



Host Versus Habitat: *Borrelia burgdorferi* sl in the Wild

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ABSTRACT *The conditioning role of habitat/biodiversity for disease development and course became lately a prominent issue for scientists. Pathogens and/or parasites are dependant on their host species therefore the increased variety of species in a habitat would "dilute" the number of efficient reservoir hosts and thus, pathogen/parasite expansion and disease transmission amongst wildlife. Lyme disease is perpetuated by enzootic maintenance cycles with multiple hosts amid reptiles, birds, and mammals, occurring simultaneously, with a peculiar pathogenesis and persistence of the bacteria in the wild. Nevertheless, the habitat, including the diversity of its specific living community, where the interaction between the agent and the host is placed, directs the outcome towards dilution or amplification.*

*Active interference of the agent and host in a specific environment delineates the persistence, transmission and infectivity of *Borrelia burgdorferi* sl. Although specific association between several closely related *Borrelia* spp and particular host and/or vector species exist, loose associations in transmission cycles in nature should be defined and considered. Decline in biodiversity diminishes the dilution effects exerted by high variability of host species in a habitat, therefore the conservation of natural ecosystems by compulsory solid systems of surveillance could provide protection against emerging pathogens by fostering biodiversity and also reducing human/domestic animals - wildlife contacts.*

Introduction

Lyme borreliosis agents, a group of tick-borne spirochaetes of genus *Borrelia*, proved to be during the last three decades an intriguing and rapidly growing complex, found in ticks as vectors and a wide range of hosts from the temperate regions of the world, including humans (Franke et al., 2013).

Interpreting the connections between ecology and development of infectious diseases for understanding the causes and consequences of diminishing biodiversity represent grand challenges in environmental sciences (Lips et al., 2004). Recent modeling of pathogens has shown that extinctions of rare species, caused by disease, may be common. The ability of pathogens to be transmitted between hosts is being expected to diminish as hosts perish. Wildlife populations have long been considered a link in the chain of pathogen emergence, by forming the potential reservoirs for zoonoses (Bengis et al., 2004; Fritz, 2009). Although most of the emerging human infectious diseases are zoonotic, some could be induced by microbial agents persistent in wildlife hosts, which rarely or never develop the disease (e.g. vector-borne diseases such as Lyme disease). Anthropogenic changes to wildlife habitat and subsequent close interaction of wildlife with civilization could increase the flow of pathogens from wildlife reservoirs and thereby foster disease emergence in humans. Similarly, habitat and behavioral changes in wildlife could lead to changes of the host-parasite relationships for vector-borne diseases (Daszak et al., 2001).

The impact of dilution effect attributed to biodiversity was promoted by several studies on vector-borne diseases (Schmidt and Ostfeld, 2001, Bouchard et al., 2013), suggesting that the more numerous the species in a habitat were, the

less pronounced the dispersal of *Borrelia burgdorferi* sl could be, therefore, the preservation of habitats, including inhabitant species at their highest variety would exert a zooprophylactic effect (Humair and Gern, 2000). Thus, the increase in numbers of non-competent hosts versus transmission-competent ones in the same habitat would logically lead to diminished numbers of infected ticks.

Nevertheless, loose epidemiological cycles, including secondary hosts or transmission incompetent ones, where the vector augmentation and/or pathogen amplification prevail should be also considered (Mannelli et al., 2012).

This review attempted an inventory of the diversity in *B. burgdorferi* sl host range and the ways it could influence the overlap between the roles of the key players: agent, host, and environment.

The agent

Lyme disease is caused by bacteria from the *Borrelia* spp., a species complex consisting of at least 19 different genomic species and several more strains which still need to be phylogenetically characterized (Franke et al., 2013) and is transmitted through ticks including four major Ixodes ticks (Sinski, 1999), *Haemaphysalis longicornis* and some soft-bodied ticks (Matuschka et al., 1998). The aetiologic agent was isolated and identified by various diagnostic tests, the disease was described and treated, and *B. burgdorferi* genome was sequenced. *B. burgdorferi sensu stricto* (ss), *B. garinii*, *B. afzelii*, *B. lusitaniae* and *B. valaisiana* were mentioned in questing I. ricinus ticks (Gray et al., 2002). While in Europe the spirochete of human Lyme disease are particularly diverse, including at least three genospecies (Baranton et al., 1992, Bykowski, et

al., 1993, Pfäffle M et al., 2011, Rudenko et al., 2011) as well as intragenospecific serotypes (Takano et al., 2010), in North America they appear to be limited to *B. burgdorferi sensu stricto* (Baranton et al., 1992, Franke et al., 2013). In Asia, *B. burgdorferi sensu stricto* appears to be absent (Masuzawa et al., 1996, Rosa et al., 2003, Hao et al., 2011). In subarctic and subantarctic regions only *Borrelia garinii* seems to be present, similarly to Europe, Russia, and Northern Asia. *Borrelia afzelii* is present in Western and Central Europe, and in Russia (Gray et al., 2002). The more close to the European center the investigated habitat is, the more intense the transmission would be (Olsen, et al., 1995).

Vectors

Various habitats influence the main vector tick species for spirochete. Thus, in Europe the principle tick vectors are *Ixodes ricinus* while *Ixodes persulcatus* (Nakao and Miyamoto, 1994a, Sato et al., 1996) is important in Eurasia and parts of Asia and *I. pacificus* and *I. scapularis* in North America. Australia has its specific *Ixodes* tick that is *Ixodes holocyclus* (Ostfeld et al., 2006).

Some of the ticks that host borreliae, although they do not infest humans, could contribute to persistence of human pathogenic species in transmission cycles (Franke et al., 2013). Besides the major epidemiological cycles, where the already mentioned *Ixodes* ticks act as vectors, very small enzootic cycles, involving a narrow host range and other tick species should be also considered. Furthermore, *B. bissettii*, *B. garinii* and *B. afzelii* were associated with *I. hexagonus* and *I. canisuga* found in hedgehogs and foxes, respectively (Wodecka et al., 2010).

After biting an infected host in the larval or nymph stage, the ticks can ingest *Borrelia* spp. Once ingested, the bacteria remain in the mid gut till the next developmental stage in the ticks' life cycle (Bykowski et al., 1993). Three host ticks are needed for transmission that is the ticks spend each stage of their two year life cycle on one host (Littman et al., 2006). Nymphs and adult ticks are capable of transmission. *Borrelia* spp. are transmitted when an infected tick bites a new host and injects the bacteria into the blood or skin. The bacteria then disseminate from the injection site throughout the body (Bykowski et al., 1993). Different developmental stages of the vectors seem to carry different *Borrelia* genospecies, in concordance to feeding habits of previous stages (ie *B. afzelii* was more frequent in questing nymphs, while *B. burgdorferi* ss and *B. garinii* were identified in questing adults – Gern et al., 1998).

The seasonal influence seems to be more pronounced on the attachment rather than on the carriage process and the seasonal pattern occurs clearly in the case of birds (Eisen et al., 2004, Reed et al., 2003). The population ecology of ticks is fundamental to the spatial and temporal variation in the risk of infection by tick-borne pathogens. Biologically, these processes involve the physiological and behavioral response of ticks to temperature, humidity stress and day length leading to specific patterns of seasonal variations in population dynamics and host relationships (Randolph, 2004). The traits of the micro-environment connecting the tick with the host rather than exclusively the host define the ecological niche for borreliae (Kurtenbach et al., 2002)

The habitat

Human activities of various kinds induce dynamic changes to the environment at either small or large scale, subjecting various ecosystems to transform. Tick population persistence along with the infectious agents they carry as well as host species are subject to adjustment to the numerous abiotic and biotic factors that are continuously appearing and interacting, therefore changing the epidemiology in well-defined areas (Pfäffle et al., 2013).

Habitat fragmentation, habitat loss, introductions of invasive

species, and climate change all have direct impacts on the many wildlife species that move across reservation boundaries. These disturbances are also likely to affect parasite communities and wildlife health (Cross et al., 2007). The conservation of natural habitats can provide protection against emerging pathogens as it does not only foster biodiversity but also helps to reduce domestic animals- wildlife and human-wildlife contacts. A previously unacknowledged benefit of biodiversity is protection against infectious diseases (Morris 2011). Currently, numerous studies are available suggesting that the presence of a variety of species in a habitat leads to a decrease in the prevalence of infectious agents including that of Lyme disease (Humair and Gern, 2000, Schmidt and Ostfeld, 2001, LoGiudice et al., 2002, Ostfeld and LoGiudice, 2003, Zavaleta et al., 2004). Nevertheless, the paradigm is being validated, even for tick born diseases, only under certain circumstances, specific community structure rather than just biodiversity directing the outcome towards dilution or amplification (Randolph and Dobson, 2012). Research in tick-borne diseases seeks to determine which environmental and behavioural factors influence the risk of human infection and disease (Heyman et al., 2010). Answering these and other questions is of both major ecological and epidemiological relevance. Both vector and reservoir populations have to be present in an area for Lyme disease to develop. The modalities that regulate the maintenance cycle (e.g., climate, phytocoenosis, zoocoenosis, vector and host diversity and density, frequency of tick-host contact, vector and host infection rates, etc.) may vary geographically and temporally and therefore influence the epidemiology of the disease in these foci. Humans accidentally enter this cycle and represent dead-end hosts for the pathogen, similarly to incompetent reservoir-vertebrates and incompetent vector ticks, since the transmission cycle of *B. burgdorferi* sl is being interrupted (Barker et al., 1993).

Agent/ vector/ reservoir host relationships

The host, the infective agent, and the environment where their interaction takes place define the epidemiological chain in infectious diseases. The role each species from an ecosystem plays in the transmission cycle of Lyme disease is defined by the reservoir competence, based on susceptibility of the host to infection and its efficacy in transmitting the borreliae to feeding ticks (LoGiudice et al., 2002). Furthermore, population density of hosts and food resources play an important role in Lyme-disease risk, as indicated by long term studies (Ostfeld et al., 2006).

Borrelia infection is maintained for a long period of time in reservoir hosts. At present, only wild small rodents were shown to remain infected during winter, outside of the tick activity period until the following spring (Gray et al., 2002, Shih et al., 1995). In order to avoid the establishment of infection, the immune system of certain species, incompetent as reservoir hosts, would fight the bacteria. In such cases, the skin of the hosts can favor the transmission of spirochetes among co-feeding ticks (Sato et al., 1996). Similarly, co-feeding allows the transmission of spirochetes from infected to uninfected ticks on hosts such as sheep and deer without generalized infection (Humair et al., 2000).

Reservoirs for Lyme disease can be numerous species of mammals (Gorelova et al., 1995, Kazmierczak et al., 1988, Krafczy et al., 2008, Sinski 1999), birds (Duneau et al., 2008, Humair 2002, Kurtenbach et al., 1998, Kurtenbach et al., 1998) and lizards (Dubska et al., 2009, Ostfeld et al., 2006). Dogs are the only animals apart from humans that are reported to experience disease (Ostfeld et al., 2006). Transmission in dogs has also been reported to occur transplacentally or through blood, urine or milk (Littman et al., 2006, Cross et al., 2007). Recent models proposed at least three major ecotypes for *B. burgdorferi sensu lato* associated with different sets of vertebrate host species (Hanincova et al., 2002) and segregation of bird- and rodent-associated ecotypes of *B. burgdorferi sensu lato* in individual questing ticks (Hanin-

cova' et al., 2003).

The main vector of *B. burgdorferi* s.l in Europe is the tick *Ixodes ricinus*, and mammals such as small rodents (*Apodemus*, *Clethrionomys*, *Microtus*) (Kurtenbach et al., 1998, Nakao and Miyamoto, 1994b, Nakao et al., 1995, Sinski 1999), rats, squirrels, dormice, shrews, hedgehogs, hares, and birds such as ground-foraging passerines and pheasants are well-known reservoirs. Rodents preferentially transmit *B. afzelii* to ticks while some songbird species (Hanincova' et al., 2003, Taragel'ova et al., 2008), seabirds (Staszewski et al., 2008), and pheasants (Kurtenbach et al., 1998) are reservoir hosts of *B. garinii* and *B. valaisiana* (Hanincova' et al., 2002, Humair et al., 1999). Some studies supported specific associations between *Borrelia* species and reservoir hosts, such as *B. afzelii* and *Apodemus* (Amore et al., 2007) mice and *Clethrionomys voles* (Humair et al., 1999), between *B. burgdorferi* ss, *B. afzelii* and squirrels (Eisen et al., 2004), and between *B. garinii*, *B. valaisiana* and birds such as *Turdus* passerines and pheasants (Dubska et al., 2009, Kurtenbach et al., 1998, Slowik et al., 2001).

In North America, Lyme disease is perpetuated by enzootic cycles involving rodents, such as white footed mice (*Peromyscus leucopus*) and is transmitted to humans and other vertebrates by *Ixodid* ticks (*Ixodes scapularis* and *Ixodes pacificus*). Several species of mammals serve as reservoirs for *B. burgdorferi* sensu lato (s.l.), i.e. by maintaining a spirochetal infection over time and infecting feeding ticks. They include the dusky-footed woodrat (*Neotoma fuscipes*), the California kangaroo rat (*Dipodomys californicus*), and three species of *Peromyscus* mice (Casher et al., 2002).

Although lizards were considered spirochete incompetent hosts, diluting the transmission of borreliae (Matuschka et al., 1991, Matuschka et al., 1992), more recent studies carried out on green lizards (*Lacerta viridis*), Balkan wall lizards (*Podarcis taurica*), and sand lizards (*Lacerta agilis*) strongly suggested that lizards, particularly *L. viridis*, could be important hosts for *I. ricinus* larvae and nymphs and therefore could be regarded as reservoirs of these important pathogen vectors (Földvári et al., 2009). Other researchers demonstrated host preference of some *Borrelia* spp, eg. *Borrelia lusitaniae* for lizards (Richter et al., 2006). *Psammotromus algirus* was able to sustain *B. lusitaniae* infection and to infect attached ticks, thus being a reservoir host competent to further transmitting the agent (Dsouli et al., 2006).

Differences in competence were observed in various lizard species. A model based on the host's ability to influence the prevalence of *B. burgdorferi* ss in the tick population indicated that five-lined skinks (*Eumeces fasciatus*) were dilution hosts, which eastern fence lizards (*Sceloporus undulatus*) were not. Thus, by incompetence as reservoirs, defined as high population densities and moderate larval tick burden, *E. fasciatus* could reduce the prevalence of the infection and associated zoonotic risk (Giery et al., 2007, Swanson et al., 2007). According to Wright et al. (1998), the western fence lizard (*Sceloporus occidentalis*) and the southern alligator lizard (*Elgaria multicarinata*) harbour a borreliacidal factor in their blood that renders them incompetent reservoirs of *B. burgdorferi* s.l. Southeastern five-lined skink (*Eumeces inexpectatus*) and the green anole (*Anolis carolinensis*) were susceptible to *B. burgdorferi* infection and remained infectious to attached ticks for at least five weeks, while *Borrelia burgdorferi* recovered from infected lizards remained infective for mammalian hosts (Levin et al., 1996).

Taxonomic differences condition the host response to borreliae. Recent investigations in Aves indicated the resident songbirds as transmitters of numerous genotypes of *Borrelia*, but meanwhile as selective amplifiers. Moreover, songbirds could simultaneously transfer more than one genotype of spirochete (Heylen et al., 2013a, Norte et al., 2013). These results stress the importance of genotype-host associations.

Further experiments pointed out that transmission cycles could be preserved by bird-specific ticks, with *I. ricinus* bridging the gap to outer hosts (Heylen et al., 2013).

Studies carried out on members of Reptilia and Mammalia (common wall lizards, *Podarcis muralis* Laurenti) and *Apodemus* spp. mice, respectively) indicated differences in infestation with developmental stages of larval and nymphal *Ixodes ricinus* stages. Nymphs that were present in high numbers in lizards tested positive for *B. lusitaniae*, whereas biopsy samples from mice remained negative (Amore et al., 2007). Furthermore, the western fence lizard (*Sceloporus occidentalis*) proved not only to be refractory to infection with *Borrelia burgdorferi* sensu stricto, but also to another member of the Lyme disease etiological complex, *Borrelia bissettii*, and caused the loss of its pathogenicity within 1 to 2 weeks after engorgement. The complement system of the lizard proved to be efficient in destroying the spirochetes in the feeding nymphs, diminishing the possibility of transmission to humans (Lane et al., 1998). As opposed to the western fence lizard, deer mice (*Peromyscus maniculatus*) developed a patent infection under similar experimental conditions (Lane et al., 2006).

Thus, the significance of host/parasite relationship involving one tick species and several host species is reinforced by the several maintenance cycles occurring simultaneously. In addition to specific cycles including certain host species and particular *Borrelia*, nonspecific maintenance cycles may occur, ie counting hedgehogs, since they were found infected by various genospecies (Pfäffle et al., 2011). The potential interaction of the three epidemiological actors of the disease chain (agent, host, environment) strictly conditions the outcome. The infection prevalence of the vector and its developmental stages control the transmission to the host, depending on the ecosystem. Geographic and temporal distribution of larvae and nymphs could be highly variable, depending on vector and hosts species (Gray et al., 2002).

Broad circulation of humans, widespread commerce with domestic and wild species, changes of habitats and global climate led to a broader spreading of parasite vectors and carried microbes (Matuschka et al., 1994). Recently, researchers reported isolation *Borrelia turcica* and unknown *Borrelia* species from imported reptiles (exotic tortoises) and their ectoparasites, which suggested these tortoises could serve as reservoir hosts. In parallel, the isolation of *Borrelia* strains from hard-bodied ticks, *Amblyomma* ticks and *Hyalomma* ticks, reaching the salivary glands, plead for the potential vector role for these ticks (Takano et al., 2010).

Little information is available on the real meaning of most vertebrate hosts as sources for *B. burgdorferi* s.l. in spite of over 200 species which could host *I. ricinus* and could therefore serve as potential reservoir hosts for *Borrelia* (Humair and Gern, 2008, Rudenko et al., 2011). Dilution effect was defined as a reduction in transmission of the infective agent by maximum densities, a diversity of species which could cause a competition with primary reservoir hosts, low attachment rate of ticks, variable competence of different host species (Mannelli et al., 2012). Using Lyme disease as an example and exploring the dilution effect, it was demonstrated that the prevalence of *Borrelia burgdorferi* in field-collected *Ixodes* ticks was dramatically lower than expected if ticks fed predominantly on highly competent reservoirs. These results highlighted the critical role of biodiversity along with host community ecology in the transmission of Lyme disease with important consequences for human health (Schmidt, 2001, Moore et al., 2010) therefore a solid system for surveillance is mandatory (Salomon et al. 2005).

Conclusions

Active interference of the agent and host in a specific environment delineates the persistence, transmission and infectivity of *Borrelia burgdorferi* s.l. Although specific association

between several closely related *Borrelia* spp and particular host and/or vector species exist, loose associations in transmission cycles in nature should be defined and considered. Decline in biodiversity, due to high variability of host species in a habitat along with the perpetually changing environment, directs the infectious pressure towards dilution or augmentation. Therefore, the conservation of natural ecosystems in their wholeness, through compulsory solid systems of surveillance, could provide protection against emerging

pathogens by fostering biodiversity and also reducing human/domestic animals - wildlife contacts.

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