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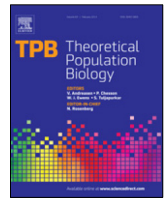Some of the authors of this publication are also working on these related projects:

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Incorporating tick feeding behaviour into R_0 for tick-borne pathogens

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ABSTRACT

Tick-borne pathogens pose a considerable disease burden in Europe and North America, where increasing numbers of human cases and the emergence of new tick-borne pathogens has renewed interest in resolving the mechanisms underpinning their geographical distribution and abundance. For *Borrelia burgdorferi* and tick-borne encephalitis (TBE) virus, transmission of infection from one generation of ticks to another occurs when older nymphal ticks infect younger larval ticks feeding on the same host, either indirectly via systemic infection of the vertebrate host or directly when feeding in close proximity. Here, expressions for the basic reproduction number, R_0, and the related tick type-reproduction number, T, are derived that account for the observation that larval and nymphal ticks tend to aggregate on the same minority of hosts, a tick feeding behaviour known as co-aggregation. The pattern of tick blood meals is represented as a directed, acyclic, bipartite contact network, with individual vertebrate hosts having in-degree, k_{in}, and out-degree, k_{out}, that respectively represent cumulative counts of nymphal and larval ticks fed over the lifetime of the host. The in- and out-degree are not independent when co-aggregation occurs such that

$$T \propto \frac{\langle k_{in} k_{out} \rangle}{\langle k_{in} \rangle},$$

where $\langle . \rangle$ indicates expected value. When systemic infection in the vertebrate host is the dominant transmission route $R_0^2 = T$, whereas when direct transmission between ticks co-feeding on the same host is dominant then $R_0 = T$ and the effect of co-aggregation on R_0 is more pronounced. Simulations of *B. burgdorferi* and TBE virus transmission on theoretical tick-mouse contact networks revealed that aggregation and co-aggregation have a synergistic effect on R_0 and T, that co-aggregation always increases R_0 and T, and that aggregation only increases R_0 and T when larvae and nymphs also co-aggregate. Co-aggregation has the greatest *absolute* effect on R_0 and T when the mean larval burden of hosts is high, and the largest *relative* effect on R_0 for pathogens sustained by co-feeding transmission, e.g. TBE virus in Europe, compared with those predominantly spread by systemic infection, e.g. *B. burgdorferi*. For both pathogens, though, co-aggregation increases the mean number of ticks infected per infectious tick, T, and so too the likelihood of pathogen persistence.

1. Introduction

The basic reproduction number of an infectious disease, denoted R_0, is a fundamental quantity in epidemiology for evaluating the likelihood of disease invasion/persistence. For single host-infections it is defined as the expected number of secondary individuals infected by a typical infectious individual in an otherwise susceptible population. For vector-borne diseases, and multi-host pathogens in general, the definition of R_0 is less straightforward as it must average over the multiple host types involved in pathogen transmission (Hartemink et al., 2008). The next generation matrix approach, introduced by Diekmann et al. (1990), is an effective method for doing so.

For tick-borne pathogens, R_0 has proved to be particularly challenging to calculate. Once more, the next-generation matrix approach has proved to be significant and influential (Matser et al., 2009; Davis and Bent, 2011; Harrison and Bennett, 2012; Dunn et al., 2013); it has been used to incorporate complexity arising from tick biology and multiple routes of transmission (Hartemink et al., 2008), seasonality in tick feeding (Davis and Bent, 2011), as well as interactions between tick-borne pathogens that share the same vertebrate host and vector (Dunn, 2014).

For some of the most important tick-borne pathogens, e.g. *Borrelia burgdorferi* (the causative agent of Lyme disease) and

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tick-borne encephalitis (TBE) virus, ticks acquire infection whilst taking their first blood meal from vertebrate hosts as larvae. Upon molting and emerging the following year as nymphs they feed a second time during which they can infect their vertebrate host (which in turn infects the next generation of larvae), as well as other ticks feeding at the same time, on the same host, and in close proximity. Hence the two immature life-stages of a tick vector, termed larvae and nymphs, are responsible for maintaining the pathogens in nature (Davis and Bent, 2011; Brunner et al., 2011). In this paper, the feeding behaviour of these two life-stages is considered, along with how these behaviours can directly influence R_0.

Aggregation refers to when most ticks (of a given life-stage) feed on a small subset of vertebrate hosts. It is a very common feeding behaviour of macroparasites, with almost all species observing a distribution known as the 80/20 rule, whereby 80% of parasites are found on approximately 20% of the hosts (Woolhouse et al., 1997; Shaw et al., 1998). Burdens of hard-bodied (Ixodes) ticks feeding on vertebrate hosts have been observed to follow a similar distribution (Brunner and Ostfeld, 2008; Ostfeld et al., 1996; Randolph et al., 1999; Craine et al., 1995; Shaw et al., 1998).

When larvae and nymphs aggregate on the *same* subset of hosts they are said to co-aggregate (Fig. 1). Co-aggregation has also been observed in the field, with studies of blacklegged ticks, *Ixodes scapularis* (the primary vector for Lyme disease in the United States), on white-footed mice, *Peromyscus leucopus*, having shown that mice with high nymphal counts tend to have high larval burdens later in the season (Brunner and Ostfeld, 2008). Similarly, studies of *Ixodes ricinus* (the tick species responsible for human cases of Lyme disease and TBE in Europe) have come to the same conclusion (Randolph et al., 1999; Craine et al., 1995).

Even though tick co-aggregation has the potential to increase pathogen transmission—by increasing the number of susceptible larvae feeding on the same hosts as infectious nymphs—only one study has investigated its influence on pathogen spread to date, namely that of Harrison and Bennett (2012). Next generation matrices for tick-borne pathogens typically incorporate co-aggregation by way of model parameterization (Hartemink et al., 2008; Matser et al., 2009; Davis and Bent, 2011). For example, to calculate the mean number of larvae infected by a nymph feeding in close proximity requires knowledge of the mean number of larvae that feed at the same time as a nymph, usually denoted C_{LN}. Large values for this parameter indicate that many larvae tend to co-aggregate on the same hosts as nymphs. But parameterization alone does not facilitate systematic investigation into the quantitative effect of co-aggregation on tick-borne pathogen emergence and persistence. To explore the nature of this relationship, at the very least the level of larval and nymphal co-aggregation, i.e. C_{LN}, needs to be varied whilst holding all other parameters constant in a sensitivity analysis of sorts.

This was the philosophy behind the approach of Harrison and Bennett (2012) where theoretical burdens of larval and nymphal ticks on hosts were generated from either a Poisson distribution (such that all hosts had similar tick burdens); from a negative binomial distribution (which captures tick aggregation on hosts), but independently of each other (such that larval and nymphal burdens on individual mice were independent); or from a negative binomial distribution, but arranged in such a way that the 20% of mice with the highest larval burdens also accounted for 80% of the nymphal tick population. This meant that estimates for parameters such as C_{LN} could be calculated from each of the three theoretical tick burdens from which estimates for the basic reproduction number R_0 (a threshold parameter that indicates an epidemic is possible whenever $R_0 > 1$) could subsequently be derived. Whilst this approach demonstrated the boosting effect

co-aggregation has on R_0, it ultimately only involved varying parameter values such as C_{LN}, and did not facilitate the derivation of an analytic relationship between R_0 and the level of larval and nymphal tick co-aggregation.

A useful way to visualize tick feeding behaviour is in the form of a tick-host contact network. Three studies have used this approach to investigate whether the epidemic threshold decreases asymptotically towards zero as the vertebrate host population size increases (Bisanzio et al., 2010; Ferreri et al., 2014, 2016), but none considered the effect that co-aggregation has on R_0. In this paper, the next generation matrix approach is combined with network theory to derive analytic expressions for R_0, and the equally useful—albeit less often considered—type-reproduction number T (Roberts and Heesterbeek, 2003; Heesterbeek and Roberts, 2007), as a function of larval and nymphal co-aggregation.

This paper begins by reviewing the relevant biological and ecological knowledge required to model the spread of *B. burgdorferi* and TBE virus. A mechanistic network model for tick and vertebrate host contact patterns is presented next and analytic formulae for R_0 and T are derived. Simulations of *B. burgdorferi* and TBE virus transmission on finite realizations of the contact network model are used to visualize the relationship between R_0 and the extent of larval and nymphal co-aggregation. Lastly, the implications of the results for the spread of *B. burgdorferi* and TBE virus are briefly discussed.

2. Background biology and ecology

2.1. Tick life cycle and phenology

The *Ixodes* tick life cycle comprises four life-stages: egg, larva, nymph, and adult (Fig. 2). Every year eggs hatch into larvae which then quest for vertebrate hosts (e.g. white-footed mice in the United States) to which they attach and take a blood meal from. Having engorged, a fed larval tick drops off its vertebrate host to molt, overwinter, and emerge as a nymph the following year. Whilst the seasonal questing behaviour of ticks (tick phenology— see below) varies across species and geographically, *I. scapularis* and *I. ricinus* nymphs tend to emerge and take a blood meal earlier or at the same time as larvae (Davis and Bent, 2011; Estrada-Peña et al., 2004; Craine et al., 1995). Female adult ticks take a third blood meal from larger vertebrate hosts (e.g. white-tailed deer in the United States) after which they lay eggs in the leaf litter. The eggs hatch into larvae the following year such that the tick life cycle is completed in a minimum of two years.

Tick phenology is the seasonal questing behaviour of the different life-stages of ticks. The phenology of *I. scapularis* larvae and nymphs in Northeast United States is illustrated in Fig. 3. The phenology of adults has not been shown as they are not important for Lyme disease transmission in this region (Davis and Bent, 2011). This may also be true of other regions given that adult *I. scapularis* ticks in the United States and adult *I. ricinus* ticks in Europe generally feed on larger hosts that are typically less competent in transmitting the pathogens (Davis and Bent, 2011; Milne, 1949; Randolph and Craine, 1995; Hartemink et al., 2008), although this is not always the case (Tälleklint and Jaenson, 1993; Milne, 1949).

The majority of nymphal *I. scapularis* ticks in Northeast United States quest in early spring. This is in contrast to larvae which have a bimodal questing pattern, with a small early peak in late spring and a large later peak two months later. The questing behaviour of *I. scapularis* in Northeast United States is not the same as that observed in Upper Midwest United States (where the majority of larvae feed in spring) (Davis and Bent, 2011). Nor is it the same as the questing behaviour of *I. ricinus* in Britain and Europe where tick questing behaviour varies dramatically
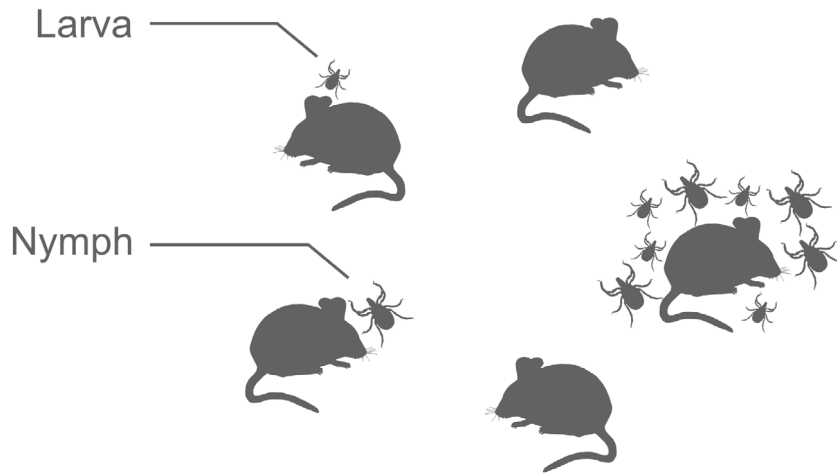
Fig. 1. Immature ticks, termed larvae and nymphs, are known to aggregate *and* co-aggregate on their vertebrate hosts, whereby the majority of both life-stages feed on the *same* minority of hosts.
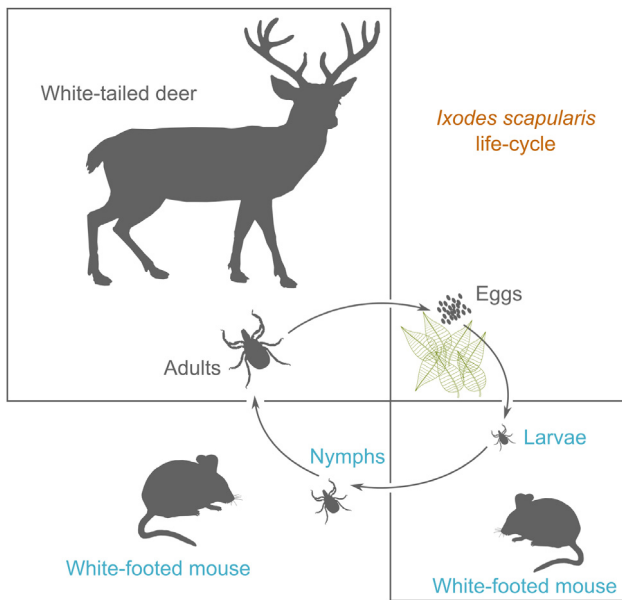

Fig. 2. The life-cycle of *Ixodes scapularis* ticks in Northeast United States. Eggs hatch into larvae which take a blood meal from vertebrate hosts, predominantly white-footed mice (*Peromyscus leucopus*). After overwintering, fed larvae molt to emerge as unfed nymphs which take a second blood meal. Later that same season, fed nymphs molt to become adults where females take a third blood meal from large vertebrates such as white-tailed deer (*Odocoileus virginianus*). Fed adult females then lay their eggs in leaf litter which become the larvae of the following season.
Source: Figure adapted from Dunn (2014).

with climate and habitat, even between localized regions within a single country. For example, the phenology of larvae and nymphs in Britain has been described as taking any one of three forms: bimodal with large early peak and small second peak, wide unimodal, or thin unimodal (Randolph and Craine, 1995; Gray, 1991; Steele and Randolph, 1985; Craine et al., 1995).

Larval and nymphal tick phenology can be described mathematically. The approach initially proposed by Brunner and Ostfeld (2008), and implemented in Davis and Bent (2011), Dunn et al. (2013) and Dunn (2014) for *I. scapularis* ticks in Northeast United States, is briefly described here. Negative binomially distributed random variables, $Z_L(t)$ and $Z_N(t)$, are defined for the number of larval and nymphal ticks respectively feeding on a vertebrate host
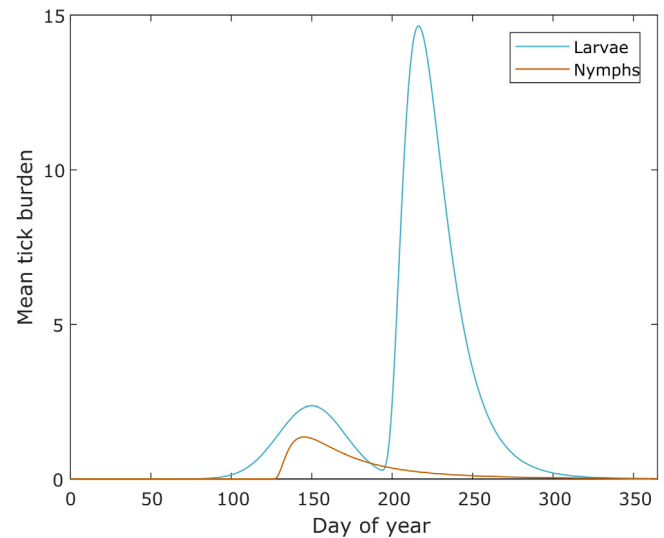

Fig. 3. Illustrative curves of the mean larval and nymphal burdens of *Ixodes scapularis* ticks on vertebrate hosts in Northeast United States. Day 0 has been assigned to 1 January. Larvae emerge in two pulses over the course of a year (blue curve), with the majority of larvae emerging in the second pulse during late spring around July. Nymphs emerge in a single pulse during early spring around May (orange curve). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

t days since the beginning of the year. The mean number of larvae and nymphs feeding on a host t days since the beginning of the year, $\bar{Z}_L(t)$ and $\bar{Z}_N(t)$ respectively, are then described by a bimodal curve comprising an early normal distribution followed by a later log-normal distribution (for larvae) and a unimodal log-normal distribution (for nymphs):

$$\bar{Z}_L(t) = \begin{cases} H_E e^{-\frac{1}{2}\left(\frac{t-\tau_E}{\mu_E}\right)^2} & \text{if } t \leq \tau_L, \\ H_E e^{-\frac{1}{2}\left(\frac{t-\tau_E}{\mu_E}\right)^2} + H_L e^{-\frac{1}{2}\left[\ln\left(\frac{t-\tau_L}{\mu_L}\right)/\sigma_L\right]^2} & \text{if } t > \tau_L, \end{cases} \tag{1}$$

$$\bar{Z}_N(t) = \begin{cases} 0 & \text{if } t \leq \tau_N, \\ H_N e^{-\frac{1}{2}\left[\ln\left(\frac{t-\tau_N}{\mu_N}\right)/\sigma_N\right]^2} & \text{if } t > \tau_N. \end{cases} \tag{2}$$

The parameters that appear in Eqs. (1) and (2) control the timing, height, and width of the phenology curves and are defined in Table 1. Estimates for each of the parameters can be obtained by

Table 1
Parameter definitions for the mathematical formulae describing *Ixodes scapularis* tick phenology in Northeast United States (Dunn et al., 2013).

Parameter	Definition
Larval phenology	
H_E	Maximum height of the early larval peak.
τ_E	Timing of the early larval peak maximum.
μ_E	Width of the early larval peak.
H_L	Maximum height of the late larval peak.
τ_L	Start of the late larval peak.
μ_L	Timing of the late larval peak maximum.
σ_L	Width of the late larval peak.
Nymphal phenology	
H_N	Maximum height of the nymphal peak.
τ_N	Start of the nymphal peak.
μ_N	Timing of the nymphal peak maximum.
σ_N	Width of the nymphal peak.

fitting the two equations to larval and nymphal tick count data using maximum likelihood techniques (Dunn, 2014).

2.2. Transmission routes

There are three ways tick-borne pathogens can be transmitted between ticks: systemically, via co-feeding, and transovarially. Systemic transmission refers to the indirect transmission of a pathogen between ticks when an infected tick takes a blood meal from a susceptible vertebrate host, a systemic infection then develops within the host, and the pathogen is transmitted to any susceptible ticks that feed on the host after a short incubation period has elapsed. For several tick-borne pathogens (including *B. burgdorferi*) systemic transmission between the immature larval and nymphal tick life-stages is the predominant route by which the pathogen spreads (Davis and Bent, 2011; Matser et al., 2009).

Co-feeding transmission refers to the horizontal (direct) transmission of a pathogen between ticks feeding on the same vertebrate host, at the same time, and in close proximity, without the involvement of a systemic infection in the host. This transmission route is particularly important for pathogens where the systemic infection of a host is cleared within a couple of days, e.g. TBE virus in Europe and some less prevalent strains of *B. burgdorferi* in the United States, but plays a smaller role in the emergence and maintenance of pathogens where host infection is lifelong, e.g. the predominant *B. burgdorferi* strains in Northeast United States (Hartemink et al., 2008; Randolph and Craine, 1995; Randolph et al., 1996; States et al., 2017).

The third possible transmission route involves an infected, engorged, adult female tick transmitting an infection to her offspring (eggs). This is referred to as transovarial or vertical transmission and may be numerically important because of the large number of eggs each adult female produces (Randolph and Craine, 1995; Hartemink et al., 2008)—consider, for example, its role in the spread of rickettsial species (Sprong et al., 2009; Moore et al., 2018; Burgdorfer and Brinton, 1975). For many other tick-borne pathogens though, especially *B. burgdorferi*, transovarial transmission is inefficient (Piesman et al., 1986; Dunn, 2014; Matuschka et al., 1998; Danielová et al., 2002; Matser et al., 2009). Consequently, this transmission route will not be considered in the network model that follows (see Section 3), and so the model and its results will only apply to those pathogens for which transovarial transmission is negligible.

3. Directed tick-host contact and transmission networks

Tick feeding behaviour can be represented as a directed, acyclic, bipartite contact network where a node represents either an immature tick or a vertebrate host, an edge represents a tick taking a blood meal from a host, and edge direction indicates the direction of *potential* pathogen transmission (Fig. 4). Each year the vertebrate host population is assumed to be replaced by a new generation of vertebrate hosts. This implies the population of hosts from which ticks feed as larvae in one season is different to the population of hosts they take a blood meal from the following season as nymphs. This is reasonable given the principal competent hosts of immature ticks are typically short-lived (≤ 12 months) small vertebrates (see Section 2.1).

In a tick-host contact network, the number of edges pointing towards and away from a vertebrate host node (i.e. its in- and out-degree) correspond to the cumulative numbers of nymphs and larvae respectively that feed on the host over its lifetime. The aggregation of nymphs is incorporated by having the in-degree of vertebrate host nodes follow a negative binomial distribution, such that a disproportionate number have high in-degree. Similarly, a negative binomially distributed out-degree for vertebrate host nodes captures the aggregation of larvae. Co-aggregation of the immature tick life-stages can then be manifested as a positive correlation, representing dependence, between the in- and out-degrees of vertebrate hosts.

A tick-borne pathogen spreading between ticks and their vertebrate hosts generates a transmission network that can be superimposed on the underlying contact network (Fig. 4). In the transmission network, nodes represent ticks or vertebrate hosts as before, but edges represent transmission events from tick-to-tick, tick-to-host, or host-to-tick depending on where they begin and end. Edges between two ticks represent co-feeding transmission, whilst edges between a vertebrate host and a tick represent systemic transmission. The inclusion of edges directly between two ticks means that, unlike contact networks, transmission networks are not bipartite. The relative importance of co-feeding transmission to tick-borne pathogen spread will vary by pathogen (Matser et al., 2009). For Lyme disease, co-feeding transmission may be more important in some geographic regions (e.g. Europe) than others (e.g. Northeast United States) (Davis and Bent, 2011).

An edge in a tick-host contact network does not indicate the time of year the associated blood meal was taken by the tick from its vertebrate host. For both systemic and co-feeding transmission, though, the timing of when ticks take a blood meal relative to one another is important. For example, if the majority of larvae and nymphs were to quest at the same time of year then co-feeding would play a larger role in the spread of tick-borne pathogens than if their questing behaviour did not overlap. To generate transmission networks from tick-host contact networks the probability an edge appears in a transmission network needs to be related to the time interval between two ticks taking their respective blood meals. This is possible if a mathematical description of tick phenology is available (see Section 2.1) and a method for doing so is described in the Appendix.

4. Deriving R_0

4.1. R_0 without co-aggregation, without co-feeding

Davis and Bent (2011) combined the next generation matrix approach and loop analysis to identify the transmission loops (repeating chains of transmission) that sustain several tick-borne pathogens in Northeast United States. Their pertinent result was that some tick-borne pathogens (including *B. burgdorferi*) rely nearly exclusively on a single transmission loop wherein larvae are infected by vertebrate hosts that were themselves infected by nymphs. Consequently, Davis and Bent (2011) proposed the
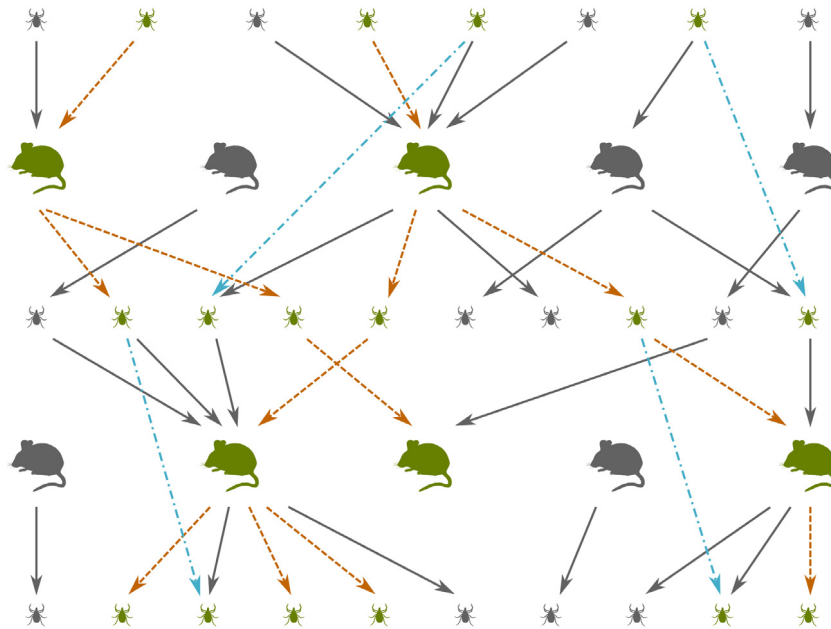
Fig. 4. A tick-borne pathogen transmission network superimposed on the underlying tick-host contact network. Each node represents either a tick or vertebrate host, with infected ticks and hosts in green. Orange (dashed) and blue (dot-dashed) edges denote tick-borne pathogen transmission: an orange edge between a tick and a host denotes systemic transmission and a blue edge between two ticks represents co-feeding transmission. A black (solid) edge between a tick and a host is a blood meal during which systemic transmission did not occur. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

following simplified next generation matrix for modelling the transmission of these pathogens

$$\mathbf{K} = \begin{bmatrix} 0 & k_{12} \\ k_{21} & 0 \end{bmatrix}, \tag{3}$$

where host type 1 is a tick infected as a larva, host type 2 is a vertebrate host infected by a nymph, and k_{ij} is the expected number of hosts of type i infected by a typical infectious host of type j in an otherwise susceptible population.

The basic reproduction number associated with Eq. (3) is the geometric mean of k_{12} and k_{21}:

$$R_0 = \sqrt{k_{12}k_{21}}. \tag{4}$$

The two non-zero k_{ij} represent systemic transmission and can be derived using epidemiological reasoning (a scenario where co-feeding transmission is non-negligible, such that $k_{11} \neq 0$, will be considered in Section 4.3). In the context of tick-host contact networks, this amounts to relating the k_{ij} to the mean in- and out-degrees of the nodes representing ticks and vertebrate hosts.

To derive k_{12}, consider an infectious vertebrate host with out-degree k_{out}. On average such a host will infect $\nu_{lh}k_{out}$ larvae, where ν_{lh} is the host-to-larva transmission probability. The underlying biology that determines the number of larvae infected by a vertebrate host is complex. For example, k_{out} will depend on the lifespan of the host, the overlap of a host's lifetime with tick questing behaviour, and the tick-host ratio. The host-to-larva transmission probability is equally complex (see Appendix). If the probability a typical infectious host has out-degree k_{out} is denoted by $P(k_{out})$, then the expected number of larvae infected by such a host is,

$$k_{12} = \sum_{k_{out}} \nu_{lh}k_{out}P(k_{out}). \tag{5}$$

There is a subtle difference between a *typical* infectious host and a host selected uniformly at random that needs to be emphasized at this point. A typical infectious host is one where the risk of the host having been infected in the first place depends on the number of nymphs that fed on it, whereas a uniformly randomly selected host is one where all hosts are at equal risk of being infected. This is equivalent to the difference between a node reached by moving along a uniformly randomly selected edge versus a node selected uniformly at random (Newman, 2010, p. 445). Here, in the context of tick-borne pathogens, this difference is captured by denoting the probability a uniformly randomly selected host has out-degree k_{out} by $p_{k_{out}}$. The equality of $P(k_{out})$ and $p_{k_{out}}$ does not hold in general, and in particular not when co-aggregation occurs (see Section 4.2). For calculating k_{12} the relevant probabilities are those for a typical infectious host.

In the absence of tick co-aggregation, though, the in- and out-degree of a vertebrate host are independent which implies $P(k_{out}) = p_{k_{out}}$. Eq. (5) can therefore be expressed as

$$k_{12} = \nu_{lh}\langle k_{out} \rangle, \tag{6}$$

where $\langle k_{out} \rangle = \sum k_{out}p_{k_{out}}$ is the mean out-degree of a uniformly randomly selected vertebrate host.

The other non-zero element of the next generation matrix, k_{21}, is equivalent to the probability a tick infected as a larva infects a vertebrate host as a nymph:

$$k_{21} = \sigma \nu_{hn}, \tag{7}$$

where σ is the probability a fed larva survives, successfully molts, and then attaches and takes a blood meal from a competent vertebrate host as a nymph the following season, and ν_{hn} is the nymph-to-host transmission probability. Substituting Eqs. (6) and (7) into Eq. (4) yields

$$R_0 = \sqrt{\sigma \nu_{lh}\nu_{hn}\langle k_{out} \rangle}, \tag{8}$$

and so R_0 is proportional to the square root of the mean lifetime larval burden.

4.2. R_0 with co-aggregation

Now consider vertebrate hosts with in-degree $k_{in} = 0$. With no infectious nymphs taking a blood meal these hosts cannot

be infected and so they cannot be typical infectious hosts. This alludes to the fact that the probability a typical infectious host has in-degree k_{in} is equal to the probability the nymph that infected it took its blood meal from a vertebrate host with in-degree k_{in}, which is

$$P(k_{in}) = \frac{k_{in} p_{k_{in}}}{\langle k_{in} \rangle}, \qquad (9)$$

where $p_{k_{in}}$ is the probability a uniformly randomly selected vertebrate host has in-degree k_{in} and $\langle k_{in} \rangle = \sum k_{in} p_{k_{in}}$ is the mean in-degree. The implication of Eq. (9) is that a typical infectious host is more likely to have high in-degree than a host that has been uniformly randomly selected. The difference between $P(k_{in})$ and $p_{k_{in}}$ is well appreciated in the complex network literature for infectious diseases (Newman, 2010; Molina and Stone, 2012; Parshani et al., 2010) and is analogous to the difference between starting an epidemic by selecting an edge versus selecting a node uniformly at random.

The co-aggregation of ticks on vertebrate hosts means that the in- and out-degree of vertebrate host nodes are positively correlated. This, together with Eq. (9), implies that a typical infectious host is more likely to have high out-degree than a uniformly randomly selected host, i.e. $P(k_{out}) \neq p_{k_{out}}$. Tick co-aggregation can be mathematically accounted for by writing

$$P(k_{out}) = \sum_{k_{in}} P(k_{out}|k_{in}) P(k_{in}), \qquad (10)$$

where $P(k_{out}|k_{in})$ is the conditional probability a vertebrate host (referring to the entire population of hosts) with in-degree k_{in} has out-degree k_{out}.

Substituting Eqs. (9) and (10) into Eq. (5) yields

$$k_{12} = \frac{\nu_{lh}}{\langle k_{in} \rangle} \sum_{k_{in}} \sum_{k_{out}} k_{out} k_{in} P(k_{out}|k_{in}) p_{k_{in}}. \qquad (11)$$

This expression can be simplified because the product of $P(k_{out}|k_{in})$ and $p_{k_{in}}$ is equivalent to the joint probability distribution that a uniformly randomly selected vertebrate host has in-degree k_{in} and out-degree k_{out}, denoted $p_{k_{in}, k_{out}}$. Specifically,

$$
\begin{aligned}
k_{12} &= \frac{\nu_{lh}}{\langle k_{in} \rangle} \sum_{k_{in}} \sum_{k_{out}} k_{out} k_{in} p_{k_{in}, k_{out}} \\
&= \nu_{lh} \frac{\langle k_{in} k_{out} \rangle}{\langle k_{in} \rangle},
\end{aligned} \qquad (12)
$$

where $\langle k_{in} k_{out} \rangle$ is the mean of the product of the in- and out-degree of vertebrate hosts.

Because tick co-aggregation does not affect k_{21} it follows from Eq. (4) that

$$R_0 = \sqrt{\sigma \nu_{lh} \nu_{hn} \frac{\langle k_{in} k_{out} \rangle}{\langle k_{in} \rangle}}. \qquad (13)$$

The increase in $\langle k_{in} k_{out} \rangle$ with co-aggregation, relative to its value when there is no co-aggregation, is a measure of the in- and out-degree correlation of vertebrate hosts (although not a formal measure such as the Pearson correlation co-efficient or the covariance) (Shtilerman and Stone, 2015). This can be illustrated with a simple example involving the larval and nymphal burdens of two hypothetical mice. Suppose the first mouse is host to 5 larvae and 1 nymph whereas the second mouse is host to 1 larva and 5 nymphs. In this scenario the product of the larval and nymphal burden of each mouse is 5 such that $\langle k_{in} k_{out} \rangle$, the mean of these two products, is also equal to 5. If instead the tick burdens on these two mice are correlated, such that the mouse with the highest larval burden is also the mouse with the highest nymphal burden, then the product of the larval and nymphal burden is 25 for the one mouse and 1 for the other, which implies $\langle k_{in} k_{out} \rangle = 13$. Although a simple example, the effect of tick

co-aggregation is clear: it increases the mean product of the lifetime larval and nymphal burdens on vertebrate hosts, and in so doing increases R_0 as well.

4.3. R_0 with co-aggregation and co-feeding

In the next generation matrix proposed by Davis and Bent (2011), i.e. Eq. (3), co-feeding transmission was explicitly ignored (since $k_{11} = 0$). This next generation matrix, though, was based on the analysis of tick-borne pathogens (including *B. burgdorferi*) spreading in Northeast United States. In parts of Britain and Europe, the contribution of co-feeding transmission to the spread of Lyme disease is understood to be important (Voordouw, 2015)—see also Nonaka et al. (2010) and Belli et al. (2017). Furthermore, States et al. (2017) have shown co-feeding transmission of *B. burgdorferi* strains—which would otherwise be rapidly cleared by the immune response of the predominant host in Northeast United States, namely *P. leucopus*—facilitates co-existence of multiple strains. This could be especially important for regions where synchronous questing behaviour of immature *I. scapularis* ticks occurs (e.g. Upper Midwest United States). Lastly, for pathogens not considered by Davis and Bent (2011), e.g. TBE virus in Europe, co-feeding transmission is known to play a critical role in the spread of the infection (Hartemink et al., 2008). Thus to incorporate co-feeding transmission would render the next generation matrix model applicable to a greater range of geographic regions and tick-borne pathogens.

For tick-host networks that incorporate both tick co-aggregation and co-feeding transmission the corresponding next generation matrix is given by

$$\mathbf{K} = \begin{bmatrix} k_{11} & k_{12} \\ k_{21} & 0 \end{bmatrix}, \qquad (14)$$

where the subscripts have the same interpretation as before, and the basic reproduction number is

$$R_0 = \frac{1}{2} \left(k_{11} + \sqrt{k_{11}^2 + 4 k_{12} k_{21}} \right). \qquad (15)$$

The inclusion of co-feeding transmission has no effect on the formulae for k_{12} and k_{21}. The additional non-zero next generation matrix element, k_{11}, is the expected number of larvae infected by a tick that was itself infected whilst feeding as a larva. A tick infected whilst feeding as a larva can only transmit the infection if it survives to take a second blood meal from a vertebrate host the following season as a nymph. This occurs with probability σ. When an infected nymph takes a blood meal from a vertebrate host with out-degree k_{out} it will infect $\nu_{ln} k_{out}$ larvae on average, where ν_{ln} is the nymph-to-larva co-feeding transmission probability. From this it follows that

$$k_{11} = \sigma \sum_{k_{out}} \nu_{ln} k_{out} P(k_{out}), \qquad (16)$$

where $P(k_{out})$ is the probability a nymph that takes a blood meal does so from a vertebrate host with out-degree k_{out}.

As in Section 4.2, tick co-aggregation means the in- and out-degree (of vertebrate hosts) are correlated. As before, Eqs. (9) and (10) are substituted into Eq. (16) to account for this which yields

$$k_{11} = \sigma \nu_{ln} \frac{\langle k_{in} k_{out} \rangle}{\langle k_{in} \rangle}, \qquad (17)$$

such that

$$R_0 = \frac{1}{2} \left(\sigma \nu_{ln} \frac{\langle k_{in} k_{out} \rangle}{\langle k_{in} \rangle} + \sqrt{\left(\sigma \nu_{ln} \frac{\langle k_{in} k_{out} \rangle}{\langle k_{in} \rangle} \right)^2 + 4 \sigma \nu_{lh} \nu_{hn} \frac{\langle k_{in} k_{out} \rangle}{\langle k_{in} \rangle}} \right). \qquad (18)$$
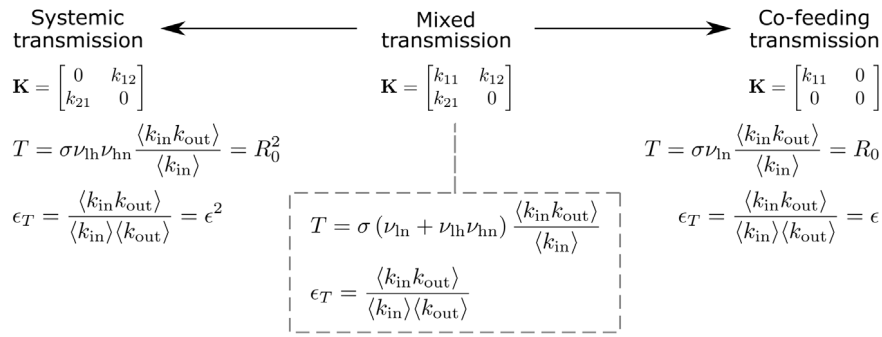
Systemic transmission	Mixed transmission	Co-feeding transmission

$$\mathbf{K} = \begin{bmatrix} 0 & k_{12} \\ k_{21} & 0 \end{bmatrix}$$

$$\mathbf{K} = \begin{bmatrix} k_{11} & k_{12} \\ k_{21} & 0 \end{bmatrix}$$

$$\mathbf{K} = \begin{bmatrix} k_{11} & 0 \\ 0 & 0 \end{bmatrix}$$

$$T = \sigma \nu_{\text{lh}} \nu_{\text{hn}} \frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle} = R_0^2$$

$$\epsilon_T = \frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle} = \epsilon^2$$

$$T = \sigma \left(\nu_{\text{ln}} + \nu_{\text{lh}} \nu_{\text{hn}} \right) \frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle}$$

$$\epsilon_T = \frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle}$$

$$T = \sigma \nu_{\text{ln}} \frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle} = R_0$$

$$\epsilon_T = \frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle} = \epsilon$$

Fig. 5. A spectrum of next generation matrix models, **K**, and their predictions regarding the relationship between the tick type-reproduction number, T, and the level of nymphal and larval co-aggregation, for tick-borne pathogens transmitted from nymphal to larval ticks feeding on the same host, either indirectly through systemic transmission, directly via co-feeding transmission, or a mixture of the two. The relationship between T and R_0 is illustrated for the two extreme cases when only systemic transmission occurs and when only co-feeding transmission occurs.

From Eq. (18) it is straight-forward to see that in the absence of systemic transmission (i.e. when $\nu_{\text{lh}} \nu_{\text{hn}} = 0$) R_0 simplifies to just k_{11}.

4.4. Relative effect

A useful way to quantify the relative effect of co-aggregation is to take the ratio of R_0 when co-aggregation is present to when co-aggregation is absent. To do this a formula for R_0 is required for when tick co-aggregation does not occur but where co-feeding transmission does. This will be denoted $R_{0,\text{nca}}$. Using arguments similar to those presented in Sections 4.1–4.3 it is not hard to show that

$$R_{0,\text{nca}} = \frac{1}{2} \left(\sigma \nu_{\text{ln}} \langle k_{\text{out}} \rangle + \sqrt{(\sigma \nu_{\text{ln}} \langle k_{\text{out}} \rangle)^2 + 4 \sigma \nu_{\text{lh}} \nu_{\text{hn}} \langle k_{\text{out}} \rangle} \right). \quad (19)$$

The relative effect of tick co-aggregation on R_0 is then obtained by dividing Eq. (18) by Eq. (19) to obtain

$$\epsilon = \frac{R_0}{R_{0,\text{nca}}}$$

$$= \frac{\sigma \nu_{\text{ln}} \langle k_{\text{in}} k_{\text{out}} \rangle + \sqrt{(\sigma \nu_{\text{ln}} \langle k_{\text{in}} k_{\text{out}} \rangle)^2 + 4 \sigma \nu_{\text{lh}} \nu_{\text{hn}} \langle k_{\text{in}} \rangle \langle k_{\text{in}} k_{\text{out}} \rangle}}{\sigma \nu_{\text{ln}} \langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle + \sqrt{(\sigma \nu_{\text{ln}} \langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle)^2 + 4 \sigma \nu_{\text{lh}} \nu_{\text{hn}} \langle k_{\text{in}} \rangle^2 \langle k_{\text{out}} \rangle}}. \quad (20)$$

If ticks co-aggregate on vertebrate hosts then $\epsilon > 1$. When ticks fail to co-aggregate, the independence of the in- and out-degree of vertebrate hosts implies $\langle k_{\text{in}} k_{\text{out}} \rangle = \langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle$ such that $\epsilon = 1$. In the unlikely event the majority of larvae feed on a different subset of hosts to the majority of nymphs, such that the in- and out-degree of vertebrate hosts are negatively correlated, then $0 < \epsilon < 1$. If co-feeding transmission is negligible (i.e. when $\nu_{\text{ln}} = 0$) the relative effect of tick co-aggregation simplifies to

$$\epsilon = \sqrt{\frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle}}. \quad (21)$$

The equivalent expression for tick-borne pathogens where co-feeding transmission is the predominant route of transmission (i.e. when $\nu_{\text{lh}} \nu_{\text{hn}} = 0$) is

$$\epsilon = \frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle}. \quad (22)$$

4.5. Type-reproduction number, T

Whilst R_0 is the more commonly investigated epidemic threshold parameter, the type-reproduction number T provides a more accurate estimate of the effort required to prevent or control an outbreak when an intervention is directed towards only a subset of the host types (Roberts and Heesterbeek, 2003; Heesterbeek and Roberts, 2007). For the tick-host networks considered here, the type-reproduction number T for a tick infected whilst feeding as a larva, is defined as the expected number of larvae infected by such a tick in an otherwise susceptible population, either directly via co-feeding transmission or indirectly through systemic transmission:

$$T = k_{11} + k_{12} k_{21}$$

$$= \sigma \left(\nu_{\text{ln}} + \nu_{\text{lh}} \nu_{\text{hn}} \right) \frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle}. \quad (23)$$

This reduces to $T = k_{12} k_{21} = R_0^2$ (see Section 4.2) when co-feeding transmission is negligible and $T = k_{11} = R_0$ (see Section 4.3) when systemic transmission is negligible. Thus, irrespective of the transmission route (systemic, co-feeding, or both), the type-reproduction number is always proportional to $\langle k_{\text{in}} k_{\text{out}} \rangle$, the mean product of the lifetime nymphal and larval burdens of vertebrate hosts. This is also true of the relative effect of co-aggregation on T:

$$\epsilon_T = \frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle}. \quad (24)$$

These results are summarized in Fig. 5.

5. Simulating R_0

To investigate the relationship between R_0 and the level of aggregation and co-aggregation in tick-host contact networks the transmission of *B. burgdorferi* and TBE virus was simulated on mechanistic tick-mouse contact networks.

5.1. Tick-mouse contact networks

Directed, acyclic, bipartite tick-mouse contact networks, similar to the one shown in Fig. 4, were constructed as follows. First, the number of seasons, $s = 2$; the number of mice per season, $M = 200$; and the mean number of larval ticks per mouse each season, $\langle k_{\text{out}} \rangle$, were set (see Table 2). To ensure both seasons had nymphal ticks, the number of nodes representing *potential* nymphs in the first season was set equal to the number of larvae (note, however, that in the first season of Fig. 4 only those nodes representing ticks that successfully took a blood meal as nymphs are shown). The values of s, M, and $\langle k_{\text{out}} \rangle$ therefore determined the number of nodes in a network.

Edges representing ticks taking blood meals from mice were generated one season at a time. An 'attractiveness' score, a_m, generated from a negative binomial distribution with mean value

Table 2

Tick-mouse contact and tick-borne pathogen transmission network parameters. Literature sources for parameter point estimates/ranges and methods used for parameter estimation:[1]Randolph and Craine (1995),[2]Dunn et al. (2013),[3]Dunn (2014),[4]unpublished data,[5]Davis and Bent (2011),[6]Hartemink et al. (2008),[7]Piesman et al. (1987),[8]Nazario et al. (1998),[9]Gern and Rais (1996),[10]Richter et al. (2002),[11]Labuda et al. (1993),[12]Labuda et al. (1997), and[13]Randolph et al. (1996).

Parameter, Symbol	Point estimate/Range (step size)	Source/Method
Tick-mouse contact networks		
No. of seasons, s	2	Arbitrarily chosen
No. of mice per season, M	200	Arbitrarily chosen
Mean lifetime larval tick burden per mouse, $\langle k_{out} \rangle$	100–300 (200)	Ref. 1–4, Eq. (A.1)
Aggregation parameter, α	0.2–4.7 (0.5)	Ref. 3, 4
Target rank correlation coefficient, ρ_{target}	0.0–0.4 (0.05)	Ref. 4
Acceptable error bound, δ	0.01	Arbitrarily chosen
Probability a fed larva feeds as nymph[a], σ	0.10	Ref. 1
No. of contact networks[b]	50	Arbitrarily chosen
Tick-borne pathogen transmission networks		
No. of transmission networks[c]	1000	Arbitrarily chosen
Nymph-to-mouse transmission probability, v_{hn}	0.83	Ref. 5, 6
Borrelia burgdorferi		
Nymph-to-larva transmission probability, v_{ln}	2.4×10^{-3}	Ref. 1–4, 7–10, Eq. (A.9)
Mouse-to-larva transmission probability, v_{lh}	7.3×10^{-2}	Ref. 1–5, 7, 8, Eq. (A.7)
Tick-borne encephalitis virus		
Nymph-to-larva transmission probability, v_{ln}	2.0×10^{-2}	Ref. 1–4, 7, 8, 10–12, Eq. (A.9)
Mouse-to-larva transmission probability, v_{lh}	1.9×10^{-3}	Ref. 1–4, 7, 8, 13, Eq. (A.7)

[a]Probability a fed larva survives and feeds as a nymph from a mouse the following season.
[b]Per aggregation and co-aggregation parameter combination.
[c]Per contact network.

$\langle k_{out} \rangle$ and aggregation parameter α, was assigned to each mouse, $m \in \{1, 2, 3, \ldots, M\}$. These scores were then converted into in-degree probability weights, $p_m = a_m/\sum_i a_i$. The process was repeated (using the same mean and aggregation parameter values) such that each mouse was also assigned an out-degree probability weight as a measure of its 'attractiveness' to larval ticks. Because the in- and out-degree probability weights were generated from negative binomial distributions they captured the required level of nymphal and larval tick aggregation on mice respectively.

To ensure the desired level of co-aggregation, ρ_{target} (see below), Spearman's rank correlation coefficient, ρ, for the in- and out-degree probability weights of the mice was calculated. If ρ was not within some acceptable error bound, δ, of ρ_{target}, then the out-degree probability weights of two uniformly randomly chosen mice were swapped. If this reduced the absolute difference between ρ and ρ_{target} the change was accepted, otherwise it was rejected 99% of the time. This out-degree probability weight swapping procedure was continued until either $|\rho - \rho_{target}| \leq \delta$ or 100,000 iterations had been performed, whichever came first.[1]

To generate edges between nymphs and mice, the in-degree probability weights of the mice were converted to cumulative in-degree probability weights, $P_m = \sum_{i \leq m} p_i$. A Bernoulli experiment was then conducted for each fed larval tick from the previous season to determine which of them survived to take a blood meal as a nymph in the current season (which occurred with probability σ). For each tick deemed to have taken a blood meal as a nymph, a uniformly distributed random number, r, between 0 and 1 was generated to determine which mouse it took a blood meal from; the mouse, m, was determined as the one with cumulative in-degree probability weight satisfying $P_m \geq r > P_{m-1}$. Having determined the mouse, a directed edge from the nymph to the mouse was generated by setting the corresponding

element of the network adjacency matrix equal to 1. The same process was used to generate edges from mice to larvae, the only difference being that all larvae took a blood meal from a mouse (since those that fail to do so play no role in the transmission of Lyme disease (Davis and Bent, 2011) and consequently need not be included in the network). To complete the tick-host network, the process of generating edges between ticks and mice was repeated for the second season.

Three parameters were varied whilst generating tick-mouse contact networks, namely $\langle k_{out} \rangle$, α, and ρ_{target} (see Table 2). The mean number of larvae per mouse each season, $\langle k_{out} \rangle$, was assigned the values of 100 or 300 to allow investigation into the relationship between R_0 and the mean lifetime larval burden of mice. A total of 50 tick-mouse contact networks were generated for every pair of values for the aggregation and co-aggregation parameters. The aggregation parameter, α, was varied from 0.2 to 4.7 in step sizes of 0.5, whilst the target level of co-aggregation, ρ_{target}, was varied from 0.0 to 0.4 in step sizes of 0.05. Consequently, 4500 tick-host contact networks were generated for each value of $\langle k_{out} \rangle$.

5.2. Tick-borne pathogen transmission networks

To calculate R_0 using Eq. (18) values for k_{11}, k_{12}, and k_{21} are required. These were obtained by counting transmission events of the three types on simulated transmission networks. For each tick-mouse contact network a total of 2000 transmission simulations were conducted: 1000 to determine k_{11} and k_{21}, and a further 1000 to determine k_{12}. Thus, in total, 50,000 transmission network realizations were used to calculate values for k_{11}, k_{12}, and k_{21} for every combination of $\langle k_{out} \rangle$, α, and ρ_{target}.

For k_{11} and k_{21}, a typical infectious tick was selected by uniformly randomly selecting from among the nodes representing potential nymphs of both seasons (i.e. fed larvae the season prior) in the tick-mouse contact network. Next, the index tick was infected and the number of larvae and mice subsequently infected by it (with probability v_{ln} and v_{hn} respectively—see Table 2) recorded. The number of larvae and mice infected by the index tick was conditional on whether it took a second blood as a nymph from a host; if it failed to do so (which occurred with

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[1] Whether or not ρ_{target} is achievable depends on the number of mice each season and the value of the aggregation parameter, α, for the negative binomial distribution used to generate tick 'attractiveness' scores for mice. In general, the more mice there are each season the more likely ρ_{target} can be achieved for a given value of α, although more mice may also mean more iterations are required for convergence.
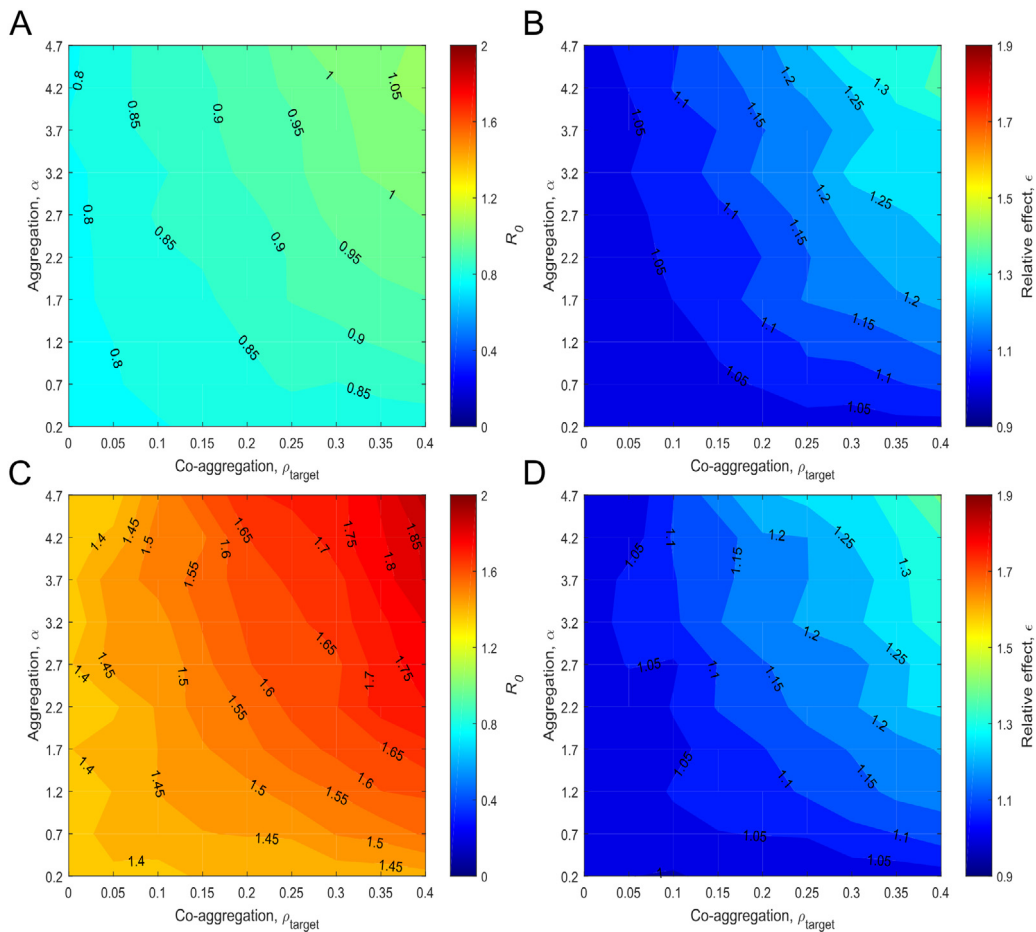
Fig. 6. The basic reproduction number R_0 (Panels A and C) and relative effect parameter ϵ (Panels B and D) for *Borrelia burgdorferi* (the causative agent of Lyme disease) as a function of larval and nymphal tick aggregation and co-aggregation, α and ρ_{target} respectively, for mean larval burdens of 100 (Panels A and B) and 300 (Panels C and D).

probability σ—see Section 5.1) then the number of larvae and mice infected by the index tick was recorded as zero. Values for k_{11} and k_{21} were obtained by calculating the average number of larvae and the average number of mice infected by the index tick over all simulations respectively.

For k_{12}, a typical infectious mouse was selected by uniformly randomly selecting an edge representing a nymph taking a blood meal in either of the two seasons, and then moving along the edge to the mouse from which it was taken. Doing so ensured the index mouse had in-degree k_{in} with probability $k_{\text{in}}p_{k_{\text{in}}}/\langle k_{\text{in}}\rangle$, whereas a mouse selected directly would have had in-degree k_{in} with probability $p_{k_{\text{in}}}$. The index mouse was infected and subsequently allowed to infect (with probability ν_{lh}—see Table 2) any ticks that took a blood meal from it as larvae. The value of k_{12} was calculated as the average number of larvae infected by the index mouse over all simulations.

Point estimates for transmission probabilities ν_{lh} and ν_{ln} were calculated for *B. burgdorferi* using Eqs. (A.7) and (A.9) respectively in the Appendix. As the tick phenology curves used to calculate these probabilities were for the questing behaviour of *I. scapularis* ticks in Northeast United States, the simulation results generated by the network model are specific to Lyme disease spread in this geographic region and may not apply to others where tick phenology is significantly different, e.g. Upper Midwest United States and Europe. The corresponding transmission probabilities for TBE virus were estimated in a similar manner. However, because the phenology of *I. ricinus* ticks in Europe can be significantly different to the phenology of *I. scapularis* ticks in Northeast United

States (see Section 2.1), the values of ν_{lh} and ν_{ln} for TBE virus should not be taken literally but rather as illustrative values for a pathogen transmitted predominantly via co-feeding.

5.3. Visualizing R_0

Fig. 6 shows the basic reproduction number R_0 (Panels A and C) and relative effect parameter ϵ (Panels B and D) for *B. burgdorferi* as a function of the aggregation and co-aggregation parameters, α and ρ_{target} respectively, for mean larval burdens of 100 (Panels A and B) and 300 (Panels C and D). Fig. 7 shows the equivalent results for TBE virus.

For a given mean larval burden, R_0 is higher for *B. burgdorferi* than it is for TBE virus. This is reasonable given that systemic transmission of *B. burgdorferi* was nearly four times more efficient than co-feeding transmission of TBE virus (see Table 2). For both pathogens though, more extreme tick co-aggregation always leads to greater values for R_0, whereas higher levels of tick aggregation only increases the value of R_0 when larvae and nymphs also co-aggregate. In addition, aggregation and co-aggregation have a synergistic effect on R_0 such that their combined effect is greater than the sum of their individual effects.

As predicted by Eq. (18), increasing the mean larval burden by a factor θ increased R_0 by a factor that lies between $\sqrt{\theta}$ and θ, the precise value being determined by the relative contributions of systemic and co-feeding transmission. Specifically, for *B. burgdorferi*, which spreads predominantly via systemic transmission, tripling the mean larval burden from 100 to 300 increased
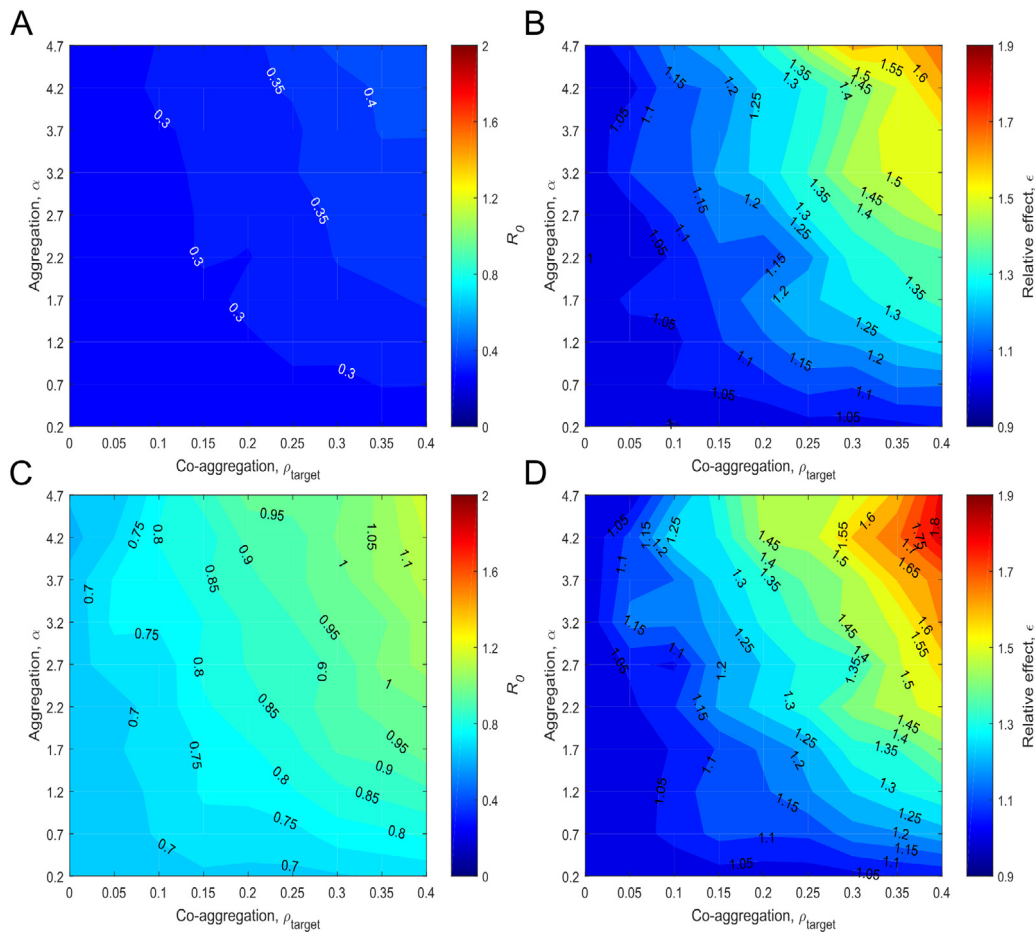
Fig. 7. The basic reproduction number R_0 (Panels A and C) and relative effect parameter ϵ (Panels B and D) for tick-borne encephalitis virus as a function of larval and nymphal tick aggregation and co-aggregation, α and ρ_{target} respectively, for mean larval burdens of 100 (Panels A and B) and 300 (Panels C and D).

R_0 by a factor close to $\sqrt{3}$ (cf. Panels A and C in Fig. 6). In contrast, for TBE virus, where the relative contribution of co-feeding transmission is much greater (see Table 2), R_0 increased by a factor closer to 3 (cf. Panels A and C in Fig. 7). These results correspond with the observation that $T = R_0^2$ when only systemic transmission occurs, $T = R_0$ when only co-feeding transmission occurs, and that T is always proportional to the mean lifetime larval burden irrespective of transmission route (see Eq. (23)).

Panels B and D in Fig. 6 reveal that, in contrast to R_0, the relative effect parameter ϵ for *B. burgdorferi* was independent of mean larval burden, a result that is in agreement with Eq. (21). For TBE virus, a more even mix of systemic and co-feeding transmission than Lyme disease (see Table 2) meant that ϵ was slightly higher for higher mean larval burdens (cf. Panels B and D in Fig. 7). On the whole though, these trends mean that, for both pathogens, co-aggregation of larvae and nymphs has a greater *absolute* effect on R_0 when the mean larval burden is high. This is confirmed by the greater number of contour lines (and hence wider range of colours) used to plot Panel C compared to Panel A in each of Figs. 6 and 7.

Lastly, for a given mean larval burden, the relative effect parameter ϵ was greater for TBE virus than *B. burgdorferi* (cf. Panel B, or Panel D, between Figs. 6 and 7). This agrees with Eqs. (21) and (22) which predict that co-aggregation will have a larger *relative* effect on R_0 for pathogens that spread predominantly via co-feeding transmission compared with those that spread mostly via the systemic infection of vertebrate hosts. It is also consistent

with the fact that the relative effect of co-aggregation on T is independent of transmission route (see Eq. (24)).

6. Discussion

By presenting tick feeding behaviour as a contact network and recognizing that co-aggregation is mathematically equivalent to the dependence of vertebrate host node out-degree on in-degree, simple equations for the dependence of R_0 and T for tick-borne pathogens on the levels of tick aggregation and co-aggregation, as well as coincident co-aggregation (when pathogens are transmitted via co-feeding), have been derived. Eq. (13) describes how R_0 is affected by the interaction between aggregation and co-aggregation and may be written as

$$R_0 = c_1 \sqrt{\frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle}} \tag{25}$$

so that R_0^2 is proportional to the mean product of lifetime larval and nymphal burdens, scaled by the mean lifetime nymphal burden. When the spread of a pathogen is dominated by the co-feeding route of transmission then Eq. (18) simplifies to

$$R_0 = c_2 \frac{\langle k_{\text{in}} k_{\text{out}} \rangle}{\langle k_{\text{in}} \rangle}, \tag{26}$$

which states that R_0, rather than R_0^2, is proportional to the mean product of lifetime larval and nymphal burdens, scaled by the mean lifetime nymphal burden. In epidemiological terms, "the

mean product of lifetime larval and nymphal burdens, scaled by the mean lifetime nymphal burden" is actually the mean lifetime larval burden of a typical infectious host. The biological interpretation of this term is that the stronger the correlation between larval burden and nymphal burden the greater the difference between a typical infectious host and one selected uniformly at random.

The difference between Eqs. (25) and (26) implies that co-aggregation will have a larger relative effect on the magnitude of R_0 for pathogens such as TBE virus in Europe that are sustained by co-feeding transmission (Randolph et al., 1996; Hartemink et al., 2008) than it will for pathogens that rely on systemic infections such as *B. burgdorferi* (Davis and Bent, 2011; Hartemink et al., 2008). This difference arises from the way R_0 is calculated for the two extremes of only co-feeding transmission (a one-step transmission system) and only systemic transmission (a two-step transmission system). Indeed, the relative effect of co-aggregation on the tick type-reproduction number T is always equal to $\langle k_{\text{in}} k_{\text{out}} \rangle / \langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle$ regardless of the transmission route (systemic, co-feeding, or both).

The formulae for R_0 and T (Eqs. (8), (13), (18), and (23)) all depend explicitly on the probability a fed larval tick survives through winter and successfully takes a blood meal as a nymph the following season, σ. In contrast, vertebrate host mortality does not appear explicitly in any of the formulae. This is because R_0 was formalized as depending directly on the lifetime larval and nymphal burdens on vertebrate hosts. Assuming vertebrate hosts live for up to one year was a clear overestimation. A more realistic lifespan on the order of months, and more generally increases in vertebrate host mortality (host population turnover), would reduce lifetime tick burdens and consequently R_0 as well. The relationship between tick burden and host mortality has not been explicitly derived but is unlikely to be a linear relationship as high host mortality may even prevent larvae from feeding on the same hosts as those which nymphs fed on earlier in the year.

In addition to the derived analytic equations for R_0, simulations of *B. burgdorferi* and TBE virus transmission on mechanistic tick-mouse contact networks were used to visualize the relationship between R_0 and the level of tick aggregation and co-aggregation. The simulation results revealed that co-feeding transmission makes minimal difference to the value of R_0 for *B. burgdorferi* in Northeast United States (not shown). This is consistent with that which has previously been reported in the literature (Davis and Bent, 2011). Furthermore, it is largely due to the small co-feeding transmission probability ν_{ln} relative to the systemic transmission probabilities ν_{hn} and ν_{lh} (see Table 2), which is a consequence of larval and nymphal ticks questing at different times of the year in this geographic region (see Fig. 3). For other regions, e.g. Upper Midwest United States, where there is a significantly greater overlap in the questing behaviour of larval and nymphal ticks (Davis and Bent, 2011), one would expect ν_{ln} to be higher, ν_{lh} to be lower, and the effect of co-feeding transmission on R_0 to consequently be greater (assuming all other parameter values remain unchanged) as shown in States et al. (2017). Similarly, for TBE virus in Europe, where the fast clearance of systemic infections in vertebrate hosts within a couple of days (Randolph et al., 1996) would render ν_{lh} relatively small, one would expect the value of R_0 to be significantly raised by the contribution of co-feeding transmission compared to if only systemic transmission occurred.

A caveat of the simulation results is that they are predicated on two assumptions, namely that the lifetime larval and nymphal burdens on hosts both follow a negative binomial distribution and that there is a monotonic relationship (representing dependence) between the counts of larvae and nymphs on individual hosts. Whilst these two assumptions are reasonable in light of the

trends typically observed for vertebrate host larval and nymphal burdens obtained from the field (Brunner and Ostfeld, 2008; Randolph et al., 1999; Craine et al., 1995), should they not apply, as has been suggested by some studies (Bisanzio et al., 2010; Ferreri et al., 2014, 2016), then the visualized relationship between R_0 and the level of tick aggregation and co-aggregation in Figs. 6 and 7 may no longer hold. Importantly, this would not render the derived analytic relationship in Eq. (18) inaccurate since this is a more general result that—whilst capturing both tick aggregation and co-aggregation—does not make any assumptions about the larval and nymphal distributions on vertebrate hosts or the relationship between them.

This paper is the first to demonstrate that co-aggregation has an effect on systemic transmission of tick-borne pathogens, not only co-feeding transmission. Whilst next generation matrix models typically incorporate co-aggregation through the parameterization of their elements relating to co-feeding transmission, they do not for the elements relating to systemic transmission (Hartemink et al., 2008; Matser et al., 2009; Davis and Bent, 2011). This is made particularly evident by the work of Harrison and Bennett (2012) where only the mean numbers of ticks infected whilst co-feeding were varied as a function of co-aggregation and not the mean numbers of ticks infected via systemic infection of the host. The novel contribution of this work can best be observed by substituting Eqs. (A.1) and (A.6) into Eq. (12) and comparing the result to the equation for next generation matrix element k_{26} from Davis and Bent (2011). The comparison reveals that the effect of co-aggregation is to raise the expected number of larvae infected by an infectious host by a factor $\langle k_{\text{in}} k_{\text{out}} \rangle / \langle k_{\text{in}} \rangle$, precisely the same factor by which the mean lifetime larval burden of a typical infectious host is greater than that of a uniformly randomly selected vertebrate host.

In conclusion, the co-aggregation of larval and nymphal ticks on vertebrate hosts raises the value of R_0 and T for tick-borne pathogens such as *B. burgdorferi* and TBE virus. The absolute increase will be greatest in geographic regions and seasons in which mean lifetime larval burden is high, whilst the relative increase in R_0 will be greater for tick-borne pathogens transmitted predominantly between co-feeding ticks, e.g. TBE virus, compared with those sustained by systemic transmission, e.g. *B. burgdorferi*. For both tick-borne pathogens, though, the effect of co-aggregation is to increase the mean number of ticks infected per infectious tick, T, and so too the likelihood of pathogen persistence.

Declaration of competing interest

Acknowledgements

Appendix. Relating tick-borne pathogen transmission probabilities to tick phenology

To generate tick-borne pathogen transmission networks from tick-host contact networks the probability an edge appears in a

transmission network needs to be related to the time interval between an infectious nymph and a susceptible larva taking their respective blood meals from the same vertebrate host. A method for doing so that makes use of mathematical descriptions of larval and nymphal tick phenology is presented here.

Recall from Eqs. (1) and (2) in Section 2.1 that the phenology of larval and nymphal ticks can be described mathematically, with $\bar{Z}_L(t)$ and $\bar{Z}_N(t)$ denoting the mean number of larvae and nymphs respectively that feed on a single host t days since the start of the year. If the average number of days larvae and nymphs remain attached to hosts whilst taking a blood meal are denoted d_L and d_N respectively, then under the gross assumption that vertebrate hosts live for a year, the mean number of *unique* larvae and nymphs that feed on a single host over its lifetime are given by

$$\langle k_{\text{out}} \rangle = \int_{\tau=0}^{365} \frac{\bar{Z}_L(\tau)}{d_L} \, d\tau \tag{A.1}$$

and

$$\langle k_{\text{in}} \rangle = \int_{t=0}^{365} \frac{\bar{Z}_N(t)}{d_N} \, dt \tag{A.2}$$

respectively.

Given that a vertebrate host is infected on day t, it follows from Eq. (A.1) that the probability any larval tick that feeds on this host does so after day t is equal to

$$\frac{\int_{\tau=t}^{365} \bar{Z}_L(\tau) \, d\tau}{\int_{\tau=0}^{365} \bar{Z}_L(\tau) \, d\tau}. \tag{A.3}$$

The unconditional probability a larval tick feeds on a vertebrate host after the host has been infected by a nymph is obtained by integrating out the dependence on t as follows:

$$\int_{t=0}^{365} a_N(t) \frac{\int_{\tau=t}^{365} \bar{Z}_L(\tau) \, d\tau}{\int_{\tau=0}^{365} \bar{Z}_L(\tau) \, d\tau} \, dt, \tag{A.4}$$

where the probability density function $a_N(t)$ is a measure of the risk that a vertebrate host will be infected on day t. Formally, this function is defined as

$$a_N(t) = \frac{\bar{Z}_N(t)}{\int_{t=0}^{365} \bar{Z}_N(t) \, dt}. \tag{A.5}$$

Vertebrate host-to-larva transmission probability, v_{lh}

The host-to-larva transmission probability v_{lh} is conditional on a larval tick having taken a blood meal from an infectious vertebrate host. A blood meal alone, though, is not the only condition required for transmission to be possible. The larval tick must also take its blood meal after a systemic infection has developed in the host (i.e. after the latent period t_L has elapsed) and before the infectious period t_I comes to an end (if the infection is not lifelong). Taking these considerations into account the host-to-larva transmission probability is given by

$$v_{\text{lh}} = v_{\text{lh}}^* \int_{t=0}^{365} a_N(t) \frac{\int_{\tau=t+t_L}^{t+t_L+t_I} \bar{Z}_L(\tau) \, d\tau}{\int_{\tau=0}^{365} \bar{Z}_L(\tau) \, d\tau} \, dt, \tag{A.6}$$

where v_{lh}^* is the average probability a vertebrate host infects a larval tick given that it takes a blood meal during the vertebrate host's infectious period. Substituting Eqs. (A.1), (A.2), and (A.5) into Eq. (A.6) means the host-to-larva transmission probability can also be written as

$$v_{\text{lh}} = \frac{v_{\text{lh}}^*}{d_L d_N \langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle} \int_{t=0}^{365} \bar{Z}_N(t) \int_{\tau=t+t_L}^{t+t_L+t_I} \bar{Z}_L(\tau) \, d\tau \, dt. \tag{A.7}$$

Nymph-to-larva transmission probability, v_{ln}

Similar to systemic transmission, a larval tick taking a blood meal from the same vertebrate host as an infectious nymph is not all that is required for co-feeding transmission to occur. For nymph-to-larva transmission there are two additional conditions: the larval tick must feed at the same time as the infectious nymph and it must also feed in close proximity to the nymph. If co-feeding transmission is assumed to occur only whilst the infectious nymph is feeding, then the nymph-to-larva transmission probability is given by

$$v_{\text{ln}} = c \, v_{\text{ln}}^* \int_{t=0}^{365} a_N(t) \frac{\int_{\tau=t}^{t+d_N} \bar{Z}_L(\tau) \, d\tau}{\int_{\tau=0}^{365} \bar{Z}_L(\tau) \, d\tau} \, dt \tag{A.8}$$

$$= \frac{c \, v_{\text{ln}}^*}{d_L d_N \langle k_{\text{in}} \rangle \langle k_{\text{out}} \rangle} \int_{t=0}^{365} \bar{Z}_N(t) \int_{\tau=t}^{t+d_N} \bar{Z}_L(\tau) \, d\tau \, dt, \tag{A.9}$$

where c is the probability a larval tick feeds near enough to a nymph such that co-feeding transmission is possible and v_{ln}^* is the nymph-to-larva transmission probability given the temporal and spatial requirements for co-feeding transmission have been satisfied.

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