Borrelia burgdorferi sensu lato, the agent of Lyme borreliosis: life in the wilds

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Summary :

In Europe, *Borrelia burgdorferi* sensu lato (sl) the agent of Lyme borreliosis circulates in endemic areas between *Ixodes ricinus* ticks and a large number of vertebrate hosts upon which ticks feed. Currently, at least 12 different *Borrelia* species belonging to the complex *B. burgdorferi* sl have been identified among which seven have been detected in *I. ricinus*: *B. burgdorferi* sensu stricto (ss), *B. garinii*, *B. afzelii*, *B. valaisiana*, *B. spielmanii* and *B. bissettii*. A few dozens of vertebrate hosts have been identified as reservoirs for these *Borrelia* species. Specific associations were rather early observed between hosts, ticks and borrelia species, like for example between rodents and *B. afzelii* and *B. burgdorferi* ss, and between birds and *B. garinii* and *B. valaisiana*. The complement present in the blood of the hosts is the active component in the *Borrelia* host specificity. Recent studies confirmed trends toward specific association between *Borrelia* species and particular host, but also suggested that loose associations may be more frequent in transmission cycles in nature than previously thought.

KEY WORDS : *Ixodes ricinus*, host DNA, *Borrelia*, reservoir hosts.

I n Europe, Lyme borreliosis endemic areas are maintained through complex interactions among different tick species, a variety of *Borrelia* strains belonging to the complex *B. burgdorferi* sensu lato (sl) and a large number of vertebrate hosts upon which ticks feed.

The tick *I. ricinus* is the classical tick vector in the European distribution of Lyme borreliosis. *I. ricinus* is very generalist feeding on numerous mammalian, avian and reptilian species in the European woodlands. This tick species is a three-host tick that goes through three developmental stages: larva, nymph, and male and female adults. To encounter its hosts, *I. ricinus* quests on the tip of low vegetation. During questing, ticks very often have to face desiccating conditions and have to quit their questing place to move to the litter zone where they regain lost body water (Randolph & Storey, 1999). Interruption of questing occurs mostly during darkness (Perret *et al.*, 2003). Interestingly, *I. ricinus* moves preferentially when desiccation risk is the lowest in nature,

that is to say at sundown. The change in light intensity that triggers tick movements is perceived by *I. ricinus* thanks to photosensitive cells (Perret *et al.*, 2003). Under moisture stress, ticks will quest for shorter periods and this will decrease the host-finding probability and thus influences tick population dynamic (*Perret et al.*, 2003). If high desiccating conditions are lasting too long, tick mortality is increased resulting in questing tick population decrease (Perret *et al.*, 2000, 2004).

Besides *I. ricinus*, other tick species have been found infected by spirochetes but only *I. hexagonus,* that feeds mainly on carnivores, and *I. uriae,* that is associated with seabirds, are able to support natural transmission of *Borreliae* and to significantly contribute to the persistence of spirochetes within endemic areas (Gern *et al.*, 1997; Olsen *et al.*, 1993). Spirochetes have also been detected or isolated from other tick species and also from insects, especially from mosquitoes and fleas but their role in the maintenance of a focus remains unknown (Gern & Humair, 2002).

At least 237 animal species are implicated, as hosts for ticks, in the maintenance of *I. ricinus* tick populations and, for this reason, are susceptible to serve as reservoir hosts for *Borrelia*. Nevertheless, compared to the large number of tick hosts, little information is available on the real significance of most animal hosts as sources for infecting *I. ricinus* with *B. burgdorferi* sl. Tick xenodiagnosis is the only way to really demonstrate the infectivity of a vertebrate species. Xenodiagnosis consists in allowing naive ticks derived from a laboratory colony free of infection to engorge on tested hosts and to analyze the ticks after the meal or after the moult to detect the pathogen. This technique also allows identify the *Borrelia* species transmitted from the vertebrate to the ticks. However, xenodiagnosis, without counting that a laboratory colony of ticks has to be available, is a very fastidious method because hosts have to be captured and maintained in captivity. Hence, it is easy to understand that small mammals are clearly the vertebrate group the most extensively studied. Currently, the infectivity of *Apodemus* mice and *Clethrionomys* voles for *I. ricinus* ticks is unanimously recognized (Gern & Humair, 2002). If the role of small

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rodents in the circulation of *Borrelia* in nature was rather easily elucidated, controversy long surrounded the role of birds as reservoirs. Today, the reservoir role of various bird species is unanimously admitted, especially concerning ground-nesting and ground-foraging birds - like thrushes, blackbirds, robins, wrens, nightingales, blue throats and pheasants. Migratory birds have also been shown through their infectivity for *I. ricinus* to participate in the enzootic maintenance of *B. burgdorferi* sl, and their role in the spread of infected ticks is now also well documented (Comstedt *et al.*, 2006; Olsen *et al.*, 1995; Poupon *et al.*, 2006). Studies on hares, squirrels and hedgehogs revealed that they contribute to *Borrelia* infection in ticks (Gern & Humair, 2002). Among large size mammals, red foxes are also able to infect ticks with *Borrelia* but the infectivity of red foxes appears as low. While the reservoir role of various tick hosts species was being identified, the role of large-sized mammals like ungulates in the contribution of the infection of ticks was denied although their role as hosts for ticks, particularly adult ticks, is considerable. In recent years, the taxonomy of *B. burgdorferi* sl spirochetes has become complicated and at least 12 different *Borrelia* species have been identified among which seven are circulating in endemic areas in Europe and have been detected in *I. ricinus*: *B. burgdorferi* sensu stricto (ss)*, B. garinii, B. afzelii, B. valaisiana, B. spielmanii* and *B. bissettii* (Rauter & Hartung, 2005; Richter *et al.*, 2006). Only one study reported *B. bissettii* in *I. ricinus* (Hanincova *et al.*, 2003) and therefore its presence in this tick vector remains to be confirmed.

The description of the various *Borrelia* species in ticks has opened up an entire new field of research in the ecology of Lyme borreliosis. A number of key issues arose from this situation and the relation existing between the various *Borrelia* species and the hosts had to be examined in more details. Specific associations were rather early observed between hosts, ticks and borreliae species that help us today to better understand the ecology of *B. burgdorferi*. First studies strongly suggested that a specific association existed between small mammals and *B. afzelii*. Then *B. burgdorferi* ss and a subtype of *B. garinii* were also associated with rodents. In addition, the frequent observations of *B. burgdorferi* ss and *B. afzelii* in the skin of grey and red squirrels and the high prevalence of these two *Borrelia* species in ticks collected from the squirrels suggested that *B. burgdorferi* ss and *B. afzelii* were transmitted from squirrels to feeding ticks. The first report of *B. garinii* in bird feeding ticks implied that *B. garinii* might be associated with birds. This was confirmed later for pheasants and blackbirds, and *B. valaisiana* was also implicated in this association with birds. The situation with *B. lusitaniae* is especially interesting. In fact, quite recently, very different vertebrate groups, such as lizards, birds and hedgehogs, were identified as

reservoirs for *B. lusitaniae* (Amore *et al.*, 2007; Dsouli *et al.*, 2006; Poupon *et al.*, 2006; Richter & Matuschka, 2006; Skuballa *et al.*, 2007). Eventually, concerning the more recent identified *Borrelia* species, *B. spielmanii,* little is known on the reservoir hosts. Only one study reported two species of dormouse as reservoirs for this species (Richter *et al.*, 2004). In other words, various studies in endemic areas throughout Europe reported associations between some *Borrelia* species and particular groups of vertebrate hosts. The explanation to these associations was given by researches summarized in Kurtenbach *et al.* (2002). The complement present in the blood of the hosts was shown to be the active component in the *Borrelia* host specificity. This information provided new insights into the dynamics of the circulation of the various *Borrelia* species among tick host populations. *In vitro* tests demonstrated that the pattern of serum sensitivity of different *Borrelia* species matched the known reservoir status of many vertebrate species for *B. burgdorferi*. For example, *B. afzelii* was resistant to rodent serum whereas *B. garinii* was destroyed by rodent serum (Kurtenbach *et al.*, 2002). The absence of generalized infection in deer correlated with the indiscriminatory borreliacidal activity of deer sera against all the *Borrelia* species tested and explained the fact that this host does not play a role in the maintenance of *Borrelia* in endemic areas as already mentioned. However, it seems that the situation is not clear-cut and that some exceptions exist, and that looser associations are present in nature. In fact, results from other studies conflicted with this situation, for example not only *B. afzelii* or *B. burgdorferi* ss but also additional *Borrelia* species and subtypes were isolated or PCR-detected in small mammals or in xenodiagnostic ticks fed on small rodents (Korenberg *et al.*, 2002). Similarly, *B. afzelii* DNA was detected in xenodiagnostic ticks fed on blackbirds (Humair *et al.*, 1999). Differences in biological and ecological factors such as different *Borrelia* subtypes, different tick vector species and different veretebrate biocenosis present in different areas could explain the discrepancy observed. The situation with *B. lusitaniae* is especially interesting. As reported by Kurtenbach *et al.* (2002), exposure of *B. lusitaniae* to lizard and bird serum resulted in killing of spirochetes. Curiously, as mentioned before, lizards and birds have been identified as reservoir hosts for *B. lusitaniae* in nature. All this strongly suggests that still much more efforts have to be put on the investigation of the role of vertebrates in the infection of ticks in local areas, keeping in mind the existence of several species and subtypes of *B. burgdorferi* sl. with apparently different host specificities. This is supported by results obtained by more sophisticated methods recently developed to assess the reservoir role of the various tick hosts. In fact, thanks to implementation of more performing molecular analysis techniques, it is currently possible to identify the hosts

that have fed the field-collected ticks in their previous developmental stages and to simultaneously detect tick-borne pathogens in the same ticks. Kirstein & Gray (1996) were the first to demonstrate the feasibility to identify host DNA in ticks by using a PCR targeting part of the cytochrome B gene. Later, studies targeting the nuclear 18S rRNA gene (Pichon *et al.*, 2003) and the 12S rDNA mitochondrial gene (Humair *et al.*, 2007) were developed. The method based on nuclear 18S rRNA gene appears as less sensitive in the sense that this method allows discrimination only among major groups of vertebrate hosts whereas the other method based on the 12S rDNA mitochondrial gene has the advantage to allow host DNA identification at the species level (Humair *et al.*, 2007). The 12S rDNA mitochondrial gene based method permits a large panel of host identification since approximately 30 different vertebrate species DNA can be detected. However, one should stress here that these methods are limited by their sensitivity. None of them globally allowed higher identification success than 50 %. Of course, the main drawbacks to these approaches are the small quantities of blood meal remnants still present in questing ticks and the time passed since the last blood meal. In fact one should keep in mind that ticks can quest for months on vegetation and hence, analysed host DNA in tick midgut is most of the time several months old. Both methods have been used to identify hosts for ticks in various areas in Europe and also to identify reservoir hosts for *Borrelia* and other tick-borne pathogens (Pichon *et al.*, 2005; 2006, Estrada-Pena *et al.*, 2005; Moràn Cadenas *et al.*, 2007). All these studies confirmed trends toward specific association between *Borrelia* species and particular host but also suggested that loose associations may be more frequent in transmission cycles in nature than previously thought.

In conclusion, it seems prudent to review the importance of the different type of associations between *Borrelia* species in various geographic areas. Clearly, additional field studies are expected in the subject of *Borrelia* host specificities, keeping in mind, particularly the rare *Borrelia* species and the huge subtype diversity hidden behind most *Borrelia* species, as well as the geographic diversity of local ecosystems. Therefore, future studies in Lyme borreliosis ecology seeking to identify the *Borrelia* species found in both ticks and reservoir hosts in various endemic areas are strongly encouraged.

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