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Ecology of *Borrelia burgdorferi* sensu lato in Europe

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Introduction

Borrelia burgdorferi, the aetiologic agent of the tick-borne zoonosis Lyme borreliosis (LB), circulates between ticks and vertebrate hosts. However, as in the case of most other vector-borne zoonoses, the presence of the pathogen in nature was revealed through clinical manifestations in humans.

It took almost 100 years after the first description, in Europe, of clinical manifestations of the disease now known as LB for biologists to discover borreliae in *Ixodes scapularis* ticks from northeastern USA (Burgdorfer *et al.*, 1982). Thus, the pathogen and the vector of this disease were discovered simultaneously. Similar spirochaetes were observed in Europe shortly thereafter in a closely related tick species, *Ixodes ricinus* (Burgdorfer *et al.*, 1983). Finally, the spirochaetes were recognized as a new species, *B. burgdorferi* (Johnson *et al.*, 1984), and as the causative agent of the new clinical entity, Lyme disease or LB. It is now known that LB is the most prevalent tick-borne disease in the northern hemisphere.

As in other vector-borne diseases, understanding the ecological relationships that exist between pathogens, vectors and wildlife hosts in LB is essential to the understanding of the epidemiology of the disease in human populations. In addition to the diversity of vector and host species in the large geographical area occupied by *B. burgdorferi* worldwide, a great diversity of *Borrelia* species within the complex *B. burgdorferi* sensu lato (s.l.) has been discovered recently.

The transmission and maintenance cycles of *B. burgdorferi* s.l. in enzootic areas show a complicated picture with regional and local pecularities, and our present view of the ecology of these spirochaetes in Europe is like a collection of temporal and local snapshots, the whole picture being still fragmentary. This chapter is a review of the current knowledge of *B. burgdorferi* s.l. ecology in Europe, but because of the relatively few publications from eastern Europe it focuses particularly on western and central regions. The maintenance of *B. burgdorferi* s.l. requires the interaction of three groups of organisms: the pathogens, vectors and hosts. These three essential components of LB will be described first, before attention is focused on their interrelationships.

The Spirochaetes

With the increasing number of isolates of *B. burgdorferi* obtained from various geographical and biological sources, it became obvious first in Europe and Asia and then also in North America that the spirochaete exhibited phenotypic and genotypic heterogeneity. This resulted in the description of 11 genomic groups or genospecies gathered under the name *Borrelia burgdorferi* s.l.: *B. burgdorferi* sensu stricto (s.s.) (Johnson *et al.*, 1984), *Borrelia garinii* (Baranton *et al.*, 1992), *Borrelia afzelii* (Canica *et al.*, 1993), *Borrelia japonica* (Kawabata *et al.*, 1993), *Borrelia andersonii* (Marconi *et al.*, 1995), *Borrelia tanukii* and *Borrelia turdi* (Fukunaga *et al.*, 1996), *Borrelia valaisiana* (Wang *et al.*, 1997), *Borrelia lusitaniae* (Le Fleche *et al.*, 1997), *Borrelia bissettii* (Postic *et al.*, 1998) and *Borrelia sinica* (Masuzawa *et al.*, 2001). In Europe, five species (*B. burgdorferi* s.s., *B. garinii*, *B. afzelii*, *B. valaisiana* and *B. lusitaniae*) have been isolated from ticks and *B. burgdorferi* s.l. has been reported from 26 countries from Italy to Iceland, and from Portugal to Russia (Hubálek and Halouzka, 1997). Two additional genospecies have been reported on the European continent from patient tissues: *B. bissettii*, a species present in North America, has been obtained from patients in Slovenia (Picken *et al.*, 1996; Strle *et al.*, 1997), and a novel *B. burgdorferi* s.l. genospecies has been cultured from an erythema migrans biopsy of a patient who contracted the disease in The Netherlands (Wang *et al.*, 1999). The European vector ticks and natural hosts of these two genospecies have not been determined as yet.

In addition to the classification of *B. burgdorferi* into 11 different species, an intraspecific heterogeneity has been documented with respect to outer surface protein (Osp) A, which is expressed by most *B. burgdorferi* s.l. spirochaetes (Will *et al.*, 1995). Wilske *et al.* (1993) developed a comprehensive serotyping system of *B. burgdorferi* s.l. by using immunoblotting and a variety of monoclonal antibodies against OspA. A strong correlation exists between OspA phenotypes and genospecific grouping of Lyme disease borreliae. This OspA serotyping was confirmed by sequence analysis of the *osp* genes (Jauris-Heipke *et al.*, 1995; Will *et al.*, 1995). These studies revealed that *B. garinii* was phenotypically the most heterogeneous genospecies with five different serotypes (serotypes 3–7).

Vectors and Carriers

In Europe, *B. burgdorferi* s.l. is mainly transmitted by *I. ricinus*. Two additional tick species, *Ixodes hexagonus* and *Ixodes uriae*, are also vectors and maintain

B. burgdorferi s.l. in secondary transmission cycles without necessarily involving *I. ricinus*.

The sheep tick or castor bean tick, *I. ricinus*, the principal vector of *B. burgdorferi* s.l., is the most common tick species in Europe. *I. ricinus* occurs from the Atlantic coast to as far east as 50–60° longitude in central Asia and from Iceland to as far south as the Atlas Mountains of North Africa (Hillyard, 1996). The eastern distribution of *I. ricinus* overlaps the western distribution of the related *Ixodes persulcatus* (Fig. 6.1).

The habitat of *I. ricinus* is situated in the leaf litter and the low strata vegetation of temperate deciduous woodlands and mixed forests. In areas with high rainfall, *I. ricinus* also occurs in high densities in coniferous forests and in open areas such as grassland (Gray *et al.*, 1998). *I. ricinus* has particular requirements regarding the humidity of its habitat and a relative humidity (RH) value of > 80% is important for the activity and survival of free-living *I. ricinus*. A low

Fig. 6.1. Distribution of *Ixodes ricinus* and *Ixodes persulcatus*, the major vectors of *Borrelia burgdorferi* s.l. in Europe. Note that, in southern areas and in Iceland and northern Scandinavia *I. ricinus* populations are sparse and scattered.

saturation deficit, which is calculated by using values of RH and temperature, can also be closely correlated with optimal conditions for *I. ricinus* (Randolph and Storey, 1999; Perret *et al.*, 2000).

Although studied for more than 50 years, knowledge of the biology of *I. ricinus* remains incomplete, partly because of the geographical and genetic heterogeneity of this tick species and also because of the diverse habitats it occupies. *I. ricinus* is an exophilic (or non-nidicolous) tick and the questing tick waits passively on the vegetation for passing hosts. A wide range of vertebrates such as reptiles, birds, small-, medium- and large-sized mammals serve as hosts for *I. ricinus* (Aeschlimann, 1972). Small mammals, ground-foraging birds and reptiles are common hosts for *I. ricinus* immatures but not for adults. Medium- to large-sized mammals are parasitized by *I. ricinus* adults (females) and immatures. Males occasionally take a blood meal but do not engorge. Immature stages and adult females can attach to humans. The life cycle of *I. ricinus* lasts 2–6 years, typically 3 years, since each stage takes about a year to develop to the next instar (Gray, 1991). The duration of the life cycle can vary from one habitat to another and also regionally, and can be affected by microclimatic factors and host density (Gray, 1991). *I. ricinus* ticks are active from spring to autumn, generally from February to November and seasonal activity of the three active stages usually shows a bimodal pattern with an activity peak in spring and another one in autumn. Tick activity in spring is usually greater than in autumn, except for larvae, which show the oppposite situation in some areas. Activity peaks can be indistinct and a pseudo-bimodal pattern may occur, as observed in Switzerland (Gigon, 1985; Perret *et al.*, 2000).

Besides the classical vector, *I. ricinus,* two other tick species, *I. hexagonus* and *I. uriae,* contribute to the circulation of *B. burgdorferi* s.l. in Europe. The hedgehog tick, *I. hexagonus*, is widespread in Europe and is also present in North Africa (Arthur, 1968). The presence of *B. burgdorferi* s.l. in *I. hexagonus* was first discovered in tick samples collected from hedgehogs (Liebisch *et al.*, 1989). The vector competence of *I. hexagonus* for *B. burgdorferi* s.l. has been demonstrated under both laboratory conditions (Gern *et al.*, 1991; Toutoungi and Gern, 1993) and field conditions (Gern *et al.*, 1997). In contrast to *I. ricinus, I. hexagonus* is an endophilic (or nidicolous) tick species and its habitat is the nest, burrow or cave of its vertebrate host. The host range of *I. hexagonus* is therefore more restricted than that of *I. ricinus* and it feeds primarily on carnivores such as foxes and mustelids, and on hedgehogs, but also, less frequently, on rodents, hares and rabbits (Arthur, 1953; Toutoungi *et al.*, 1991). *I. hexagonus* has occasionally been collected from birds (*Pica pica*, *Falco tinnunculus*) and deer (*Capreolus capreolus*) (Toutoungi *et al.*, 1991; Hubbard *et al.*, 1998). Domestic animals such as cats, dogs, horses, goats and cows have also been found to be infested (Arthur, 1968; Toutoungi *et al.*, 1991; Bernasconi *et al.*, 1997). *I. hexagonus* also bites humans but apparently less frequently than *I. ricinus* (Arthur, 1953; Liebisch and Liebisch, 1996; Liebisch *et al.*, 1998a). The occurrence of *I. hexagonus* in the urban environment is due to the presence of suitable hosts such as hedgehogs, cats and dogs in gardens and public parks (Gern *et al.*, 1991, 1997).

The seabird tick, *I. uriae*, has been found to be infected by *B. garinii* (Olsen *et al.*, 1993; Nuttall *et al.*, 1994; Hubbard *et al.*, 1998) and is the third ixodid species implicated in the circulation of *B. burgdorferi* s.l. in Europe. *I. uriae* infests more than 50 seabird species living in high latitude areas in both hemispheres (Olsen, 1995). In Europe, *I. uriae* is present on the coasts of France, Great Britain, Ireland, Denmark, Norway, Sweden and Iceland (Olsen *et al.*, 1995a; Hillyard, 1996). Apparently, *I. uriae* can infest other avian hosts such as passerines resting in marine habitats (Arthur, 1968). Mammals such as seals, river otters and humans can also occasionally be parasitized by *I. uriae* (Arthur, 1968; Eley, 1977; Mehl and Traavik, 1983; Hubbard *et al.*, 1998). People engaged in ornithological activity, such as bird-ringing, or in exploitation of colonial seabirds, as in the Faeroe Islands, are likely to be exposed to *I. uriae* bites (Olsen *et al.*, 1995a; Hubbard *et al.*, 1998; Gylfe *et al.*, 1999). The vector competence of *I. uriae* for *B. burgdorferi* s.l. has never been demonstrated under laboratory conditions, but its involvement as a vector of borrelial spirochaetes in transmission cycles in seabird colonies has been shown (Olsen *et al.*, 1993, 1995a).

The presence of *B. burgdorferi* s.l. in vector arthropods results from blood meals taken from infectious vertebrate hosts. In competent vectors, borrelial infection persists in the tick throughout the moulting process to the next instar. This phenomenon, called transstadial transmission, or more appropriately transstadial maintenance, is fundamental to the long-term survival of *B. burgdorferi* s.l. in nature and its efficiency is one of the factors determining vector competence. Transstadial maintenance has been demonstrated in two European tick vectors, *I. ricinus* and *I. hexagonus* (Monin *et al.*, 1989; Gern *et al.*, 1991; Toutoungi and Gern, 1993; Bellet-Edimo, 1997), and apparently occurs also in *I. uriae*, since unfed *I. uriae* have been found to be infected (Hubbard *et al.*, 1998) and since a cycle has been described between seabirds and *I. uriae* (Olsen *et al.*, 1993).

B. burgdorferi s.l. is ingested during the tick blood meal, colonizes the tick midgut and usually remains there after the moult. In some cases, spirochaetes may migrate to other tick organs, causing generalized or systemic infections in unfed *I. ricinus* ticks (Lebet and Gern, 1994; Leuba-Garcia *et al.*, 1994). In female ticks, *B. burgdorferi* s.l. may be present in ovaries and in the developing embryos (eggs). This mode of infection of the larval progeny through the ovarian tissue of the mother tick is called transovarial or vertical transmission. Experimental transovarial transmission of *B. burgdorferi* s.s. (isolate Sta14, now identified as NE14) in *I. ricinus* was successfully demonstrated (Monin *et al.*, 1989). Studies on the efficiency of transovarial transmission showed that this phenomenon rarely occurs in *I. ricinus*, as only about 1% of field-collected females transmitted *B. burgdorferi* s.l. to *I. ricinus* larvae (Bellet-Edimo, 1997). However, when transovarial transmission does occur, the prevalence of infection in *I. ricinus* eggs or subsequent larvae is high (43–100%) (Burgdorfer *et al.*, 1983; Bellet-Edimo, 1997). Transovarial transmission of *B. burgdorferi* s.s. (isolate B31) also occurs in *I. hexagonus* and has been shown experimentally (Gern *et al.*, 1991). In contrast to *I. ricinus*, many more *I. hexagonus* females can transmit *B. burgdorferi* s.l. to their progeny, but infection rates within batches of infected progenies are lower than in *I. ricinus*. This discrepancy might be related to tick biological and behavioural features: in the case of the endophilic *I. hexagonus*, a high prevalence of infection in larvae is less important than in the case of the exophilic *I. ricinus*, because of closer contacts in a confined environment between endophilic ticks and hosts. However, the prevalence of inherited infection in field-derived *I. hexagonus* larvae remains unknown. At present, no data are available concerning the existence of transovarial transmission of *B. burgdorferi* s.l. in *I. uriae*.

B. burgdorferi s.l. is reportedly present in all *I. ricinus* populations examined so far in Europe (Hubálek and Halouzka, 1998). Consequently, the infection risk for humans seems to be present in any *I. ricinus* habitat. The infection prevalence of questing *I. ricinus* ticks in Europe averages 1.9% for larvae (range 0–11%), 10.8% for nymphs (range 2–43%) and 17.4% for adults (range 3–75%) (Hubálek and Halouzka, 1998; de Michelis *et al.*, 2000) and varies geographically and also according to the methods used for the detection of *B. burgdorferi* s.l. in unfed *I. ricinus* ticks. Cultivation in BSK medium seems to be the least sensitive method compared with microscopy (dark-field, phase-contrast or immunofluorescence) or PCR (Hubálek and Halouzka, 1998; Gern *et al.*, 1999).

The prevalence of *B. burgdorferi* s.l. in unfed *I. hexagonus* is unknown. Concerning *I. uriae*, the prevalence of *B. garinii* was reported by Olsen (1995), who detected borreliae in 1/100 unengorged ticks, and by Hubbard *et al.* (1998), who detected infection in 13/13 field-collected engorged or semi-engorged museum specimens. Given the biology of *I. hexagonus* and *I. uriae*, inspection of host nests is needed for the collection of additional data for unfed free-living ticks of these two species.

As already mentioned, five genospecies of *B. burgdorferi* s.l. (*B. burgdorferi* s.s., *B. garinii*, *B. afzelii*, *B. valaisiana* and *B. lusitaniae*) are present in European ixodid vectors. The three pathogenic species (*B. burgdorferi* s.s., *B. garinii* and *B. afzelii*) have been frequently isolated from or detected in *Ixodes* spp. ticks, whereas *B. valaisiana* and *B. lusitaniae* have rarely been reported. Since the discovery of *B. burgdorferi* in *I. ricinus* ticks, valuable data concerning the occurrence of *B. burgdorferi* s.l. genospecies have been collected throughout Europe. However, it is difficult to draw a precise map of the distribution and frequency of the different *B. burgdorferi* s.l. genospecies on the European continent until more data have been collected. Current knowledge shows that *B. garinii* is the most frequent genospecies found in ticks, followed by *B. afzelii* and *B. burgdorferi* s.s. (Hubálek and Halouzka, 1997; Saint Girons *et al.*, 1998). *B. garinii* and *B. afzelii* occur throughout the European continent from the Atlantic coasts to the Ural mountains. In contrast, the frequency of *B. burgdorferi* s.s. seems to decrease from west to east (Saint Girons *et al.*, 1998) and appears to be absent in *I. persulcatus*. *B. valaisiana* and *B. lusitaniae* appear to be far less common and have been reported from certain European countries only. *B. valaisiana* occurs in Switzerland (Péter and Bretz, 1992; Péter *et al.*, 1995; Humair *et al.*, 1998), The Netherlands (Rijpkema *et al.*, 1995), Germany (Liebisch *et al.*, 1998a), Croatia (Rijpkema *et al.*, 1996), Great Britain (Cutler *et al.*, 1989; Kurtenbach *et al.*, 1998b), Ireland (Kirstein *et al.*, 1997), Italy (Cinco *et al.*, 1998) and Slovakia (Gern *et al.*, 1999).

B. lusitaniae has been reported in Portugal (Nuncio *et al.*, 1993; de Michelis *et al.*, 2000), Spain (Escudero *et al.*, 2000), Slovakia (Gern *et al.*, 1999), Belorussia, the Czech Republic (Le Fleche *et al.*, 1997), Moldavia, Ukraine (Postic *et al.*, 1997) and interestingly in North Africa (Zhioua *et al.*, 1999; Younsi *et al.*, 2001). It is true that *B. valaisiana* and *B. lusitaniae* have been described only recently but this cannot explain their lower frequency compared with pathogenic *Borrelia* spp. Very few isolates of *B. valaisiana* and *B. lusitaniae* have been obtained to date, and cultivation in the usual BSK medium seems to be problematic for the isolation of these two genomic groups. However, PCR-based studies have demonstrated a high prevalence of *B. valaisiana* in unfed *I. ricinus* ticks in Ireland (Kirstein *et al.*, 1997) and of *B. lusitaniae* in ticks in Portugal (de Michelis *et al.*, 2000). The question remains whether the DNA detected reflects the presence of viable borreliae. Further studies are needed to determine if *B. valaisiana* and *B. lusitaniae* are indeed patchily distributed in Europe or if they have been relatively undetected previously.

Infections by multiple *B. burgdorferi* s.l. genospecies have been observed in ticks in many parts of Europe, including The Netherlands (Rijpkema *et al.*, 1995), Croatia (Rijpkema *et al.*, 1996), Switzerland (Leuba-Garcia *et al.*, 1994), France (Pichon *et al.*, 1995), Belgium (Misonne *et al.*, 1998), Estonia, Kirghizia, Moldavia, Russia and Ukraine (Postic *et al.*, 1997), Ireland (Kirstein *et al.*, 1997), Italy (Cinco *et al.*, 1998) and Germany (Liebisch *et al.*, 1998a; Hu *et al.*, 2001). Different combinations of mixed infection with two or three genospecies have been detected. *B. burgdorferi* s.s. and *B. lusitaniae* are the two *Borrelia* spp. least involved in mixed infections. In most cases, mixed infections in ticks have been detected using PCR methods. Detection of mixed infections using cultivation might be difficult because one genospecies may overgrow another.

Certain tick or insect species found to be infected, but without evidence of vector competence, are considered to be carriers (Table 6.1). Transstadial transmission appears to occur in some tick carrier species since unfed ticks have been found infected by borreliae. It should be noted that spirochaetes in these insect and tick carriers have not always been clearly characterized as *B. burgdorferi* s.l.

Vertebrate Hosts

B. burgdorferi s.l. is maintained in natural cycles involving not only vectors but also wild vertebrates. The vertebrate hosts that are required to maintain tick populations may also act as reservoir hosts for tick-transmitted pathogens. Reservoir hosts as defined by Kahl *et al.* (Chapter 2) have mainly been identified in Europe by xenodiagnosis and by comparison of infection rates in questing ticks with those in ticks removed from hosts.

These reservoir hosts are among the large spectrum of different host species of the three competent tick vectors, *I. ricinus*, *I. hexagonus* and *I. uriae*. *I. ricinus* feeds on the largest variety of hosts on the European continent, infesting more than 300 different species including mammals, birds and reptiles (Anderson,

Table 6.1. Arthropod carrier species of *Borrelia burgdorferi* sensu lato in Europe.

Species	Countries	References
ACARI		
Ixodidae		
Ixodes trianguliceps	France	Doby *et al.* (1990)
	Russia	Postic *et al.* (1997)
	UK	Hubbard *et al.* (1998)
Ixodes acuminatus	France	Doby *et al.* (1990)
Ixodes canisuga	France	Doby *et al.* (1991)
	Germany	Liebisch *et al.* (1998b)
	Spain	Estrada-Peña *et al.* (1995)
Ixodes frontalis	France	Doby *et al.* (1995)
	Spain	Estrada-Peña *et al.* (1995)
Dermacentor reticulatus	Germany	Kahl *et al.* (1992)
	France	Doby *et al.* (1994)
	UK	Hubbard *et al.* (1998)
	Germany	Liebisch *et al.* (1998b)
Haemaphysalis punctata	Spain	Marquez and Constan (1990)
	UK	Nuttall *et al.* (1994)
	Sweden	Tälleklint (1996)
	UK	Hubbard *et al.* (1998)
Haemaphysalis inermis	France	Macaigne and Perez-Eid (1991)
Haemaphysalis concinna	France	Doby *et al.* (1994)
	Czech Republic	Hubálek *et al.* (1998a)
Haemaphysalis marginatum	Portugal	de Michelis *et al.* (2000)
Rhipicephalus sanguineus	UK	Hubbard *et al.* (1998)
Argasidae		
Argas reflexus	Italy	Stanek and Simeoni (1989)
Argas vespertilionis	UK	Hubbard *et al.* (1998)
INSECTA		
Siphonaptera (fleas)		
Ctenophtalmus baeticus arvernus	France	Doby *et al.* (1990)
Ctenophtalmus agyrtes	Czech Republic	Hubálek *et al.* (1998a)
Ctenophtalmus solutus	Slovakia	cited in Hubálek *et al.* (1998a)
Megabothris turbidus	France	Doby *et al.* (1990)
	Slovakia	cited in Hubálek *et al.* (1998a)
Spilopsyllus cuniculi	France	Doby *et al.* (1991)
Anoplura (sucking lice)		
Haematopinus suis	France	Doby *et al.* (1994)

Table 6.1. Continued

Species	Countries	References
Diptera		
Aedes vexans	Czech Republic	Halouzka (1993)
	Czech Republic	Halouzka *et al.* (1998)
	Czech Republic	Hubálek *et al.* (1998a)
Aedes cantans	Czech Republic	Halouzka *et al.* (1998)
	Czech Republic	Hubálek *et al.* (1998a)
Aedes sticticus	Czech Republic	Halouzka *et al.* (1998)
	Czech Republic	Hubálek *et al.* (1998a)
Culex pipiens pipiens	Czech Republic	Halouzka *et al.* (1998)
	Czech Republic	Hubálek *et al.* (1998a)
Culex pipiens molestus	Czech Republic	Halouzka (1993)
	Czech Republic	Halouzka *et al.* (1998)
	Czech Republic	Halouzka *et al.* (1999)
	Czech Republic	Hubálek *et al.* (1998a)
Lipoptena cervi	France	Doby *et al.* (1994)

1991). Consequently, each of these hosts could potentially be a reservoir of *B. burgdorferi* s.l. and therefore the reservoir status of each host species must be individually evaluated.

Of about 300 vertebrate species that are hosts for *I. ricinus*, only a small number have been examined so far for their borrelial infectivity to ticks. Small mammals are certainly the vertebrate group that has been the most extensively investigated up to now, mainly because they can be easily captured, handled and maintained in the laboratory. Several species of mice, voles, rats and shrews have been shown to be competent reservoirs of *B. burgdorferi* s.l. in Europe (Gern *et al.*, 1998). In particular, strong evidence for reservoir competence has been obtained in many European countries for the mice, *Apodemus flavicollis*, *Apodemus sylvaticus*, *Apodemus agrarius* and for the vole, *Clethrionomys glareolus*. These rodents transmit *B. burgdorferi* s.l. to a large number of *I. ricinus* larvae (Aeschlimann *et al.*, 1986; Matuschka *et al.*, 1992; de Boer *et al.*, 1993; Humair *et al.*, 1993a, 1999; Gern *et al.*, 1994a; Kurtenbach *et al.*, 1994a, 1995, 1998b; Tälleklint and Jaenson, 1994; Randolph and Craine, 1995; Hu *et al.*, 1997; Richter *et al.*, 1999).

More limited information has been obtained for other small- and medium-sized mammals. An additional species of vole, *Microtus agrestis*, has been described as a reservoir in Sweden by Tälleklint and Jaenson (1994), and black rats (*Rattus rattus*) and Norway rats (*Rattus norvegicus*) contribute to the transmission of *B. burgdorferi* s.l. in urbanized environments in continental Europe (Matuschka *et al.*, 1996, 1997). In an urban park in Germany, for example, *R. norvegicus* rats were less abundant than *A. flavicollis*, but showed a higher infestation by *I. ricinus* subadults and a greater infectivity for ticks (Matuschka *et al.*, 1996). Black rats and Norway rats also perpetuate LB spirochaetes in Madeira, a subtropical island where native terrestrial mammals are absent (Matuschka *et al.*, 1994b).

Edible dormice (*Glis glis*) (Matuschka *et al.*, 1994a) and garden dormice (*Eliomys quercinus*) (Matuschka *et al.*, 1999) have been shown to be reservoir hosts in Germany and in France, respectively. Edible and garden dormice were more heavily infested by larval and nymphal *I. ricinus* than were mice and voles in the studied habitats. This suggests that in certain habitats where dormice are abundant, these rodent species could be the primary reservoirs (Matuschka *et al.*, 1994b, 1999). Other rodents like grey squirrels (*Sciurus carolinensis*) in England (Craine *et al.*, 1997) and red squirrels (*Sciurus vulgaris*) in Switzerland (Humair and Gern, 1998) also contribute to the amplification of the infection in vector ticks. Grey squirrels, which were introduced into Great Britain from North America, were shown to be more important hosts than small rodents for *I. ricinus* larvae and nymphs (Craine *et al.*, 1995). Moreover, the abundance of grey squirrels makes this species an important source of infection for ticks wherever it is present. Similarly, in continental Europe red squirrels have also been found to be heavily infested with ticks (Matuschka *et al.*, 1996; Humair and Gern, 1998) and feeding ticks showed a high prevalence of infection (69%) (Humair and Gern, 1998). Although red squirrel density has not been evaluated, it is evident that they are potent reservoir hosts and that they contribute to the maintenance of *B. burgdorferi* s.l. in enzootic areas (Humair and Gern, 1998).

Among insectivores, the reservoir competence of three species of shrews (*Neomys fodiens*, *Sorex minutus* and *Sorex araneus*) has been demonstrated in Sweden (Tälleklint and Jaenson, 1994). Evidence of reservoir competence of hedgehogs has been obtained from Ireland (Gray *et al.*, 1994), Germany (Liebisch *et al.*, 1996) and Switzerland (Gern *et al.*, 1997). In Switzerland, an enzootic transmission cycle has been described in an urban environment involving hedgehogs and *I. hexagonus* in the absence of *I. ricinus* (Gern *et al.*, 1997).

Lagomorphs such as the brown hare (*Lepus europaeus*) and the varying hare (*Lepus timidus*) also contribute to the maintenance of *B. burgdorferi* s.l. in nature, as observed in Sweden in habitats where hares coexist with small mammals (Tälleklint and Jaenson, 1993, 1994), and on islands where hares are the only terrestrial mammal species permanently present (Jaenson and Tälleklint, 1996). Although the European rabbit (*Oryctolagus cuniculus*) can apparently function as a reservoir host for *B. afzelii* (Matuschka *et al.*, 2000), the reservoir capacity of this species seems limited since only one out of seven rabbits (14%) was infective to ticks.

Among large-sized mammals, two studies in Germany (Kahl and Geue, 1998; Liebisch *et al.*, 1998b) have implicated red foxes as reservoirs, but these animals did not appear to be very potent reservoirs since the borreliae were apparently poorly transmitted to ticks.

Birds in Europe were initially identified as non-reservoirs (Matuschka and Spielman, 1992). They were described not only as incompetent reservoirs, but also as having a zooprophylactic role. Zooprophylactic hosts are considered as dead-end hosts for *B. burgdorferi* s.l. because they eliminate borrelial infection in feeding ticks, which interrupts the maintenance cycle of the spirochaete. Although the involvement of birds has remained controversial for a long time, their role as reservoir hosts is now clearly established. Infected ticks have been collected from various bird species in Sweden (Olsen *et al.*, 1993, 1995a,b), Switzerland (Humair *et al.*, 1993b, 1998), England (Craine *et al.*, 1997; Kurtenbach *et al.*, 1998b) and the Czech Republic (Hubálek *et al.*, 1996). Ground-foraging birds such as thrushes (*Turdus* spp.), blackbirds (*Turdus merula*), robins (*Erithacus rubecula*) and pheasants (*Phasianus colchicus*) were particularly involved, as well as colonial seabirds.

B. burgdorferi s.l. was isolated from *I. uriae* ticks fed on seabirds (Olsen *et al.*, 1993, 1995a; Gylfe *et al.*, 1999), from *I. ricinus* ticks fed on blackbirds (Hubálek *et al.*, 1996; Humair *et al.*, 1998), from the skin of blackbirds (*T. merula*) (Humair *et al.*, 1998) and from the blood of puffins (*Fratercula artica*) (Gylfe *et al.*, 1999), suggesting that live spirochaetes can survive in birds and in bird-feeding ticks. Strong evidence of reservoir competence was eventually obtained for two bird species: blackbird (*T. merula*) (Humair *et al.*, 1998) and pheasant (*P. colchicus*) (Kurtenbach *et al.*, 1998a) were investigated by tick xenodiagnosis, which clearly demonstrated the efficient transmission of borrelial infection from birds to ticks. *B. burgdorferi* s.l. also circulates between seabirds such as razorbills (*Alca torda*) and puffins (*F. artica*) and the seabird tick *I. uriae* in isolated environments (Olsen *et al.*, 1993, 1995a; Gylfe *et al.*, 1999). Borrelia-infected *I. uriae* ticks have been observed in seabird colonies in the northern and southern hemispheres (Olsen *et al.*, 1995a). The similarity of DNA sequences of *B. burgdorferi* s.l. from *I. uriae* of various locations strongly suggests that seabirds transfer borrelial spirochaetes between seabird colonies (Olsen *et al.*, 1995a). Reactivation of latent borrelial infection has been observed with passerines experimentally submitted to stressful conditions that simulate migration (progressive decrease of photoperiod) (Gylfe *et al.*, 2000). This suggests that migrating birds could spread *B. burgdorferi* s.l. spirochaetes to ticks encountered along the migration route.

The determination of the reservoir competence of a host species is important but should also include quantification of the contribution of a particular host species in a particular habitat. The immune status of the host may have an impact on the circulation of *B. burgdorferi* s.l. in nature by affecting the ability of ticks to acquire infection from reservoir hosts. *Apodemus* spp. mice and *Clethrionomys* spp. voles are a good illustration of this phenomenon. These host species seem to have developed different responses towards tick infestation and borrelial infection, and this influences their respective reservoir importance. Kurtenbach *et al.* (1994b) showed in a laboratory study that *C. glareolus* voles express a low immunity to spirochaetes and as a consequence develop high levels of infection and show high transmission rates to ticks. In contrast, *Apodemus* spp. mice control spirochaete infection more effectively by a specific immune response that maintains their borrelial infection at a low level and also at a low transmission rate to ticks (Kurtenbach *et al.*, 1994b). In parallel, Dizij and Kurtenbach (1995) demonstrated experimentally that voles, but not mice, acquire resistance to *I. ricinus* ticks with consecutive infestations. Interestingly, some of the phenomena demonstrated by these laboratory results have been confirmed under natural conditions. Spirochaetes are more easily isolated from ear biopsies of *Clethrionomys* spp. voles than of *Apodemus* spp. mice (Petney *et al.*, 1996; Humair *et al.*, 1999). This high level of *B. burgdorferi* infection in voles also allows a higher transmission rate of spirochaetes from voles to the ticks feeding on them (Humair *et al.*, 1999). However, voles progressively acquire resistance to ticks after several tick infestations. Ticks that feed on these resistant voles suffer a retarded development due to a reduction of their engorgement weights as well as an increased mortality since they do not moult successfully. The low moulting success of ticks fed on voles lessens the relative contribution of voles in endemic areas, despite their high infection rate and their high infectivity for ticks (Humair *et al.*, 1999).

Reservoir potential (as defined by Mather *et al.*, 1989) or coefficient of transmission (Randolph and Craine, 1995) is an attempt to quantify the contribution as a reservoir of a particular host species in a particular habit. The reservoir potential is influenced by three main factors: (i) host infectivity to ticks; (ii) degree of tick–host contact (mean infestation rate by larval ticks); and (iii) host density in the habitat (Mather *et al.*, 1989). The higher the values of these three parameters, the higher the reservoir potential of the host and the more important its contribution as a reservoir in the studied habitat. The evaluation of reservoir potential is not an easy task since it requires the assessment of various factors (Tälleklint and Jaenson, 1994), but this multifaceted approach should be encouraged in the future.

The determination of a vertebrate species as a reservoir is obviously important in understanding the ecology of LB. The identification of a host as a non-reservoir or as a zooprophylactic host is also important since the circulation of *B. burgdorferi* s.l. in nature is impaired by such hosts. The identification of a non-reservoir is in fact much more difficult than that of a reservoir host, since repeated investigations are required. Until now, only a few vertebrate species have been described as non-reservoirs or zooprophylactic hosts in Europe.

The lizard (*Lacerta viridis*) was reported to have a zooprophylactic effect on the island of Madeira since spirochaetes were destroyed in the midgut of ticks feeding on this host (Matuschka *et al.*, 1994b). Several other vertebrates such as blackbirds (Matuschka and Spielman, 1992) and some ungulates (roe deer, *C. capreolus*; red deer, *Cervus elaphus*; sika deer, *Cervus nippon* (cited by Gern *et al.*, 1998); moose, *Alces alces*; fallow deer, *Dama dama*; cattle, *Bos taurus*; sheep, *Ovis aries*) (Gray *et al.*, 1992, 1995; Jaenson and Tälleklint, 1992; Tälleklint and Jaenson, 1994) have been reported as zooprophylactic or non-reservoir hosts. However, further studies have shown that some of these host species are in fact reservoir-competent for some genospecies of *B. burgdorferi*, for example blackbirds (Humair *et al.*, 1998), and others, for example sheep (Ogden *et al.*, 1997) and sika deer (Kimura *et al.*, 1995), may also contribute to the circulation of *B. burgdorferi* s.l. in nature.

Various factors may lead to the conclusion that a host is non-reservoir or is zooprophylactic. For example, most methods for the determination of the reservoir competence of a host are based on detection of systemic infections. However, co-feeding transmission, in which uninfected engorging ticks may acquire borrelia infections from localized sites where infected ticks feed simultaneously (Gern and Rais, 1996; Randolph *et al.*, 1996), has been reported in sheep in Britain (Ogden *et al.*, 1997) and sika deer in Japan (Kimura *et al.*, 1995). Even if some ungulates do not sustain systemic borrelia infection, those in which co-feeding transmission occurs can be considered as amplifying hosts. Quantification of co-feeding transmission in ungulates, particularly in deer, and its importance in maintaining disease foci remain to be evaluated.

Another factor complicating the determination of reservoir competence of a vertebrate host is the possibility of host specificity of different *Borrelia* genospecies. This aspect has been neglected for a long time and even now knowledge of the relationships between the different *B. burgdorferi* s.l. species and their hosts is poorly documented. Indications of this phenomenon in Europe were first provided by the research of Humair *et al.* (1995) in Switzerland, who showed that borrelial isolates from 14 ear biopsies taken from ten *C. glareolus* voles and one *A. flavicollis* mouse (three isolates were obtained from two recaptured voles) captured in two endemic areas belonged exclusively to *B. afzelii*, although *B. burgdorferi* s.s. and *B. garinii* were present in questing ticks in the same areas. Interestingly, the first borrelial isolate obtained from a rodent in Sweden (Hovmark *et al.*, 1988) was characterized as *B. afzelii* (Postic *et al.*, 1994). More recently, *B. afzelii* was once again isolated from ear biopsies of mice and voles collected in a third study site in Switzerland, suggesting that a specific association occurs between *B. afzelii* and small rodents (Humair *et al.*, 1999). The use of tick xenodiagnosis on mice and voles showed that *B. afzelii* is clearly the dominant genospecies transmitted from small rodents to *I. ricinus* ticks in two enzootic areas in Switzerland (Hu *et al.*, 1997; Humair *et al.*, 1999). Out of 124 positive xenodiagnostic ticks, 120 ticks (97%) were infected by *B. afzelii*, two ticks contained *B burgdorferi* s.s. (one with a co-infection with *B. afzelii*) and two ticks contained uncharacterized spirochaetes. The isolation of *B. afzelii* from xenodiagnostic ticks demonstrates the viability of spirochaetes discovered in rodent-feeding ticks, which is a crucial point in the transmission of *B. burgdorferi* s.l. from rodents. All these results strongly suggest that an association exists between *Apodemus* spp. mice, *Clethrionomys* spp. voles and *B. afzelii*, since this genospecies is almost exclusively transmitted to *I. ricinus* larvae from these animals. Dolan *et al.* (1998) obtained similar results with *I. ricinus* ticks and laboratory mice in a Centres for Disease Control and Prevention (CDC) laboratory, where *B. afzelii* was isolated in all ear biopsy cultures from outbred laboratory mice that had been infested with *I. ricinus* ticks collected from the field near Neuchâtel (Switzerland). Additionally, xenodiagnostic *I. ricinus* larvae fed on outbred mice inoculated with other strains of *B. burgdorferi* s.l. (*B. afzelii* strain PGau.C3, *B. garinii* strains VS286 and VSBP, *B. burgdorferi* s.s. strains B-31 and B-31.D1) acquired all three *Borrelia* genospecies. However, the prevalence of *B. afzelii* infection in ticks was particularly high (90%) compared with low infection prevalences (3–5%) with *B. garinii* and *B. burgdorferi* s.s. Further, only *B. afzelii* survived the transstadial maintenance to nymphs (Dolan *et al.*, 1998). Additional observations were obtained by Hubálek *et al.* (1998a), who isolated *B. afzelii* from two *A. flavicollis*, one *C. glareolus* and two fleas (*Ctenophthalmus agyrtes*) fed on *C. glareolus* in the Czech Republic.

In parallel with the observations made by Humair *et al.* (1995) on *B. afzelii* and small rodents, Olsen *et al.* (1995b) described the predominant presence of *B. garinii* in ticks feeding on migrating birds collected in Sweden and Denmark: *B. garinii* DNA was found in 70% of *I. ricinus* larvae (23/33), *B. burgdorferi* s.s. DNA in 20% (7/33) and *B. afzelii* DNA in 9% (3/33). *B. garinii* was detected in all *I. ricinus* larvae (*n* = 11) collected from autumn-migrating birds and was also most prevalent in *I. ricinus* larvae collected from spring-migrating birds. In addition to migrating passerines, the existence of an association between birds and *B. garinii* was clearly illustrated by Olsen *et al.* (1995a) in the case of seabirds and the vector *I. uriae*. In the isolated maintenance cycle involving seabirds and the seabird tick (Olsen *et al.*, 1993), *B. garinii* was isolated and characterized from *I. uriae* ticks and more recently from seabird tissues (Olsen *et al.*, 1995a; Gylfe *et al.*, 1999).

In pheasants, more than 50% of *I. ricinus* ticks were infected by borreliae, particularly *B. garinii* (27%) and *B. valaisiana* (16%) (Kurtenbach *et al.*, 1998b). Hubálek *et al.* (1996) in the Czech Republic obtained a *B. garinii* isolate from a nymphal *I. ricinus* fed on *T. merula*. Shortly afterwards, *B. garinii* and *B. valaisiana* infections (seven isolates and four DNA detections) were revealed in five *T. merula* and one *Turdus philomelos* (Humair *et al.*, 1998). No infection with *Borrelia* genospecies other than *B. garinii* and *B. valaisiana* has been observed in European *Turdus* spp.

Characterization of borreliae from xenodiagnostic ticks fed on blackbirds in Switzerland demonstrated that *B. valaisiana* was most prevalent in these ticks (30%) when compared with *B. garinii* (3%) and *B. afzelii* (3%). Co-infections with *B. valaisiana* and *B. garinii* or *B. afzelii* were observed in 4% of the ticks. Interestingly, only *B. valaisiana* and *B. garinii* were isolated successfully from these xenodiagnostic ticks, whereas the presence of *B. afzelii* was revealed by DNA detection only (Humair *et al.*, 1998). This shows that blackbirds transmitted viable *B. valaisiana* and *B. garinii* to ticks feeding on them whereas the viability of *B. afzelii* spirochaetes in bird-feeding ticks remains uncertain.

More recently, Gylfe *et al.* (2000) demonstrated that *B. garinii* latent infection can be reactivated in experimentally infected redwing thrushes (*Turdus iliacus*) placed under stressful conditions simulating migration and causing migratory restlessness. This suggests that *Turdus* spp. passerines can sustain *B. garinii* infection for a long time and that migrating birds placed under conditions of autumnal migration transmit *B. garinii* to ticks. This corroborates the previous observation of *B. garinii* in all examined *I. ricinus* ticks from autumnal migrant birds (Olsen *et al.*, 1995b). Birds have a high body temperature, and Hubálek *et al.* (1998b) reported that the optimal growth temperature of *B. garinii* is higher than that of the other two pathogenic species, *B. afzelii* and *B. burgdorferi* s.s. Overall, these data strongly suggest that an association exists between birds and *B. garinii* and also *B. valaisiana*, a more recently described genospecies. Interestingly, an association between *B. garinii* and birds has been observed in Japan (Nakao *et al.*, 1994; Miyamoto *et al.*, 1997; Ishiguro *et al.*, 2000). However, in areas outside the geographical distribution of *B. garinii*, such as North America, *B. burgdorferi* s.s. is the *Borrelia* sp. circulating between birds and ticks (Richter *et al.*, 2000).

In addition to the associations of small rodents with *B. afzelii*, and birds with *B. garinii* and *B. valaisiana*, another association has been described in Switzerland and in the UK. As previously mentioned, grey and red squirrels (*S. carolinensis* and *S. vulgaris*) are reservoirs for *B. burgdorferi* s.l. In Switzerland, *B. burgdorferi* s.s. and *B. afzelii* were the only two genospecies isolated from skin samples of red squirrels (*S. vulgaris*) and were the two most prevalent genospecies found in *I. ricinus* ticks engorging on red squirrels, representing 96% of the isolates (Humair and Gern, 1998). The high frequency of *B. burgdorferi* s.s. and *B. afzelii* in the skin of squirrels as well as in squirrel-feeding *I. ricinus* suggests that both genospecies are preferentially transmitted from squirrels to ticks. Similar findings were obtained by Craine *et al.* (1997) in the UK: *B. afzelii* was identified in *I. ricinus* nymphs fed on a naturally infected grey squirrel and another grey squirrel sustained an experimental infection with *B. burgdorferi* s.s.

However, other borrelia–host associations that may depend on the local ecosystems have been described. In some parts of Europe, in addition to *B. afzelii*, *B. garinii* was also observed to be associated with rodents. Thus, data from Austria, Germany and Russia showed that different genospecies can be isolated from or DNA detected (by PCR) in the urinary bladder, heart and spleen of small mammals or in xenodiagnostic ticks fed on rats and yellow-necked mice (Khanakah *et al.*, 1994; Gorelova *et al.*, 1995; Richter *et al.*, 1999). Differences in biological and ecological factors occurring in some parts of Europe, such as the presence of different tick and rodent species or of different *B. garinii* subtypes, could explain the observed discrepancy. For example, *B. garinii* serotype 4, which has only been described in the cerebrospinal fluid of patients in Germany, The Netherlands, Denmark and Slovenia (Wilske *et al.*, 1993, 1996; van Dam *et al.*, 1997), is apparently rare in nature and has so far only been found in *I. ricinus* ticks collected in Germany (Eiffert *et al.*, 1995; Hu *et al.*, 2001). The difficulty in obtaining infected ticks from laboratory mice infected with *B. garinii* (usually associated with birds) has been documented previously (Gern *et al.*, 1994b; Dolan *et al.*, 1998). However, it was shown recently that laboratory mice infected with the serotype 4 of *B. garinii* easily transmitted this serotype to ticks (Hu *et al.*, 2001), which could be explained by the resistance of this serotype to complement (van Dam *et al.*, 1997). Therefore, one explanation for the observation of *B. garinii* in rodents in Austria, Germany and Russia may be the presence of *B. garinii* serotype 4 or related serotypes in these areas. In Japan, a similar association has been described between rodents and *B. garinii* ribotype IV, since this particular ribotype was predominant in rodents (*Apodemus speciosus* and *Clethrionomys rutilus*) and rodent-feeding *I. persulcatus* ticks (Nakao *et al.*, 1994). These observations show how diverse the ecology of *B. burgdorferi* s.l. can be.

Although some of the reservoirs of *B. afzelii*, *B. burgdorferi* s.s., *B. garinii* and *B. valaisiana* have been documented, those of *B. lusitaniae* still remain to be identified. De Michelis *et al.* (2000) suggested that avian and rodent species might be reservoir hosts for *B. lusitaniae* because DNA of this genospecies has been detected in adults of *Hyalomma marginatum*, a tick species that mainly feeds on birds and rodents as subadults (Hillyard, 1996). Other borrelia types identified in European patients by Picken *et al.* (1996) and Wang *et al.* (1999) have not been described in either ticks or wild animals.

Specific associations between reservoirs and *Borrelia* spp. spirochaetes imply that in certain cases *B. burgdorferi* s.l. genospecies are also transmitted to inadequate hosts. The specific association between birds and *B. garinii/B. valaisiana* in Europe may explain why the reservoir role of birds has remained controversial for a long time and why they were first described as zooprophylactic hosts. The first European studies on birds were performed before the description of different genospecies within the *B. burgdorferi* s.l. species complex. The existence of specific associations explains why blackbirds (*T. merula*) were first described as incompetent reservoirs and even as zooprophylactic hosts for LB spirochaetes by Matuschka and Spielman (1992). In this experiment, blackbirds were exposed to infected *I. ricinus* nymphs that acquired infection after feeding as larvae on black-striped mice (*A. agrarius*). Thus, considering the association of small rodents and *B. afzelii*, it is most probable that the *I. ricinus* nymphs used to infect blackbirds carried mouse-adapted *Borrelia* genospecies. Consequently, blackbirds were effectively inadequate hosts for the *Borrelia* spp. to which they had been exposed, because they are incompetent reservoirs and zooprophylactic hosts for rodent-adapted genospecies.

The existence of specific spirochaetal transmission patterns, of zooprophylactic and of non-reservoir hosts can be explained by the specific borreliacidal effect of host complement (Kurtenbach *et al.*, 1998c). This effect may prevent infection of the vertebrate host by some *Borrelia* species and may result in loss of spirochaetes by ticks that feed on inadequate hosts. The pattern of serum sensitivity of different *Borrelia* genospecies matches the known reservoir status of many vertebrate species for *B. burgdorferi* s.l. For example, *B. afzelii* was resistant to rodent sera whereas a strain of *B. garinii* was readily lysed, and the absence of systemic infection in deer correlates with the borreliacidal activity of deer sera against all the genospecies tested (Kurtenbach *et al.*, 1998c).

Conclusions

The ecological picture of LB is particularly complex because of the existence of diversity within the three main components of this zoonosis: pathogens, vectors and reservoirs. At present in Europe, five *B. burgdorferi* s.l. genospecies, three vector species and about 35 reservoir host species are linked in a web of relationships between each other. By focusing on this web, it can be seen that the links are organized, since each element of the web has privileged relationships with some others. For example, the seabird tick, *I. uriae*, is only infected by *B. garinii*, whereas *I. ricinus* may be infected by any of the five genospecies present in Europe. Similarly, *I. uriae* exclusively infests seabirds, whereas *I. ricinus* infests a large range of mammals and birds. It is obvious from this example that *B. garinii* is associated with seabirds, whereas similar associations between hosts of *I. ricinus* and *B. burgdorferi* s.l. genospecies are less obvious.

Although *I. ricinus* infection by *B. burgdorferi* s.l. genospecies and host infestation by ticks are generally well documented, the relationships between *I. ricinus* hosts and borreliae needs clarification in many respects. Additionally, the web varies geographically as well as temporally.

A huge amount of knowledge has been collected on the ecology of *B. burgdorferi* s.l. in Europe but further studies are needed for a better understanding of the situation. Emphasis has to be put on the distribution of the various *Borrelia* species and subtypes throughout Europe, the identification of additional reservoir hosts, their relationships with the various *Borrelia* species and the immune response of hosts towards ticks and borreliae. Because of regional and local pecularities, these studies should be performed in as many habitats as possible. The collection of these local snapshots will help towards a better picture of the ecology of *B. burgdorferi* s.l. in Europe.

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