. ricinus, and our calculations support the widely held idea that small rodents are the most important hosts in feeding larval I. ricinus, that birds and rodents feed most of the nymphs, and that ungulates are the main hosts for adult I. ricinus (e.g. Gray 1998, Mannelli et al 2012). We found that rodents and thrushes contribute most to the pool of B. burgdorferi infected nymphs. We suggest two different maintenance strategies for B. burgdorferi, which are correlated to high host densities or high infection prevalence of the hosts. These might explain how some tick-borne pathogens can be maintained with very low prevalence in questing ticks. We show that using a simple framework and a systematic data search can be used to calculate the relative importance of host species for tick species, and tick-borne pathogens, which can be used in research on other tick species and other tick-borne pathogens. These results can aid selection of host species to target for intervention strategies (Perkins et al 2003).

Rodents, thrushes and deer, that are the most important host groups feeding I. ricinus and infecting I. ricinus larvae with B. burgdorferi, have increased in distribution and abundance in recent decades due to changes in land use and forest management (Gregory et al 2007, Apollonio et al 2010, van Strien et al 2015), which could be the main driver behind increased disease incidence with tick-borne diseases in Europe.

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