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Natural history of *Borrelia burgdorferi* sensu lato

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Summary. Lyme borreliosis is a zoonosis: its causative agent, *B. burgdorferi*, circulates between ticks and a large range of vertebrates. Identification of the hosts which are responsible for the infection of the vectors is extremely important to determine the potential risk of infection in an habitat. Various small mammals and bird species are considered reservoirs for the Lyme disease spirochetes. Grey and red squirrels, hedgehogs as well as hares and rabbits can develop an infection and transmit *B. burgdorferi sensu lato* to feeding ticks.

In Eurasian endemic areas, many different *Borrelia* species circulate between ticks and vertebrate hosts. Studies have shown that European and Asian genospecies are associated with specific groups of vertebrate hosts, such as *B. valaisiana* and *B. garinii* with birds, *B. afzelii* with small mammals and *B. burgdorferi* ss and *B. afzelii* with red squirrels. However, such associations are not always observed as in Japan where *B. garinii*, *B. afzelii* and unidentified Borrelia species are found in small mammals.

Some enzootic cycles involving tick species which do not feed at all on humans or which rarely feed on humans have been described in Europe and USA. It is likely that many existing enzootic foci have yet to be discovered. The circulation of *B. burgdorferi* in silent foci does not have important implications for human health, but it demonstrates the complexity of the ecology of this microorganism and the variety of ecological niches this spirochete can occupy.

Key words: *Borrelia burgdorferi*, spirochetes, ticks, ecology, reservoir hosts.

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 geographic areas. However, Lyme borreliosis is above all a zoonosis: its causative agent, *Borrelia burgdorferi*, circulates between ticks and a large variety of animals which act as hosts for ticks. Humans are not involved in the circulation of *B. burgdorferi* in nature since humans come into contact with the pathogen only accidentally and serve as dead-end hosts. Consequently, the success of any understanding of human Lyme borreliosis is dictated by a good knowledge of the biology and ecology of *B. burgdorferi* which include pathogen-vector-reservoir interactions.

Four tick species have been recognized as main vectors of *B. burgdorferi*: *I. ricinus* in Europe and North Africa, *I. persulcatus* in Asia, *I. scapularis* in northeastern America and *I. pacificus* in the western part of the United States. All these vectors have a similar life cycle involving three blood meals on three different hosts. They all feed on a large variety of animals, but the number of hosts varies depending on the tick species: *I. ricinus* and *I. persulcatus* parasitize the greatest number of host species by infesting more than 200 vertebrate species, whereas the American tick species are only found on 50-80 vertebrate species. Feeding is a particular event in the life of ticks since it implies a relatively long-lasting close contact between the tick and the host. From a biological viewpoint, exchanges occur during the feeding process: tick saliva is injected in the host skin and the blood is sucked by the tick. From an eco-epidemiological viewpoint, these exchanges of biological fluids allow exchanges of pathogens (spirochetes) from ticks to hosts and/or from amplifying and reservoir hosts to ticks. Ticks acquire spirochetes while feeding on infected reservoir hosts and spirochetes persist in the tick to the next instar through transstadial transmission. This transmission is a prerequisite for the maintenance of the cycle in a natural focus. In female ticks, the ovary can be infected and consequently the eggs and the derived larvae can acquire the infection. This transovarial transmission is rare in most vector ticks. The prevalence of infection in larval ticks is usually low, reaching less than 10%, whereas 25 to 50% of nymphs and adults are infected. The difference in the infection rate between larvae and nymphs and adults is the result of an infectious blood meal taken by larvae on reservoir hosts.

In fact, spirochetes present in the ticks have their origin in the amplifying and reservoir hosts. Compared to the large number of hosts for ticks, little information is available on the significance of these hosts as sources of infection for tick vectors. Xenodiagnosis, which is the most valuable method for the identification of reservoir hosts, is rarely used to obtain the evidence that specific hosts are reservoirs and that they can transmit borreliae to naive feeding ticks. Small mammals are the most extensively studied group of vertebrates, studied, because these animals can be easily captured, handled and maintained in the laboratory. Hence, several species of mice, voles, rats and shrews have been shown to be competent reservoirs of *B. burgdorferi* in Asia, Europe and the United States. Various bird species, especially ground-foraging birds - like thrushes, blackbirds, robins, wrens and pheasants - are incriminated as reservoirs for the Lyme disease spirochetes. Grey and red squirrels, hedgehogs as well as hares and rabbits can develop an infection and transmit *B. burgdorferi* sl to feeding ticks.

In Europe, a great heterogeneity is observed among *B. burgdorferi* sl isolates obtained from humans, animals and ticks whereas isolates seem to be more homogeneous in North America. Two or more genospecies are present in USA: *B. burgdorferi* sensu stricto (ss), *B. andersonii* and a third larger group of isolates related to strain 25015 obtained from various sources nationwide, with a greater heterogeneity observed in southeastern United States. On the other hand, European isolates have been classified into five genospecies: *B. burgdorferi* ss, *B. garinii*, *B. afzelii*, *B. valaisiana* and *B. lusitaniae*. In Asia, *B. garinii*, *B. afzelii*, *B. japonica*, *B. turdae* and *B. tanuki* have been described, the last 3 species being confined to Japan.

The heterogeneity among spirochetes circulating in Eurasian endemic areas has prompted scientists to reconsider the relationships existing between the pathogens of Lyme borreliosis and the various vertebrate hosts inhabiting endemic areas. In Europe, the first *Borrelia* isolate from rodents was obtained by Hovmark et al. [1] in Sweden and was characterized as *B. afzelii*. In Russia and in Austria, Gorelova et al. [2] and Khanakha et al. [3] have shown that different genospecies can be isolated or PCR-detected in the urinary bladder, heart and spleen of small mammals. However, only *B. afzelii* could be isolated from ear biospies taken from small mammals captured in different endemic areas in Switzerland [4]. Later, Hu et al. [5] showed that rodents exclusively transmitted *B. afzelii* to feeding ticks. These results strongly suggested a specific association between the type strain *B. afzelii*, small rodents and *I. ricinus*.

B. garinii has been described in bird-feeding ticks by Nakao et al. [6] in Japan and by Olsen et al. [7] in Sweden. Recently, Humair et al. [8] succeeded in isolating *B. garinii* and *B. valaisiana* from the skin of blackbirds. A xenodiagnosis performed on these birds showed that blackbirds transmitted *B. garinii* and *B. valaisiana* to naive feeding ticks. In England, pheasants were also found to be infected by these two *Borrelia* species [9]. The *Borrelia* species infecting red squirrels were investigated in Switzerland and the genotypic identification by RFLP showed that isolates obtained from skin samples were *B. burgdorferi* ss and *B. afzelii*. [10]. *B. afzelii* and *B. burgdorferi* ss were also the two most frequent genospecies found in ticks feeding on squirrels. This is in accordance with results obtained by Craine et al. [11] with grey squirrels in the UK, where *B. afzelii* was observed in the skin of this animal and where infection of the grey squirrel with *B. burgdorferi* ss was achieved in an experimental setting. These observations indicate that, in various endemic areas through Europe and Asia, genospecies are associated with particular groups of vertebrate hosts, such as *B. valaisiana* and *B. garinii* with birds, *B. afzelii* with small mammals and *B. burgdorferi* ss and *B. afzelii* with red squirrels.

Such strict associations between hosts and *Borrelia* species are not always observed: in Japan, for example, *B. garinii*, *B. afzelii* and unidentified *Borrelia* species are found in small mammals [12,13,14]. This absence of a specific association in Asia might be related to various ecological factors such as different *B. garinii* subtypes, different tick vector species as well as different rodent species occurring in Asia.

According to current knowledge, vertebrates can be classified into different types of hosts in the tick-vertebrate-*Borrelia* relationships. Classically a distinction is made between vertebrates which act as reservoir hosts (since spirochetes not only multiply and disseminate but also persist for a long period of time) and vertebrates which are not reservoirs. The identification of a host species as a reservoir is extremly important but not easily demonstrated. In fact, negative results can be obtained for various reasons and quick interpretation may obscure the true natural situation, meaning that repeated investigations in different situations are necessary. Interestingly, most attempts to determine the ability of a vertebrate species to support *B. burgdorferi* infection and to transmit the infection to feeding ticks have been designed to detect disseminated infection. However, *B. burgdorferi* spirochetes can remain localized at the inoculation site in the skin for many weeks before dissemination. Moreover co-feeding transmission of spirochetes can occur between infected and uninfected ticks feeding simultaneously at the same site on a host presenting no generalized infection [15]. Some tick hosts, like deer and other ungulates for example, have been intensively investigated and were thought not to be reservoirs for a long time. However, recent reports show that these hosts may be capable of supporting localized infection and transmitting *Borrelia* to ticks without developing disseminated infection. These hosts include sika deer in Japan [16] and sheep in England [17]. It is now well documented that a host can transmit *B. burgdorferi* to ticks even in the absence of generalized infection. Other vertebrate hosts have been suspected to have a zooprophylactic effect on infected feeding ticks by destroying spirochetes present in the tick midgut. One of these is the western fence lizard whose blood contains a thermolabile, borreliacidal factor, probably a protein, which destroys spirochetes in the midgut diverticula of feeding *I. pacificus* nymphs [18].

The associations described above between *Borrelia* species and vertebrate hosts raise the question of what happens to spirochetes transmitted to inadequate hosts. According to what was observed in endemic areas, it appears that the maintenance of *B. burgdorferi* sl in inadequate hosts as well as the transmission to feeding ticks are not ensured, although some additional laboratory experiments are required to confirm this observation. However, this explains why some hosts like blackbirds have been described as incompetent reservoirs [19]. In the experiment, blackbirds were infected via the bites of ticks previously fed on infected rodents. In view of the association known today between rodents and *B. afzelii*, ticks used in this experiment harbored *B. afzelii* and transmitted it to blackbirds. Birds which are known to be associated with *B. garinii* and *B. valaisiana* were apparently unable to maintain and transmit *B. afzelii* to feeding ticks. Such a phenomenon may also occur in nature and *B. afzelii* can disappear from a focus when a large number of birds are artificially introduced in an endemic area. In England for example, Kurtenbach et al. [9] observed that *B. afzelii* may be completely absent in some areas and that small mammals transmit *B. burgdorferi* ss to xenodiagnostic ticks but only at an extremely low rate. The absence of *B. afzelii* in these foci might be related to a large and dense pheasant population which substancially reduces the basic reproduction number of *B. afzelii*: this genospecies is then taken out of the ecosystem by phaesants which act as zooprophylactic hosts. In this context, the observation of a specific association between genospecies of *B. burgdorferi* sl and different vertebrate species must be considered when investigating the role of vertebrates as reservoirs not only in nature but also in the laboratory setting.

Direct evidence of the presence of Lyme borreliosis has only been reported from the northern hemisphere, however *B. burgdorferi*-infected *I. uriae* have also been reported from the southern hemisphere. In fact, Olsen et al. [20] described the presence of *B. garinii* in *I. uriae* ticks in different locations in both the northern and southern hemispheres. These authors demonstrated that *I. uriae* and their associated hosts, seabirds, can maintain transmission cycles and they suggested that spirochetes may be exchanged by seabirds between both hemispheres.

In addition to the classical maintenance cycle in which humans are accidentally implicated through infected tick bites and which are frequently revealed by humans developing clinical manifestation after a tick bite, there are other maintenance cycles which remain silent because humans are not bitten, or rarely bitten, by the tick species responsible for the transmission of the spirochetes. Such an enzootic cycle was discovered in the foothills of Colorado between *I. spinipalpis* and the rodent *Neotoma mexicana* [21] and in the southern United States [22]. Another silent cycle was also observed in Switzerland in an urban area where *B. burgdorferi* is circulating between *I. hexagonus* and hedgehogs [23]. In addition, if a bridge vector like *I. ricinus, I. scapularis* or *I. persulcatus*, is absent, the focus will remain completely silent. Such silent transmission cycles certainly occur through the entire distribution area of *B. burgdorferi* sensu lato. Nevertheless most of these enzootic silent foci have yet to be discovered.

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