
Life Cycle of *Borrelia burgdorferi sensu lato* and Transmission to Humans

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Abstract

Lyme borreliosis is a zoonosis: its causative agent, *Borrelia burgdorferi sensu lato*, circulates between *Ixodes ricinus* ticks and a large variety of vertebrates. *I. ricinus* has a wide geographical distribution throughout Europe within the latitudes of 65° and 39° and from Portugal into Russia. Enzootic cycles in Europe involve at least 7 *Borrelia* species. Apparently, associations exist in nature between *Borrelia* species and hosts. *B. afzelii* and *B. burgdorferi sensu stricto* are associated with rodents, and *B. garinii* and *B. valaisiana* with birds. *B. lusitaniae* may be transmitted to ticks by some lizard species and birds. *B. spielmanii* appears to be associated with dormice and hedgehogs. Less strict associations also exist. Transmission of *Borrelia* infection by *I. ricinus* to their hosts, including humans, does not occur immediately when ticks attach to host skin. A delay is observed, which may depend on the *Borrelia* species infecting the tick. *B. afzelii* can be transmitted during the first 24 h, whereas *B. burgdorferi* needs 48 h of tick attachment before its transmission begins. Nothing is known about the other *Borrelia* species; however, success of transmission always increases with tick attachment duration. Therefore, careful visual examinations of the body for at least 2 successive days are recommended after visiting an endemic area.

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Among diseases due to vector-borne pathogens in Europe, Lyme borreliosis, which is transmitted by the tick *Ixodes ricinus*, is the most widespread and has a big impact on human health. Lyme borreliosis is a zoonosis: its causative agent, *Borrelia burgdorferi sensu lato* (s.l.), circulates between ticks and a large variety of vertebrates that act as hosts for ticks. By acquiring the infection through infected tick bites and by developing clinical manifestations of Lyme borreliosis, humans reveal the presence of the microorganism in various geographical areas. Humans are not involved in the transmission cycle of *B. burgdorferi* s.l. in nature. They act as dead-end hosts.

Biology of *I. ricinus*

I. ricinus has a very wide geographical distribution throughout Europe. It has been found within the latitudes of 65° and 39° and from Portugal into Russia, and also in North Africa (Tunisia, Algeria and Morocco) [1]. In continental Europe, *I. ricinus* is mainly present in deciduous woodlands and mixed forests. Ticks colonize biotopes offering a high relative humidity. In fact, *I. ricinus* only survives where the relative humidity in its microhabitat does not fall under 80%. The duration of its life cycle can vary regionally and from one habitat to another, and can be affected by climatic factors and host density. The large geographical distribution of *I. ricinus* implies that this tick has to survive under various environmental conditions, i.e. throughout this large geographical area, temperatures vary considerably. Since temperature is known to have an effect on tick questing activity and on tick development rates, it is an important parameter in the dynamic of seasonal activity. Several papers described that the seasonal activity of questing *I. ricinus* presents different patterns under different climatic conditions. This seasonal activity pattern may be unimodal with a major peak of tick activity in spring or in winter, or may be bimodal with 2 peaks of tick activity, one in spring and another one in autumn [2]. This is important information because seasonal questing activity of *I. ricinus* influences the risk of being bitten by ticks, geographically and temporally.

The vertical distribution limit of *I. ricinus* differs throughout Europe according to geographical position. However, recently many studies reported a shift in this limit to higher altitudes, most probably due to the increase in temperature observed during these last decades [2]. Interestingly, due to the vertical distribution limit observed in tick distribution, it is frequently believed that the higher the altitude, the less ticks. This should not be considered as a rule; various reports have recently shown that in some habitats the opposite has been observed: the higher the altitude, the more ticks. However, it is important to note that the tick densities described at the highest altitudes were usually rather low.

In many aspects, ticks differ from insects. One way is that each of their developmental stages (larvae, nymphs and adult females) feeds once on a host, and this lasts for several consecutive days (fig. 1). Each blood meal is followed by a developmental phase, except for the females that will lay eggs after their blood meal and then die. Male ticks may take up a very small quantity of blood, but they never take large blood meals. The total duration of blood meals of *I. ricinus* is short, and does not last more than 12–20 days. Larvae feed for 2 to 4 days, nymphs for 4 to 6 days and females for 6 to 10 days. Ticks can survive for years in their biotopes; however, they spend only a small part of their life in a parasitic phase. Most of the *I. ricinus* lifetime is spent outside of the hosts, either on the ground or in vegetation. To find a host, *I. ricinus* climbs onto low vegetation and waits at the tip where they quest for a host for time-limited periods. During these periods of questing, *I. ricinus* ticks stay mainly immobile at the tip of the vegetation. When ticks are questing, they respond to mechanical and chem-

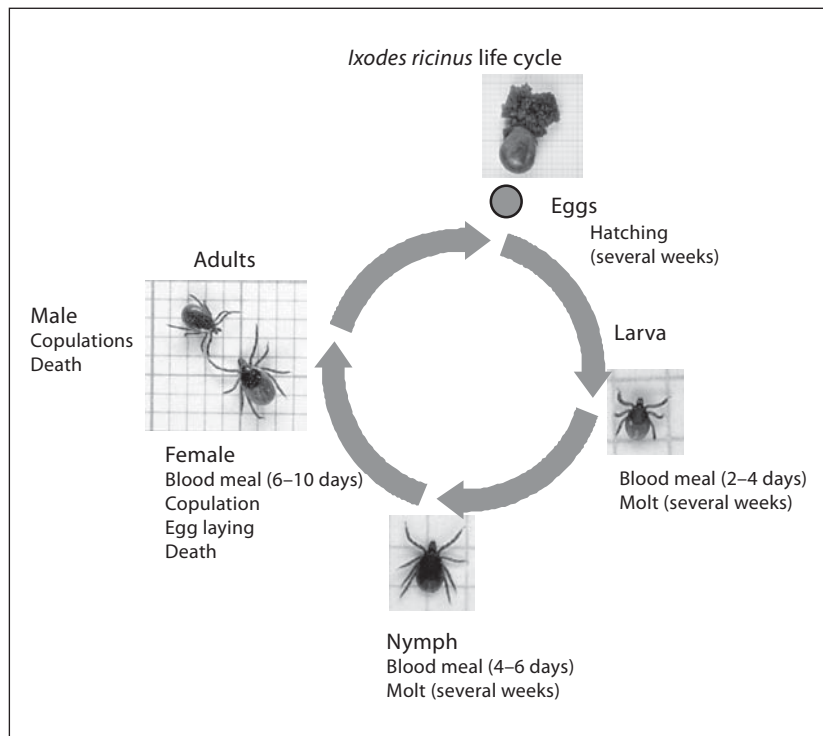


Fig. 1. Complete life cycle of the *I. ricinus* tick.

ical stimuli produced by hosts, including humans. When hosts pass close enough, questing ticks grab their hosts. This behaviour of *I. ricinus* is important since it implies that hosts, including humans, take some active part in the tick-host encounter.

During questing periods, *I. ricinus* often experiences desiccating conditions. As already mentioned, *I. ricinus* ticks are susceptible to desiccation when questing for hosts on vegetation, and high humidity is a prerequisite for tick survival. The atmosphere is often unsaturated, and this represents a net water loss for the ticks. Therefore, questing ticks have to rehydrate, and to do so they regularly leave their questing place and move to the litter zone. There, to maintain their water balance, ticks actively absorb water from the subsaturated atmosphere. High humidity is found at the base of vegetation, where ticks uptake atmospheric water. One aspect of the life cycle of ticks is that they do not have unlimited time to find their hosts. Indeed, their survival is limited by the amount of energy they gain with blood meals and by their ability to maintain their water content in a desiccating atmosphere. For example, if highly desiccating conditions develop, ticks reduce their questing duration and move more often to the soil to rehydrate; eventually, their energy reserves will run out before they find a host and they will die. In nature, abrupt declines in questing tick populations have been reported to coincide with abrupt increases in saturation deficit

(measurement of the drying power of the air that includes relative humidity and temperature) [3–5]. Long-lasting high saturation deficit may influence the evolution of seasonal questing tick density, and also impair tick population maintenance in some areas [2, 6]. If highly desiccating conditions are lasting and they coincide with tick questing activity period, tick populations may greatly suffer from this moisture stress and may be dramatically reduced. It was observed that under warmer episodes in spring and summer, when synchrony of weather conditions with the tick life cycle occurred – e.g. in spring, when many ticks quest and long-lasting highly desiccating conditions are present – questing duration was reduced and tick mortality was increased, leading to a lower questing tick population [4, 5].

Life Cycle of *B. burgdorferi* s.l.

At the time of its discovery in the beginning of the 1980s, the causative agent of Lyme borreliosis, *B. burgdorferi*, was thought to be a uniform organism. Currently, 12 *Borrelia* species are included in the complex *B. burgdorferi* s.l., and 7 of them have been reported in *I. ricinus* in Europe: *B. burgdorferi* sensu stricto (s.s.), *B. garinii*, *B. afzelii*, *B. valaisiana*, *B. lusitaniae*, *B. bissettii* and *B. spielmanii* [7, 8]. *B. bissettii* has been reported only once in *I. ricinus* ticks in Europe. This was in a report from Slovakia, where 1 tick was found to be reactive with probes specific for *B. bissettii* [9]; this tick was also reactive with probes for 2 other species of *B. burgdorferi*, which complicated the specific identification of the spirochetes present in this tick, and, as a result, the presence of *B. bissettii* in *I. ricinus* has to be confirmed by additional reports.

In Europe, *B. burgdorferi* s.l. has been reported from Italy to Iceland and from Portugal to Russia [7]. The reported mean rates of *B. burgdorferi* in *I. ricinus* are 1.9% for larvae, 10.8% for nymphs and 17.4% for adults [10]. Occasionally, higher infection rates have been reported, mainly using PCR, as for example in Portugal where *B. burgdorferi* DNA in *I. ricinus* ticks reached 75% [7]. Local and temporal variations in the infection prevalences of *Borrelia* in ticks have been recorded.

B. garinii and *B. afzelii* are the most frequent and most widely distributed species, whereas *B. burgdorferi* s.s. and *B. valaisiana* are less common [7]. *B. lusitaniae* presents an interesting geographical distribution. In fact, *B. lusitaniae*, first isolated from *I. ricinus* ticks in Portugal, has been reported in various European countries, for example Bulgaria, Portugal, Slovakia, Switzerland, the Czech Republic, Moldavia, Ukraine, Poland and Spain [1, 7]. Its presence has also been described in North Africa [11]. Interestingly, *B. lusitaniae* is very common and greatly exceeds the other species in *I. ricinus* ticks in Portugal and North Africa, whereas this *Borrelia* species is only sporadically reported in ticks from the other areas. Rauter and Hartung [7] in their meta-analysis give a detailed distribution of the main *Borrelia* species in different parts of Europe. However, it is important to repeat here that the distribution of the various species of *B. burgdorferi* s.l. and their frequency vary in endemic areas

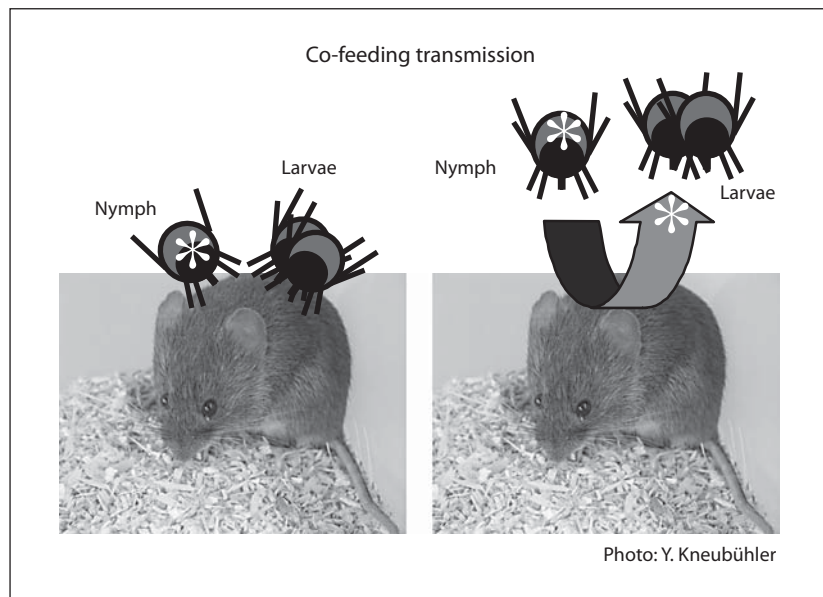


Fig. 2. Co-feeding transmission.

over time. For example, *B. afzelii* may alternatively dominate in an area with *B. garinii*. The recently described species, *B. spielmanii*, has been reported in *I. ricinus* from The Netherlands, Denmark, Hungary, Slovenia, Germany and France [8]. The reported geographical distribution of the different *Borrelia* species and their frequency, and especially of those which are less frequently reported, may greatly change in the future due to the implementation of more molecular analysis techniques.

Since, in some endemic areas in Europe, at least 6 *Borrelia* species may circulate, mixed infection with more than 1 species in ticks can be observed. Infections by multiple *B. burgdorferi* s.l. species have been observed in ticks in many parts of Europe [7]. Different combinations of mixed infections with 2 or 3 species have been detected in *I. ricinus*. *B. garinii* and *B. valaisiana* constitute the majority of mixed infections, followed by mixed infections with *B. garinii* and *B. afzelii*. Such mixed infections are reported less frequently than single infections, and are often detected by PCR methods. Rauter and Hartung [7], in their analysis of data collected throughout Europe, reported 13% mixed infections in *I. ricinus* ticks. These multiple infections may result from the feeding of ticks on a host infected by more than 1 *Borrelia* species or from infected ticks feeding simultaneously with uninfected ticks on a host and exchanging the *Borrelia* species through co-feeding transmission from infected to uninfected ticks (fig. 2) [12]. Moreover, ticks may acquire various *Borrelia* species through their successive blood meals on various hosts, and maintain the infection to the subsequent stage via transstadial transmission. Transovarial transmission of *Borrelia* from in-

fecting *I. ricinus* females to their progeny is also possible, but it represents a rare phenomenon [1]. Nevertheless, transovarially transmitted spirochetes may also contribute to mixed infections in ticks.

The efficient persistence of the borreliae in endemic areas requires the involvement of reservoir hosts. Potential hosts for ticks are numerous, and more than 300 vertebrate species have been identified as hosts for *I. ricinus*, including small mammals, birds, larger mammals and reptiles. Among these hosts, some act as blood meal sources and as reservoir hosts for pathogens, others as blood meal sources only. Natural hosts do not seem to develop clinical manifestations of the disease, although it is difficult to evaluate the impact of *Borrelia* infection on their health, and minor clinical manifestations may escape our attention.

Only a few dozen of the hosts for ticks have been currently identified as reservoir hosts for *B. burgdorferi* s.l. in Europe. Globally, little information is available on the real significance of most animal hosts as sources for infecting ticks with *B. burgdorferi* s.l. At present, several species of mice, voles, rats and shrews are recognized as reservoirs of *B. burgdorferi* s.l. in Europe [1]. In particular, it was evidenced that the mice *Apodemus flavicollis*, *A. sylvaticus*, *A. agrarius* and the vole, *Clethrionomys glareolus*, play key roles in the ecology of Lyme borreliosis as reservoirs for *B. burgdorferi* s.l. in many European countries. Once infected by an infectious tick bite, some reservoir hosts, like *Apodemus* mice, have been shown to persistently remain infectious for ticks. Small rodents are frequently parasitized by larval and nymphal *I. ricinus*, and this also contributes to their importance as reservoirs. Less information has been obtained on the roles of other small mammal species in the maintenance cycles of *Borrelia* in nature. Nevertheless, another species of vole (*Microtus agrestis*) in Sweden, and black rats (*Rattus rattus*) and Norway rats (*R. norvegicus*) in urbanized environments in Germany and in Madeira, may serve as sources of infection for *I. ricinus* ticks. Similarly, only few data have been collected on *B. burgdorferi* s.l. in shrews (*Sorex minutus* and *S. araneus* and *Neomys fodiens*) or in ticks attached on them. Observations in endemic areas of Germany and France showed that edible dormice (*Glis glis*) and garden dormice (*Eliomys quercinus*) are reservoir hosts for *Borrelia*. Other rodent species, like grey squirrels (*Sciurus carolinensis*) in the UK and red squirrels (*S. vulgaris*) in Switzerland, also contribute to the amplification of *Borrelia* in the tick population. Red and grey squirrels are usually very heavily infested with ticks, and 1 study reported a high prevalence of infection (69%) in ticks feeding on red squirrels. In other investigations in Ireland, Germany and Switzerland, it was reported that the European hedgehog (*Erinaceus europaeus*) also perpetuates *B. burgdorferi* s.l. [7]. In Switzerland, an enzootic transmission cycle of *B. burgdorferi* s.l. involving hedgehogs and another tick vector, *I. hexagonus*, has been observed in an urban environment. This shows that gardens can also represent zones at risk of Lyme borreliosis as further discussed below. Examination of the role of lagomorphs (*Lepus europaeus*, *L. timidus*, and *Oryctolagus cuniculus*) in the support of the enzootic cycle of *B. burgdorferi* s.l. has also elucidated their roles as reservoirs [1].

When attention was first directed at the role of birds in the ecology of Lyme borreliosis, their role was minimized. However, at the beginning of the 1990s, the reservoir role of birds was clarified in Europe, and now it is unanimously accepted that some bird species are reservoirs for *B. burgdorferi* s.l. In 1998, 2 studies clearly defined the reservoir role of birds, one on a passerine bird, the blackbird (*Turdus merula*), the other one on a gallinaceous bird species, the pheasant (*Phasianus colchicus*) [1]. Both studies examined the reservoir role of these bird species using xenodiagnosis. Tick xenodiagnosis consists of infecting uninfected ticks – usually larvae – during feeding on the animal suspected to be reservoir host. These results and others have evidenced the contribution of birds to the circulation of *Borrelia* in endemic areas. Interestingly, a transmission cycle of *B. burgdorferi* s.l. was discovered in environmental settings other than the biotopes where *I. ricinus* usually live. In fact, it was demonstrated, on a Swedish island, that *B. burgdorferi* spirochetes could be maintained in seabird colonies among razorbills (*Alca torda*) by an associated tick species, *I. uriae*. Of course, interest in birds was also focused on the potential role of migrating birds in transporting infected ticks. This approach turned out to be justified, and spirochetes were reported in ticks collected from migratory birds in various studies. The involvement of seabirds and *I. uriae* (in the marine environment) in the transport of infected *Borrelia* between the northern and the southern hemispheres was described. In this context, it is interesting to mention that in a laboratory study, reactivation of latent *Borrelia* infection could be induced in passerines experimentally submitted to stressful conditions simulating migration. This implies that during their migration, birds can infect ticks all along their migration route. Bird migration also allows the transfer and establishment of particular *Borrelia* species, as described for *B. lusitaniae*. In fact, birds migrating between south-west Europe/North Africa to north-western Europe have been suggested to be responsible for the transfer of *B. lusitaniae* from North Africa and south-west Europe, where this *Borrelia* species clearly dominates, to north-west Europe where it is much less frequent [13].

Assessment of the reservoir competency of large mammals is clearly a difficult task. It necessitates, if xenodiagnosis is applied, capture of the animals and maintenance in a laboratory structure. The consequence of this is that the role of medium-sized and large mammalian species has been studied less and is not yet clearly understood. Red foxes seem to be implicated in the maintenance of *Borrelia* in nature, as described in Germany. However, these animals do not appear to be very potent reservoirs, since spirochetes were poorly transmitted to ticks feeding on them. According to various reports, ruminants appear to act primarily as sources of blood for ticks. Controversy long surrounded the exact role of large animals, particularly cervids, in the maintenance cycle of *Borrelia* in endemic areas. Currently, most studies seem to indicate that they do not play a role as reservoirs. In fact, studies undertaken in Sweden and in the UK on roe deer (*Capreolus capreolus*), moose (*Alces alces*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) suggested that these species do not infect feeding ticks with *B. burgdorferi* s.l. However, according to some recent devel-

opments, the possibility exists that they may act as supports for co-feeding transmission of *Borrelia* between infected and uninfected ticks, and therefore may represent amplifying hosts.

As previously mentioned, in Europe, at least 6 *Borrelia* species may circulate between vertebrate hosts and ticks. This raises a fundamental question: how do the different *Borrelia* species interact with the different host species in endemic areas? The first findings showing an association between a *Borrelia* species and some host species date back to the middle of the 1990s. At that time, it was shown that *Borrelia* species isolated from *Apodemus* spp. captured in 2 sites in Switzerland all belonged to *B. afzelii*, whereas *Borrelia* species diversity in ticks collected by flagging vegetation in these sites displayed heterogeneity. Later, it was shown that small rodents of the genus *Apodemus* and of the genus *Clethrionomys* as well as red (*Sciurus vulgaris*) and grey squirrels (*S. carolinensis*) were usually infected by *B. afzelii* and less frequently by *B. burgdorferi* s.s. and that they transmitted these 2 *Borrelia* species to ticks feeding on them. On the other hand, at the same time, *B. garinii* was shown to be associated with migratory birds, and *B. garinii* and *B. valaisiana* with blackbirds and pheasants. *B. garinii* was also described as the *Borrelia* species involved in marine environments – in seabird colonies and in the tick *I. uriae* – located in both the northern and southern hemispheres.

As far as less common *Borrelia* species are concerned, like *B. lusitaniae* and *B. spielmanii*, recent works identified associations with some vertebrate hosts as well. Thus, Dsouli et al. [11] demonstrated the reservoir role of the lizard *Psammodromus algirus* for *B. lusitaniae* in North Africa (Tunisia), Richter and Matuschka [14] the roles of the common wall lizard *Podarcis muralis* and sand lizard *Lacerta agilis* in Germany, and, finally, Amore et al. [15] reported that *P. muralis* was a reservoir for this *Borrelia* species in Italy. Poupon et al. [13] observed *B. lusitaniae* in *I. ricinus* larvae collected from birds that were migrating between southwest Europe/North Africa and northwestern Europe. These authors strongly suspected the role of migratory birds in the dispersal of *B. lusitaniae*. Concerning *B. spielmanii*, the garden dormouse, *E. quercinus* [16], and the hedgehog *E. europaeus* [17] have been described as contributing the majority of *B. spielmanii*-infected ticks in areas endemic for this *Borrelia* species.

At this point, one might justifiably ask: What element is behind this host-specificity of *B. burgdorferi* s.l.? Explanation for this observation came from studies showing that determinants for the described phenomenon were linked to the host complement system [18]. It was demonstrated in vitro that *B. burgdorferi* s.s., *B. garinii*, *B. valaisiana* and *B. afzelii* showed different patterns of resistance or sensitivity to serum according to host species, corresponding to the host specificity observed in nature [18]. The main disadvantage of this in vitro system is that a great heterogeneity is present among *Borrelia* strains in nature, and therefore a very large number of various *Borrelia* strains have to be tested in relation to a very large number of host sera to be able to mimic situations encountered in nature. An illustration of this is

B. lusitaniae. Kurtenbach et al. [18] reported that *B. lusitaniae* is sensitive to the complement of some bird and lizard species, and hence is destroyed by these host sera. However, as reported before, *B. lusitaniae* has been found to be associated with some lizard and bird species in nature. Further research in this field is required to better understand all subtleties governing these interactions. This is particularly important because besides these strict associations between *Borrelia* and vertebrate hosts, loose associations between *Borrelia* and hosts have also been described in the natural environment. *B. garinii* has occasionally been described as associated with rodents, and *B. afzelii* has been detected in xenodiagnostic ticks that fed on birds. The existence of such loose associations between hosts and *Borrelia* was confirmed recently in studies using less classical methods to identify host reservoirs. In fact, the use of molecular tools upon field-collected ticks – that allow the identification of host DNA remaining in the tick midgut from the previous blood meal, along with the detection of *Borrelia* – tended to show that in parallel to the strict associations between *Borrelia* species and hosts, less strict associations also exist [19]. All this goes to show that in nature strict and loose associations probably occur between *Borrelia* species and host species. Additional studies are required to really understand the relationships between the various *Borrelia* species and strains and their hosts in nature.

It is striking that among the 300 vertebrate species serving as hosts for ticks, only a few have been identified as reservoir hosts. We have already touched on the difficulties in assessing the reservoir competency of vertebrates, particularly large mammals. This can be mainly attributed to the fact that, as a gold standard, reservoir identification implies tick xenodiagnosis. This necessitates animal trappings and temporary maintenance of these animals in captivity. It is obvious that most tick hosts are difficult to capture and to maintain in a laboratory. That is one of the reasons why researchers have recently developed molecular tools allowing identification of hosts that have fed the field-collected ticks in their previous developmental stages. This method coupled with the simultaneous detection of pathogens in ticks, mainly in nymphs, has been developed and applied in the field. Two main host genes have been targeted in these studies, the nuclear 18S rRNA gene [20] and the 12S rDNA mitochondrial gene [21]. The method based on the nuclear 18S rRNA gene appears to be less sensitive, in the sense that it allows the discrimination of only major groups of vertebrate hosts [20]. The other method, based on the 12S rDNA mitochondrial gene, has the advantage of allowing identification of host DNA to the species level, narrowing down host identification [21]. The use of these molecular tools may help to elucidate the maintenance and the circulation of *B. burgdorferi* s.l. among their different hosts throughout the large geographical distribution of *I. ricinus* ticks in Europe and North Africa.

Transmission of *B. burgdorferi* to Humans

Let us first remember here that the encounter between ticks and their hosts, including humans, comprises a tick that is immobile on vegetation waiting for a host that is moving; this means that the encounter between these 2 elements of the eco-epidemiological chain is based mainly on the active part of the host. Once the encounter has taken place, the tick will move on its host to look for an adequate place to introduce its mouthparts into the skin of its host. In humans, it may take a few minutes to hours before the tick attaches to the skin. The duration of attachment of the tick *I. ricinus* to its hosts, as we mentioned before, can vary between 3 and 10 days depending on the developmental stage. *B. burgdorferi* s.l. spirochetes are transmitted to their hosts orally while ticks are taking their blood meal. It took a few years after the discovery of *B. burgdorferi* in ticks in North America in the 1980s for the mechanism of how the spirochetes were transmitted to the host to be elucidated. This was mainly due to the fact that before blood meal, in unfed ticks, spirochetes are located in the tick midgut. Thus, for some years, regurgitation of midgut content was considered as the mode of transmission of *B. burgdorferi* s.l., before the transmission route was elucidated. Currently, it is well established that *B. burgdorferi* s.l. is transmitted to the host via infected saliva during the blood meal. Very few studies have investigated the transmission dynamic of *B. burgdorferi* s.l. by *I. ricinus*; however, these studies showed that, in the majority of infected *I. ricinus* ticks, spirochetes (that are present in the midgut of ticks before blood meal begins) migrate during blood feeding to the salivary glands, from which they are transmitted to the host via saliva. Furthermore, microscopic examination of unfed nymphal and adult *I. ricinus* ticks collected in endemic areas demonstrated that spirochetes may also be present in the salivary glands of ticks even before any blood uptake [22].

When unfed *I. ricinus* attaches to a vertebrate host, *Borrelia* transmission does not occur at the beginning of the blood uptake but later on, and transmission efficiency increases with the duration of the blood meal [23, 24]. The uptake of blood seems to trigger spirochetes to migrate from tick midgut to the salivary glands. The delay in transmission observed during the first hours of the blood meal might be due to this phenomenon, the migration of the spirochetes. In a laboratory study, an early transmission of borreliae with high efficiency was described for *I. ricinus*. In fact, Kahl et al. [23] reported that 50% of laboratory animals were infected by *B. burgdorferi* s.l. after only 16.7 h of tick attachment. The observations of high infection rates in salivary glands of unfed *I. ricinus* suggest that systemically infected ticks may transmit *Borrelia* early after attachment to hosts [22], and this might be a factor that might reduce the delay in transmission after attachment of the ticks to the hosts. Crippa et al. [24], comparing transmission dynamic of spirochetes by *B. burgdorferi* s.s.- and *B. afzelii*-infected ticks, reported that this delay might also be influenced by the *Borrelia* species infecting the ticks. In fact, earlier transmission by *I. ricinus* occurred when ticks were infected by *B. afzelii* rather than by *B. burgdorferi* s.s. These authors

reported that during the first 48 h of attachment to the host, *B. burgdorferi* s.s.-infected ticks did not infect the 18 exposed mice, whereas *B. afzelii*-infected ticks transmitted infection to 33% of the mice [24]. This study not only showed that *I. ricinus* transmits *B. afzelii* earlier than *B. burgdorferi* s.s., but also that *I. ricinus* is a more efficient vector for *B. afzelii* than for *B. burgdorferi* s.s. Unfortunately, nothing is known on the transmission delay for other pathogenic *Borrelia* species infecting *I. ricinus*, such as *B. garinii*, *B. valaisiana* and the recently described species *B. spielmanii*. All this indicates that ticks should be removed as soon as they are found attached to the skin.

The migration of *Borrelia* from the midgut to the salivary glands during tick feeding is associated with variable protein expression. From studies mainly on the North American tick vector, *I. scapularis*, but also on *I. ricinus*, it is known that in unfed ticks, before the beginning of blood uptake, spirochetes located in the midgut express outer surface protein A (OspA). On its surface, OspA possesses a receptor for plasminogen of the host organism. After tick feeding starts on the host, plasminogen changes into plasmin, which facilitates migration through the midgut wall to the salivary glands. During blood feeding, OspA synthesis is repressed and OspC synthesis is induced. In *I. ricinus*, very few studies addressed this point. Leuba-Garcia et al. [22] observed that *B. afzelii* spirochetes expressing OspA and OspC were present in the midgut of unfed ticks, and that spirochetes expressing OspA were not detected in ticks attached to the host for more than 24 h. In salivary glands of engorged ticks, *B. afzelii* spirochetes expressed OspC. This study also reported that in the skin of mice infected by *B. afzelii*-infected nymphs, spirochetes expressed OspC. Later, Fingerle et al. [25], using different *B. afzelii* and *B. garinii* strains, demonstrated that in capillary-infected *I. ricinus* ticks, OspA was expressed in the tick midgut and that the proportion of OspC-positive borreliae was usually greater when the borreliae reached the salivary glands. In this study, a *B. afzelii* strain unable to produce OspC was unable to disseminate and to induce infection in salivary glands, showing the role of OspC in *Borrelia* dissemination in *I. ricinus*. The degree of strain specificity on the dynamics of Osp expression and the dissemination of spirochetes in the vector is an interesting topic. The interactions of the various *Borrelia* species and strains with *I. ricinus* are clearly extremely complex and insufficiently studied.

We cannot end this chapter without adding some words on another tick species, the hedgehog tick (*I. hexagonus*), that may transmit *Borrelia* infection to humans. Its vector competence has been demonstrated under laboratory conditions, and confirmed under field conditions. This tick species is one of the most widespread tick species in Europe. *I. hexagonus* is a nidicolous species, which means that this tick lives in the nest, burrow or cave of its hosts. Hosts for this tick species are mainly recorded among carnivores. In view of its habitats, *I. hexagonus* rarely comes in contact with humans. However, hedgehogs are also frequent hosts for *I. hexagonus*, and since hedgehogs are frequent hosts in our gardens, humans can come into contact with this tick (particularly when they handle nests of hedgehogs, which have surface nests,

when gardening). *I. hexagonus* bites humans, although less frequently than *I. ricinus*, and may transmit *Borrelia* infection to them. In addition to *I. ricinus* and *I. hexagonus*, other tick species and even insect species have been found to be infected by *B. burgdorferi* s.l., but without evidence of vector competence. A list of these insect and tick species can be found in a report by Gern and Humair [1].

We have seen that once on their host, *I. ricinus* ticks do not attach immediately to the skin, but look for a suitable place. We have also reported that the risk of transmission of *Borrelia* by feeding ticks increases with attachment duration. Both these elements are important in the prevention of Lyme borreliosis. It means that careful visual examinations of body may prevent tick bites as well as *Borrelia* infection. Body examination is recommended not only during and immediately after stays in tick biotopes, but also during the following days.

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