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Effect of deer density on tick infestation of rodents and the hazard of tick-borne encephalitis. II: Population and infection models

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ABSTRACT

Tick-borne encephalitis is an emerging vector-borne zoonotic disease reported in several European and Asiatic countries with complex transmission routes that involve various vertebrate host species other than a tick vector. Understanding and quantifying the contribution of the different hosts involved in the TBE virus cycle is crucial in estimating the threshold conditions for virus emergence and spread. Some hosts, such as rodents, act both as feeding hosts for ticks and reservoirs of the infection. Other species, such as deer, provide important sources of blood for feeding ticks but they do not support TBE virus transmission, acting instead as dead-end (i.e., incompetent) hosts. Here, we introduce an eco-epidemiological model to explore the dynamics of tick populations and TBE virus infection in relation to the density of two key hosts. In particular, our aim is to validate and interpret in a robust theoretical framework the empirical findings regarding the effect of deer density on tick infestation on rodents and thus TBE virus occurrence from selected European foci. Model results show hump-shaped relationships between deer density and both feeding ticks on rodents and the basic reproduction number for TBE virus. This suggests that deer may act as tick amplifiers, but may also divert tick bites from competent hosts, thus diluting pathogen transmission. However, our model shows that the mechanism responsible for the dilution effect is more complex than the simple reduction of tick burden on competent hosts. Indeed, while the number of feeding ticks on rodents may increase with deer density, the proportion of blood meals on competent compared with incompetent hosts may decrease, triggering a decline in infection. As a consequence, using simply the number of ticks per rodent as a predictor of TBE transmission potential could be misleading if competent hosts share habitats with incompetent hosts.

1. Introduction

Tick-borne infections are mostly caused by pathogens that are transmitted among several competent hosts by ticks that become infected following a blood meal. Among the zoonotic infections transmitted by *Ixodes ricinus*, tick-borne encephalitis, Lyme disease, rickettsiosis and anaplasmosis are emerging as international human health threats (Parola, 2004; Jensenius et al., 2006; Stanek, 2009).

Ticks belonging to the *I. ricinus* complex can feed on a wide range of host species, some of which are competent for the transmission of a given pathogen. In the case of Lyme disease in the north eastern USA, where the white-footed mouse is the main competent host, detailed studies have shown that the tick burden per mouse and the fraction of ticks infected with *Borrelia burgdorferi* is inversely correlated with the abundance of incompetent host species, such as the chipmunk, grey squirrel and white-tailed deer (Ostfeld and Keesing, 2000; Schmid and Ostfeld, 2001; Ostfeld et al., 2006). This phenomenon is known as the dilution effect, since the presence of incompetent hosts diverts tick bites from competent hosts, reducing disease prevalence (Van Buskirk and Ostfeld, 1995; Norman et al., 1999). In this way, biodiversity loss is believed to increase pathogen transmission (Keesing et al., 2010). LoGiudice et al. (2003) pointed out that the dilution effect may not exclusively be an effect of species diversity, but could be a result of the positive correlation between diversity and the relative abundance of incompetent hosts in a community.

In the case of tick-borne encephalitis (TBE) in Europe, other host species are involved in transmission, but their functional role in TBE virus (TBEV) circulation is similar to the case of Lyme disease in the USA. Deer species (mainly roe and red deer) represent an important source of blood for all *I. ricinus* tick stages (e.g., Carpi et al., 2008; Kiffner et al., 2010), but are not competent for TBEV transmission, while wild rodents, which are responsible for much of the transmission, are generally hosts for the immature tick stages only (Perkins et al., 2003, 2006; Rizzoli et al., 2009). An empirical comparison among sites with contrasting TBEV occurrence in Italy and Slovakia has assessed the association between

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TBE hazard in the enzootic cycle and deer abundance (Cagnacci et al., 2012). Deer density has been shown to have an initial positive effect on the number of feeding ticks on rodents, to then reach a threshold value above which the effect becomes negative, as deer apparently divert ticks from feeding on rodents (Cagnacci et al., 2012). On the other hand, in the same study it was shown that the probability of TBEV occurrence in rodents and ticks is a monotonically decreasing function of deer density.

In this paper, we develop mathematical models that explore the dynamics of both tick populations and TBEV infection with the aim of interpreting the empirical results obtained by Cagnacci et al. (2012) in a robust theoretical framework. Specifically, the objective of this exercise is to understand the mechanisms that link the relative abundance of deer species (family Cervidae) to the tick population dynamics, in particular to the number of ticks feeding on rodents, which has been often used as a primary predictor of TBEV maintenance in the natural cycle (Randolph et al., 1996, 1999). In addition, the analysis of a TBEV infection model allows us to quantify, through computation of the basic reproduction number, the conditions that would lead to TBEV persistence, and to evaluate the effect of competent and incompetent host densities on the threshold of persistence. We use the basic reproduction number as a measure of TBEV transmission potential within the enzootic cycle, which represents (together with human exposure) a fundamental component of the overall risk of human infection (Randolph, 2010).

The ultimate purpose of this research is to compare model outputs with empirical results obtained from experiments carried out in Italy and Slovakia in order to explain the variation in tick abundance and TBEV occurrence observed at different experimental sites, and to provide a useful tool for identifying potential TBEV circulation foci. Finally, the infection model allows us to make helpful predictions of pathogen dynamics under different ecological scenarios.

Tick-borne infection systems incorporate interesting complexities as a result of a series of heterogeneities coupled with non-linear phenomena operating in the transmission processes between ticks, hosts and pathogens (Randolph et al., 2002a). The investigation of these complexities has required the development of several theoretical models. The first step has been the development of mathematical models for tick population dynamics by Sandberg et al. (1992), Kitron and Mannelli (1994), and Randolph and Rogers (1997), followed by the implementation of specific models for tick-borne infections. Further models, based on ordinary differential equations (ODE), have been developed by several authors (see for instance Norman et al., 1999, 2004; Gilbert et al., 2001; Rosà et al., 2003; Rosà and Pugliese, 2007). One of the advantages in using ODE models is the possibility of estimating threshold conditions which permit a pathogen to persist, such as the basic reproduction number. However, despite their simplicity, these models usually require the estimation of several ecological and epidemiological parameter values. Basic models describe the tick population through a single dynamical variable (see Norman et al., 2004), while more realistic models include detailed information of the tick life cycle including tick life stages (larvae, nymphs and adults) with stage progression occurring through blood meals on different vertebrate hosts. For instance, two-vertebrate host systems including tick life stages have been developed in Norman et al. (1999) and Rosà et al. (2003), while a system with a three-host community has been investigated in Gilbert et al. (2001). In even more complex models, the explicit modelling of the different phases of tick activity has been introduced: specifically, the questing phase, where ticks seek a host on the vegetation, and the feeding phase, where ticks take their blood meal on the host (Rosà and Pugliese, 2007).

Other sources of complexity in the description of tick-borne infections arise from the different possible transmission pathways. One route of transmission takes place from an infected tick to a susceptible host and vice versa, often denoted as 'systemic transmission'. This route of transmission takes place via the host's blood circulatory system where a viraemia is produced; when the viraemia is sufficiently high, a biting tick will acquire the virus. An important advance in the understanding of how tick-borne pathogens persist in natural systems was the discovery of non-systemic transmission through co-feeding ticks taking place on some host species, such as rodents (Labuda et al., 1993a).

This route of transmission takes place via the host's skin, thereby avoiding the circulatory system, and viraemia is not needed. Specifically, non-systemic transmission occurs horizontally between ticks feeding together on the same host, and is considered crucial for the persistence of some infections, notably the TBEV complex (Randolph et al., 1996, 2002a). Trans-ovarial transmission, from adult ticks to offspring, is also known to occur, but its frequency is very low, and its contribution to TBEV transmission is generally thought to be negligible (Nuttall and Labuda, 2003).

2. Materials and methods

Here we consider simple deterministic models both for the dynamics of the tick *I. ricinus* and the transmission of the TBEV. The models consider two classes of hosts: wild rodents (named as H_R) and deer (named as H_D). Tick dynamics is based on the biology of *I. ricinus* which is the main vector for TBEV transmission. *Ixodes ricinus* has a life cycle that develops from the egg through two immature stages (larva and nymph) to the adult. Each immature stage requires a blood meal from a suitable vertebrate host. The adult female requires a meal before producing eggs once and then dying. Adult females can only obtain a feed from large mammals i.e., deer, whilst the immature stages will also feed on smaller warm-blooded vertebrates such as rodents.

We built a model that explicitly takes into account the different tick activity phases (i.e., questing and feeding on different hosts). Such a framework follows the empirical analyses by Cagnacci et al. (2012), where feeding ticks on rodents are related to deer abundance. On the other hand, we prefer to keep the model as simple as possible in order to provide general analytical results and, at the same time, to avoid hyper-parameterisation. Then, following Norman et al. (2004) and Porter et al. (2011), we added together all tick stages to obtain the equations for total tick dynamics. In addition, to suitably describe some biological processes that involve specific tick stages, such as the production of larvae (sustained only by adults) and non-systemic transmission (sustained only by nymphs), we assumed a fixed proportion of tick individuals within each developmental stage. Randolph (2004) showed that the latter assumption could be considered biologically acceptable as ixodid ticks display a remarkably constant population size and maintain a quite regular proportion of individuals within each developmental stage.

2.1. Tick population dynamics

Here we introduce the model for the dynamics of the tick vector *I. ricinus*. The model consists of three coupled differential equations describing the changes in the abundance of questing ticks, T_q, feeding ticks on rodents, T_{fR}, and feeding ticks on deer T_{fD}. The equations of the resulting model are as follows:

$$\frac{dT_q}{dt} = b_{TR}a_R\sigma T_{fR} + b_{TD}a_D\sigma T_{fD} - d_T T_q - (\beta_R H_R + \beta_D H_D)T_q + (1 - a_R)\sigma T_{fR} + (1 - a_D)\sigma T_{fD}$$

$$\frac{dT_{fR}}{dt} = \beta_R H_R T_q - \sigma T_{fR} \quad (1)$$

$$\frac{dT_{fD}}{dt} = \beta_D H_D T_q - \sigma T_{fD}$$

A full list of parameters with their biological interpretation is given in Table 1. Questing ticks die at rate d_T and move into the feeding tick compartments by encountering hosts of either type H_R and H_D at rate β_R and β_D, respectively. Once the blood meal is completed, ticks drop off their hosts at rate σ (corresponding to an average duration of a blood meal of $1/\sigma$ days). While dropped adult ticks (measured by the fraction a_i) reproduce at rate b_{Ti} and die, dropped immature stages $(1 - a_i)$ return to the questing compartments (with $i = R,D$). We assume a density-dependent birth rate that can be written as $b_{Ti} = r_T \exp(-s_T T_{fi})$, where r_T is the maximum egg production of adult ticks and s_T is the strength of density-dependence.

2.2. TBEV infection dynamics

The model for TBEV infection is an extension of the model proposed by Norman et al. (2004) for tick-borne transmitted virus, deriving explicitly the equations for the activity phases (questing and feeding) of a tick population. The model consists of nine coupled differential equations describing the temporal variation in the abundance of ticks and hosts, subdivided into compartments with respect to the state of infection as in a classical Susceptible-Infected-Recovered (SIR) model. The first six equations describe the temporal variations of susceptible (questing, T^s_q, feeding on rodents, T^s_{fR}, and deer, T^s_{fD}) and infected ticks (T^i_q, T^i_{fR}, T^i_{fD}). Empirical investigations on TBEV transmission have found that rodents are the most competent hosts (both for systemic and non-systemic transmission), while for deer no evidence of virus transmission has been observed (Labuda et al., 1993a). Hence, the last three equations describe the infection dynamics of susceptible (H^s_R), infected (H^i_R) and immune (H^r_R) rodents while deer are assumed to be at constant density (H_D). The equations of the resulting model are as follows:

$$\frac{dT^s_q}{dt} = b_{TR} a_R \sigma T^{s+i}_{fR} + b_{TD} a_D \sigma T^{s+i}_{fD} - dT^s_q - (\beta_R H_R + \beta_D H_D) T^s_q + (1 - a_R)\sigma T^s_{fR} + (1 - a_D)\sigma T^s_{fD}$$

$$\frac{dT^i_q}{dt} = -dT^i_q - (\beta_R H_R + \beta_D H_D) T^i_q + (1 - a_R)\sigma T^i_{fR} + (1 - a_D)\sigma T^i_{fD}$$

$$\frac{dT^s_{fR}}{dt} = (1 - p)\exp[-n_R \theta T^i_{fR}]\beta_R H^i_R T^s_q + \exp[-n_R \theta T^i_{fR}]\beta_R (H^s_R + H^r_R) T^s_q - \sigma T^s_{fR}$$

$$\frac{dT^i_{fR}}{dt} = \beta_R H_R T^i_q + p\beta_R H^i_R T^s_q + (1 - p)(1 - \exp[n_R \theta T^i_{fR}])\beta_R H^i_R T^s_q + (1 - \exp[n_R \theta T^i_{fR}])\beta_R (H^s_R + H^r_R) T^s_q - \sigma T^i_{fR}$$

$$\frac{dT^s_{fD}}{dt} = \beta_D H_D T^s_q - \sigma T^s_{fD} \quad (2)$$

$$\frac{dT^i_{fD}}{dt} = \beta_D H_D T^i_q - \sigma T^i_{fD}$$

$$\frac{dH^s_R}{dt} = b(H_R) H_R - d_R H^s_R - q\beta_R H^s_R T^i_q$$

$$\frac{dH^i_R}{dt} = q\beta_R H^s_R T^i_q - (d_R + \gamma_R + \alpha_R) H^i_R$$

$$\frac{dH^r_R}{dt} = \gamma_R H^i_R - d_R H^r_R$$

A full list of parameters with their biological interpretation is given in Table 1. Since TBEV infection may induce extra mortality (α_R, disease-related death rate) in some rodent species (Labuda et al., 1993b), we need to introduce its population dynamics into the model. Precisely, we define d_R as the rodent natural mortality rate and $b(H_R) = b_R H_R - [(d_R - b_R)/K_R] H^2_R$, as its density-dependent fertility function, where b_R represents competent host birth rate at low densities and K_R represents its carrying capacity. Moreover, infected rodents may recover from TBEV infection at rate γ_R.

Ticks feeding on infected competent hosts become infected, through the systemic transmission route, with probability p, while a competent host fed on by an infected tick has probability q of becoming infected. Here, we assume that systemic infection occurs at the beginning of the blood meal, so that the infection rate of ticks [hosts] will be proportional to the encounter rate between

Table 1
Numerical values and biological interpretation of parameters used in the models.

Description	Symbol [units]	Mean (Ref.)
Well-estimated parameters		
Average egg production per fed adult tick	r_T [# eggs]	2000 (Randolph and Craine, 1995)
Death rate of questing ticks	d_T [day^{-1}]	0.02 (Randolph et al., 2002b)
Detachment rate of feeding ticks	σ [day^{-1}]	0.256 (Hartemink et al., 2008)
Density-dependent birth rate of ticks	s_T [ha tick^{-1} day^{-1}]	0.025 (Rosà and Pugliese, 2007)
Efficiency of systemic transmission from competent host to tick	p	0.8 (Hartemink et al., 2008)
Efficiency of systemic transmission from tick to competent host	q	0.9 (Hartemink et al., 2008)
Efficiency of non-systemic transmission from tick to tick	θ	0.55 (Labuda et al., 1993b)
Natural birth rate of rodent host	b_R [day^{-1}]	0.0082 (Pugliese and Rosà, 2008)
Natural death rate of rodent host	d_R [day^{-1}]	0.0037 (Pugliese and Rosà, 2008)
Recovery rate of rodent host	γ_R [day^{-1}]	0.3 (Pugliese and Rosà, 2008)
Disease-related death rate of rodents	α_R [day^{-1}]	0.33 (Pugliese and Rosà, 2008)
Fraction of adults in feeding ticks on rodents	a_R	0 (Perkins et al., 2003, 2006)
Uncertain parameters		Mean ± S.E. (Ref.)
Carrying capacity of rodents	K_R [host ha^{-1}]	7.6 ± 0.62 (Rosà et al., 2007)
Encounter rate between questing ticks and rodents	β_R [ha host^{-1} day^{-1}]	0.0035 ± 1.76e-4 (Rosà et al., 2007)
Encounter rate between questing ticks and deer	β_D [ha host^{-1} day^{-1}]	0.097 ± 0.03 (Pugliese et al., 2003)
Fraction of adults in feeding ticks on deer	a_D	0.007 ± 0.003 (Carpi et al., 2008;Kiffner et al., 2010)
Fraction of nymphs in feeding ticks on rodents	n_R	0.013 ± 0.001 (Rosà et al., 2007)

questing susceptible ticks T_q^s [questing infected ticks T_q^i] and infected hosts H_R^i [susceptible hosts H_R^s], i.e., $p\beta_R T_q^s H_R^i$ [$q\beta_R T_q^i H_R^s$].

As in Rosà and Pugliese (2007), we introduced a mechanism for non-systemic transmission (also knows as co-feeding) specific for TBEV. Precisely, we assumed the rate of non-systemic transmission to be proportional to the density of infected feeding ticks, T_{fR}^i, the density of susceptible questing ticks, T_q^s, and competent hosts, H_R (precisely, $1-\exp[n_R\theta T_{fR}^i]\beta_R T_q^s H_R$). The proportion $n_R\theta$ describes the co-feeding transmission term, where θ is the probability of non-systemic transmission, and n_R is the fraction of nymphs against the total number of ticks feeding on H_R (since only nymphs may infect other ticks through co-feeding).

2.3. Model parameter estimation

Models (1) and (2) are calibrated with parameter values estimated from *I. ricinus* and TBEV infection, considering rodents and deer as the main hosts. Parameter values are summarised in Table 1. While some of the parameters describing tick ecology and TBE epidemiology have been robustly estimated in the scientific literature, other parameter values – which are strongly related to the local ecological conditions, such as encounter rates (β_R, β_D), rodent density (H_R), and the fractions of ticks in different stages (i.e., n_R, a_D) – are more subject to site-specific variability. To overcome this uncertainty, we performed sensitivity analyses of model results assuming the uncertain parameters (β_R, β_D, H_R, a_D, and n_R) as realisations from Gaussian distributions. To generate a plausible collection of parameter values, we used the Latin Hypercube Sampling method with 10,000 extractions (Hoare et al., 2008). Means and standard errors of the uncertain parameter distributions are derived from previous field studies, all conducted in the same study site in Trentino, northern Italy (Table 1). In particular, estimates of rodent density (H_R), feeding ticks on rodents (T_{fR}) and questing ticks (T_q) are derived from a long-term study, where live-trapping rodent monitoring has been implemented using capture-mark-recapture technique, while the density of questing ticks was estimated from vegetation using a dragging technique (Rosà et al., 2007). Rodent density was estimated using a standard open population Jolly-Seber model (Krebs, 1989). For each rodent captured, a careful assessment of the number of different tick life stages feeding on that rodent was carried out (Rosà et al., 2007). These data also allow us to estimate the fraction of feeding ticks in each stage and, in particular, the fraction of nymphs feeding on rodents (n_R).

Imposing $dT_{fR}/dt = 0$ in model (1) and rearranging, we obtained the following relationship for calculating the encounter rate between ticks and rodents at the non-trivial equilibrium:

$$\beta_R = \frac{T_{fR}}{H_R}\frac{\sigma}{T_q}. \quad (3)$$

Inserting in (3) the observed values of feeding ticks per rodent (T_{fR}/H_R), questing ticks (T_q) and the tick detachment rate (σ) we obtained the mean and standard error of β_R reported in Table 1.

No comparable measures existed for the same study area to estimate the encounter rates of ticks with deer (β_D). However, an experiment with tracer animals (domesticated goats) was carried out at the same study site in Trentino (northern Italy), obtaining the numerical values reported in Pugliese et al. (2003) and summarised in Table 1.

Several empirical works performed in Trentino (northern Italy) show that wild rodents are generally hosts for the immature tick stages only, while adults can only obtain a suitable feed from large mammals (Perkins et al., 2003, 2006; Rizzoli et al., 2009). Thus, we assumed that the fraction of adult ticks feeding on rodents is negligible (i.e., we set $a_R = 0$). In addition, Rosà and Pugliese (2007) showed that the assumption of neglecting the small fraction of adult ticks on rodents in the population model does not critically affect tick dynamics. Finally, the fraction of adult ticks feeding on deer (a_D) was estimated using tick collection data from deer legs in Carpi et al. (2008). Since Kiffner et al. (2010) showed that the proportion of adult ticks feeding on legs is lower in comparison with the entire body, we corrected our estimate by assuming the legs-to-body tick ratio observed in Kiffner et al. (2010).

Table 2
Comparison between observed tick-borne encephalitis virus occurrence, column [TBE virus], and R_0 estimates in TBE virus-positive and TBE virus-negative sites, columns [R_0^{TBE}] and [R_0^{stand}]. Columns [T_{fR}/H_R] and [H_D] report the observed values of feeding ticks per rodent and deer density, respectively, used for R_0 computation as in Eq. (7) in Section 3.2, column [R_0^{TBE}]. Columns [L_{fR}/H_R] and [*Cof*] report the observed values of larval feeding ticks per rodents and number of co-feeding groups, respectively, used for standard R_0 computation as in Randolph et al. (1999), column [R_0^{stand}]. Data are from Cagnacci et al. (2012).

Site (Country)	$\frac{T_{fR}}{H_R}$	H_D	$\frac{L_{fR}}{H_R}$	*Cof*	TBE virus	$R_0^{TBE} \pm$ S.E.	R_0^{stand}
Belluno (Italy)	24.13	4.6	23.12	31.83	Yes	4.95 ± 0.67	7.52
Grosotto (Italy)	1.09	6.8	1.09	0.00	No	0.71 ± 0.18	0.11
Lamar (Italy)	16.93	2.2	16.17	21.14	Yes	3.80 ± 0.54	5.07
Mazzo (Italy)	0.53	3.1	0.42	0.43	No	0.49 ± 0.074	0.11
Rozhanoce (Slovakia)	1.63	15.5	1.60	8.3	No	0.89 ± 0.10	1.53
Topolcianky (Slovakia)	4.40	2.0	3.90	1.00	Yes	1.60 ± 0.48	0.55

2.4. Empirical assessment of the effect of deer on tick abundance and TBEV occurrence

Following model selection results from Cagnacci et al. (2012) we selected the best generalised linear mixed-model (GLMM), with negative binomial error distribution, that included the effect of deer (linear and quadratic terms) and region on the number of feeding ticks on rodents (see Table 2 in Cagnacci et al., 2012). Then, to obtain a robust estimate of the observed value of deer density at which the peak of feeding ticks on rodents occurs ($\hat{H}_D^{ticks}$), and to compute its standard error, we took into account the uncertainty in deer densities empirically detected at each study site (see Table 1 in Cagnacci et al., 2012). We assigned to deer density estimates a truncated Gaussian distribution representing the degree of uncertainty associated with empirical values. Specifically, by randomly extracting deer density values from a probability distribution constrained at 95% by the confidence intervals reported in Cagnacci et al. (2012) (see Table 1), we ran the best GLMM 1,000 times generating output distributions for each model coefficient (precisely: deer, D_1; deer^2, D_2; and region, *Region*). In particular, from the GLMM coefficients related to deer we estimated the distribution of the deer density at which the peak of feeding ticks on rodents occurs ($\hat{H}_D^{ticks}$) as $D_1/(2 * D_2)$.

In order to compare infection model prediction with empirical results on TBEV occurrence, we used the field estimates of the average number of larvae (L_{fR}/H_R) and total tick (T_{fR}/H_R) feedings on rodents, the number of co-feeding groups on rodents (*Cof*) and deer density (H_D) in Cagnacci et al. (2012) to compute, through model (2), the expected basic reproduction number for TBEV (R_0^{TBE}) at each study site. The basic reproduction number measures the number of secondary cases produced by a single infection in a completely susceptible population. By definition, the pathogen can persist in the population when the mean number of infected hosts produced by a single infected host is larger than 1 ($R_0^{TBE} > 1$). Finally, we compared the empirical results on TBEV

occurrence with the expected R_0 computed through classical procedures for tick-borne infections (Randolph et al., 1999).

Statistical analyses were performed with statistical packages [R] 2.10.1 (2010, The R Foundation for Statistical Computing).

3. Results

3.1. Tick population dynamics: model results and comparison with empirical data

Before comparing model outputs with empirical results obtained from experiments described in Cagnacci et al. (2012), we briefly reviewed the main features of model (1). We considered tick dynamics in the absence of adult ticks feeding on rodents (i.e., $a_R = 0$), since they generally feed on large mammals only (see Section 2.3 for details). Then, using next-generation matrix techniques (Diekmann et al., 1990), we computed for model (1) the following basic reproduction number of ticks:

$$R_0^{\text{ticks}} = \frac{r_T a_D \beta_D H_D}{(d_T + a_D \beta_D H_D)}. \tag{4}$$

R_0^{ticks} represents the expected number of ticks produced by a single tick, when density-dependent effects are absent, and defines the threshold for tick population to persist ($R_0^{\text{ticks}} > 1$). Similar to the model of Norman et al. (2004), Eq. (4) shows that R_0^{ticks} in model (1) is an increasing, saturating function of deer density (H_D), and does not depend on rodent host density (H_R).

From model (1), we analytically computed the mean number of feeding ticks per rodent host (T_{fR}/H_R) at the non-trivial equilibrium, finding the following function of H_D:

$$\frac{T_{fR}}{H_R} = \frac{\beta_R}{s_T \beta_D H_D} \log(R_0^{\text{ticks}}). \tag{5}$$

In Fig. 1 we show how T_{fR}/H_R, computed as in (5), changes with deer density (solid curve). In particular, the solid curve is computed as the average of 10,000 extractions by using Latin Hypercube Sampling techniques from Gaussian distributions of the uncertain parameters with means and standard errors in Table 1, while the grey area represents the 95% confidence interval.

In the same figure we display the mean value of the empirical results obtained from the GLMM re-sampling procedure that allows for variability in deer densities estimates (dashed curve).

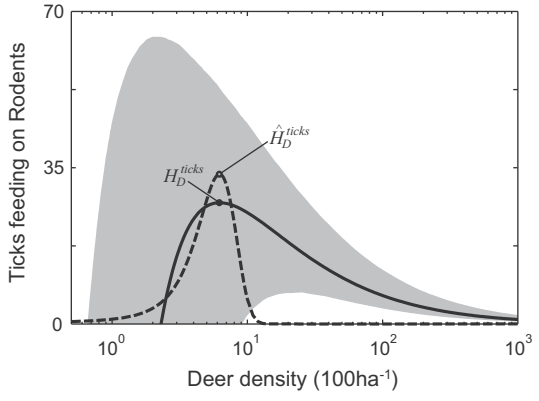

Fig. 1. Effect of deer density on the number of feeding ticks per rodent (T_{fR}/H_R) as in Eq. (5) in Section 3.1 (solid curve) and from the empirical results obtained through the generalised linear mixed-model re-sampling procedure (dashed curve). The solid curve is obtained as the average of 10,000 extractions sampled from uncertain parameter distributions with means and standard errors as in Table 1, while the grey region represents the 95% confidence interval. Filled (H_D^{ticks}) and open ($\hat{H}_D^{\text{ticks}}$) circles represent the predictions by model (1) and the observed values of deer density at which the peak of the number of ticks feeding per rodent takes place. Other parameters are as in Table 1.

Theoretical and empirical results showed a qualitatively similar pattern of feeding ticks on rodents, reaching their peaks (H_D^{ticks} and $\hat{H}_D^{\text{ticks}}$, respectively) for intermediate levels of deer density.

Through algebraic manipulations of model (1), we computed the predicted value of deer density at which the peak of feeding ticks on rodent occurs (H_D^{ticks}) at the non-trivial equilibrium, obtaining:

$$H_D^{\text{ticks}} = \frac{d_T}{(r_T/\tilde{R}_0^{\text{ticks}} - 1) a_D \beta_D} \tag{6}$$

where $\tilde{R}_0^{\text{ticks}}$ is the solution of the implicit equation $R_0^{\text{ticks}}/r_T + \log(R_0^{\text{ticks}}) = 1$ (see Proposition 1 in Supplementary data S1 for the proof). Since $R_0^{\text{ticks}} > 1$ is a necessary condition for tick persistence, the solution of the previous expression satisfies the condition $\tilde{R}_0^{\text{ticks}} < r_T$ for every values of r_T. This implies that H_D^{ticks} in (6) is always positive and, consequently, the relationship between deer and ticks on rodents is always hump-shaped for every combination of parameter values.

While values for adult tick egg production (r_T) and death rate (d_T) have been robustly estimated in several ecological surveys (Randolph and Craine, 1995; Randolph et al., 2002b), the fraction of adult ticks (a_D) and the encounter rate between ticks and deer (β_D), essential to quantify H_D^{ticks}, present a wide range of uncertainties. In order to evaluate the ranges of deer density values at which the peak of tick feeding on rodents occurs, a sensitivity analysis of these two parameters has been preformed and summarised in Fig. 2. Black dots represent H_D^{ticks} values as in (6), where the pairs (β_D,a_D) are sampled using Latin Hypercube Sampling techniques; Fig. 2 visualises the sampling along the β_D axis (Fig. 2A) and a_D axis (Fig. 2B). Black line $\bar{H}_D^{\text{ticks}}$ defines the average value of H_D^{ticks} resulting from the 10,000 (β_D,a_D) combinations, while dashed lines indicate the confidence interval of the observed peak estimated from the GLMM re-sampling procedure. Fig. 2 shows that the values predicted by model (1) of deer density at which the peak of feeding ticks occurs are in good agreement with empirical data. Indeed, the average value $\bar{H}_D^{\text{ticks}} = 7.51$ host 100 ha^{-1} (black line), falls within the confidence interval of the observed peak estimate ($4.8 < \hat{H}_D^{\text{ticks}} < 10.5$ host 100 ha^{-1}).

3.2. TBEV infection dynamics: model results and comparison with empirical data

From TBEV infection model (2), we computed the basic reproduction number (R_0^{TBE}) with next-generation matrix technique (Diekmann et al., 1990) as the larger root of the following quadratic function (see Proposition 2 in Supplementary data S1 for details):

$$R_0^{\text{TBE}} = \max_{\lambda}(\text{roots}(\lambda^2 - \phi(H_D)\lambda - \varphi(H_D) = 0)) \tag{7}$$

where $\varphi(H_D)$ [$\phi(H_D)$] represents the contribution of infection spread from the systemic [non-systemic] transmission. By definition, the infection can persist in the population when the mean number of infected hosts produced by a single infected host, through both systemic and non-systemic transmission, is larger than 1 ($R_0^{\text{TBE}} > 1$). From (7), we derive the threshold value for pathogen invasion as follows:

$$\begin{aligned}\varphi(H_D) + \phi(H_D) &= \frac{q p \beta_R^2 T_q H_R}{(d_R + \gamma_R + \alpha_R)(d_T + a_D \beta_D H_D)} \\ &\quad + \frac{n_R \theta \beta_R T_q H_R (d_T + \beta_R H_R + a_D \beta_D H_D)}{\sigma (d_T + a_D \beta_D H_D)} \\ &= 1. \end{aligned} \tag{8}$$

Fig. 3 shows the region in the two-dimensional parameter space of deer (H_D) and rodent (H_R) densities where the basic reproduction number R_0^{TBE} is greater than 1, taking into account the result of the

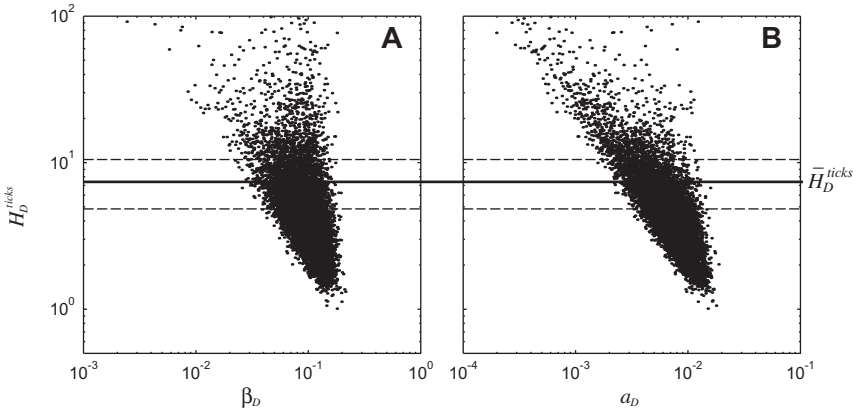

Fig. 2. Effect of encounter rate between questing ticks and deer (β_D) and fraction of ticks feeding on deer in the adult stage (a_D) on the value of deer density at which the peak of ticks feeding on rodents occurs (H_D^{ticks}) as in Eq. (6) in Section 3.1. Black dots represent H_D^{ticks} values for each pair (β_D, a_D) sampled through Latin Hypercube Sampling techniques; (A) visualises the sampling along the β_D axis; (B) visualises the sampling along the a_D axis. The black line $\bar{H}_D^{ticks}$ defines the average value of H_D^{ticks} resulting from the 10,000 (β_D, a_D) combinations. Dashed lines define the confidence interval of the observed peak estimated through the generalised linear mixed-model re-sampling procedure. Other parameters are as in Table 1.

sensitivity analysis of uncertain parameters from Table 1. The black curve represents the median value of R_0^{TBE} in 10,000 extractions, while the grey area boundaries represent the 95% confidence interval of the distribution.

We were interested to quantitatively validate model (2) results with data obtained empirically by Cagnacci et al. (2012) in six different TBE-negative and TBE-positive European locations. In Table 2, we provide model (2)'s estimated values of R_0^{TBE} at the six sampling sites by using mean values of field data for T_{fR}/H_R and H_D (see also Table 1 in Cagnacci et al., 2012), and using the parameter set, with their standard errors, as reported in Table 1. These estimates were in very good agreement with observed data of presence and absence of TBEV recorded at the six locations (see columns [TBE virus] and [R_0^{TBE}] in Table 2). In addition, we showed that a standard procedure for R_0 computation, tailored by Randolph et al. (1999) for infection within Slovakian regions, may fail to predict TBEV presence/absence in some of our study sites (see column [R_0^{stand}] in Table 2). More precisely, this procedure overestimates R_0 for sites with very high deer densities (as in Rozhanoce) since it does not take into account the deer dilution effect. On the other hand, it underestimates TBEV transmission potential where the low level of rodent tick burden (as in Topolcianky) is compensated by a deer density close to the value at which R_0^{TBE} is maximised.

In Fig. 4 we show the effect of deer density on the abundance of infected questing ticks (T_q^i, grey curve) and competent hosts positive to TBEV ($H_R^i + H_R^r$, black curve) as predicted by model (2). While the abundance of infected questing ticks displays a hump-shaped behaviour that follows TBEV basic reproduction number values, as defined in (7), the abundance of positive rodents displays a more complex bi-modal relationship with deer density. This is due to the effect of the disease-related death (α_R) which depresses the total rodent population (and consequently its TBE-positive fraction) when TBEV is higher in ticks. Indeed, simulating model (2) in the absence of disease-related death, $\alpha_R = 0$ (not shown here), we found a pattern in positive rodents similar to that showed for infected questing ticks.

Comparing expressions obtained through models (1) and (2), in particular (6) and (7), it is possible to analytically prove that the peak of TBEV basic reproduction number, H_D^{TBE}, predicted by model (2) always occurs at a lower deer density than the peak for feeding ticks on rodents, H_D^{ticks}, predicted by model (1), which translates in the following mathematical inequality:

$$H_D^{TBE} = \arg\max_{H_D}[R_0^{TBE}] < \arg\max_{H_D}[T_{fR}/H_R] = H_D^{ticks}$$

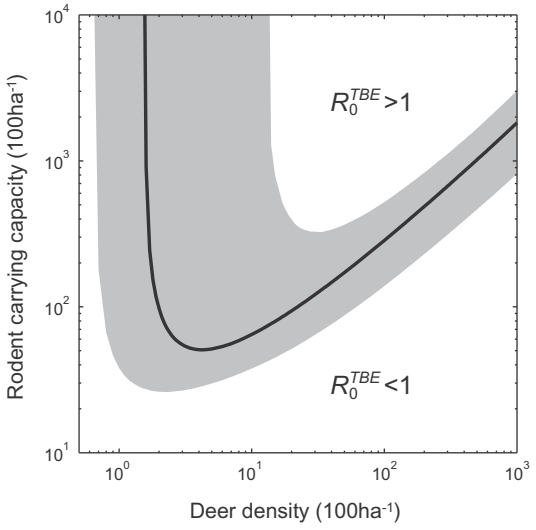

Fig. 3. Effect of deer density (H_D) and rodent carrying capacity (K_R) on R_0^{TBE} as in Eq. (7) in Section 3.2. The black curve is obtained as the median of 10,000 extractions sampled from uncertain parameter distributions with means and standard errors as in Table 1, while the grey region represents the 95% confidence interval. Other parameters are as in Table 1.

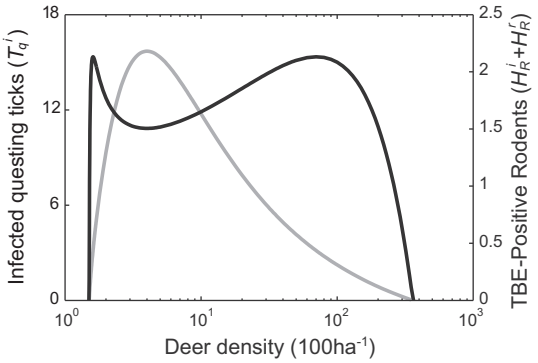

Fig. 4. Effect of deer density on the abundance of infected questing ticks (T_q^i, grey curve) and the abundance of rodents positive to TBE virus ($H_R^i + H_R^r$, black curve) as in model (2) in Section 2.2. Simulation parameters are as in Table 1.

(see Proposition 3 in Supplementary data S1 for details). This implies that the number of secondary infections (measured by R_0^{TBE}) may decline with deer density while the number of feeding ticks on rodents is still increasing.

4. Discussion

In this analysis, we investigated the contribution of incompetent hosts (such as deer) on tick vector abundance and on the persistence of TBEV. In particular, we were interested in providing a robust theoretical framework for the experimental results of Cagnacci et al. (2012) on the effect of deer density on TBEV transmission potential in some European countries, and to give insightful ecological interpretations of their results.

Tick life-cycle depends on the presence of large mammalian hosts for blood meals, and deer play a crucial role in the persistence of tick populations in different European regions (Rizzoli et al., 2009). On the other hand, deer are incompetent hosts for the transmission of TBEV, and their role in pathogen spread depends on whether the increase in vector density is sufficient to compensate for virus lost in 'wasted bites' on dead-end incompetent hosts (Hudson et al., 1995; Dobson and Foufopoulos, 2001).

In Italian and Slovakian study sites, Cagnacci and co-authors (2012) found a hump-shaped relationship between incompetent host density and the abundance of ticks feeding on competent hosts. They explained this result by suggesting that an initial increase in deer density amplifies the vector population by increasing the ratio of vector to susceptible rodent hosts, while a further increase 'dilutes' the number of ticks per rodents because deer at higher densities divert tick bites from them.

The analysis of the tick population model (1) agrees with the experimental findings, both qualitatively (Fig. 1) and quantitatively (Fig. 2), showing a peak of feeding ticks on competent hosts for intermediate densities of incompetent hosts. This pattern is the result of two competing effects driven by deer: on one hand, deer act as vector amplifiers; on the other hand, they also act as tick bite diverters from rodents. As deer are crucial for the tick life-cycle completion, an increase in their density will produce an increase in the total tick population. Initially, as the total number of ticks increases, we might expect more ticks feeding on rodents. However, when total ticks reach sufficiently high levels of abundance, density-dependent constraints would begin to take place, slowing down tick growth and saturating the tick population. Thus, a further increase in deer will have only a marginal effect on the increase in the tick population, while their effect on diverting bites from other hosts becomes more relevant, triggering the decrease of feeding ticks on rodents.

The exact nature of density-dependent regulation in ticks is not yet well understood. However, it is generally thought that the regulation is due to host immunity rather than direct competition or predation (Randolph, 2004). Indeed, Wikel (1996) and Brossard and Wikel (2004) showed that hosts acquire resistance to tick feeding as a result of repeated infestations. Their results are also supported by observations both in tick-cattle and tick-rodent interactions (Sutherst et al., 1979; Hughes and Randolph, 2001). Effects of acquired resistance in hosts have been observed, especially in tick fecundity, such as the increased duration of feeding, decreased number of eggs and reduced viability of those eggs (Bowessidjaou et al., 1977; Wikel, 1996; Brossard and Wikel, 2004). Hudson and Dobson (1995) showed that a decrease in the average blood meal might affect tick fecundity, as it varies directly with meal size. Here, we introduce the density-dependent regulation in our models, letting adult tick fecundity depend on the instantaneous total tick load, as proposed by Rosà and Pugliese (2007). The same authors also showed that the choice of shape of the density-dependent regulation function does not critically affect the conclusions for the role of deer on TBEV epidemiology (Rosà and Pugliese, 2007).

By analysing TBEV persistence through infection model (2), we found a pattern consistent with that of feeding ticks. In particular, we showed that the TBEV basic reproduction number, R_0^{TBE}, is larger than one for intermediate levels of deer density (see Fig. 3) when the abundance of ticks on rodents is sufficient to sustain pathogen transmission, while for higher deer densities the infection dies out, supporting the 'wasted bites' hypothesis. This finding is in agreement with several theoretical results on tick-borne diseases, such as on TBEV in northern Italy (Rosà et al., 2003, 2007) and the louping ill virus system in upland Britain (Gilbert et al., 2001; Norman et al., 2004) showing this is not an artefact of this specific system. However, the comparison between tick population model (1) and TBEV infection model (2) outputs shows that the mechanism of 'wasted bites' seems more complex than a simple reduction of the number of feeding ticks on rodents. Indeed, we proved that the peak of TBEV basic reproduction number occurs at lower deer densities (H_D^{TBE}) than the peak of feeding ticks on rodents (H_D^{ticks}), highlighting that incompetent host density may depress the efficiency of pathogen transmission while still amplifying the tick burden on competent hosts. This result does not depend on the specific assumptions made in our models. Indeed, in more complex models where tick stage progression is included (see Rosà and Pugliese, 2007; Pugliese and Rosà, 2008), a similar pattern, with the peak of infection occurring at lower deer densities than the peak for feeding ticks on rodents, has been found. The biological explanation derives from the definition of basic reproduction number (R_0^{TBE}) as found in (7). Indeed, the peak of R_0^{TBE} occurs when the maximum number of ticks, which were previously infected on rodents, return to feed on rodents. Then, while the number of ticks on rodents may increase with deer density, as a consequence of the amplification of total tick population, the ratio of blood meals on competent hosts with respect to those on incompetent hosts may decrease, triggering an infection decline. Thus, using an estimation of the number of ticks per rodent as a predictor for TBEV transmission potential could be misleading, while taking into account the deer host density could increase the reliability of this prediction (see also Rizzoli et al., 2009). We showed that including the presence of incompetent deer hosts in the computation of the basic reproduction number is crucial to assess TBEV persistence at different sites, while simpler techniques based only on the tick burden on rodents may fail to assess it accurately (see Table 2 and Fig. 3). However, an in-depth host blood meal identification in questing ticks through molecular methods should provide a precise estimate of the ratio of blood meals on competent with respect to incompetent hosts that would represent a better proxy for the assessment of TBE transmission potential in the enzootic cycle.

Moreover, the results that emerged from the comparison between models (1) and (2) can also provide a possible explanation for the differences observed by Cagnacci et al. (2012) in the effect of deer density on the abundance in ticks feeding on rodents and TBEV occurrence. Cagnacci et al. (2012) found a hump-shaped relationship between feeding ticks on rodents and deer density, while they observed a monotonical decrease of TBEV occurrence probability with deer density. This discrepancy may be due to the deer density estimates obtained in their empirical assessment study. While the range of the estimated deer densities is well-centred to cover both the increasing and decreasing phases of the relationship between deer and feeding ticks on rodents, the same range of data could be not centred to catch the increasing phase of the relationship between deer and TBEV occurrence that model (2) predicts to occur at lower deer densities; as a consequence, only the decreasing phase is observed.

Model (2) could also explain the inconsistency in TBEV infection between ticks and rodents found by Cagnacci et al. (2012) (see

Table 1). As shown in Fig. 4, the pattern of infection in rodents can differ dramatically from those in ticks, with the latter mimicking the behaviour of the basic reproduction number and the former shaped by the effect of the disease-related deaths. Labuda et al. (1993b) showed that disease-related death is strongly species-specific and can be high for certain rodent species (e.g., *Pitymys subterraneus*) whereas other species (e.g., *Apodemus flavicollis*) may develop low viraemias and are much more likely to survive infection. This result can have substantial consequences on TBE surveillance, where rodents (which are simpler to test) have been frequently used as sentinel animals (Hayasaka et al., 1999; Achazi et al., 2011), while pathogen persistence and the overall risk for human transmission seems better represented by the infection in ticks.

In the case of other tick-borne diseases, the role of tick hosts may be different from the TBE case. For instance, the lizard *Sceloporus occidentalis*, acts as an incompetent reservoir for Lyme disease (such as deer in the TBE system), while acting as a blood meal host for immature tick stages only (such as rodents in the TBE system). Swei et al. (2011) found experimentally that removing incompetent lizards may reduce the overall tick density and, thus, decrease disease risk.

Here we used deterministic models with constant coefficients, disregarding seasonality, a crucial feature in tick population dynamics in temperate regions (Randolph et al., 2002b). In the specific case of TBEV, the synchronisation of the annual tick cycle with those of competent rodents is one of the key factors for infection persistence (Randolph et al., 1999). However, the aim of this work was to highlight the mechanisms linking TBEV transmission to incompetent host density, so adding seasonal details would have obscured our main goal. Interestingly, Ghosh and Pugliese (2004) found a dilution effect triggered by incompetent host densities even in a discrete growing season model. In addition, seasonal models for vector-borne (Ogden et al., 2007; Nonaka et al., 2010), as well as directly transmitted diseases in wildlife (Bolzoni et al., 2008; Duke-Sylvester et al., 2011), are considerably more complex to investigate, but their predictions may allow us to understand intra- and inter-annual variability in disease infections in the near future.

Finally, the evaluation of the relative abundance of the key hosts involved in the pathogen transmission and the degree of host diversity within TBEV endemic areas is essential for a better evaluation of TBE risk. These issues, in particular the functional role of biodiversity, will be our focus in further investigations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2012.02.006.

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