

# Lyme Disease: Vectors and Reservoirs

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## INTRODUCTION

Lyme disease, also termed Lyme borreliosis, is caused by tick-transmitted spirochetes classified into the *Borrelia burgdorferi* species complex (*B. burgdorferi sensu lato* (s.l.)) [1], which is composed by at least nineteen genospecies distributed in North America, Europe and Asia (Table 1) [2,3]. *B. burgdorferi sensu stricto* (s.s.) constitute the main etiological agent of the disease in North America, whereas *Borrelia garinii*, *Borrelia afzelii*, *Borrelia spielmanii* and *Borrelia bavariensis* also occur in Europe or Asia (Table 1). The Center for Disease Control and Prevention (CDC) record about 30,000 cases of Lyme disease each year in the USA, however most of the cases are not reported, leading to estimates of a 10-fold increase in the incidence of the disease [3,4]. Moreover, it is estimated that 85,000 cases occur in Europe each year, but follow up of the incidence of the disease is difficult due the absence of a uniform reporting system [3]. Genospecies of these pathogens as well as their tick-vectors present particularities such as

predilections to specific hosts or even elicitation of different clinical manifestations, rendering complex mechanisms of maintenance, transmission and interactions between pathogens, vectors and hosts [5]. In view of those facts, diverse aspects of vectors and reservoirs of Lyme borreliosis should be considered to understand the dynamics of disease transmission and innovate guidelines for diagnosis, control and prevention of infections with *B. burgdorferi* s.l. In this chapter we will explore features from vectors, reservoirs and non-reservoir hosts that are implicated in the risk of transmission to humans and incidence of the disease such as distribution, ecology, epidemiology, seasonality and other factors with relevance for the comprehension of this vector-borne disease.

## IXODES TICKS: THE VECTORS OF LYME DISEASE

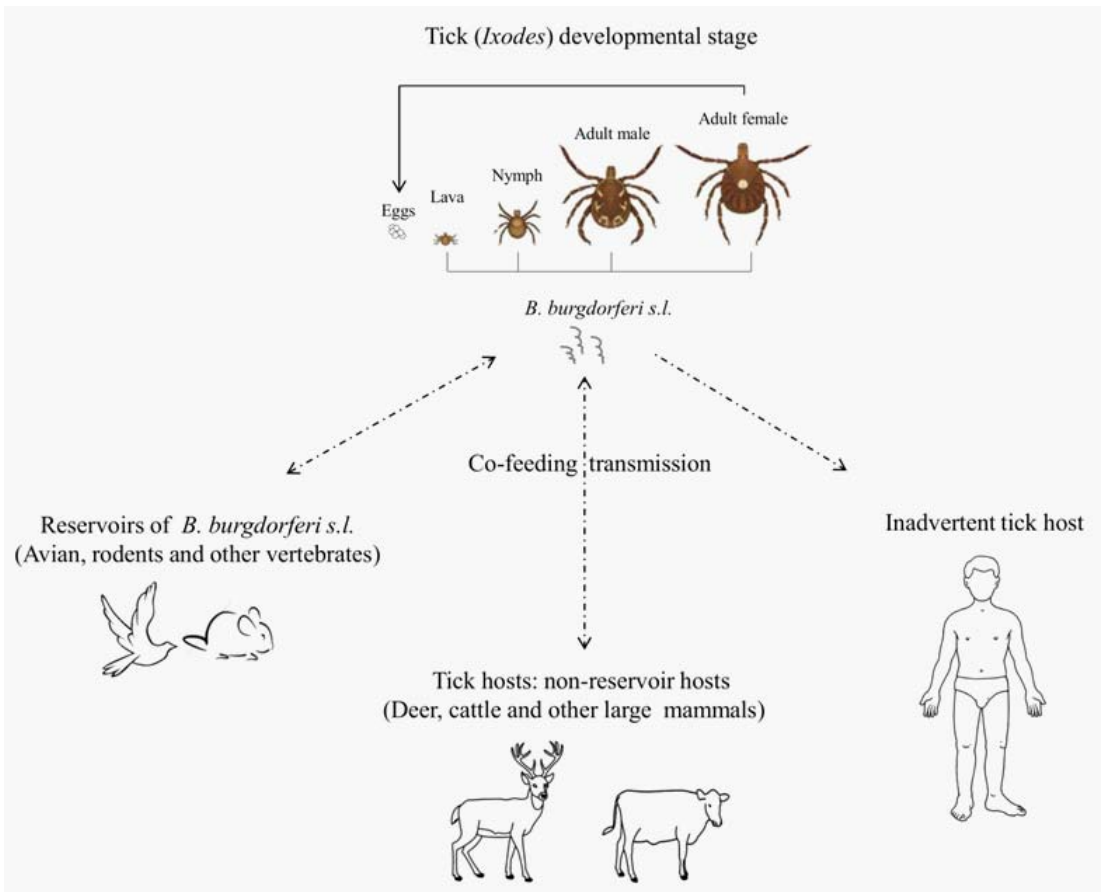
Competent vectors of Lyme borreliosis include hard ticks belonging to the genus *Ixodes*, whereby the species of ticks responsible for transmitting the pathogens are distributed according to geographical locations [6]. Indeed, the main vector of Lyme borreliosis in the upper Midwestern and northeastern of USA is *Ixodes scapularis*, whereas, *I. pacificus* has been found to be the vector in the western of USA [1].

Furthermore, *I. ricinus* is the main vector of the disease in Europe and to a less extend in Asia [2], while *I. persulcatus* is the tick transmitting Lyme borreliosis in most of Asia [3] (Table 1).

**Table 1:** Vectors and reservoirs associated with Lyme disease worldwide [1,3].

Vectors	Reservoirs	<i>B. burgdorferi</i> s.l.	Geographic Distribution
<i>Ixodes pacificus</i> ; <i>I. ricinus</i> ; <i>I. scapularis</i>	Avian species; Rodents	<i>Borrelia burgdorferi</i> s.s.	Europe; USA
<i>I. persulcatus</i> ; <i>I. ricinus</i>	Rodents	<i>B. afzelii</i>	Asia; Europe
<i>I. persulcatus</i> ; <i>I. ricinus</i>	Avian species; Rodents	<i>B. garinii</i>	Asia; Europe
<i>I. persulcatus</i> ; <i>I. ricinus</i>	Rodents	<i>B. bavariensis</i>	Asia; Europe
<i>I pacificus</i> ; <i>I minor</i>	Birds	<i>B. americana</i>	USA
<i>I. dentatus</i>	Rabbit	<i>B. andersonii</i>	USA
<i>I. minor</i> ; <i>I. pacificus</i> ; <i>I. ricinus</i> ; <i>I. scapularis</i>	Birds; Rodents	<i>B. bissettii</i>	Europe; USA
<i>I. ricinus</i>	Rodents	<i>B. spielmanii</i>	Europe
<i>I. granulatus</i> ; <i>I. ricinus</i>	Birds	<i>B. valaisiana</i>	Asia; Europe
<i>I. ricinus</i>	Rodents	<i>B. lusitaniae</i>	Africa; Europe

To reproduce and develop, *Ixodes* ticks rely on blood-feeding in vertebrate hosts that include small rodents, birds, ungulates, humans, among several others (Figure 1). The life cycle of those arthropods is composed by four stages (egg, larvae, nymph and adult), whereas three blood meals are necessary to complete their cycle (Figure 1). *Ixodes* are heteroxenous parasites, meaning that these ticks usually feed in two or more hosts during their life cycle (Figure 1). Interestingly, male ticks rarely feed and never engorge, but they might be important to maintain the females attached to their hosts during blood-meals.



**Figure 1:** Developmental stages of *Ixodes* ticks and dynamics of transmission of *Borrelia burgdorferi* sensu lato to reservoir, non-reservoir hosts and inadvertent hosts. *Ixodes* ticks present four stages, which range from eggs to adults. During this process, ticks may become infected with spirochetes of the complex *B. burgdorferi* s. l. by feeding in previous infected reservoir hosts as rodents, birds and other vertebrates. Depending on the availability of hosts, ticks present preferences which include large mammals. Many of those present the innate ability to kill *Borrelia* spirochetes and thus, they are considered non-competent hosts, in other words, hosts that do not transmit the bacteria directly to vectors. Despite of this fact, those same hosts play a role in the dynamics of tick populations and transmission of *B. burgdorferi* s. l., as they may carry all stages of ticks, implicated on the co-feeding transmission process. Thus, depending of several factors, *Borrelia*-infected ticks occasionally encounters inadvertent hosts as humans, whereas the interface between pathogen, tick and host impacts the further development of Lyme disease.

The development of ticks from larvae into adult stages might take 2 to 6 years, which will depend on several environmental factors, availability of hosts and the effects of development-delaying diapause mechanisms [7]. Generally, the *Ixodes* life cycle begins with a vertebrate host passing through vegetation, such as leaves and grass that harbor newly hatched larvae. While questing (behavior of host seeking), larvae recognize biochemical signals such as rising carbon dioxide levels from potential hosts and climb to the skin of an animal, where they will attach with their specialized mouthparts, or even look for thinner locations in the host skin (Figure 1) [1]. Approximately 3 days after feeding, larvae detach from the host and molt (shed their outer skin layer) to become nymphs (Figure 1). Of note, after ticks drop off their host, they need a minimum relative humidity of 80% for survival. During this stage, the nymphs wait for a second host to begin a new blood-meal that takes about 5 days, following detachment from the host and molting to finally become adults (Figure 1). After attachment to hosts, female adults feed for about 7 days, drop off to lay the eggs and restart the cycle (Figure 1). Of importance, after detaching from hosts, ticks may

take several months to develop into specific developmental stages, or to lay the eggs in the case of engorged adult females, a feature that can influence the risk of infection and development of Lyme disease. Despite of evidences for transovarial transmission by *B. Miyamotoi* [8], acquisition of *B. burgdorferi* occurs by feeding on vertebrate hosts harboring the pathogens, in other words, the reservoirs of the disease. Once ticks become infected, they retain the bacteria through developmental stages (transstadial transmission), making them capable of transmitting the spirochetes during the next blood meal [3]. In addition, a mode of transmission known as co-feeding has been described. In this process, infected ticks that are feeding in close proximity to uninfected ticks in the same host are able to transmit the bacteria. Indeed, this was demonstrated in transmission of *B. burgdorferi* by *I. scapularis* [9,10] and *I. ricinus* [11], *B. afzelii* by *I. ricinus* [12] and *B. garinii* by *I. persulcatus* [13]. The importance of co-feeding is related to the potential for nymph-to-larva transmission events, whereby in Europe, those tick stages are searching and questing for hosts during the same periods ranging from spring to autumn [2]. In contrast, those two stages are highly active during different periods of the year in North America [14]. Because some large vertebrate hosts may carry all stages of ticks at the same time, they might play a role by providing a site for co-feeding transmission and amplification of the pathogen even though they may not be infected themselves [15]. After feeding on infected animals, spirochetes are present in the midgut of ticks and to a less extent in the hindgut and rectal ampulla [16]. Other studies identified the presence of the bacteria in the hemolymph [17] and latter in the salivary glands [18], however confirmation of the salivary gland route of transmission occurred after spirochetes were found in tick saliva [19]. Interestingly, when these pathogens are ingested by tick larva, they exhibit a fast multiplication rate until molting into nymphs, when the number of spirochetes drops [20]. However, after nymphs begin to feed, spirochetes also start multiplying and may increase > 300-fold during this period [21]. A study conducted by Piesman, J. [22] revealed that during

early tick feeding, spirochetes are occasionally detected in salivary glands, however sufficient numbers of bacteria that cause infection in experimental hosts were found to localize into salivary glands only 60 hours after tick attachment [22]. However, transmission of *B. burgdorferi* by *I. pacificus* or *I. scapularis* was shown to require a blood-feeding period of at least 36h [23,24]. In contrast, *B. afzelli* might be rapidly transmitted of by *I. ricinus*, in which transmission occurred 17h after ticks were attached to gerbils [25]. During early feeding stages, small populations of spirochetes expressing outer surface protein A (OspA) are present in salivary glands and in the skin of the host, however, those bacteria do not seem to be infective [26]. Following 2 days of blood feeding, two major populations of spirochetes appear in large numbers in both the salivary glands and in the feeding site at the host's skin: those expressing OspC and those that do not express OspA nor OspC [26]. Therefore, infection might be determined by the number infective bacteria, their phenotype regarding Osp while entering the host, or both [27]. Indeed, the expression of those proteins are consistent with their function, whereby OspA is implicated in the colonization of ticks, while OspC is required for infecting the host[28]. In this infection, bacteria multiply intensely and amplify in the host's skin [29], which thus function as a "barrier" to select distinct clones of the bacteria that further spread in the host [30]. After lacerating the epidermis, the tick mouth parts penetrate the dermis and form the hemorrhagic pool. Thus, the pathogens are inoculated, multiply and disseminate to target organs. After their removal or spontaneously detachment from host skin, a common sign caused by skin inflammation appears, which is denominated the erythema migrans [31]. Notably, about 25% to 30% of *I. scapularis* at the nymph stage are infected with *B. burgdorferi* s.l. in the northeastern of USA, however, only 1% to 2% of individuals bitten by *I. scapularis* become infected [27]. This low rate of infection may be explained by the fact that most of ticks are identified and removed before they transmit the pathogens. Studies about the dynamics of *B. burgdorferi* transmission by nymphs identified that transmission do not occur during the first day of feeding, which begin to improve in the second day of blood-feeding, whereby transmission is highly efficient during the third day after tick attachment [32]. Indeed, those observations correlate with multiplication, Osp switching and spread of spirochetes within the tick. Tick salivary glands constitute a site for development and replication of many pathogens, however, saliva play critical roles in promoting vector-borne diseases, as it contains several pharmacologically active molecules that facilitate tick feeding and consequently, pathogen transmission [33,34]. Besides their role in blood-feeding, salivary glands are also implicated in ion and water metabolism. In ticks that are not attached to a host, salivary glands help ticks to stay hydrated while waiting for a host. This process may take years and thus salivary glands produce hygroscopic saliva that is secreted on the surface of the hypostome, whereby atmospheric moisture is captured by the salty saliva and incorporated back into the body of the tick [34]. A complex cocktail of salivary components evolved to help the parasite to overcome their host's defense response against blood loss as for the mechanical injury caused by those arthropods, ranging from inflammatory reactions at the feeding site to inhibit blood flow as for stimulation of defensive behavior by the sensation of itching and pain [34]. Thus, tick

saliva is constituted with molecules that inhibit homeostasis; block pain and itch responses and behavior; influence angiogenesis and extra-cellular matrix remodeling; and modulate the innate and adaptive immune response [35,36]. Moreover, the expression of several families of proteins present in tick saliva is influenced by the developmental stage or even the host they feed-in [37]. Indeed, the phagocytosis and killing of *B. afzelli* by murine macrophages were impaired in the presence of salivary gland extracts from *I. ricinus* [38,39]. Furthermore, when naïve mice were inoculated with *B. burgdorferi* in the presence or absence of salivary gland extracts from *Ixodes*, it was demonstrated that those receiving the pathogens along with salivary gland extracts presented with a higher burden of spirochetes in several tissues [40,41]. In addition, a study showed that the ID50 of *B. burgdorferi* in tick salivary gland was 10 times lower than in the tick midgut [42]. Taken together, those studies indicate that tick salivary components enhance pathogen transmission and, in a natural context of infection with *B. burgdorferi* s.l., it is conceivable that saliva might even be necessary for the development of Lyme disease.

## RESERVOIRS OF *BORRELIA BURGDORFERI* SENSU LATO

As ticks are hematophagous ectoparasites, they depend on the uptake of blood from hosts to develop and complete their lifecycle. As discussed in the previous section, during the course of the blood meal, ticks inoculate their saliva into the host skin, whereas this interface presents with several symbiotic, commensal or pathogenic microorganisms (such as virus, protozoan and bacteria) harbored by the ticks [37,43,44] and/or its host [45,46]. As a consequence, many of these tick hosts can serve as reservoirs of several microorganisms, including *Borrelia* spirochetes [47] (Table 1). In this section we will discuss the role of *Borrelia* reservoirs, which are determined by the capacity of some vertebrate animals such as rodents, sheep or birds to accommodate species of *Borrelia* without developing symptoms or disease, as well as for their ability to disseminate these spirochetes to vectors (ticks from the genus *Ixodes*), which transmit the pathogens to other hosts [48,49]. *Ixodes* ticks can feed in more than three hundred different animals, including small and large mammals as mice, chipmunks, shrews, deer (*Odocoileus heionus*, *Odocoileus virginianus*, *Capreolus capreolus* and *Cervus elaphus*), cattle and sheep; as well as several birds, lizards and skinks [50–55]. However, these ticks appear to have preferences for certain hosts, which vary geographically worldwide. In addition, few of these hosts can be considered reservoirs of *Borrelia* spirochetes. Among the reservoirs of *Borrelia* spirochetes that cause Lyme disease we highlight the white-footed mouse (*Peromyscus leucopus*), which is considered the main competent reservoir of *B. burgdorferi* in North America, followed by chipmunks and shrews [51]. Therefore, in regions where white-footed mice are abundant, those small mammals are the main contributor to prevalence of *Borrelia*-infected ticks [56,57], which may indicate a high risk for Lyme disease. However, if *P. leucopus* coexists with other potential reservoirs, as the chipmunks and shrews, the number of ticks that acquire the spirochetes from white-footed mouse is significantly decreased [56,58,59]. In contrast, there are evidences that in the western United States, the *Ixodes* ticks display different feeding behaviors, where the immature stages of *I. scapularis* are found preferentially

feeding on lizards, skinks and/or western gray squirrel (*Sciurus griseus*) [52,53,60,61]. Therefore, the vast majority of infected ticks acquire *Borrelia* spirochetes from the above mentioned hosts, whereas the dynamics of their population dynamics is directly associated with the prevalence of Lyme disease in the United States [59]. In Europe, *B. burgdorferi* s.l. is maintained into the several species of wild animals, such as mice, voles, squirrels, woodland birds and pheasants [62-65]. *B. afzelii* is frequently detected in small rodents, while *B. garinii* and *B. valaisiana* can be detected in some rodents, but they are mainly prevalent in birds [66,67], including several species of seabirds that may be important contributors to the expansion of these bacteria [68,69] (Table 1). On the other hand, several large mammals serve as tick hosts, but they are not considered competent reservoirs for *B. burgdorferi* s.l., due their innate ability to kill the bacteria. Those animals include: large hoofed mammals of the family Cervidae as the white-tailed deer, mule deer, roe deer, red deer and other deer species, as well as sheep and cattle [70-75]. Despite of that, these non-competent hosts are essential for the maintenance of tick populations due to a high burden of all tick stages that they are able to accommodate during repeated infestations. Furthermore, these non-competent hosts can facilitate the co-feeding transmission process, and therefore, their prevalence in determined regions is directly associated to infections of ticks with *B. burgdorferi* s.l. [15].

## RISK OF EXPOSURE TO TICKS AND DEVELOPMENT OF LYME DISEASE

The risk assessment is an essential strategy for the implementation of measures to prevent Lyme disease. A key factor that contributes to the prevalence of Lyme disease in a given region is the high number of reservoirs of *B. burgdorferi* s.l., whereby infection with those pathogens do not influence their survival and might even persist for their entire life [56,58,76-78]. The increase of the reservoirs are favored by harmonic interaction between ticks and vertebrate hosts, since nymphs infected with *B. burgdorferi* s.l. can infect 90% of the vertebrate hosts which become a reservoir for life [51,77]. Added to this, approximately 95% of ticks fed in a reservoir become infected and, therefore, can infect new hosts as mentioned previously [57]. High risk of Lyme disease is increased in areas that exhibit the simultaneous presence of tick populations infected with pathogenic strains of *Borrelia* and of their reservoir hosts, which can be either peri-urban or rural areas, especially those that are used for forestry and recreational activities [79]. Although the presence of many non-competent reservoir hosts as cattle and deer facilitates the co-feeding transmission process, high numbers of those hosts also promote the decrease the risk of Lyme disease, because ticks present preferences for those hosts when compared to inadvertent tick hosts, as humans. In view of those facts, some studies propose the vaccination of reservoirs of *B. burgdorferi* s.l. as an ecological approach to prevent infections of humans with these bacteria and development of Lyme disease. [56,80].

Overall, the presence of *Borrelia*-infected ticks; the pathogenicity of the spirochete strains; the high density of reservoir hosts to maintain and transmit the bacteria to ticks; the availability of these reservoirs for questing ticks and, the low number of non-competent reservoir hosts in a given region are indicative of high-risk situations for Lyme disease [3,49]. Therefore, beyond the strategies of control for both ticks and reservoirs of *B. burgdorferi* s.l., several factors as knowledge about factors associated to development of Lyme disease and behavior of people in high-endemic areas are critical for prevention of the disease [1]. As *Ixodes* ticks can take up to 6 years to reach its final developmental stage [81], they are able to acquire and transmit *B. burgdorferi* s.l. to several hosts during this period. Therefore, preventive actions such as the correct use of acaricides combined with a constant maintenance of the local vegetation and delimitation of tick competent and non-competent reservoirs are fundamental to control and reduce tick populations and the incidence of Lyme disease. Also, other strategies as avoiding environments with a high activity of ticks, use of tick repellents, covering and conducting inspections of the entire surface of the skin to find possible ticks and, their complete removal from skin are recommended to prevent and reduce the risk of the transmission of Lyme borreliosis [82-84].

## THE EFFECTS OF CLIMATE CHANGES AND SEASONALITY OF VECTORS OF LYME DISEASE

Additional factors such as climate changes and environmental seasonality are also associated with incidence of Lyme disease. Recent studies shows that climate changes affect vector-borne diseases because arthropods (vectors of numerous diseases, including the Lyme borreliosis) are ectothermic and, therefore, dependent widely on the environmental temperature [85]. Regarding the Lyme disease, changes in temperature influence the reproduction and activity of *Ixodes* ticks and, consequently, the incidence of this disease [85-87]. Recently, it was shown that climate warming increases the rate of reproduction of ticks, which increases their population and favor the spread of *B. burgdorferi* s.l., leading to an elevated risk for transmission of those pathogens to humans [85]. Moreover, there are strong evidences that transmission of *B. burgdorferi* s.l. is affected by seasonality, reinforcing the role of temperature in this process [14,87]. Also, climate changes might influence the geographical distribution of ticks, which have been reported in regions where those arthropods were not present before [85]. Importantly, it is known that the peak of the activity of tick nymphs begins in the spring and intensifies in early summer, coinciding with both the increase of tick host activities and outdoor recreational activities for humans, especially the younger and older people [88-90]. For this reason tick nymphs are considered the most important stage for transmission of Lyme disease. Since the climate changes can also affect the seasonality of questing nymphs by extending their active period during autumn and winter, the risk of infection and transmission of *B. burgdorferi* s.l. can also increase and, consequently the seasonality patterns of the incidence of Lyme disease may also be subjected to changes [87,91]. For instance, in Europe, nymphs and larvae of *I. ricinus* present intense activities during late spring until early summer, followed by a second period in the autumn, while in northeastern of



USA, the *I. scapularis* larvae peak in late summer until early autumn [14]. Moreover, adult stages of *I. pacificus* become active during the autumn followed by a peak in the winter, while the larvae and nymphs become active during early spring and extends until the autumn [92].

## SUMMARY

In this chapter, we highlight that the spreading of Lyme disease is based on tick's population and various interactions between *B. burgdorferi* s.l., *Ixodes* ticks and reservoir and non-reservoir hosts. In view of that, detailed investigations focused into the biology and interactions between pathogens, vectors and their hosts are required to prevent and innovate further aspects as treatment of Lyme borreliosis. The distribution and abundance of ticks depends on the presence of a proper environment for tick development as vegetation type and the presence of hosts. Moreover, abiotic factors such as the climate influence the development of ticks in short and long terms. Moreover, the preference of ticks for particular hosts, as the balance between reservoir and non-reservoir hosts might account for the risk of infection with *Borrelia* spirochetes. Taken together, the information presented here point for the need of a global overview into factors influencing vectors and reservoirs by combining diverse strategies, ranging from genomics to ecological perspectives, as well as insights into the immune response at the interface of *B. burgdorferi*, ticks and hosts.

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