Original article

Low probability of a dilution effect for Lyme borreliosis in Belgian forests

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ABSTRACT

An increasing number of studies have investigated the consequences of biodiversity loss for the occurrence of vector-borne diseases such as Lyme borreliosis, the most common tick-borne disease in the northern hemisphere. As host species differ in their ability to transmit the Lyme borreliosis bacteria *Borrelia burgdorferi* s.l. to ticks, increased host diversity can decrease disease prevalence by increasing the proportion of dilution hosts, host species that transmit pathogens less efficiently. Previous research shows that Lyme borreliosis risk differs between forest types and suggests that a higher diversity of host species might dilute the contribution of small rodents to infect ticks with *B. afzelii*, a common *Borrelia* genospecies. However, empirical evidence for a dilution effect in Europe is largely lacking. We tested the dilution effect hypothesis in 19 Belgian forest stands of different forest types along a diversity gradient. We used empirical data and a Bayesian belief network to investigate the impact of the proportion of dilution hosts on the density of ticks infected with *B. afzelii*, and identified the key drivers determining the density of infected ticks, which is a measure of human infection risk. Densities of ticks and *B. afzelii* infection prevalence differed between forest types, but the model indicated that the density of infected ticks is hardly affected by dilution. The most important variables explaining variability in disease risk were related to the density of ticks. Combining empirical data with a model-based approach supported decision making to reduce tick-borne disease risk. We found a low probability of a dilution effect for Lyme borreliosis in a north-western European context. We emphasize that under these circumstances, Lyme borreliosis prevention should rather aim at reducing tick-human contact rate instead of attempting to increase the proportion of dilution hosts.

1. Introduction

Biodiversity loss, driven by anthropogenic and environmental changes, is known to affect changes in the functioning of ecosystems and the delivery of several ecosystem goods and services (Cardinale et al., 2012). Recently, research focusing on disease control has linked biodiversity loss with increased infectious disease risk (Johnson et al., 2013; Naeem et al., 2012). Many infectious diseases are vector-borne (i.e. transmitted to humans via a vector such as insects or ticks) and zoonotic (i.e. the source of the pathogen is a non-human host species) (Jones et al., 2008; Taylor et al., 2001). The transmission potential usually varies between host species, with some species transmitting the pathogen efficiently (competent host species), and others rarely or not (non-competent hosts or 'dilution hosts') (LoGiudice et al., 2003; Matuschka et al., 1992). A competent host species transmits the

pathogen to the tick but does not need to be (permanently) infected. A reservoir host species on the other hand can permanently maintain the pathogen and can transmit the pathogen to the tick. A reservoir host has the ability to transmit the pathogen to the feeding ticks, but is not necessarily infected itself. In an effort to elucidate the relationship between biodiversity and disease risk, the 'dilution effect hypothesis' has been formulated (Norman et al., 1999; Ostfeld and Keesing, 2000). A dilution effect occurs when increased host species diversity decreases vector-borne zoonotic disease risk by decreasing the relative abundance of competent hosts and increasing the relative abundance of dilution hosts. Ticks that have access to a host community with many different species are, stochastically, less likely to make contact with a host that transmits the pathogen compared to ticks in a species-poor host community lowering the average pathogen prevalence (Keesing et al., 2006; Ogden and Tsao, 2009). However, the dilution

effect relies on the assumption that less diverse host communities are dominated by competent host species (LoGiudice et al., 2003; Ostfeld and Keesing, 2000) and the generality of the dilution effect, thus, remains controversial. An increasing number of studies argue that the dilution effect is in fact a local phenomenon and relies on the specific composition of the host communities, the pathogens, the vectors and their specific ecology, rather than on species diversity itself (Ogden and Tsao, 2009; Salkeld et al., 2013).

Lyme borreliosis is the most prevalent vector-borne disease in the temperate regions of the northern hemisphere, with important medical and economic consequences (World Health Organization, 2004; Wormser et al., 2006). It is caused by some genospecies of the *Borrelia burgdorferi* sensu lato bacterial complex (Stanek et al., 2012), which are to be distinguished from the relapsing fever causing bacterium *Borrelia miyamotoi*. *Borrelia burgdorferi* sensu lato ('*Borrelia*') is vectored by *Ixodes ricinus* (Linnaeus, 1758) ticks in Europe, which have three mobile life stages (larva, nymph and adult). Each stage ascends the vegetation and waits for a passing host (Gray, 1998). Transmission of *Borrelia* from adult female to eggs and larvae occurs only exceptionally (Barbour and Fish, 1993; Parola and Raoult, 2001). Infected nymphs and adults are therefore the main threat to human health. *Ixodes ricinus* predominantly occurs in forests, because of the humid microclimate and availability of vertebrate hosts for their blood meals (Gray et al., 1998; Lindstrom and Jaenson, 2003). Structure-rich deciduous forests usually harbor higher densities of ticks, compared to open fields and structure-less coniferous forests (Jaenson et al., 2009; Ruyts et al., 2016; Tack et al., 2012). A recent European meta-analysis (Hofmeester et al., 2016) showed that small rodents, such as the wood mouse (*Apodemus sylvaticus* Linnaeus, 1758) and the bank vole (*Myodes glareolus* Schreber 1780) are generally the most important feeding hosts for larval *I. ricinus*, based on the amount of larvae they feed, while thrushes are the most important hosts for nymphs. Roe deer (*Capreolus capreolus* Linnaeus, 1758) are generally the most important feeding hosts for adults in Europe and are important in the maintenance and reproduction of *I. ricinus* populations (Gray, 1998; Hofmeester et al., 2016; Ruiz-Fons and Gilbert, 2010). Many studies have already shown the association between small rodents and the *Borrelia* genospecies *B. afzelii* (Hanincová et al., 2003a; Humair et al., 1999). Birds have been reported to transmit *B. garinii* and *B. valaisiana* (Hanincová et al., 2003b; Heylen et al., 2014). Roe deer are unable to transmit any of the *Borrelia* genospecies (Jaenson and Tälleklint, 1992). Some host species, such as the Eurasian red squirrel (*Sciurus vulgaris* Linnaeus, 1758) and the European hedgehog (*Erinaceus europaeus* Linnaeus, 1758) have been suggested to transmit *B. afzelii* as well (Jahfari et al., 2017b; Pisanu et al., 2014; Ruyts et al., 2017; Skuballa et al., 2012). Apart from being associated with different host species, the pathogenic *Borrelia* genospecies cause different disease symptoms in humans (Balmelli and Piffaretti, 1995). The most common genospecies isolated from Western-European Lyme borreliosis patients is *B. afzelii*, which generally causes skin manifestations (Jahfari et al., 2017a).

The dilution effect hypothesis has been postulated, tested and elaborately discussed for the case of Lyme borreliosis in North American ecosystems (Norman et al., 1999; Ostfeld and Keesing, 2000). Lyme borreliosis ecology in North America, however, differs from the European situation. In general, *Borrelia burgdorferi* sensu stricto (s.s.) is the only *Borrelia* genospecies in North America (Kurtenbach et al., 2006; Stanek et al., 2012; but see Pritt et al., 2016) and is transmitted to ticks by few reservoir species. In contrast, in Europe, there are multiple distinct pathogenic *Borrelia* genospecies, which are associated with specific ranges of hosts, and most of the host communities probably consist of multiple reservoir species, that transmit one or several *Borrelia* genospecies (Craine et al., 1995; Estrada-Peña et al., 2016; Rauter and Hartung, 2005; Tälleklint and Jaenson, 1994). Recently, Hofmeester et al. (2017) and Ruyts et al. (2016) have indirectly tested the dilution effect hypothesis for the different *Borrelia* genospecies in Europe. They found indications that Lyme borreliosis risk can differ

between different forest types. Nymphs were more likely infected with *B. afzelii* in pine forests than in oak forests, independent from the density of nymphs, and infections with other genospecies tended to occur more often in oak forests. A higher diversity of host species in oak forests might have diminished the influence of small rodents to infect ticks with *B. afzelii*, the most common *Borrelia* genospecies found in ticks in many parts of Western Europe (Rauter and Hartung, 2005; Ruyts et al., 2016). We thus expect in this study to see a dilution effect for *B. afzelii* in oak forests, caused by a lower proportion of rodents in the host community in oak forests compared to pine forests. However, the relations between forest types, host community composition and disease risk have not been adequately studied so far. This research gap contributes to the difficulty of verifying the validity of the dilution effect hypothesis for Lyme borreliosis in Europe.

Our study aims to unravel the relationship between host community composition and Lyme borreliosis risk. The risk for human exposure to Lyme borreliosis depends on the density of host-seeking infected ticks (acarological risk) and on the human-tick contact rate (Jaenson et al., 2009). Here, we study acarological risk and test the dilution effect hypothesis for *B. afzelii* in a European context. This study investigates the impact of the proportion of dilution hosts in the host community on the density of ticks infected with *B. afzelii* and identifies the key drivers determining the density of infected ticks, a widely applied disease risk measure (Ogden and Tsao, 2009). In this study, we use empirical models to investigate relationships in the field and a literature-based Bayesian belief network model to (1) gain more insights into the mechanisms that drive these field observations in different forest types, and to (2) position our study within the full range of conditions that can be observed in the field.

2. Methods

2.1. Study site

Questing ticks were already collected in the framework of other studies that investigated the risk of tick-borne pathogens in northern Belgium (Ruyts et al., 2016; unpublished data). We focused on forest stands of four forest types in study sites Averbode-Hertberg (AH) and Postel (P), located at a distance of approximately 32 km apart. The stands were dominated by oak (*Quercus robur* L.) or pine (*Pinus sylvestris* L. or *Pinus nigra* Arnold subsp. *laricio* (Poiret) Maire), and did (> 50% of the forest ground covered with shrub layer) or did not (< 25% of shrub layer) contain a well-established shrub layer. In site AH, we investigated 10 stands: two pine stands without a shrub layer, three pine stands with a shrub layer, three oak stands without a shrub layer, and two oak stands with a shrub layer. In site P, we sampled nine stands: three pine stands without a shrub layer, three pine stands with a shrub layer, and three oak stands with a shrub layer. Oak stands with a shrub layer are supposed to contain the highest host diversity, and pine stands without a shrub layer supposedly the lowest (Carnus et al., 2006; Du Bus de Warnaffe and Deconchat, 2008). The stands ranged from 0.5 to 4 ha and were on average 1 ha large (for the exact description of the study area and composition of the stands, see Ruyts et al. (2016)). In the center of each stand, we chose a sampling plot of 0.2 ha.

2.2. Data collection

2.2.1. Ticks

Ticks were collected by dragging a 1 m^2 flag through the vegetation along six transects of 25 m in the sampling plot in each stand in June, July and September 2013 and 2014. In 2013, we visually estimated the total amount of larvae attached to the flag at the end of the six transects, while in 2014, we collected all nymphs attached to the flag at the end of each transect. We used forceps to remove nymphs from the blanket and stored them in vials containing 70% ethanol at − 22 °C. Nymphs from the different sampling occasions were pooled per forest