Lyme disease

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Summary

Lyme borreliosis, the most common vector-borne disease in the northern hemisphere, is caused by bacteria belonging to the *Borrelia burgdorferi* complex. The disease is multisystemic, affecting mainly the skin, nervous system, heart and joints. In Europe, the vector of the disease is the tick *Ixodes ricinus,* whereas in the United States of America, two primary tick vectors exist, namely: *I. scapularis* in the north-eastern and mid-western regions and *I. pacificus* on the west coast. Several species of small and medium-sized mammals and ground-feeding birds serve as reservoirs for the bacteria in endemic areas. The prognosis for patients with Lyme borreliosis is excellent, particularly when diagnosed and treated early in the course of infection. Prevention of Lyme borreliosis can be achieved using two approaches, either prevention of infection by immunisation, or prevention of tick bites through avoidance, personal protection and tick control.

Keywords

Borrelia burgdorferi – Clinical manifestations – Ecology – Epidemiology – Ixodes – Lyme disease – Prevention – Public health – Ticks – Zoonoses.

Introduction

Lyme borreliosis is a multisystemic disease caused by spirochaete bacteria belonging to the *Borrelia burgdorferi* sensu lato (sl) complex. These spirochaetes are transmitted by ticks of the genus *Ixodes*. Lyme disease is a zoonosis which is maintained in nature in enzootic cycles involving various tick species and some of the hosts of these ticks. The distribution of *B. burgdorferi* sl is world-wide but covers mainly the Northern hemisphere, where well-defined clinical cases have been reported.

Clinical manifestations of Lyme borreliosis have been described in Europe for many years. However, the aetiology of the disease remained unknown until W. Burgdorfer discovered the causative bacteria in North America less than twenty years ago (19). In fact, the first description of Lyme borreliosis was made in 1883 by a German physician, A. Buchwald, who described an inflammatory skin lesion, *Acrodermatitis Chronica Atrophicans* (ACA) (18). Several years later, in 1909, at the Swedish Dermatological Society, A. Afzelius demonstrated a migrating annular skin lesion that had developed at the site of an *I. ricinus* tick bite (2). He called this lesion *Erythema Chronicum Migrans* (ECM). In 1922, Garin and Bujadoux (44) described neurological disorders, such as facial palsy, which developed after ECM, and in 1941, Bannwarth reported many cases of chronic lymphocytic meningitis (6). Various aetiologies for skin manifestations, neurological disorders and joint problems observed in countries of Europe were suspected. The infective nature of ECM and ACA was proven in 1955 by Binder *et al.* and Götz (16, 51). Successful treatment by antibiotics was also reported by Hollström for ECM (55), and by Thyresson for ACA (131). However, the real aetiology of these clinical manifestations remained unknown until arthritis and ECM were reported in the United States of America (USA) and investigated further. Weber and Pfister provide a more detailed review of the history of Lyme borreliosis in Europe (133).

In 1975, Polly Murray and Judith Mensch from Lyme, in Connecticut, USA, reported to Dr David Snydman at the Connecticut State Health Department that their children, as well as other children living in Lyme, were thought to have juvenile arthritis. This epidemic of arthritis was recognised as a new disease and was named Lyme arthritis by Steere *et al.* (120). Epidemiological studies among populations living in this area showed that this form of arthritis was associated with skin lesions and neurological and cardiac manifestations (119). In 1978, studies incriminated the tick *I. scapularis* in the epidemiology of Lyme disease (121). Later, links were made with clinical manifestations described in Europe and treatment of patients from Lyme with antibiotics was successful (123). In 1982, the infectious origin of Lyme borreliosis was demonstrated by Burgdorfer, who identified spirochaetes in *I. scapularis* ticks which reacted with immune sera from patients (19), and by Barbour who isolated and cultured spirochaetes from ticks (8). Several months later, spirochaetes were also isolated from ticks collected in Europe (10, 20). These spirochaetes were named *B. burgdorferi* in honour of the discoverer, W. Burgdorfer (68).

Based on the deoxyribonucleic acid (DNA), DNA/DNA hybridisation of a small number of strains, a single species was initially thought to be responsible for Lyme disease (also called Lyme borreliosis) in the USA and Europe. Later, DNA/DNA hybridisation, ribotyping and 16S ribosomal ribonucleic acid (rRNA) sequencing of isolates obtained from different geographic origins in North America and Europe showed that at least three different species were responsible for Lyme disease, namely: *B. burgdorferi sensu stricto* (ss), *B. afzelii* and *B. garinii* (7, 22, 82, 101). Additional *Borrelia* species have been described in the USA and Eurasia, but the role of these species in the pathogenicity of Lyme borreliosis is still uncertain, as discussed below.

Aetiological agent

Borrelia burgdorferi, the aetiological agent of Lyme borreliosis, is a motile, long, slender, helix-shaped bacterium. *Borrelia* spirochaetes are Eubacteria in the order Spirochaetales (56). Analysis of 16S rRNA sequences has shown that the phylogeny of the spirochaetes consists of the following six major groups: *Treponema*, *Spirochaeta*, *Borrelia*, *Serpulina*, *Leptospira* and an undefined strain (99). The DNA of *Borrelia* has a low guanine-cytosine content, ranging from 27.1% to 30.5%. Other genera, such as *Leptospira* and *Treponema*, contain between 35% and 53% of guanine-cytosine in the genomic DNA (12, 65). *Borreliae* are agents of relapsing fevers and Lyme borreliosis and are transmitted by ticks, with the exception of *B. recurrentis*, the agent of louse-borne relapsing fever, which is transmitted by lice.

Borrelia burgdorferi measures between 4 µm and 30 µm in length, 0.18 µm to 0.25 µm in diameter and presents from three to ten irregular coils (67). The cell is surrounded by a fluid outer envelope which contains outer surface proteins (66). Between the protoplasmic cylinder and the outer membrane is the periplasmic space. In this periplasmic space, *B. burgdorferi* has several flagella which are inserted subterminally at each end of the protoplasmic cylinder (seven to eleven flagella at each end) and overlap in the centre of the spirochaete (57). The protoplasmic cylinder contains the genomic material.

Spirochaetes can be observed by dark-field microscopy or phase contrast microscopy. Cells can be visualised after staining with Gram, Giemsa or carbofuchsine (104) stains, as well as by immunological reactions using anti-*Borrelia* antibodies labelled with fluorescein, for example.

Borrelia burgdorferi can be cultivated *in vitro* in Barbour-Stoenner-Kelly (BSK) medium (10), in modified Kelly medium (MKP) (103), or in a medium described by Sinsky and Piesman (116). All these media are complex, containing amino acids, vitamins, inorganic salts, N-acetyl glucosamine, serum albumin and rabbit serum. The optimal temperature for growth is between 33°C and 34°C. The generation time is between 7 hours and 20 hours, and a concentration of 10^6-10^8 borreliae/ml can be reached.

Immunochemical analysis of *B. burgdorferi* isolates has revealed more than thirty polypeptides in protein profiles (12). One of the most important proteins is flagellin, a major constituent of flagella (13). Other proteins have been identified which are located on the surface of *B. burgdorferi* and named outer surface proteins (Osp), all of these proteins are lipoproteins (17). OspA (30 kDa-33.5 kDa), OspB (34 kDa-35 kDa) and OspC (20 kDa-23 kDa) of the different genospecies are variable in size. The expression of these proteins varies during subcultures. Wilske *et al.* observed that quantitative expression of OspA and OspB in culture is inversely proportional to that of OspC (136). Schwan *et al.* also observed the variation in the expression of these proteins in ticks (109). In unfed ticks, spirochaetes express OspA and not OspC. During the feeding of ticks, expression of OspA is switched off and OspC is expressed (109). However, in some cases, OspC is already expressed in the midgut of unfed *I. ricinus* ticks (39, 79). The variation in expression of OspA and OspC in the tick midgut during feeding is probably related to the temperature increase and the presence of mammalian blood (109). Interestingly, antibodies to OspA are rarely detected in humans or animals infected by tick bites, whereas animals infected by syringe inoculation of cultured spirochaetes develop high levels of anti-OspA antibodies (46, 105). Other Osp proteins have been described in the outer membrane, such as p39, OspD (28 kDa) (94), OspE (19 kDa) and OspF (26 kDa) (75).

The genomic structure of *B. burgdorferi* is unique among prokaryotes. The genome consists of a single linear chromosome of approximately 1,000 kilobases (kb) in size with both linear and circular plasmids of various sizes (14, 15, 38, 107). Four to nine plasmids are present and range in size from 8 kb to 140 kb (9). The peculiarity of the genome of *B. burgdorfèri* resides in the small size and the linearity of the chromosome and some of the plasmids. Recently, the sequence of the entire genome of *B. burgdorferi* has been determined (41). The arrangement and the organisation of the rRNA genes in *B. burgdorferi* is unusual because two copies of *rrl* (23S) and *rrf* (5S) are present, but only one copy of *rrs* (16S) (29, 42, 110). The *Osp* genes encoding the different Osp proteins are situated on linear or circular plasmids. The *OspA* and *OspB* genes of *B. burgdorferi* ss are carried, as an operon, on a 49-kb linear plasmid (14), whereas the operon is located on a 55-kb and 56-kb linear plasmid in *B. garinii* and *B. afzelii*, respectively (108). The *OspE* and *OspF* genes are also organised in an operon on a 45-kb linear plasmid (75). The *OspD* gene is present on a 38-kb linear plasmid (94). In contrast, the *OspC* gene is localised in a 27-kb circular plasmid (83, 106).

Borrelia burgdorferi has been isolated from various vertebrates, including man, and from ticks and insects. Isolates of *B. burgdorferi* show considerable phenotypic diversity, first described among European isolates (11, 135). Later, on the basis of analysis of rRNA gene restriction patterns, protein electrophoresis patterns, and monoclonal antibody reactivity, *B. burgdorferi* was separated into ten species, all identified under the broader name *B. burgdorferi* sl: *B. burgdorferi* ss, *B. andersonii* (84), *B. bissettii* (102), *B. garinii*, *B. afzelii* (22), *B. valaisiana* (132), *B. lusitaniae* (78), *B. japonica* (70), *B. tanukii* (43) and *B. turdae* (43). In the USA, three *Borrelia* species have been reported, as follows: *B. burgdorferi* ss, *B. andersonii* and *B. bissettii*. In Europe, five species, namely: *B. burgdorferi* ss, *B. garinii*, *B. afzelii*, *B. valaisiana* and *B. lusitaniae*, have been identified. *Borrelia burgdorferi* ss seems to be absent in Asia, where *B. garinii*, *B. afzelii*, *B. japonica*, *B. tanukii* and *B. turdae* have been isolated. The last three species have never been isolated outside Japan.

Some of these *Borrelia* species appear to have a very specific association with their tick vectors, implicating a well-defined geographic distribution. *Borrelia andersonii* is associated with *I. dentatus* in North America, *B. lusitaniae* with *I. ricinus* in Europe and North Africa, and three species, *B. japonica*, *B. tanukii* and *B. turdae*, have been isolated in Japan from *I. ovatus*, *I. tanuki* and *I. turdi*, respectively. The remaining *Borrelia* species, *B. burgdorferi* ss, *B. garinii*, *B. afzelii*, *B. bissettii* and *B. valaisiana*, are transmitted by a larger number of tick species, depending on their geographic distribution, namely: *I. scapularis*, *I. pacificus* and *I. spinipalpis* in the USA, *I. ricinus* and *I. hexagonus* in Europe and *I. persulcatus* in Eurasia. The distribution of *B. garinii* has been reported world-wide and is linked to that of a tick associated with sea birds, *I. uriae*, from which the species has been isolated.

The division into genomic groups appears to have some clinical relevance. Until now, only *B. burgdorferi* ss, *B. afzelii* and *B. garinii* have been clearly associated with clinical manifestations of Lyme borreliosis. The pathogenic potential of the other *Borrelia* species remains unknown.

Clinical manifestations and diagnosis

The clinical manifestations of Lyme borreliosis are diverse, due to the fact that infection with *B. burgdorferi* may affect a variety of organs, including the skin, heart, nerves and joints. In common with syphilis, another spirochetal disease, the progression of symptoms is often discussed in terms of stages (5), with the late stages of Lyme borreliosis being generally more severe and more difficult to treat than early stage infection (111). However, classification of Lyme borreliosis by stages can present problems, as symptoms may overlap and all stages do not necessarily occur in every patient (90). Another factor which complicates the clinical presentation of Lyme borreliosis is that differences in disease manifestation and severity occur throughout the world, with a wider range of disease presentations reported from Europe than from North America (118). This probably reflects the wider variety of *B. burgdorferi* genotypes found in Europe.

Early localised infection

One of the hallmark symptoms of early Lyme borreliosis is an expanding skin rash, erythema migrans (EM, formally ECM), which occurs in approximately 90% of patients with objective findings of *B. burgdorferi* infection (45, 137). This rash typically commences a few days to several weeks after the bite of an infected tick. The initial presentation of EM is usually as an erythematous macule or papule at the site of a tick bite, which expands over days to weeks in a circular or oval pattern as the spirochaetes migrate along the leading edge. The erythema may contain bands of skin which appear normal within its border (91) (Fig. 1a). Although early descriptions of this rash stressed the appearance of annular bands with central clearing (124), recent studies in the USA demonstrate that the appearance may vary, including irregularity in shape, central vesiculation and localised pruritus (91, 92) (Fig. 1b). However, central clearing appears to be more common in Europe than in North America (93). Although EM is often asymptomatic, some patients experience localised itching, irritation, burning or heat (4). The EM will eventually fade, even without treatment. Secondary lesions may also develop, located anywhere on the body except the palms and soles, and these are a symptom of disseminated infection (124).

Early disseminated infection

If early infection spreads beyond the primary skin lesion, a variety of systemic symptoms can occur. Such symptoms may include fatigue, arthralgia, myalgia, headache, fever, stiff neck and, less commonly, dizziness and nausea (92). In the USA, up to 80% of patients presenting with EM have associated systemic complaints, and these may occur before, during, or after resolution of the rash (91). Systemic symptoms associated with EM appear to be more common in the USA than in Europe, with patients in Europe generally having a milder early course of disease (93, 128).

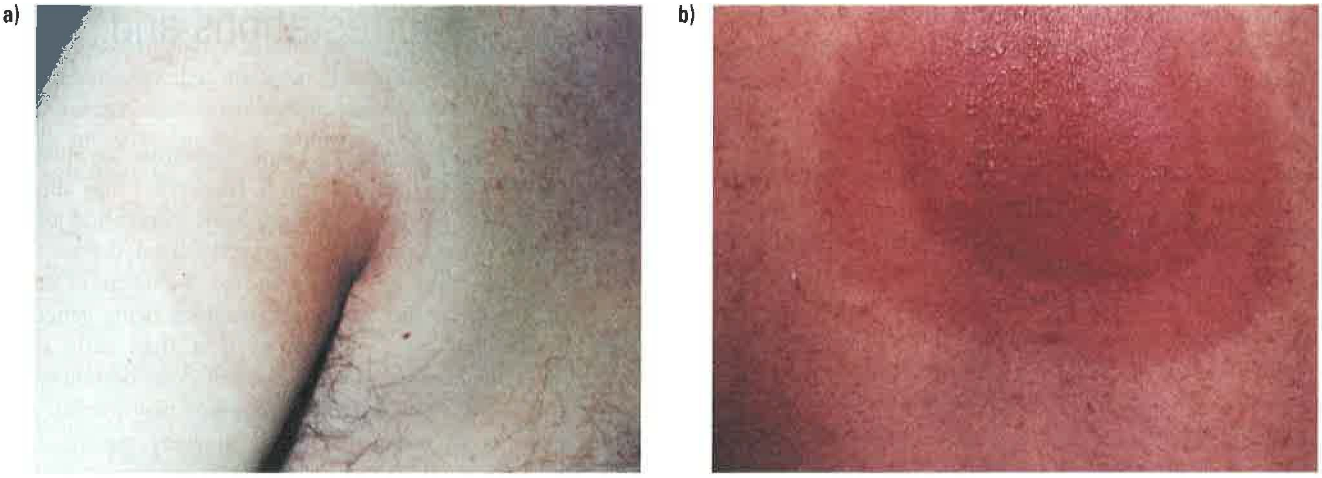

Fig. 1
a) Culture-confirmed erythema migrans showing typical banding pattern
b) Atypical culture-confirmed erythema migrans showing vesiculation
Photographs: courtesy of Dr G.P. Wormser, New York Medical College

In untreated patients, more severe systemic manifestations may occur weeks to months after disease onset and may involve the neurological, cardiac and rheumatological systems. Neurological manifestations usually include cranial nerve palsy, meningitis and radiculoneuritis, alone or in combination. Cardiac involvement may occur in 4% to 8% of cases, with atrioventricular block being the most commonly described abnormality (122). Acute arthritis may succeed arthralgia in cases of disseminated infection, with 60% of patients in the USA reporting asymmetric oligoarticular arthritis in large joints, especially the knee (126).

Late infection

Late, persistent infection may begin months to years after the onset of disease and may follow a period of intermittent symptoms (124). Manifestations of late Lyme disease include chronic neurological and rheumatological abnormalities. Neurological manifestations include progressive encephalomyelitis, encephalopathy, peripheral neuropathy and a variety of central nervous system (CNS) disorders (72). Rheumatological abnormalities are seen primarily in the form of chronic arthritis, affecting only one or a few large joints, such as the knee. Although historically it has been suggested that joint manifestations were more common in North America and neurological involvement more prevalent in Europe, studies suggest that the neurological and arthritic manifestations in both regions are remarkably similar (53, 54). The chronic and progressive skin disorder known as ACA is the most common chronic manifestation of late Lyme borreliosis in Europe and may occur many years after the initial infection (4). This condition is characterised by red or bluish-red lesions, usually on the extremities, which may become permanently atrophic or indurated (118). This disorder is not common in North America.

Diagnosis of Lyme borreliosis in endemic areas is based largely on the clinical presentation, depending heavily on recognition of EM or, less commonly, a 'flu-like' illness during the summer months (112). However, diagnosis can be more difficult in the absence of a recognised EM lesion or in the case of later-stage infection. In such cases, appropriate laboratory tests may be helpful.

Culture of *B. burgdorferi* from infected patients can be difficult. Consequently, diagnostic tests for Lyme borreliosis rely primarily on the measurement of antiborrelial antibodies present in blood, cerebrospinal fluid, or synovial fluid. In the USA, the recommended serological testing is a two-step process. A positive or equivocal result to enzyme-linked immunosorbent assay (ELISA) is followed by an immunoblot on the same sample, which can detect immunoglobulin (Ig) M and IgG antibodies against individual *B. burgdorferi* antigens that have been separated by electrophoresis (93). If the immunoblot is positive, the diagnosis of Lyme borreliosis is supported. Generally, serological testing is less effective during early, localised infection and becomes more sensitive in disseminated and chronic disease. Strategies for the use of serology to assist in the diagnosis of the disease in Europe are under investigation (118).

Epidemiology and surveillance

Lyme borreliosis has been reported from the USA, Canada, large areas of Europe and northern Asia. Although a Lyme borreliosis-like illness has been reported in Australia, Africa and South America, *B. burgdorferi* has not yet been isolated from patients in these regions and, therefore, the disease should not be considered endemic in these areas (93).

Lyme borreliosis is the most common vector-borne disease in the USA, with the number of cases increasing annually from a low of approximately 500 cases in 1982 to an average of almost 15,000 cases per year in recent years (24; K. Orloski, unpublished findings) (Fig. 2).

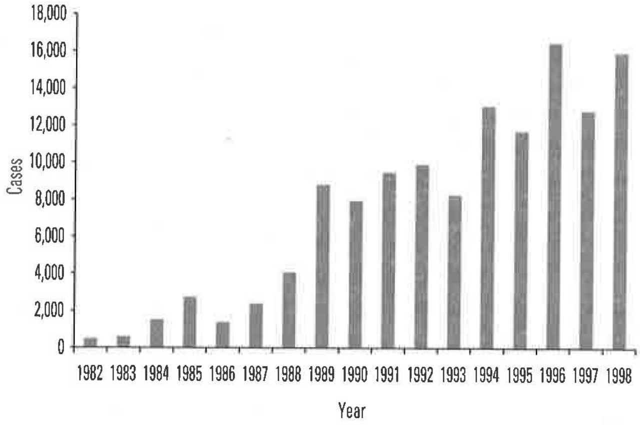

Fig. 2
Cases of Lyme borreliosis reported in the United States of America between 1982 and 1998
Source: Centers for Disease Control and Prevention, Atlanta

Most cases in the USA are reported from the mid-Atlantic, north-east, and north central regions, with some Counties in these areas reporting incidence rates greater than 100 cases per 100,000 population (24). Furthermore, recent studies have shown that the disease may be significantly under-reported by physicians in these endemic areas, and that the actual number of cases may be several-fold higher than suggested by the current passive reporting system (21).

Demographic data from the USA indicate that females account for 51% of reported Lyme borreliosis cases, and that incidence rates are highest for children under fifteen years of age and for people between twenty-five and forty-four years of age (89). In Europe, early manifestations of Lyme borreliosis are distributed nearly equally among all age groups (117).

Epidemiological data for Lyme borreliosis are typically derived from case information reported to health officials by local physicians. For surveillance purposes, Lyme borreliosis in the USA is defined as the presence of an EM rash of more than 5 cm in diameter, or laboratory confirmation of infection with *B. burgdorferi* and at least one objective sign of musculoskeletal, neurological or cardiovascular disease (23). In Europe, a general case surveillance system comparable to that in the USA has not been established and, at present, each country evaluates the prevalence of Lyme borreliosis using individual methods. However, a standardised case definition suitable for epidemiological use throughout Europe is currently being developed (118). Reliance on reporting by physicians can nevertheless be problematic. Under-reporting, as mentioned above, in addition to overdiagnosis, may confound efforts to determine the true incidence of Lyme borreliosis or assess risk in an area (114). For this reason, alternative methods of assessing environmental risk may be preferable to case reporting in many circumstances.

In the USA, a strong correlation exists between the seasonal distribution of nymphal *I. scapularis* and the seasonal onset of EM, suggesting that the peak risk period for Lyme borreliosis is the time when nymphal *I. scapularis* are most active (37). For this reason, sampling for nymphs is a reliable method of evaluating the risk of Lyme borreliosis and has been used as an alternative to human case surveillance (28, 32). One sampling method, known as 'drag sampling', involves pulling a 1 m^2 piece of heavy, white cloth (flannel or corduroy) over vegetation; this is the most effective method of collecting host-seeking *I. scapularis* in forested areas (33). This sampling method allows the investigator to determine the density of host-seeking nymphs in an area. Collected nymphs may then be tested using a variety of methods (e.g. polymerase chain reaction [PCR]), to determine the rate of *B. burgdorferi* infection. In Europe, the correlation between nymphal *I. ricinus* activity and the risk of acquiring Lyme borreliosis is not as great, since the host-seeking activities of nymphs and adults are almost simultaneous and, therefore, both stages must be considered when evaluating the risk of infection in an area.

Another effective surveillance method is the study of ticks which parasitise humans. Such studies, which rely on the identification of ticks found attached to people, can provide valuable information on the nature and frequency of tick bites and the risk for Lyme borreliosis in a region. For example, studies on tick bites in the north-eastern USA have demonstrated that almost 70% of victims of tick bites were exposed near the home, that children of 10 years of age and younger receive more tick bites than any other age class, and that nymphal ticks remain attached to victims longer than adult ticks (30, 36). In Europe, such studies have shown that the proportion of tick bite victims who become ill after a known bite is below 1% (1). Health officials can use data from these studies to supplement human case report data to determine the nature of risk and identify areas of high risk for Lyme borreliosis.

The goal of Lyme borreliosis surveillance should be to obtain epidemiological data and to assess environmental risk so that prevention and control measures can be targeted effectively. Such a programme should therefore include both human case reporting and the use of tick surveillance methods. The use of sentinel animals, such as dogs, has also been demonstrated to accurately assess risk for Lyme borreliosis and may be incorporated into a comprehensive surveillance programme (27, 35). Lyme borreliosis risk, in common with other tick-borne diseases, is closely linked to the ecology of an area.

Inclusion of a component to measure environmental risk, is therefore strongly recommended for any surveillance programme.

Ecology

The endemic status of Lyme borreliosis is maintained through complex interactions among different tick species, a variety of strains of *Borrelia* and a large number of vertebrate hosts. The tick *I. ricinus* is the classic vector of Lyme borreliosis in Europe (Fig. 3). Two additional tick vectors, *I. hexagonus*, a species which frequently infests carnivores, and *I. uriae*, which is associated with sea birds, are implicated in *Borrelia* transmission in endemic areas, but both species only rarely bite humans. Spirochaetes have also been detected or isolated from insects (mosquitoes and fleas), but *B. burgdorferi* transmission by non-tick vectors has never been demonstrated and the role of insects in the maintenance of a focus remains unknown.

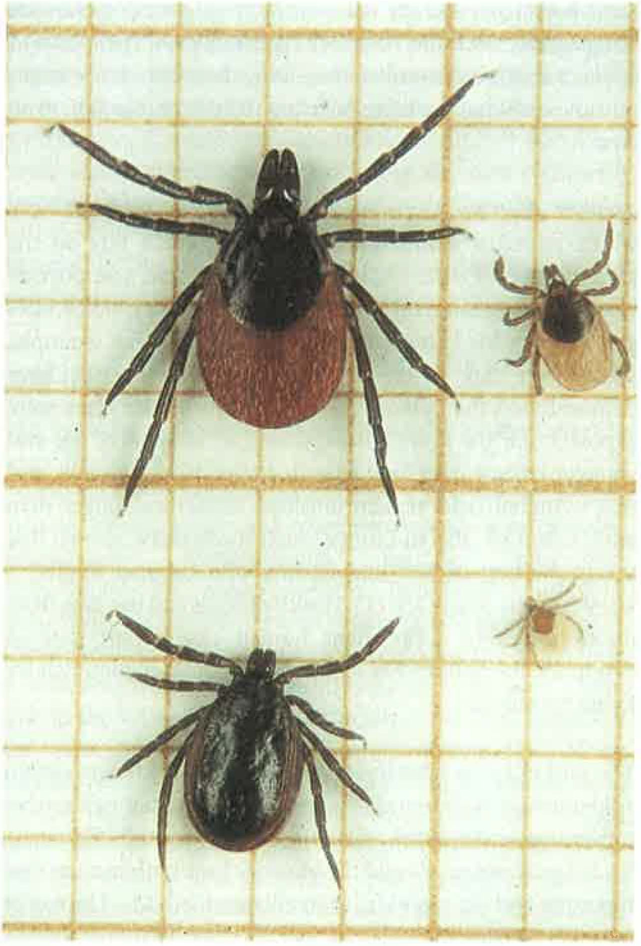

Fig. 3
***Ixodes ricinus*: female (top left) and male (bottom left) and nymph and larva on the right**
Source: Institut de Zoologie, Neuchâtel, Switzerland

In the USA, the two primary tick vectors of Lyme borreliosis are *I. scapularis* (formerly *I. dammini* [96]) in the north-eastern and mid-western regions, and *I. pacificus* on the west coast. As is the case in Europe, other *Ixodes* species (e.g. *I. dentatus*), may have the ability to transmit *B. burgdorferi* to wildlife, and to maintain enzootic cycles in nature (3), but these ticks rarely bite humans (31).

The ecology of vector ticks is an important factor in determining the risk for Lyme borreliosis in an area. For example, in the north-eastern USA, where the incidence of Lyme borreliosis is high, *I. scapularis* is abundant in wooded areas which support suitable wildlife hosts. However, this tick can also be found in the proximity of homes that are located near woodlands, increasing the risk for residents (30). The peridomestic nature of the tick, as well as the increasing annual tick numbers, are factors that contribute to the increase in incidence of Lyme borreliosis (37).

Studies of the ecology of Lyme borreliosis have demonstrated that the persistence of *Borrelia* pathogens in endemic areas requires the involvement of various reservoir hosts. Although *I. ricinus* feeds on a large number of hosts, little information is available on the real significance of most host animals as sources for *B. burgdorferi* sl infection of the vectors. The presence of *Borrelia* in a host does not mean that this host is infective to ticks and acts as a reservoir. The reservoir role of a vertebrate is determined by the success of the transmission of spirochaetes from host to ticks and the persistence of the infection in the tick and in the host. Tick xenodiagnosis is the most effective method to assess the reservoir status of a vertebrate species. Xenodiagnosis consists of allowing naïve ticks, derived from a laboratory colony free of infection, to engorge on the host in question. These ticks are then analysed after the meal or after the moult, to detect the pathogen. This method is applicable to animals which can easily be kept in captivity such as small rodents.

Among tick hosts, small mammals are the vertebrate group which has been studied the most extensively. As a result, several species of mice, voles, rats, shrews and the edible dormice (50), have been shown to be competent reservoirs of *B. burgdorferi* in Europe. Hosts acting as reservoirs must maintain the infection for a long period of time, even during non-transmission periods, which has been demonstrated for *Clethrionomys* voles and *Apodemus* mice (64). However, these rodents seem to have developed different strategies towards tick infestation and *Borrelia* infection. *Clethrionomys* voles develop high levels of *Borrelia* infection which results in a more elevated transmission rate to the ticks (64). In contrast, *Apodemus* mice control spirochaetes more effectively by specific immune responses which maintain the *Borrelia* infection at a low level (64). In fact, spirochaetes are more easily detectable in ear biopsies of *Clethrionomys* voles than of *Apodemus* mice (64). However, vole-fed ticks do not readily engorge or moult successfully because of the acquired resistance to ticks developed by voles (64). Considering the moulting success of ticks, the relative contribution of *Apodemus* mice can be greater than that of *Clethrionomys* voles, despite the high infection rate of the voles. This indicates that the reservoir competence of hosts is modulated by the immune response towards the vector and towards the pathogen.

In contrast to small mammals, the involvement of birds in the ecology of Lyme borreliosis in Europe was controversial for many years, mainly because a study had demonstrated that the blackbird (*Turdus merula*) failed to transmit spirochaetes to the ticks which feed on these birds (87). Blackbirds were also observed to have a zooprophylactic role, since infected ticks lost their infection when feeding on blackbirds. These findings contrast with those of field studies showing that infected ticks can be collected from various bird species, especially ground foraging birds, such as thrushes, blackbirds, robins, wrens and pheasants (60, 61, 97, 98). More recently, *B. burgdorferi* sl has been isolated from the skin of birds and from ticks collected from birds (63). Xenodiagnosis performed on blackbirds and pheasants clearly demonstrated that these species of bird transmit *Borrelia* infection to ticks (63, 73). In addition to small mammals and birds, competent reservoirs were also found among medium-sized mammals. Tàlleklint and Jaenson observed that hares (*Lepus timidus* and *L. europaeus*) can maintain *B. burgdorferi* in endemic areas in Sweden (129, 130). Hares are among the only hosts of *I. ricinus* which are known to be both reservoirs for *B. burgdorferi* and a source of blood meal for all stages of *I. ricinus*. Grey and red squirrels also contribute to *Borrelia* infection in ticks, as shown by Craine *et al.* in England and by Humair and Gern in Switzerland (25, 62). Hedgehogs have also been demonstrated to serve as reservoirs (49, 52, 80). Among large-sized mammals, foxes have been shown to transmit spirochaetes to ticks but only at a low rate (69, 81). In contrast, large-sized mammals, such as ungulates, are apparently incompetent as reservoirs.

Both small and large mammals, as well as birds, are involved in the ecology of *I. scapularis* and *B. burgdorferi* in the USA. Immature larval and nymphal ticks feed on a wide variety of mammalian hosts, including white-footed mice (*Peromyscus leucopus*), chipmunks, racoons and skunks. These mammals may also serve as reservoirs for *B. burgdorferi* (40, 85), as may several species of ground-feeding birds. In contrast, adult *I. scapularis* feed primarily on deer, which are necessary to maintain high tick populations. However, as in Europe, deer seem to be incompetent reservoirs for *B. burgdorferi* (85). In the western USA, rodents and lizards serve as important hosts for immature *I. pacificus* (76).

The presence of various *Borrelia* species in ticks has opened up an entire new field of research in the ecology of Lyme borreliosis. For instance, defining which *Borrelia* species infects which host species and the ticks feeding on those hosts remained enigmatic for a long time, since very few isolates were obtained from hosts and from host-feeding ticks until recently. However, specific associations have been observed between hosts, ticks and *Borrelia* species, which helps to explain why some tick hosts have been described as incompetent reservoir hosts for *Borrelia* or as having a zooprophylactic role.

In Europe, the first *Borrelia* isolate from rodents was obtained by Hovmark *et al.* in Sweden and was characterised as *B. afzelii* (58). Later, *Borrelia* isolates obtained from ear biopsies of infected rodents captured in Switzerland and from xenodiagnostic ticks fed on these rodents were shown to belong to *B. afzelii* (59, 64). These results strongly suggest that a specific association exists between the rodents and *B. afzelii*.

The presence of *B. garinii* in bird-feeding ticks described by Olsén *et al.* in Sweden and Hubálek *et al.* in the Czech Republic suggested that *B. garinii* might be associated with birds (60, 98). Recently, Humair *et al.* showed that *B. garinii* and *B. valaisiana* infected the skin of five blackbirds and one song thrush (*Turdus philomelos*) (63). Xenodiagnosis using blackbirds showed that these birds transmitted *B. garinii* and *B. valaisiana* to the ticks feeding on them. In England, Kurtenbach *et al.* reported that pheasants were also infected by these two species of *Borrelia* (73). Recently, the genotypic identification by restriction fragment length polymorphism (RFLP) of isolates obtained from skin samples from red squirrels in Switzerland showed that the isolates belonged to *B. burgdorferi* ss and *B. afzelii* (62). These two genospecies also dominated among isolates from ticks feeding on squirrels. This observation suggests that these two genospecies are transmitted from squirrels to feeding ticks. Interestingly, this is in accordance with results obtained by Craine *et al.* with grey squirrels in the United Kingdom (UK) where *B. afzelii* was observed in the skin of this animal and where successful infection of grey squirrels with *B. burgdorferi* ss was obtained experimentally (25). Vertebrate reservoirs for *B. lusitaniae* have not yet been detected. Thus, in some endemic areas of Europe, genospecies are associated with particular groups of vertebrate hosts, for instance, *B. valaisiana* and *B. garinii* are associated with birds, *B. afzelii* with small mammals and *B. burgdorferi* ss and *B. afzelii* with red squirrels.

The identification of host species that are not reservoir competent is also extremely important, although difficult to achieve. In fact, negative results can be obtained for a variety of reasons, thereby obscuring the true situation and requiring repeated investigations in different locations. 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, it was demonstrated that co-feeding transmission of spirochaetes can occur between infected and uninfected ticks feeding together on a host presenting no generalised infection (47). As mentioned earlier, the role of some tick hosts, such as deer and other ungulates, although intensively investigated, remains controversial. Recently Kimura *et al.* in Japan and Ogden *et al.* in England reported that sika deer (*Cervus nippon yesoensis*) and sheep, respectively, are capable of supporting localised transmission and that these hosts can serve as support for infection when both infected and uninfected ticks are feeding in clusters (71, 95). In fact, uninfected ticks may acquire infection from a localised site at which they feed simultaneously with infected ticks and may continue to acquire infection after the infected ticks have dropped from the host. In this case, the infection seems to remain confined to specific parts of the body to which infected ticks have attached.

The preferential association observed between *Borrelia* species and some vertebrate hosts raises the question of the fate of spirochaetes transmitted to inappropriate hosts. For example, birds which are associated with *B. garinii* and *B. valaisiana* infection, might be inappropriate hosts for *B. afzelii* and would be unable to maintain and transmit *B. afzelii* to feeding ticks. Such a phenomenon may also occur in nature. In England, Kurtenbach *et al.* observed that *B. afzelii* was absent in an area and that small mammals in this focus transmitted *B. burgdorferi* ss to xenodiagnostic ticks but only at an extremely low rate (73). The lack of *B. afzelii* in these foci might be related to a large and dense pheasant population, which substantially reduces the number of *B. afzelii*, since this species is an inappropriate host for *B. afzelii*. A significant number of pheasants had been artificially introduced into this area. Kurtenbach *et al.* suggested that birds in this ecosystem had a zooprophylactic role for *B. afzelii* and that *B. afzelii* had been eradicated from the area. Other tick hosts, such as the sand lizard (*Lacerta agilis*) in Europe and the western fence lizard (*Sceloporus occidentalis*) in the USA, have been suspected of having a zooprophylactic effect on infected ticks feeding on them by destroying spirochaetes present in the feeding ticks (77, 86).

An explanation of the specific association observed between hosts and *Borrelia* species, and of the zooprophylactic role of some animals, may be found in the results of Kurtenbach *et al.* who showed that the pattern of serum sensitivity of different *Borrelia* genospecies matched the known reservoir status of many vertebrate species for *B. burgdorferi* (74).

The existence of several genospecies of *B. burgdorferi* sl with apparently different host specificities must be considered when investigating the role of vertebrates in the infection of ticks in certain areas. If possible, future studies should seek to identify the genospecies found in both ticks and reservoir hosts.

Treatment and prevention

The prognosis for patients with Lyme borreliosis is excellent, particularly when diagnosed and treated early in the course of infection. Penicillin and tetracycline preparations are effective treatments, usually administered for a course of two weeks (93). More specifically, doxycycline, amoxycillin, cefuroxime, and ceftriaxone have been shown to be the most effective antibiotics for the treatment of the initial stages of Lyme borreliosis (134). Treatment of later stage infection, which may include cardiac, neurological and arthritic complications, may be more aggressive and include the use of either oral or intravenous antibiotics (93, 124, 125, 134). However, the administration of long-term antibiotic therapy for suspected late stage Lyme disease (e.g. for a period of months or years), is not recommended and remains controversial (93, 113).

Another controversial issue is the administration of prophylactic antibiotics for *I. scapularis* bites. However, due to the low risk of acquiring infection from a recognised tick bite and the possibility of adverse allergic reaction to antibiotics, routine prophylactic therapy after a tick bite is generally not recommended (93, 134).

While treatment of Lyme borreliosis is usually successful, the potential for serious complications resulting from infection makes prevention preferable. Prevention of Lyme borreliosis may take two approaches, namely: prevention of infection by immunisation, or prevention of tick bites through avoidance, personal protection or tick control. The recent development and marketing of a vaccine in the USA has focused attention on the former method. This vaccine, which targets the OspA of *B. burgdorferi*, works by killing the spirochaetes while in the tick midgut, and is administered in three doses over the course of a year. Although this vaccine has been shown to be safe and almost 80% effective in adults (115, 127), cost-effectiveness studies indicate that the vaccine should be limited to high-risk individuals (88). Given the genetic variation of *B. burgdorferi* strains, the vaccine developed in the USA would not be expected to be effective in Europe (48). Studies to develop a vaccine for use in Europe are currently underway.

Recent progress in vaccine development should not replace aggressive measures to prevent tick bites in areas that are endemic for Lyme borreliosis. It must be remembered that the current vaccine available in the USA is not 100% effective, is not licensed for use in children under fifteen years of age, is less effective in older adults, and will not prevent other infections transmitted by *I. scapularis*, such as babesiosis, human granulocytic ehrlichiosis (HGE), or tick-borne encephalitis (TBE).

When the risk of acquiring tick bites is recreational in nature (i.e. due to visiting recreational parks or engaging in recreational activities in areas inhabited by ticks), personal protection measures should be employed. These measures include tucking trouser legs in socks or boots to keep ticks away from the skin, use of repellents, and checking clothing frequently to remove crawling ticks. Additionally, nightly checks of both adults and children for attached ticks should be conducted. These checks may prevent infection if the tick is promptly removed because transmission of *B. burgdorferi* by a tick may take up to 48 h (100).

When the risk is residential in nature, as in many areas of the north-eastern USA (30), tick bite prevention should employ personal protection measures, as outlined above, in addition to reduction of tick numbers through habitat modification and the use of insecticides to control *I. scapularis*. Habitat modification includes mowing lawns, cutting back vegetation, and clearing leaf litter. Such practices may reduce the risk of tick bites by rendering the environment inhospitable for host-seeking ticks, which require relatively high humidities to survive. However, when ticks are abundant and the risk for Lyme borreliosis is high, habitat modification should be combined with chemical control for increased protection.

Since the annual incidence of Lyme disease in the USA is closely correlated with the abundance of nymphal *I. scapularis* in an area, the nymphal stage should be the target of chemical control efforts (37). Many studies have demonstrated the effectiveness of chemicals such as carbaryl, chlorpyrifos, and pyrethrin-based insecticides in killing *I. scapularis* (26). One application of an approved chemical, applied during peak nymphal activity, can achieve over 90% control of nymphal ticks throughout the summer months. In residential areas, where exposure is constant and the effectiveness of personal protection methods may be diminished (34), chemical control of nymphs may significantly reduce the risk of Lyme borreliosis. For this reason, judicious use of insecticides in selected areas of high-risk recreational parks with high public usage, should also be considered (32). To date, ground application of insecticides has proven superior to alternative methods of tick control, such as host reduction and host targeted acaricides, in reducing Lyme borreliosis risk in the suburban habitats that typically harbour *I. scapularis*. However, studies on alternative approaches to the control of *I. scapularis* are ongoing and will probably result in the development of methods that rely less on insecticide use and incorporate an integrated approach to the management of Lyme borreliosis risk.

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Maladie de Lyme

L. Gern & R.C. Falco

Résumé

La borréliose de Lyme qui est, dans l'hémisphère nord, la plus répandue des maladies à transmission vectorielle, est due à des bactéries appartenant au genre *Borrelia burgdorferi*. Il s'agit d'une maladie plurisystémique qui affecte essentiellement la peau, le système nerveux, le cœur et les articulations. En Europe, le vecteur de la maladie est la tique *Ixodes ricinus*; aux États-Unis d'Amérique les deux vecteurs principaux sont la tique *I. scapularis* dans les régions du nord-est et du centre-ouest et *I. pacificus* sur la Côte Ouest. Plusieurs espèces de petits et moyens mammifères ou d'oiseaux terrestres servent de réservoirs à la bactérie dans les zones endémiques. Le pronostic est excellent, surtout lorsque la maladie de Lyme est diagnostiquée en phase initiale et traitée immédiatement. La prophylaxie repose sur deux méthodes : l'immunisation préventive, d'une part, et la mise en œuvre de mesures permettant d'éviter les piqûres de tiques et de lutter contre ces vecteurs, d'autre part.

Mots-clés
Borrelia burgdorferi – Écologie – Épidémiologie – Ixodes – Maladie de Lyme – Prophylaxie – Santé publique – Signes cliniques – Tiques – Zoonoses.

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La enfermedad de Lyme

L. Gern & R.C. Falco

Resumen

De todas las enfermedades vehiculadas por vectores, la borreliosis de Lyme, causada por bacterias pertenecientes al complejo *Borrelia burgdorferi*, es la más común en el hemisferio norte. Esta enfermedad de carácter multisistémico afecta principalmente la piel, el sistema nervioso, el corazón y las articulaciones. En Europa, el vector de la enfermedad es la garrapata *Ixodes ricinus*. En los Estados Unidos de América, por su parte, existen dos garrapatas que ejercen de vectores primarios: *I. scapularis* en las regiones del noreste y el medio oeste, e *I. pacificus* en la costa oeste. En las áreas endémicas varias especies de mamíferos pequeños y medianos y de aves de alimentación terrestre sirven de reservorio de las bacterias. Los pacientes afectados de borreliosis de Lyme presentan un pronóstico excelente, sobre todo cuando se diagnostica y trata la infección en sus fases iniciales. Para prevenir esta enfermedad caben dos procedimientos distintos: prevenir la infección con medidas de inmunización; o impedir la mordedura de la garrapata previniendo esta posibilidad, protegiéndose del contacto y luchando contra sus poblaciones.

Palabras clave
Borrelia burgdorferi – Ecología – Enfermedad de Lyme – Epidemiología – Garrapatas – Ixodes – Salud pública – Signos clínicos – Prevención – Zoonosis.

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