The importance of the aggregation of ticks on small mammal hosts for the establishment and persistence of tick-borne pathogens: an investigation using the R_0 model

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SUMMARY

Aggregation of parasites amongst hosts is important for the epidemiology of vector-borne diseases because hosts that support the majority of the vector population are responsible for the majority of pathogen transmission. *Ixodes ricinus* ticks transmit numerous pathogens of medical importance including *Borrelia burgdorferi* s.l. and tick-borne encephalitis virus. One transmission route involved is 'co-feeding transmission', where larvae become infected via feeding alongside infected nymphs. The aggregation of ticks on hosts leads to an increase in the number of larvae feeding alongside nymphs, increasing the transmission potential via this route. The basic reproduction number, R_0, can be used to identify whether a pathogen will become established if introduced. In the current study we use previously published tick, and pathogen, specific data to parameterize an R_0 model to investigate how the degree of aggregation of ticks on hosts affects pathogen persistence. The coincident aggregated distribution permitted the establishment of tick-borne encephalitis virus but did not influence whether *B. burgdorferi* s.l. became established. The relationship between the k-exponent of the negative binomial distribution and R_0 was also defined. Therefore, the degree of aggregation of ticks on small mammal hosts has important implications for the risk to human health in a given area.

Key words: vector-borne diseases, aggregation, transmission, *Ixodes ricinus*, *Borrelia burgdorferi* s.l., epidemiology.

INTRODUCTION

Parasites are often aggregated among their hosts such that a large proportion of the parasite population is present on only a small proportion of the host population (Crofton, 1971; Dobson and Merenlender, 1991; Shaw and Dobson, 1995). This type of aggregation follows a statistical trend found across a wide range of disciplines known as the 'Pareto principle' or '80/20 rule' (80% of parasites are found on 20% of the host population) (Woolhouse et al. 1997; Koch, 1999). The aggregated distribution of parasites can often be modelled using the negative binomial distribution, a discrete probability distribution with a k-exponent that defines the degree of aggregation (Crofton, 1971; Shaw and Dobson, 1995). Heterogeneities in thse abundance of parasites among hosts arise due to variability in individuals in their rate of exposure to parasites and in their susceptibility to parasites once they have been exposed (Wilson et al. 2002). The aggregation of parasites has important implications for the epidemiology of vector-borne pathogens; if a small fraction of the host population supports the majority of the

vector population then those individuals will also be the most important hosts for the transmission of pathogens (Woolhouse et al. 1997; Perkins et al. 2003).

Ticks are vectors of numerous bacterial, viral and protozoan pathogens of medical importance (Jongejan and Uilenberg, 2004) and small mammals are reservoirs of many of these pathogens (Goodman et al. 2005). The transmission of pathogens from one tick to another, a pre-requisite for the establishment of cycles of infection, may occur via 3 pathways (see Randolph (1998) for a review). Firstly, female ticks may transmit the pathogen to eggs via transovarial transmission. Secondly, nymphs may feed on, and infect, a host leading to a systemic infection; larval ticks may then acquire an infection by ingesting the blood of the infected host, maintain the infection transstadially, and infect an alternative host during a subsequent bloodmeal as a nymph. Thirdly, ticks may become infected by co-feeding, spatially or temporally, alongside infected ticks. This 'co-feeding transmission' does not require the host to have a systemic infection but instead pathogens are passed from one tick to another as they feed together. Vertebrate hosts may vary in their competency to support transstadial and co-feeding transmission. For example, red grouse (*Lagopus lagopus scoticus*) and sheep become viraemic with louping-ill and can

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transmit it transstadially, hares (*Lepus timidus*), however, do not develop a systemic infection but play a crucial role in the persistence of the disease via non-viraemic, co-feeding transmission (Hudson *et al.* 1995; Norman *et al.* 2004). In the case of *Borrelia burgdorferi* s.l., small mammals are competent for both transstadial transmission and co-feeding transmission. In contrast, sheep do not develop a systemic infection and therefore do not permit transstadial transmission, however, they do support co-feeding transmission (see Randolph *et al.* (1996) for a review).

Larvae and nymphs of *Ixodes ricinus*, the vector of numerous pathogens, have been found to be highly aggregated among small mammal hosts across much of Europe, typically having a k-exponent of the negative binomial distribution between 0 and 1 (Randolph *et al.* 1999). Often larvae and nymphs of *I. ricinus* are not independently aggregated but follow a coincident aggregated distribution, i.e. those individuals with large numbers of larvae also support nymphs (Randolph *et al.* 1999; Harrison *et al.* 2011). For example, Harrison *et al.* (2011) found that the same 20% of wood mice in Ireland fed 72% of *I. ricinus* larvae and all nymphs found on mice, a pattern corresponding closely to the 80/20 rule. This level of aggregation has important implications for the efficiency of co-feeding transmission as it may allow roughly twice as many larvae to feed alongside nymphs than would be possible with an independent aggregated distribution (Randolph *et al.* 1999).

The basic reproduction number, denoted as R_0, has a number of important applications in epidemiology. It 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). It has a threshold value such that if $R_0 > 1$ a pathogen will persist when introduced, but a value of $R_0 < 1$ suggests that it will die out. In the case of tick-borne pathogens it can be used to identify the average number of ticks infected in the second generation after 1 infected tick enters the population, therefore, if $R_0 > 1$, the pathogen will perpetuate within the tick-host system. Heterogeneities in the efficiency of different transmission routes of tick-borne pathogens will affect R_0 and, therefore, the likelihood of a pathogen becoming established within a system (Woolhouse *et al.* 1997). As expected, due to the applicability of the basic reproduction number, many attempts have been made to estimate R_0 for tick-borne pathogens (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). Indeed, Rosa *et al.* (2003) previously investigated the role of tick aggregation on the persistence of tick-borne pathogens; however, many of these approaches were lacking in their relevance to the tick life cycle, assuming bite rates akin to insect vector models, or used derived expressions that could

not be strictly interpreted as a biologically meaningful value of R_0 (Hartemink *et al.* 2008).

The current study uses the most up-to-date and biologically relevant next-generation model of the basic reproduction number for tick-borne pathogens (Hartemink *et al.* 2008) to investigate how the degree of aggregation of *I. ricinus* ticks on small mammal hosts (ranging from dispersed to independent aggregated to coincident aggregated distribution) affects the ability of tick-borne pathogens to become established in enzootic small mammal cycles. We use previously published tick-related and pathogen-specific data as well as simulated data to parameterize the R_0 model specific for *Borrelia burgdorferi* s.l., and tick-borne encephalitis virus (TBEV), 2 of the most important tick-borne pathogens of humans in Europe. The k-exponent of the negative binomial distribution was varied while other parameters were held static so that the effect of the aggregation of ticks on the persistence of tick-borne pathogens in small mammal hosts could be observed. This method also allowed the relationship between the k-exponent of the negative binomial distribution and R_0 to be defined. It has also been shown that increasing the diversity of vertebrate hosts in an ecosystem can reduce the risk of disease as only some hosts are competent for disease transmission and infective tick bites may be 'wasted' on non-transmission competent hosts, the so called 'dilution effect' (Ostfeld and Keesing, 2000; Schmidt and Ostfeld, 2001). Therefore, we also investigated how the degree of aggregation of ticks on small mammal hosts affects the ability of these pathogens to become established in relation to the proportion of transmission-competent hosts on which ticks are feeding.

MATERIALS AND METHODS

Data generation

Theoretical datasets for larvae, nymphs and adults were generated from the negative binomial distribution using the 'rnbinom' function in package 'base' within the R statistical software environment, available from www.r-project.org. Three parameters were required to simulate the data; the 'number of observations', n, (the host population size, set at 500 for all simulations), 'size' (also known as the dispersion parameter or the k-exponent of the negative binomial distribution, hereafter referred to as the 'k-exponent' or 'k') and 'mu' (the mean, in this case the mean number of each tick life stage present on the small mammal population). For each life stage, datasets were created assuming 3 distributions using a range of values of the k-exponent. The first dataset had a starting value of $k = 0.1$ and increased in increments of 0.1 until $k = 1.0$, thus representing natural levels of aggregation of ticks on small mammals in Europe (Randolph *et al.* 1999). The

second dataset represented a dispersed independent distribution of ticks on hosts beginning at $k = 1{\cdot}5$ and increasing in increments of $0{\cdot}5$ until $k = 30{\cdot}5$ at which point the variance was approximately equal to the mean and the negative binomial distribution became Poisson distributed (mean $= 6$, variance $= 6{\cdot}67$, goodness of fit to the Poisson distribution $\chi^2 = 18{\cdot}5$, D.F. $= 14$, $P = 0{\cdot}184$). The third dataset represented a coincident aggregated distribution and utilized data generated for the independent aggregation of ticks in dataset 1 ($0{\cdot}1 \leqslant k \leqslant 1{\cdot}0$), but in this case 80% of nymphs that were previously independently distributed among the host population were re-distributed randomly among the 20% of individuals most parasitized by larval ticks, thus conforming to the 80/20 rule and achieving a coincident aggregated distribution with values of k ranging between $0{\cdot}1$ and $1{\cdot}0$. As adult ticks were so rare among the datasets ($mu = 0{\cdot}001$), all adults were re-distributed randomly among the top 20% of individuals most parasitized by larval ticks for the coincident aggregated dataset. Therefore, 86 individual datasets were created each for larval, nymphal and adult ticks, 10 for the independent aggregated distribution ($0{\cdot}1 \leqslant k \leqslant 1{\cdot}0$), 66 for dispersed distribution ($1{\cdot}5 \leqslant k \leqslant 30{\cdot}5$) and 10 for the coincident aggregated distribution ($0{\cdot}1 \leqslant k \leqslant 1{\cdot}0$). Increments of $0{\cdot}1$ were used from $0{\cdot}1$ to $1{\cdot}0$ to increase the sample size for this range of values of the k-exponent, as they represented natural levels of aggregation as previously stated.

Values of 'mu', the average number of each tick life stage were set as 6, $0{\cdot}2$ and $0{\cdot}001$ for larvae, nymphs and adults respectively (cited by Hartemink *et al*. 2008). Although the presence of adult ticks is rare among small mammals the model is parameterized to indicate whether the pathogen is likely to become established or persist in small mammal cycles and we therefore assume that the tick population is supported by larger mammals within the system e.g. sheep, hares or deer. The data generated for larvae, nymphs and adults were used to calculate parameters within the R_0 model of Hartemink *et al*. (2008) that are affected by the degree of aggregation of ticks on hosts i.e. those involved with co-feeding: C_{LL}, C_{NL}, C_{AL}, C_{LN}, C_{NN}, C_{AN}, C_{LA}, C_{NA}, C_{AA} (for example, C_{LL} is 'the mean number of larvae co-feeding with a larva', see Table 1 for a full list of parameter descriptions). A set of these parameters was produced for each of the 86 individual datasets across the range of the dispersed distribution ($1{\cdot}5 \leqslant k \leqslant 30{\cdot}5$), the independent aggregated distribution ($0{\cdot}1 \leqslant k \leqslant 1{\cdot}0$) and the coincident aggregated distribution ($0{\cdot}1 \leqslant k \leqslant 1$).

The next generation matrix

When diseases are transmitted directly, estimating R_0 is straightforward but vector-borne diseases are more complex as they utilize different 'infected types' i.e. hosts and vectors. This is made increasingly complex

Table 1. Ecological parameters for *Ixodes ricinus*

(Adapted from Hartemink *et al*. 2008.)

Parameter	Description	Estimate†
E	Eggs per adult	2000[1,2]
s_L	Survival probability from egg to feeding larvae	$0{\cdot}05$[1]
s_N	Survival probability from feeding larvae to feeding nymph	$0{\cdot}1$[1]
s_A	Survival probability from feeding nymph to feeding adult	$0{\cdot}1$[1]
C_{LL}	Mean number of larvae co-feeding with a larva	6–20*
C_{NL}	Mean number of nymphs co-feeding with a larva	$0{\cdot}1$–$0{\cdot}2$*
C_{AL}	Mean number of adults co-feeding with a larva	0*
C_{LN}	Mean number of larvae co-feeding with a nymph	$4{\cdot}2$–$7{\cdot}5$*
C_{NN}	Mean number of nymphs co-feeding with a nymph	2–$3{\cdot}7$*
C_{AN}	Mean number of adults co-feeding with a nymph	0–$0{\cdot}01$*
C_{LA}	Mean number of larvae co-feeding with an adult	0–22*
C_{NA}	Mean number of nymphs co-feeding with an adult	0*
C_{AA}	Mean number of adults co-feeding with an adult	0*
N_{LH}	Average number larvae on competent host	6[3,4,5]
N_{NH}	Average number nymphs on competent host	$0{\cdot}2$[4,5]
N_{AH}	Average number adults on competent host	$0{\cdot}001$[6]
D_L	Days of attachment of larva	$2{\cdot}5$[4]
D_N	Days of attachment of nymph	$3{\cdot}5$[6]
D_A	Days of attachment of adult	12[6]

† Numbers in superscript refer to the following sources cited by Hartemink *et al*. (2008): [1]Randolph and Craine (1995); [2]Randolph (2004); [3]Randolph *et al*. (1999); [4]Gray (2002): [5]Humair *et al*. (1999); [6]S.E. Randolph, *unpublished manuscript*. *Parameters calculated in the current study across a range of values of the k-exponent. Please refer to the Supplementary data (online version only) for equations used to calculate each element within the next generation matrix and for the structure of the matrix.

when additional transmission routes and host and vector types are present; however, the next-generation matrix provides a method to incorporate these complexities. The matrix is based on the concept of types at birth i.e. the different states at which individuals become infected. Hartemink *et al*. (2008) identified 5 types at birth for tick-borne infections, (1) eggs (via transovarial transmission), (2) larvae, (3) nymphs, and (4) adults (via bloodmeals) and (5) vertebrate hosts. For a system with 5 types at birth the next generation matrix will be a 5×5 matrix of elements with every type at birth being infected by another (see Supplementary material for elements and the structure of the next generation matrix, online

Table 2. *Ecological parameters for* Borrelia burgdorferi *s.l. and tick-borne encephalitis virus*

(Taken from Hartemink *et al*. 2008.)

Parameter	Description	*B. burgdorferi* s.l.	TBE virus†
i	Systemic infection duration	120 days[1]	2 days[1]
θ	Efficiency from tick to tick	0·56[2]	0·55[3,4]
p_L	Efficiency from competent host to larva	0·5[5]	0·8[6]
p_N	Efficiency from competent host to nymph	0·5[6]	0·8[6]
p_A	Efficiency from competent host to adult	0·4[7]	0·8[6]
q_L	Efficiency from larva to competent host	0·8[6]	0·9[6]
q_N	Efficiency from nymph to competent host	0·8[6]	0·9[6]
q_A	Efficiency from adult to competent host	0·8[6]	0·9[6]
r_A	Efficiency from adult to egg	0·1[8]	0·001[9]

† Numbers in superscript refer to the following sources cited by Hartemink *et al*. (2008): [1]Randolph *et al*. (1996); [2]Gern and Rais (1996); [3]Labuda *et al*. (1993); [4]Labuda *et al*. (1997); [5]Randolph and Craine (1995); [6]S.E. Randolph, *unpublished manuscript*; [7]Kurtenbach *et al*. (1994); [8]Hubálek and Halouzka (1998). Please refer to the Supplementary data (online version only) for equations used to calculate each element within the next generation matrix and for the structure of the matrix.

version only). If we consider the element k_{31}: the number of nymphs (type at birth 3) infected by a tick infected as an egg (type at birth 1), which has the formula $k_{31} = (s_L \o_{LN} C_{NL} + s_L s_N \o_{NN} C_{NN} + s_L s_N s_A \o_{AN} C_{NA}) h_c$, it has 3 terms as ticks infected as eggs can infect nymphs after their first, second and third bloodmeals while the survival to each stage (s), the transmission efficiency from tick to tick ($\o$) and the aggregation of ticks (C) is taken into account. Of course, some of the elements of the matrix are not possible and therefore equal 0, for example, k_{15}, as tick eggs cannot be infected by a vertebrate host.

Calculation of R_0

Values of R_0 were calculated for *B. burgdorferi* s.l. and tick-borne encephalitis virus (TBEV) using the next generation matrix of Hartemink *et al*. (2008). Tick-related parameters involved with co-feeding transmission derived from datasets described above (C_{LL}-C_{AA}, Table 1) and previously published pathogen-specific parameters (Tables 1 and 2) were entered into equations to calculate each element of the matrix (please see Supplementary material for equations used to calculate each element, online version only). Previously published parameters were held constant while each of the 86 parameter sets (C_{LL}-C_{AA}) were substituted into the model in turn, thus allowing values of R_0 for each pathogen to be calculated as a function of the degree of aggregation. R_0 was calculated via the spectral decomposition of the parameterized next-generation matrix using the eigen(matrix) function in package 'base' in R. Values of R_0 were plotted as a function of the k-exponent and non-linear regression performed (Sigmaplot 10, Systat Software Inc.) for 2 datasets, one as the dispersed distribution approached the independent aggregated distribution and the second as dispersed distribution approached the coincident aggregated distribution.

To give an indication of the mean effect each degree of aggregation had on the basic reproduction number in relation to the proportion of transmission-competent hosts on which *I. ricinus* is feeding, values of R_0 were averaged across the datasets for each of the 3 levels of aggregation and the difference between the means of each distribution (ΔR_0) calculated for a range of values of h_c, (10–100% at 10% intervals). In the current study we define small mammals as 'competent hosts' as they facilitate both transstadial and co-feeding transmission for both pathogens and are considered to be the most important reservoirs of these pathogens in Europe. Although sheep support co-feeding transmission of *B. burgdorferi* s.l., they are not thought to be important for the epidemiology of this pathogen in most of its range (Hartemink *et al*. 2008).

RESULTS

Increasing levels of aggregation of ticks on hosts led to an increase in values of R_0 for both pathogens. When values of R_0 were plotted as a function of k the effect of aggregation on R_0 values could be clearly seen. The independent aggregated distribution formed at values of the k-exponent between 0·1 and 1·0 caused values of R_0 to increase in comparison to values observed when ticks were dispersed, leading to an inverse first-order polynomial relationship between k and R_0 when the dispersed distribution converged on the independent aggregated distribution (non-linear regression, $R^2_{adj} = 0.87$, F = 478·66, D.F. = 68, $P < 0.0001$, $f = 3.87 + (0.02/x)$, (*B. burgdorferi* s.l.) and $R^2_{adj} = 0.47$, F = 115·94, D.F. = 68, $P < 0.0001$, $f = 0.73 + (0.001/x)$ (TBEV), adjusted R^2, F-value, degrees of freedom, P-value and relationship of the line of the first order polynomial, Fig. 1a and b). The coincident aggregated distribution also caused an increase in R_0 relative to the dispersed distribution but to a greater degree than the independent
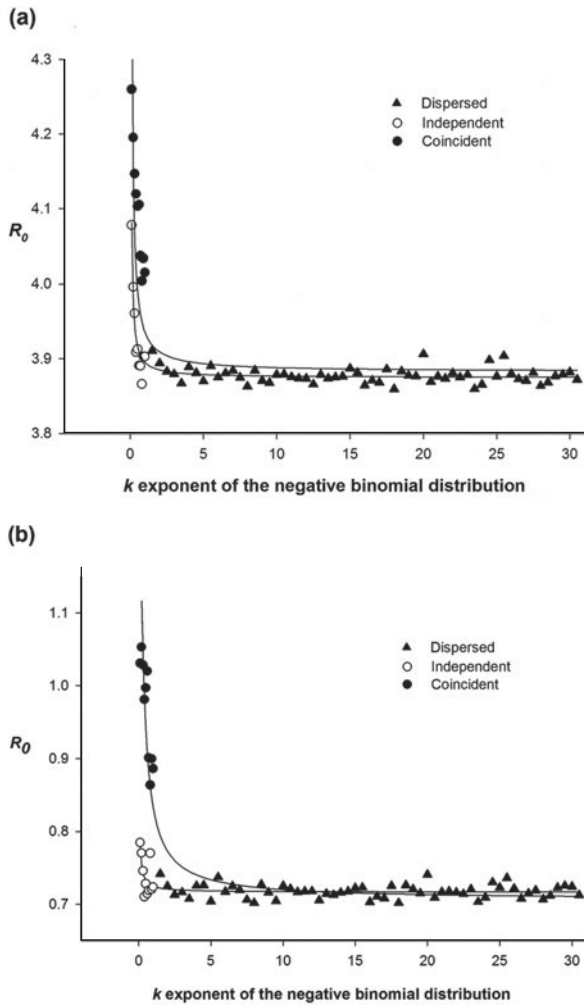
Fig. 1. Values of R_0 calculated for (a) *Borrelia burgdorferi* s.l. and (b) tick-borne encephalitis virus, plotted as a function of the degree of aggregation of ticks on small mammal hosts (*k*-exponent of the negative binomial distribution) ranging from dispersed ($1 \cdot 5 \leqslant k \leqslant 30 \cdot 5$) to independent aggregated ($0 \cdot 1 \leqslant k \leqslant 1$) and from the dispersed ($1 \cdot 5 \leqslant k \leqslant 30 \cdot 5$) to the coincident aggregated distributions ($0 \cdot 1 \leqslant k \leqslant 1$). Small values of the *k*-exponent indicate high levels of aggregation, larger values indicate low levels of aggregation. Plots of first-order polynomial relationships between *k* and R_0 for the dispersed distribution converging on the independent aggregation and for the dispersed distribution converging on the coincident aggregated distribution for each pathogen are also presented.

aggregated distribution. This relationship also followed an inverse first-order polynomial ($R^2_{adj} = 0 \cdot 77$, F $= 231 \cdot 66$, D.F. $= 68$, $P < 0 \cdot 0001$, $f = 3 \cdot 88 + (0 \cdot 05/x)$, (*B. burgdorferi* s.l.) and $R^2_{adj} = 0 \cdot 62$, F $= 60 \cdot 74$, D.F. $= 68$, $P < 0 \cdot 0001$, $f = 0 \cdot 72 + (0 \cdot 001/x)$ (TBEV).

Average values of R_0 were increased for both pathogens with increasing levels of aggregation relative to those obtained when ticks followed a dispersed distribution, these values also increased in relation h_c (Table 3.). For *B. burgdorferi* s.l., the average value of R_0 increased by a minimum of $0 \cdot 012$ ($0 \cdot 75\%$) from $1 \cdot 594$ to $1 \cdot 606$ at $h_c = 10\%$ and by a maximum of $0 \cdot 066 (1 \cdot 49\%)$ from $4 \cdot 444$ to $4 \cdot 510$ at $h_c = 100\%$ when ticks followed the independent aggregated distribution relative to the dispersed distribution. When ticks followed a coincident aggregated distribution the average value of R_0 increased by $0 \cdot 033 (2 \cdot 07\%)$ from $1 \cdot 594$ to $1 \cdot 627$ at $h_c = 10\%$ and by $0 \cdot 297 (6 \cdot 68\%)$ from $4 \cdot 444$ to $4 \cdot 741$ at $h_c = 100\%$ relative to the dispersed distribution. Therefore, the threshold value of $R_0 > 1$ was achieved for *B. burgdorferi* s.l. regardless of the level of aggregation of ticks on hosts and even when the proportion of competent hosts on which *I. ricinus* had fed was very low (10%). In the case of TBEV, the average value of R_0 increased by $0 \cdot 003 (1 \cdot 32\%)$ from $0 \cdot 227$ to $0 \cdot 230$ at $h_c = 10\%$ and by $0 \cdot 028 (3 \cdot 26\%)$ from $0 \cdot 858$ to $0 \cdot 886$ at $h_c = 100\%$ when ticks followed an independent aggregated distribution relative to a dispersed distribution. When ticks followed a coincident aggregated distribution, the effect was much greater, the average R_0 value increased by $0 \cdot 029$ ($12 \cdot 78\%$) from $0 \cdot 227$ to $0 \cdot 256$ at $h_c = 10\%$ and by $0 \cdot 342$ ($39 \cdot 86\%$) from $0 \cdot 858$ to $1 \cdot 200$ at $h_c = 100\%$. In contrast to *B. burgdorferi* s.l., values of R_0 for TBEV only achieved the threshold value when h_c reached 80% and only when ticks followed a coincident aggregated distribution. In this case the coincident aggregated distribution of ticks on hosts as well as ticks feeding on a high proportion of transmission competent hosts is essential for the threshold value of R_0 to be achieved and the pathogen to persist.

DISCUSSION

Using different levels of aggregation from the dispersed to the independent aggregated to the coincident aggregated we showed that values of R_0 became elevated with progressive levels of aggregation, making it increasingly likely for these pathogens to become established and to persist. In the case of *Borrelia burgdorferi* s.l., values of R_0 were always above the threshold value for the establishment of the disease and therefore the level of aggregation of ticks on hosts and the proportion of competent hosts on which ticks are feeding has little effect on whether this pathogen becomes established or not. In contrast, the threshold value of R_0 for TBEV was only achieved when the proportion of transmission-competent hosts on which ticks were feeding reached 80% and only when the ticks followed a coincident aggregated distribution. This suggests that any decrease in the proportion of competent hosts on which ticks are feeding, for example through vaccination or increasing the diversity of potential vertebrate hosts (assuming they are less competent transmission hosts), will cause a reduction in R_0 and the pathogen will become less likely to become established or to persist. For TBEV, the distribution of ticks on hosts has a much more definitive role in whether the pathogen becomes established or not.

Table 3. Mean values of R_0 for *Borrelia burgdorferi* s.l. and tick-borne encephalitis virus for different distributions of *Ixodes ricinus* ticks on small mammal hosts in relation to the proportion of transmission-competent hosts on which *I. ricinus* is feeding (h_c)

(k_d = dispersed distribution with values of the k exponent between 1·5 and 30·5, k_i = independent aggregated distribution (0·1 ≤ k ≤ 1) and k_c = coincident aggregated distribution (0·1 ≤ k ≤ 1). Differences between means for aggregated distributions and the dispersed distrubution are also presented (ΔR_0).)

| | *Borrelia burgdorferi* s.l. | | | | | Tick-borne encephalitis virus | | | | |
h_c	k_d = 1·5 to 30·5	k_i = 0·1 to 1	k_c = 0·1 to 1	$\Delta R_0\ k_i{-}k_d$ (%)	$\Delta R_0\ k_c{-}k_d$ (%)	k_d = 1·5 to 30·5	k_i = 0·1 to 1	k_c = 0·1 to 1	$\Delta R_0\ k_i{-}k_d$ (%)	$\Delta R_0\ k_c{-}k_d$ (%)
10	1·594	1·606	1·627	0·012 (0·75)	0·033 (2·07)	0·227	0·230	0·256	0·003 (1·32)	0·029 (12·78)
20	2·137	2·158	2·201	0·021 (0·98)	0·064 (2·99)	0·332	0·336	0·391	0·004 (1·20)	0·059 (17·77)
30	2·552	2·580	2·645	0·028 (1·10)	0·093 (3·64)	0·417	0·423	0·509	0·006 (1·44)	0·092 (22·06)
40	2·903	2·937	3·025	0·034 (1·17)	0·122 (4·20)	0·493	0·500	0·618	0·007 (1·42)	0·125 (25·35)
50	3·213	3·253	3·365	0·040 (1·24)	0·152 (4·73)	0·562	0·576	0·721	0·014 (2·49)	0·159 (28·29)
60	3·494	3·540	3·675	0·046 (1·32)	0·181 (5·18)	0·627	0·637	0·821	0·010 (1·59)	0·194 (30·94)
70	3·754	3·805	3·964	0·051 (1·36)	0·210 (5·59)	0·688	0·699	0·918	0·011 (1·60)	0·230 (33·43)
80	3·997	4·053	4·236	0·056 (1·40)	0·239 (5·98)	0·747	0·760	1·014	0·013 (1·74)	0·267 (35·74)
90	4·226	4·287	4·495	0·061 (1·44)	0·269 (6·37)	0·803	0·818	1·107	0·015 (1·87)	0·304 (37·86)
100	4·444	4·510	4·741	0·066 (1·49)	0·297 (6·68)	0·858	0·886	1·200	0·028 (3·26)	0·342 (39·86)

Without the increased transmission efficiency afforded to the pathogen via this enhanced potential for the co-feeding transmission route this pathogen would not become established in small mammal hosts at all. Results of the current study are consistent with those of Hartemink *et al*. (2008) and Randolph *et al*. (1996) who also found that the threshold value of R_0 was easily achieved for *B. burgdorferi* s.l. with or without co-feeding transmission but the threshold value for TBEV was only achieved when co-feeding transmission was present.

The differential response of these two pathogens to co-feeding transmission lies in differences in their systemic infection duration (Randolph *et al*. 1996). The systemic infection of *B. burgdorferi* s.l. in a rodent host is 120 days but only 2 days for TBEV (Randolph *et al*. 1996). When considering transstadial transmission, rodents infect ticks with *B. burgdorferi* s.l. over a long period of time, furnishing the system with large numbers of infected ticks that in turn infect large numbers of hosts. This leads to a greater overall level of transmission between hosts and vectors and allows values of R_0 to be maintained above the threshold value for establishment. In contrast, rodents can only infect ticks with TBEV over a short period. Thus many fewer ticks become infected with TBEV than with *B. burgdorferi* s.l. and values of R_0 for TBEV are reduced so that they do not reach the threshold value for establishment of the pathogen. However, the extra boost in overall transmission efficiency supplied by co-feeding transmission, driven by the coincident aggregated distribution of ticks, is enough in some cases to increase values of R_0 above the threshold value. This explains why natural cycles of *B. burgdorferi* s.l. are widespread while those of TBEV are highly focal and patchy in distribution; only certain locations provide the correct climatic condition for the seasonal synchrony of larvae and nymphs on small mammal hosts that contribute to the coincident aggregated distribution that drives co-feeding transmission (Randolph *et al*. 1996; Randolph and Rogers, 2000). Warm summers allow the rapid development of ticks while rapid autumn cooling sends larvae into winter diapause causing them to emerge in synchrony with nymphs in spring and facilitates the coincident aggregation of ticks on hosts and, therefore, co-feeding transmission (Randolph and Rogers, 2000; Gatewood *et al*. 2009). In contrast to TBEV, locations with climatic conditions that promote the activity of nymphs prior to larvae often have the strongest transmission cycles for *B. burgdorferi* s.l (Kurtenbach *et al*. 2006). As co-feeding transmission is less important for *B. burgdorferi* s.l, this activity profile promotes the persistence of this pathogen as it allows time for vertebrate hosts to become infected by nymphs, develop a systemic infection, and to subsequently infect larvae. In addition, genospecies of *Borrelia* that persist for longer in vertebrate hosts

may be favoured by climate-driven asynchrony of immature ticks while those genospecies that have a shorter systemic may be driven by seasonal synchrony (Kurtenbach *et al*. 2006; Gatewood *et al*. 2009). This can lead to geographical variations in the distribution of *Borrelia* genospecies driven by climate (Gatewood *et al*. 2009). While the degree of aggregation of ticks on hosts at a given point in time has implications for pathogen transmission, there is also a temporal aspect due to the seasonality of immature ticks in response to climate.

While the coincident aggregated distribution caused an increase in R_0 so did the independent aggregated distribution, albeit to a lesser extent. In this case, it is not that individuals have an increased chance of hosting both larvae and nymphs but that the independent aggregation of larvae and nymphs is such that occasionally some individuals will host nymphs and, by chance, large numbers of larvae at the same time. This will lead to an 'accidental' increase in R_0 but to a lesser extent than when individuals consistently host nymphs and larvae together as under the coincident aggregated distribution.

In addition to the climatic conditions that allow synchronous feeding of larval and nymphal ticks, a number of other biotic factors are involved. Questing larvae are themselves clumped in their environment as they emerge from egg masses laid down by females (Randolph and Steele, 1985; Daniels and Fish, 1990). Sexually active adult male hosts often cover wider ranges than other individuals within the population and so may encounter more ticks in the environment (Randolph, 1977; Boyer *et al*. 2010). These individuals are often the largest within a population (due to maturity and sexual size dimorphism) and there is evidence to suggest that larger individuals have greater tick loads than smaller individuals (Gallivan and Horak, 1997; Harrison *et al*. 2010). Sexually active males also have higher levels of testosterone that may depress innate and acquired resistance to ticks in small mammal hosts and lead to increased attachment rates (Hughes and Randolph, 2001). As a result of these factors affecting the exposure and susceptibility of individuals to parasites, adult males are often the '20%' in the '80/20 rule' i.e. they are the fraction of the host population that contributes most to the transmission of parasites and vector-borne diseases (Perkins *et al*. 2003; Ferrari *et al*. 2004; Bouchard *et al*. 2011). Perkins *et al*. (2003) demonstrated that within a population of *Apodemus flavicollis*, 20% of the hosts were responsible for 74% to 94% of transmission of TBEV to ticks and that adult males of high body mass were the most likely members of this group. Similarly, Ferarri *et al*. (2004) found that the 20% of *A. flavicollis* most infected with the nematode, *Heligmosomoides polygyrus,* (consisting of 62% males) accounted for 73% of the eggs expelled in faeces.

Although the aggregation of ticks on certain individuals, such as adult males, is common there may be exceptions and this will have consequences for the persistence of tick-borne pathogens. If tick life stages aggregate on different hosts temporally, for example, nymphs on adult males early in the season as a result of elevated testosterone or increased movement, and larvae on pregnant females or juveniles later in the season due to reduced immunity, then pathogens such as *B. burgdorferi* s.l would be less likely to persist (see Mannelli *et al*. 2012).

Identifying the most important demographic for the transmission of pathogens within a population has important implications for their control. By targeting the fraction of the population most accountable for the transmission of parasites, transmission potential can be reduced. Conversely, targeting individuals in the population outside the important '20%' of key hosts may have little or no effect on the overall epidemiology pathogens (Perkins *et al*. 2003; Ferrari *et al*. 2004). The development of vaccines for *B. burgdorferi* s.l. in natural rodent hosts, such as white-footed mice, *Peromyscus leucopus*, has focussed on outer surface protein A (Osp A) which is involved with the attachment of spirochetes in the midgut of ticks (Pal *et al*. 2000). Recent field studies have indicated that vaccination of small mammal reservoirs may be a viable option for controlling Lyme borreliosis. Tsao *et al*. (2004) demonstrated that the prevalence of *B. burgdorferi* s.l. infection in *I. scapularis* could be reduced by between 19% and 25% via vaccination of white-footed mice while Telford *et al*. (2011) suggested that over half the population of white-footed mice could be targeted with nest-box deployed bait that could be used for vaccination. Although these measures are likely to reduce the number of nymphs infected with *B. burgdorferi* s.l. in the environment and, therefore, the risk to public health, data from the current study suggest that they are unlikely to eliminate the pathogen from the system altogether.

The current study uses the most up-to-date and biologically relevant model of the basic reproduction number, R_0, for tick-borne pathogens to investigate how the degree of aggregation of ticks on small mammal hosts, from the dispersed to the independent aggregated to the coincident aggregated distribution, affects the persistence of tick-borne pathogens in enzootic small mammal cycles. In addition, the relationship between the *k*-exponent of the negative binomial distribution and R_0 is defined for the first time.

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