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Investigating the persistence of tick-borne pathogens via the $R_0$ model

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SUMMARY

In the epidemiology of infectious diseases, the basic reproduction number, $R_0$, has a number of important applications, most notably it can be used to predict whether a pathogen is likely to become established, or persist, in a given area. We used the $R_0$ model to investigate the persistence of 3 tick-borne pathogens; Babesia microti, Anaplasma phagocytophilum and Borrelia burgdorferi sensu lato in an Apodemus sylvaticus-Ixodes ricinus system. The persistence of these pathogens was also determined empirically by screening questing ticks and wood mice by PCR. All 3 pathogens behaved differently in response to changes in the proportion of transmission hosts on which I. ricinus fed, the efficiency of transmission between the host and ticks and the abundance of larval and nymphal ticks found on small mammals. Empirical data supported theoretical predictions of the $R_0$ model. The transmission pathway employed and the duration of systemic infection were also identified as important factors responsible for establishment or persistence of tick-borne pathogens in a given tick-host system. The current study demonstrates how the $R_0$ model can be put to practical use to investigate factors affecting tick-borne pathogen persistence, which has important implications for animal and human health worldwide.

Key words: tick-borne disease, zoonosis, basic reproduction number, Ixodes ricinus.

INTRODUCTION

In infectious disease epidemiology, the basic reproduction number, $R_0$, is defined as the average number of secondary cases caused by one infected individual entering a population consisting solely of susceptible individuals (Anderson and May, 1990; Diekmann et al. 1990; Hartemink et al. 2008). $R_0$ has a number of important applications. It has a threshold value such that if $R_0 > 1$, a pathogen will persist should it be introduced, whilst $R_0 < 1$ suggests it will die out. $R_0$ is also a measure of the risk that an outbreak may occur and, when an outbreak does occur, it gives a measure of the initial rate of exponential increase of infected individuals. The proportion of a population that requires vaccination in order to prevent an outbreak is also determined using $R_0$ (Anderson and May, 1990; Diekmann et al. 1990; Hartemink et al. 2008). $R_0$, however, is difficult to define in natural systems due to indeterminate variability in susceptibility, infectivity and contact rates among individuals. This problem is often compounded by the presence of multiple host species and transmission routes (Hartemink et al. 2008). Given the importance of $R_0$ in the epidemiology of infectious diseases there have been many attempts to define $R_0$ for tick-borne infections (Randolph, 1998; Norman et al. 1999; Randolph et al. 1999; Caraco et al. 2002; Rosa et al. 2003; Ghosh and Pugliese, 2004; Rosa and Pugliese, 2007). More recently, next generation matrix methods have been employed to address the complexities of infections in natural systems (Hartemink et al. 2008) which has resulted in the most comprehensive and biologically correct estimation of $R_0$ for tick-borne infections.

Tick species of the genus Ixodes are important vectors of numerous pathogens worldwide (Parola and Raoult, 2001). Throughout Europe, I. ricinus is the vector of Babesia microti, Anaplasma phagocytophilum and Borrelia burgdorferi sensu lato, the agents of human babesiosis, human granulocytic anaplasmosis and Lyme borreliosis respectively (Duhr et al. 2001; Parola, 2004; Stanzak et al. 2004). To be a competent vector, a tick must acquire a bloodmeal from a given host species. For trans-stadial transmission, larvae and nymphs that feed on an infected host, develop to the next instar, and infect a new host during their subsequent feed as nymphs or adults, thereby maintaining a cycle of infection. (Randolph and Storey, 1999). In some cases, ticks can also acquire an infection by feeding alongside infected ticks, without the need for systemic infection of the host (Jones et al. 1987; Randolph et al. 1996). In Europe, rodents host both larvae and nymphs of I. ricinus (Milne, 1949; Gern et al. 1998; Liz et al. 2000; Karbowiak, 2004) and are competent transmission hosts of B. microti, A. phagocytophilum and B. burgdorferi s.l. B. microti
is a small mammal specific pathogen whilst *A. phagocytophilum* infects both small mammals and large mammals such as deer, although it is thought that separate *A. phagocytophilum* strains exist in discrete small mammal and large mammal cycles (Bown et al. 2009). Members of the *B. burgdorferi* s.l. complex utilize a range of vertebrate transmission hosts, for example, the *B. valaisiana* genospecies is associated with birds and *B. afzelii* with rodents (Kurtenbach et al. 2002). Deer are not considered competent transmission hosts of *B. burgdorferi* s.l. (Telford et al. 2006). In some locations, as in Ireland, nymphs of *I. ricinus* may be found in extremely low numbers or be completely absent from small mammals (Gray et al. 1999, 2000; Harrison et al. 2010). This has led to the suggestion that small mammals may not always be important transmission hosts of tick-borne infections (Gray et al. 1999, 2000).

We used empirical data from Ireland, where the incidence of nymphs of *I. ricinus* on small mammals is low, and previously published tick, and pathogen-specific, data to parameterize the $R_0$ model of Hartemink et al. (2008). This model was then used to predict whether infections of *B. microti, A. phagocytophilum,* and *B. burgdorferi* s.l. were likely to persist in small mammal populations. The model was also used to investigate how changes in the proportion of transmission-competent hosts on which *I. ricinus* had fed, the transmission efficiency of pathogens to and from ticks and hosts, and the abundance of larvae and nymphs on hosts, affects pathogen persistence in small mammals. Predictions of the model were validated by screening small mammals and ticks for pathogens by PCR.

**MATERIALS AND METHODS**

**Calculation of $R_0$**

In the current study, $R_0$ was calculated as a function of $h_c$, the proportion of competent hosts on which *I. ricinus* is feeding, for *B. microti, A. phagocytophilum* and *B. burgdorferi* s.l. using the next-generation matrix method of Hartemink et al. (2008). Each element in the matrix was calculated using previously published tick-related and pathogen-specific parameters and tick-related parameters describing the distribution of life stages of *I. ricinus* on *A. sylvaticus* specific to the current study (Tables 1 and 2). As there is a paucity of literature regarding the transmission efficiency of *B. microti* and *A. phagocytophilum* from *I. ricinus* to small mammal hosts ($\beta_{T \rightarrow V}$) and from small mammal hosts to *I. ricinus* ($\beta_{V \rightarrow T}$), $R_0$ was calculated using low, medium and high transmission efficiency scenarios for these pathogens using transmission coefficients of 0·1, 0·5, and 0·9 respectively. In the case of *B. burgdorferi* s.l. previously published transmission coefficients were used.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$</td>
<td>Eggs per adult</td>
<td>20001,2</td>
</tr>
<tr>
<td>$s_L$</td>
<td>Survival probability from egg to feeding larvae</td>
<td>0·053</td>
</tr>
<tr>
<td>$s_N$</td>
<td>Survival probability from feeding larva to feeding nymph</td>
<td>0·11</td>
</tr>
<tr>
<td>$s_A$</td>
<td>Survival probability from feeding nymph to feeding adult</td>
<td>0·11</td>
</tr>
<tr>
<td>$C_{LL}$</td>
<td>Mean number of larvae co-feeding with a larva</td>
<td>11·993</td>
</tr>
<tr>
<td>$C_{NL}$</td>
<td>Mean number of nymphs co-feeding with a larva</td>
<td>1</td>
</tr>
<tr>
<td>$C_{CA}$</td>
<td>Mean number of adults co-feeding with a larva</td>
<td>0</td>
</tr>
<tr>
<td>$C_{CN}$</td>
<td>Mean number of nymphs co-feeding with a nymph</td>
<td>0</td>
</tr>
<tr>
<td>$C_{CA}$</td>
<td>Mean number of adults co-feeding with an adult</td>
<td>0</td>
</tr>
<tr>
<td>$N_{LM}$</td>
<td>Average number of larvae on a competent host</td>
<td>7·875</td>
</tr>
<tr>
<td>$N_{NM}$</td>
<td>Average number of nymphs on a competent host</td>
<td>0·025</td>
</tr>
<tr>
<td>$N_{MA}$</td>
<td>Average number of adults on a competent host</td>
<td>0</td>
</tr>
<tr>
<td>$D_L$</td>
<td>Days of attachment of larva</td>
<td>2·5</td>
</tr>
<tr>
<td>$D_N$</td>
<td>Days of attachment of nymph</td>
<td>3·5</td>
</tr>
<tr>
<td>$D_A$</td>
<td>Days of attachment of adult</td>
<td>12</td>
</tr>
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To investigate what impact the abundance of larval and nymphal stages on hosts may have on the ability of these tick-borne pathogens to become established, or persist, in the current study, $R_0$ was calculated using a fixed value of $h_c$ (corresponding to the value obtained for *A. sylvaticus* from bloodmeal analysis) across a range of mean loads of larval and nymphal ticks using a medium level transmission coefficient of 0·5 for *B. microti* and *A. phagocytophilum* and previously published transmission coefficients for *B. burgdorferi* s.l.

$R_0$ was calculated via the spectral decomposition of the parameterized next-generation matrix that yields a set of eigenvalues, the largest of which is $R_0$. The matrix was decomposed using the eigen (matrix) function in package base of the R software.
Table 2. Ecological parameters for B. microti, A. phagocytophilum and B. burgdorferi s.l. (adapted from Hartemink et al. (2008).)


<table>
<thead>
<tr>
<th>Description</th>
<th>B. microti</th>
<th>A. phagocytophilum</th>
<th>B. burgdorferi s.l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$i$</td>
<td>2·5 days$^1$</td>
<td>40 days$^2$</td>
<td>120 days$^7$</td>
</tr>
<tr>
<td>$\theta$</td>
<td>$0^\circ$</td>
<td>$0^\circ$</td>
<td>$0^\circ$</td>
</tr>
<tr>
<td>$p_L$</td>
<td>$0·1/0·5/0·9^b$</td>
<td>$0·1/0·5/0·9^b$</td>
<td>$0·5^9$</td>
</tr>
<tr>
<td>$p_N$</td>
<td>$0·1/0·5/0·9^b$</td>
<td>$0·1/0·5/0·9^b$</td>
<td>$0·5^9$</td>
</tr>
<tr>
<td>$p_A$</td>
<td>$0·1/0·5/0·9^b$</td>
<td>$0·1/0·5/0·9^b$</td>
<td>$0·4^3$</td>
</tr>
<tr>
<td>$q_L$</td>
<td>$0^{1,2}$</td>
<td>$0^{5,6,8}$</td>
<td>$0·8^2$</td>
</tr>
<tr>
<td>$q_N$</td>
<td>$0·1/0·5/0·9^b$</td>
<td>$0·1/0·5/0·9^b$</td>
<td>$0·8^2$</td>
</tr>
<tr>
<td>$q_A$</td>
<td>$0·1/0·5/0·9^b$</td>
<td>$0·1/0·5/0·9^b$</td>
<td>$0·8^2$</td>
</tr>
<tr>
<td>$r_A$</td>
<td>$0^{1,2,3}$</td>
<td>$0^{5,6,8}$</td>
<td>$0·112$</td>
</tr>
</tbody>
</table>

package available under GNU licence from www.r-project.org.

Study sites

Five sites supporting mixed broadleaf and coniferous woodland sites in Northern Ireland were sampled over 8 weeks from May until July 2007. Sites were selected on the basis that they had resident populations of red deer, Cervus elaphus, (2 sites) or fallow deer, Dama dama, (3 sites) and were therefore likely to have ticks present.

Small mammal samples

In total, 180 Self-set snap traps were deployed in pairs at 15 m intervals in vegetation adjacent to forest tracks. Traps were set after 6 pm in the evening and collected before 8 am the following morning. Each mouse was stored separately in a sealed sample bag and a 1·5 ml microcentrifuge tube at $-20^\circ$C prior to DNA extraction.

Sampling of questing ticks

The abundance of questing ticks was assessed using a standardized drag sampling technique. A 1 m × 1 m square piece of towelled material, weighted and spread out with bars at the leading and rear edge was dragged along a 15 m transect of trackside grass at 1 ms$^{-1}$ with a total of 20 transects per forest site. Ticks were removed from the drag after each transect using fine forceps and stored in 70% ethanol. Ticks were identified to species level using standard keys, counted, and the developmental stage recorded.

In addition to ticks collected from standardized drag sample transects, additional drag samples were conducted to increase the sample size of ticks available for screening for tick-borne pathogens. All sites were sampled for questing ticks at the same time as small mammal trapping (May, June and July, 2007).

DNA extraction

DNA was extracted from blood by alkaline digestion (Bown et al. 2003). First, 0·5 ml of 1·25% ammonia solution was added to 50 μl of blood in a Sure-Lock microcentrifuge tube (Fisher Scientific, Loughborough, UK) and heated to 100 °C for 20 min. Tubes were centrifuged, opened and heated until half the initial volume remained. The solution was diluted 1 in 10 with sterile, deionized distilled water. The same method was used to extract DNA from ticks that had first been macerated using a pipette tip. DNA extracts of ticks were not diluted. Only nymphal and adult ticks were tested for the presence of pathogens.

Detection of pathogens via polymerase chain reaction (PCR)

An Apicomplexa-specific PCR targeting the 18S rRNA gene was used to test for the presence of Babesia microti (Simpson et al. 2005). A. phagocytophilum and B. burgdorferi s.l. infections were detected using a real-time PCR assay as previously described by Courtney et al. (2004). Samples positive for A. phagocytophilum were subjected to a second,
nested PCR assay targeting the msp4 gene for sequence determination (De La Fuente et al. 2005; Bown et al. 2007). Samples positive for *B. burgdorferi* s.l. were subjected to a second, nested PCR targeting the 5S-23S intergenic spacer region (Rijpkema et al. 1995). All PCRs included negative controls in a ratio of 1:5 and positive controls. Amplification products were purified using a Qiaquick PCR purification kit (Qiagen) and sequences determined using a commercial sequencing service (Macrogen, Korea). Sequence data from successfully sequenced amplification products were used to search for other closely related sequences using the NCBI nucleotide BLAST database. Sequences were aligned and compared using BioEdit v7.0.9© (Ibis Biosciences, California, USA).

**Bloodmeal analysis**

Bloodmeal analysis, to identify hosts that questing *I. ricinus* nymphs had fed on as larvae, was conducted using a published reverse line blot (RLB) protocol (Humair et al. 2007). Five probes were used (‘*Apodemus*, ‘*bird*, ‘*Capreolus*, ‘*Sciurus*’ and ‘*Sorex*’) as they represent the most likely vertebrate hosts present at study sites, targeting *Apodemus sylvaticus*, birds, deer, squirrel spp. and *Sorex minutus* respectively.

**RESULTS**

**The basic reproduction number, R₀**

Values of R₀ plotted as a function of hₐ (the proportion of competent hosts on which *I. ricinus* is feeding), for *B. microti*, *A. phagocytophilum* and *B. burgdorferi* s.l. are presented in Fig. 1.

In the case of *B. microti*, the threshold value for R₀ was never reached regardless of the proportion of competent hosts on which *I. ricinus* had fed or the transmission efficiency scenario employed. This was also the case for *A. phagocytophilum* under low transmission efficiency. However, for medium and high transmission scenarios the proportion of competent hosts on which *I. ricinus* was required to feed upon in order for the threshold value to be reached were 30% and 9% respectively. When hₐ was fixed at 11.45% (representing 11 out of 96 positive reactions obtained for *A. sylvaticus* during bloodmeal analysis) the transmission coefficient required to produce a value of R₀>1 for *A. phagocytophilum* was 0.795. In contrast, the threshold value of R₀ was rapidly achieved for *B. burgdorferi* s.l. with only 2.55% of competent hosts required to be feeding *I. ricinus* for the threshold to be reached.

A plot of the interaction between mean number of larvae and nymphs on a competent host and R₀ for each pathogen is presented in Fig. 2. In the case of *B. microti*, increasing the mean number of larvae and nymphs on the host slowly increased the value of R₀, but even at unrealistically high tick burdens (80 larvae and 80 nymphs) the threshold value of R₀ was not reached. In the case of *A. phagocytophilum*, however, the threshold value was achieved much more rapidly, requiring, only a single larvae and 30 nymphs or 20 larvae and a single nymph for the threshold value to be achieved. Similarly, in the case of *B. burgdorferi* s.l. the value of R₀ increased rapidly with increasing tick load, requiring only a single larvae and a single nymph for the threshold value of R₀ to be reached.

**Tick distribution**

A total of 233 questing ticks consisting of 100 larvae, 129 nymphs and 4 adults were collected from standardized drag samples. The only tick species identified was *I. ricinus*. Densities were generally low with a mean abundances per m²±s.e. for larvae, nymphs and adults of 0.086±0.019, 0.067±0.014, and 0.003±0.001 respectively. A total of 1168 ticks consisting of 1165 larvae, 3 nymphs and 0 adults were collected from wood mice, giving an overall nymph:larvae ratio of 1:388. Again, the only tick species recovered was *I. ricinus*. Mean tick burdens per mouse±s.e. for larvae, nymphs and adults were 7.871±1.087, 0.020±0.011 and 0 respectively. The distribution of ticks on wood mice was over-dispersed, with a small proportion of the host population (20%) feeding the majority of larvae (72%) and all nymphs.

**Pathogen detection**

In addition to the 100 nymphs and 4 adult ticks collected by standardized drag samples, a further 167 nymphs and 6 adults were collected by non-standardized drags. In total, 137 wood mice and 277 ticks (267 nymphs and 10 adults) were tested for the presence of *B. microti* and *A. phagocytophilum* whilst the 277 ticks were also tested for *B. burgdorferi* s.l. Three *I. ricinus* nymphs tested positive for the presence of *A. phagocytophilum* but no wood mice or adult ticks were positive. Of the 277 ticks screened for the presence of *B. burgdorferi* s.l. 20 nymphs were positive. No samples were positive for the presence *Babesia microti*.

**Sequence analyses**

(a) *A. phagocytophilum*. Of the 3 tick samples that tested positive for *A. phagocytophilum*, 2 (R14 and R49) were sequenced successfully. R14 and R49 were not identical but shared 96.3% similarity. R14 was identical to a strain found in a dog in Slovenia (GenBank Accession no. EF442004), whilst R49 was most closely related to strains recovered from red
Fig. 1. $R_0$ plotted as a function of $h_c$, the fraction of bloodmeals taken on a competent host, for (a) Babesia microti, (b) Anaplasma phagocytophilum (both under low, medium and high transmission efficiency scenarios) and (c) Borrelia burgdorferi s.l. using previously published transmission coefficients (cited by Hartemink et al. 2008).
and roe deer in Slovakia and a lamb from Norway sharing 98.0% sequence similarity (EU180065, EF442003 and EU240474, respectively). All percentage similarities are across 301 base pairs.

(b) B. burgdorferi s.l. Of the 20 ticks that tested positive for B. burgdorferi s.l. 13 were sequenced successfully. R6, R17, R21, R57, T5, T61 and TC64 were most closely related to the B. garinii genospecies (AB178361) sharing 96.4%–98.2% sequence similarity. TC33 and TC19 were most closely related to the B. afzelii-type strain (GQ369937) with 93.2% and 98.6% sequence similarity and L1, TC16, R64 and T1 were most closely related to the B. valaisiana genospecies (L30134) with 93.5%–97.3% similarity. Therefore, 85% of B. burgdorferi-positive samples successfully sequenced were bird-associated genospecies whilst 15% were associated with rodents. All percentage similarities are across 225 base pairs.

Bloodmeal analysis

A total of 170 questing I. ricinus nymphs collected from 4 sites were included in bloodmeal analysis, 83 of which yielded positive reactions. DNA from more than 1 host was found in 13/83 positive reactions resulting in 96 host identifications made from 83 positive reactions. Birds were the most important hosts for I. ricinus nymphs feeding as larvae and were present in 51 out of 96 host identifications. Deer were the second most important hosts (18/96) followed by wood mice and pygmy shrews (both 11/96). Squirrels were the least important hosts for larval ticks, present in only 5 out of 96 host identifications. None of the ticks that tested positive for A. phagocytophilum yielded reactions in the bloodmeal analysis. Sixteen of the 20 nymphs that tested positive for B. burgdorferi s.l. were included in bloodmeal analysis. Of the 8 B. burgdorferi s.l. positive samples identified as the B. valaisiana genotype by sequence analysis, 4 gave positive reactions all of which indicated that the ticks had previously fed on birds. Of the 3 B. burgdorferi s.l. positive samples identified as B. garinii, 1 gave a positive host identification indicating that this tick had also fed on a bird. Neither of the samples identified as B. afzelii by sequence analysis gave positive host identifications. Two samples which gave positive host identifications were of mixed origin, both of which included a deer and shrew signal.

Published transmission coefficients (cited by Hartemink et al. 1998) for (c) and $h_r=11.45\%$ for all 3 pathogens.

Hatching indicates areas of the plot where $R_0<1$. 

Fig. 2. Interaction of mean larval and nymphal abundance of Ixodes ricinus on Apodemus sylvaticus and $R_0$ for (a) Babesia microti, (b) Anaplasma phagocytophilum and (c) Borrelia burgdorferi s.l. assuming a medium level transmission efficiency of 0.5 for (a, b), previously
DISCUSSION

The basic reproduction number, $R_0$, responded differently for each pathogen in response to the proportion of competent hosts on which $I. ricinus$ fed and the mean abundance of larval and nymphal ticks on hosts. Values of $R_0$ suggested that $B. microti$ could not persist given the distribution of life-history stages of ticks on wood mice, even if the transmission coefficients were high, if ticks fed solely on competent reservoir hosts, or if tick larval and nymphal tick burdens were unrealistically high. This was supported by the absence of $B. microti$ in wood mice and questing ticks when screened by PCR. The inability of $B. microti$ to become established or persist in this system is likely to be a product of the short period of infectivity that this pathogen has for ticks of 1–4 days (Randolph, 1995).

In the case of $A. phagocytophilum$, the threshold value of $R_0$ was achieved, but only when the proportion of competent hosts on which $I. ricinus$ had fed was greater than that of the current study or when the transmission coefficient was unrealistically high. $A. phagocytophilum$ was not detected in small mammals but $A. phagocytophilum$ was found in questing ticks. However, sequence analysis revealed that the strains were most closely related to those recovered from large mammals across Europe suggesting that other, large mammal, hosts of $I. ricinus$ present at the study site were responsible for these infections. Moreover, Bown et al. (2009) observed that different $A. phagocytophilum$ strains exist in discrete enzootic small mammal and large mammal cycles. The prevalence of infection of $I. ricinus$ nymphs was low (1·12%) and the probability of a mouse feeding a nymph was also low (2·02%). Even if different strains of $A. phagocytophilum$ were capable of utilizing both large and small mammals the probability of a nymph infected with $A. phagocytophilum$ feeding on a mouse was extremely low (0·02% or 1 in 5000) making the spillover of $A. phagocytophilum$ from larger to small mammals highly unlikely. Therefore, it is highly probable that the $A. phagocytophilum$ strains present in the current study were involved in an ungulate-tick cycle and that no $A. phagocytophilum$ cycles were present in wood mice.

In contrast to $B. microti$ and $A. phagocytophilum$, the threshold value of $R_0$ for $B. burgdorferi$ s.l. was achieved rapidly, requiring $I. ricinus$ to feed on a much smaller proportion of competent hosts than encountered in the current study (2·55%). This threshold value was reached using realistic transmission coefficients and required fewer larval and nymphal tick abundances to feed on mice than that recorded in the current study. Values of $R_0$ indicated that small mammals alone could maintain cycles of infection of $B. burgdorferi$ s.l. without the need for alternative transmission hosts. s.l. This suggestion was at least partially supported by the identification of $B. afzelii$, a rodent-associated Borrelia genospecies (Kurtenbach et al. 2002), in questing ticks. However, the origin of the $B. afzelii$ infections could not be determined by bloodmeal analysis. Squirrels are also competent reservoirs of this Borrelia genospecies (Craine et al. 1997) and it is possible that they were the origin of the infection.

Differences in the response of $R_0$ between $B. microti$ and $A. phagocytophilum$ most likely lie in differences in the systemic infection duration. Clinical infections of $B. microti$ have been detected for up to 31 days post-infection by PCR in the USA (Vannier et al. 2004). As previously mentioned, Randolph (1995) observed that in the actual period of infectivity for ticks feeding on an infected host is 1–4 days using British strains. $A. phagocytophilum$ infections have been detected by PCR for up to 40 days post-infection (Telford et al. 1996) but the actual period of infectivity is unknown. If, like $B. microti$, the period of infectivity is much less than the period where the infection can be detected by PCR then the threshold value of $R_0$ would be more difficult to achieve and infection cycles of $A. phagocytophilum$ less likely to develop.

The ability of $B. burgdorferi$ s.l. to become established more readily in the wood mouse-tick system than other pathogens is a product of its relatively long systemic infection duration and the secondary route of infection available via efficient co-feeding transmission (Randolph et al. 1996).

As expected, wood mice were infected almost exclusively with larvae and only 3 nymphs were recovered. The resultant small nymph to larvae ratio (1:388) is comparable to those found elsewhere in Ireland (1:∞ and 1:650 (Gray et al. 1992) and 1:105 (Gray et al. 1999)) but is generally much smaller than those recorded across the rest of Europe (min = 1:7, max = 1:185, mean = 1:44, n = 19) (Matuschka et al. 1991; Humair et al. 1993; Talleklint and Jaenson, 1994; Kurtenbach et al. 1995; Humair et al. 1999; Randolph and Storey, 1999; Randolph et al. 1999)). It has been suggested that climatic conditions, such as humidity and temperature, can determine the distribution of tick life stages on hosts (Randolph and Storey, 1999). Ticks are prone to desiccation and immature stages are more susceptible than adults due to their smaller surface area to volume ratio, higher metabolic rate and limited fat reserves (Randolph and Storey, 1999). As a result, different life stages quest at different heights in vegetation, with larvae questing close to the moist litter layer and nymphs and adults questing progressively higher (Gigon, 1985). Experimental data have shown that nymphs, when confronted by increasingly dry conditions, quest lower in vegetation and feed more frequently on small mammals (Randolph and Storey, 1999). Ireland has a temperate maritime climate and generally has higher levels of precipitation and lower temperatures
than other locations across Europe (BIOCLIM variables; BIO12-annual precipitation and BIO1-annual mean temperature, www.worldclim.org/bioclim). Therefore, it is likely that nymphs in Ireland quest higher in vegetation than individuals in drier locations and, as a result, do not encounter small mammals as frequently. Low nymph to larvae ratios may limit the development of enzootic tick-borne pathogen cycles in small mammals. However, the distribution of *I. ricinus* on small mammals is often over-dispersed and this must be taken into account when assessing if tick-borne pathogen cycles are likely to be present, or develop, in a given area (Nilsson and Lundqvist, 1978; Craine et al. 1995; Randolph et al. 1999). For example, Randolph et al. (1999) found that the same 20% of small mammal hosts fed 61% of larvae and 72% of nymphs whilst a similar observation was made in the current study (20% of hosts fed 72% of larvae and all nymphs). This coincident aggregated distribution has important implications for the transmission of tick-borne pathogens as it allows small numbers of nymphs to feed alongside, and potentially infect, large numbers of larvae (Randolph et al. 1999). Therefore, even small nymph to larvae ratios, such as those found in Ireland may be epidemiologically significant.

Sequence analysis indicated that 2 bird-associated genospecies of *B. burgdorferi* s.l. were also present in *I. ricinus* nymphs, *B. valaisiana* and *B. garinii* (Kurtenbach et al. 2002). Bloodmeal analysis revealed that birds were the most important hosts of larval *I. ricinus* and that ticks infected with *B. valaisiana* and *B. garinii* had previously fed on birds. Therefore, it is not surprising that bird-associated *Borrelia* genospecies were the most common infections present. Present data suggest that birds are important hosts of larval *I. ricinus* and have a more important role in the epidemiology of *B. burgdorferi* s.l. in Ireland than small mammals. This suggestion is supported by previous studies in Ireland that also found bird-associated *Borrelia* genospecies to be the most common *Borrelia* infections present in questing ticks and that wood mice were rarely infected with *B. burgdorferi* s.l. (Kirstein et al. 1997; Gray et al. 1999, 2000).

The current study highlights how individual variation in the ecological parameters of tick-borne pathogens and their vectors can greatly affect the probability of establishment and persistence of pathogens within a system. We believe the R0 model of Hartemink et al. (2008) and the methods currently presented provide a potentially valuable tool in the control of tick-borne pathogens, allowing the identification of factors responsible for tick-borne pathogen persistence which could be utilized in management decisions. The view that small mammals have a more limited role in the epidemiology of tick-borne infections where nymphs of *I. ricinus* are rare on small mammals is supported.


APPENDIX

Structure of the next generation matrix (a), a schematic version of the matrix indicating the location of the various transmission routes used by pathogens (b) and a list of equations used to calculate each element within the matrix (c) (taken from Hartemink et al. 2008). Equations utilize tick- and pathogen-specific parameters derived from the literature and the current study (Tables 1 and 2).

(a) \[
K = \begin{pmatrix}
k_{11} & k_{12} & k_{13} & k_{14} & 0 \\
k_{21} & k_{22} & k_{23} & 0 & k_{25} \\
k_{31} & k_{32} & k_{33} & 0 & k_{35} \\
k_{41} & k_{42} & k_{43} & 0 & k_{45} \\
k_{51} & k_{52} & k_{53} & 0 & 0
\end{pmatrix}
\]

(b) \[
\begin{pmatrix}
\text{transovarial} & \text{transovarial} & \text{transovarial} & \text{transovarial} & 0 \\
\text{cofeeding} & \text{cofeeding} & \text{cofeeding} & 0 & \text{host } \rightarrow \text{ L} \\
\text{cofeeding} & \text{cofeeding} & \text{cofeeding} & 0 & \text{host } \rightarrow \text{ N} \\
\text{cofeeding} & \text{cofeeding} & \text{cofeeding} & 0 & \text{host } \rightarrow \text{ A} \\
\text{tick } \rightarrow \text{ host} & \text{tick } \rightarrow \text{ host} & \text{tick } \rightarrow \text{ host} & 0 & 0
\end{pmatrix}
\]

(c) \[
k_{11} = s_L s_N s_A E r_A, \\
k_{12} = s_N s_A E r_A, \\
k_{13} = s_A E r_A, \\
k_{14} = E r_A, \\
k_{15} = 0, \\
k_{21} = (s_L q_L C_{LL} + s_L s_N q_N C_{LN} + s_L s_N s_A q_A C_{LA}) \ h_t, \\
k_{22} = (s_N q_N C_{LN} + s_N s_A q_A C_{LA}) \ h_t, \\
k_{23} = (s_A q_A C_{LA}) \ h_t, \\
k_{24} = 0, \\
k_{25} = p_L i N_L \ h_t, \\
k_{31} = (s_L s_A q_A C_{NL} + s_L s_N q_N C_{NN} + s_L s_N s_A q_A C_{NA}) \ h_t, \\
k_{32} = (s_N q_N C_{NN} + s_N s_A q_A C_{NA}) \ h_t, \\
k_{33} = (s_A q_A C_{NA}) \ h_t, \\
k_{34} = 0, \\
k_{35} = p_N i N_N \ h_t, \\
k_{41} = (s_L s_A q_A C_{AL} + s_L s_N q_N C_{AN} + s_L s_N s_A q_A C_{AA}) \ h_t, \\
k_{42} = (s_N q_N C_{AN} + s_N s_A q_A C_{AA}) \ h_t, \\
k_{43} = (s_A q_A C_{AA}) \ h_t, \\
k_{44} = 0, \\
k_{45} = p_A i N_A \ h_t, \\
k_{51} = (s_L q_L + s_L s_N q_N + s_L s_N s_A q_A) \ h_t, \\
k_{52} = (s_N q_N + s_N s_A q_A) \ h_t, \\
k_{53} = s_A q_A h_t, \\
k_{54} = 0, \\
k_{55} = 0.
\]