Part 1

Infectious Diseases of the Fetus and Newborn Infant

FIFTH EDITION

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Contents

CHAPTER 11

Lyme Disease

TESSA GARDNER, M.D.

Lyme disease, or Lyme borreliosis, is a tickborne zoonosis of both children and adults caused by the spirochete *Borrelia burgdorferi*.[1, 2] It has a worldwide geographic distribution and has been reported from more than 40 countries and 6 continents; the geographic distribution and number of cases reported continue to increase (Figs. 11–1 and 11–2). It is now the most common tickborne infection in the United States,[3–6] where 16,800 cases were reported to the Centers for Disease Control and Prevention (CDC) in 1998 (Fig. 11–3); in Europe,[8–10] where 2100 cases were reported to the European Union Concerted Action of Risk Assessment in Lyme Borreliosis (EUCALB) in 1994, and more than 60,000 cases were estimated to occur annually as of 1998[9]; and possibly in the world.[11–13]

Lyme borreliosis is a fairly recently recognized infection, although erythema migrans (EM), the characteristic skin lesion of early Lyme borreliosis, was first described in a Swedish woman in 1909 by Afzelius, who proposed that it was related to a zoonosis transmitted by a tick bite.[14] In 1975, Steere and associates recognized an outbreak of infectious arthritis and unusual rash similar to European EM in Old Lyme, Connecticut; they proposed that transmission occurred via an arthropod vector and named the disease Lyme arthritis.[15] Eventually, it was found to be associated with ixodid tick bites and later, when its multisystem involvement was recognized, became known as Lyme disease.

In 1981, Burgdorfer and colleagues discovered a new species of *Borrelia* in *Ixodes* ticks associated with Lyme disease, and this became known as *Borrelia burgdorferi*.[1, 16, 17] This spirochete was found to be the causative agent of North American Lyme disease[18] and of European EM,[19] as well as other European syndromes such as acrodermatitis chronica atrophicans (ACA),[20] Bannwarth's syndrome,[21] and lymphadenosis benigna cutis[22]; the entire disease complex is now known as Lyme borreliosis.

As worldwide reporting of Lyme borreliosis increases, a geographically defined "Lyme Belt" is emerging between 30 and 65 degrees North latitude in the Eastern Hemisphere, and between 25 and 50 degrees North latitude in the Western Hemisphere; there may also be a belt developing between 30 and 40 degrees South latitude in the Eastern Hemisphere. This is reminiscent of the "Malaria Belt," which has been defined by climatic conditions and the distribution of another major arthropod vector of human disease, the *Anopheles* mosquito.

A

FIGURE 11–1 *A*, The geographic distribution of Lyme borreliosis in Europe. Europe is the main area outside North America from which Lyme borreliosis has been reported. This map shows European countries from which cases of Lyme borreliosis have been reported either to the World Health Organization,[501] to the European Union Concerted Action on Risk Assessment in Lyme Borreliosis,[9, 10] or in the medical literature.[11, 12, 41–44, 48, 83, 85–87, 90, 162, 251, 268, 275, 276, 305, 310, 352, 370, 371, 381, 387, 389, 402–405, 409, 422, 432–435, 448, 503, 504, 507–523, 525–537, 539–550, 552, 553] Reliable statistics on incidence by country are not available, as reporting of cases is voluntary in most countries. The highest incidences (either 1000–20,000 cases/country or 15–140 cases/100,000 population annually) of European Lyme borreliosis have been reported from Austria, Slovenia, Poland, Sweden, Bulgaria, Denmark, Hungary, the Netherlands, Finland, the Czech Republic, Switzerland, Germany, Italy, and France; lower incidences (either <500 cases/country, or <5 cases/100,000 population annually) have been reported from Belgium, Croatia, Estonia, Greece, Ireland, Latvia, Lithuania, Luxembourg, Moldavia, Norway, Romania, Russia, Spain, the United Kingdom, and the former Yugoslavia.

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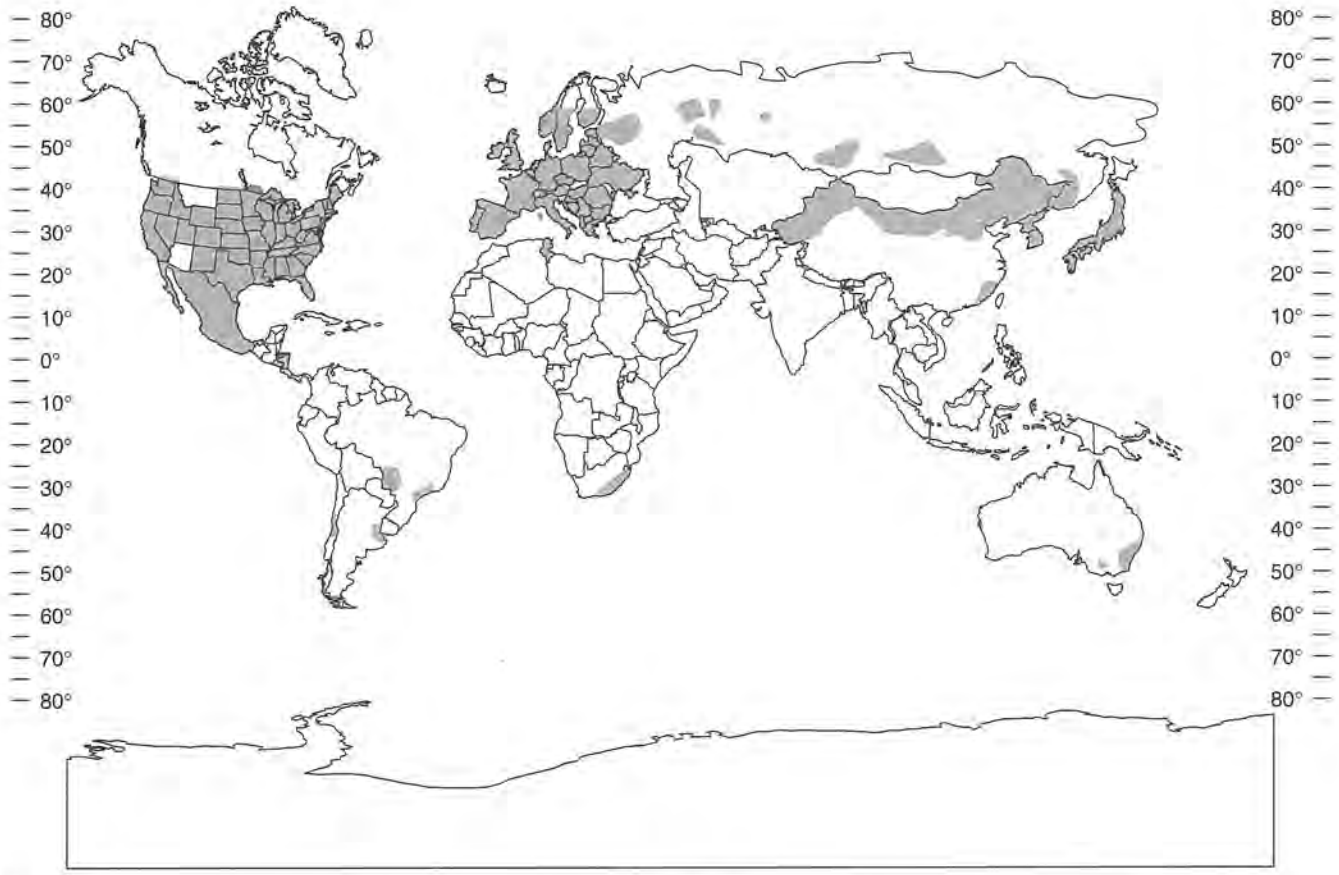
Lyme borreliosis is a multisystem infection that initially emerged as a new "great imitator"[14] because of the diversity of its clinical presentations, which comprise both early and late stages and include dermatologic, cardiac, neurologic, arthritic, and ocular manifestations.[23] However, more than 20 years since its recognition as a new disease,[15] the spectrum of its clinical manifestations has been extensively characterized, resulting in gradual loss of this reputation.[24] The existence of congenital borreliosis was suspected because of clinical similarities between the two spirochetoses Lyme borreliosis and the classic "great imitator" syphilis,[599] and the well-known association of gestational syphilis with miscarriage, early congenital infection, and late congenital infection.

Maternal-fetal transmission of *B. burgdorferi* was first reported in 1985 by Schlesinger and co-workers.[25] As the number of reported cases of Lyme disease continues to increase, there have been increasing reports of gestational Lyme disease associated with adverse outcomes and suspected congenital Lyme borreliosis.[25–48] Although a homogeneous congenital Lyme borreliosis syndrome has not yet emerged, there are several features that are common among the 66 adverse outcomes of pregnancies complicated by gestational Lyme borreliosis reviewed later in this chapter (including miscarriage during the



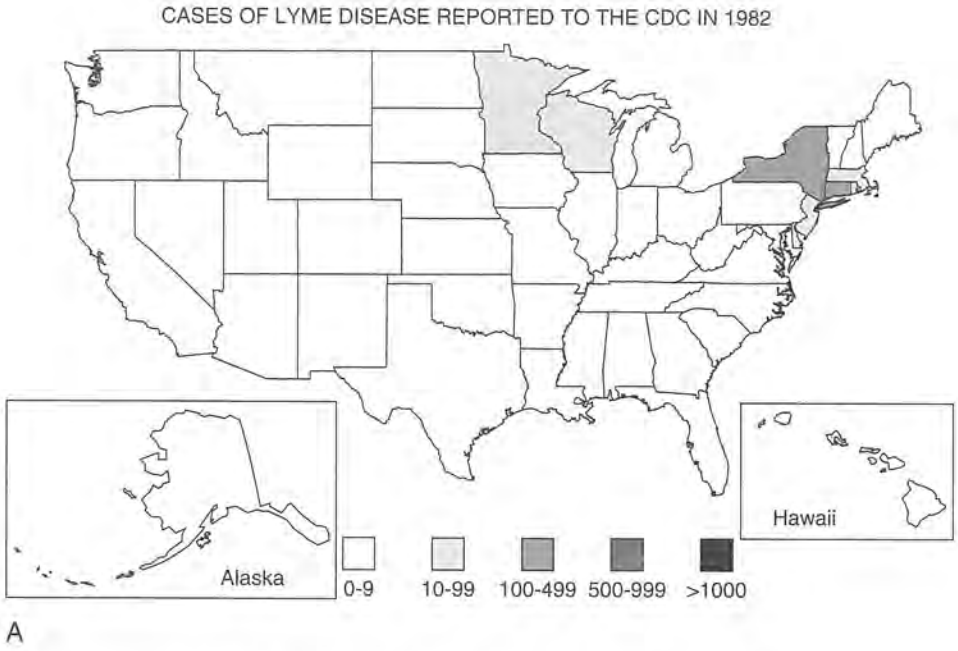
B

FIGURE 11–1 *Continued. B,* The worldwide geographic distribution of Lyme disease in temperate zone "Lyme Belts." In addition to North America and Europe, Lyme borreliosis is also endemic in Asia, mainly in China and Japan, and it has been reported from countries on three other continents and the Caribbean, including Argentina, Australia, Brazil, Chile, Egypt, Honduras, Israel, Mexico, Mozambique, Puerto Rico, South Africa, Taiwan, and Tunisia, although some of these cases may not have been indigenously acquired. The existence of indigenous cases in Central and South America, the Caribbean, Australia, and central and southern Africa is still uncertain.[164, 165, 303, 344, 349–351, 374, 388, 439, 448, 449, 451–453, 455, 456, 506, 554–561, 563, 566–569] Ixodid ticks infected with *Borrelia burgdorferi* have been found in Korea, and in several subarctic and subantarctic circumpolar islands (Egg and St. Lazaria Islands of Alaska, Flatey Island of Iceland, Campbell Island of New Zealand, and the Crozet Islands), but no cases of Lyme borreliosis have been reported yet from these areas.[165, 555] The geographic distribution of Lyme disease cases forms two belts—a 35-degree-wide northern temperate zone belt between 30 and 65 degrees North latitude in the Eastern Hemisphere, and another one slightly more southerly between 15 and 50 degrees North latitude in the Western Hemisphere. These include the majority of the Asian, European, North African, and North American cases. In addition, the cases from Australia, southern Africa, and South America appear to be clustered in a temperate zone belt between 10 and 40 degrees South latitude, but more cases are needed to determine if this is a true Southern Hemisphere "Lyme Belt."

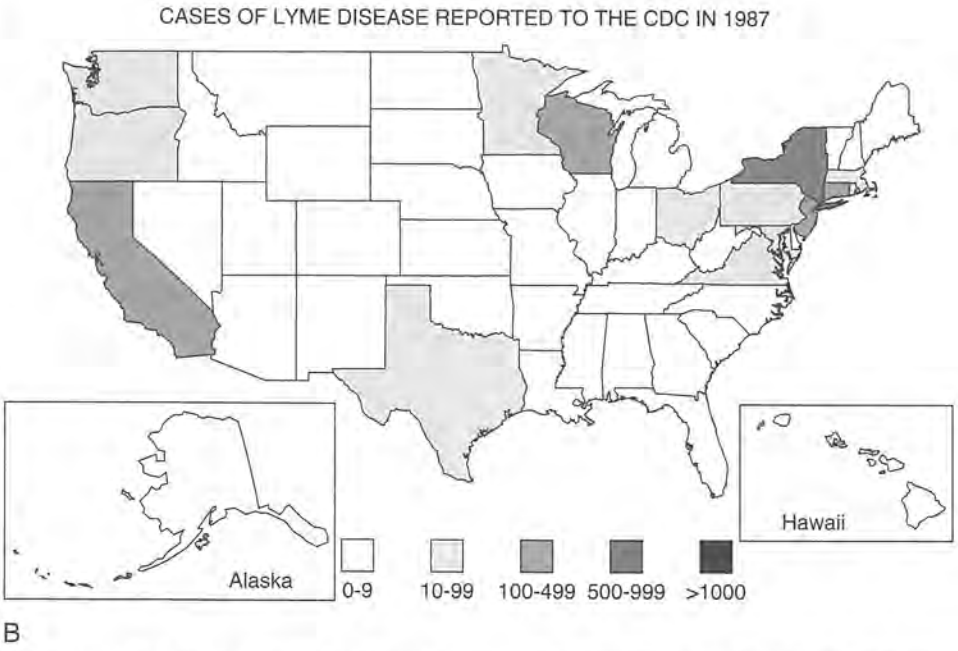
first 20 weeks of gestation with a high frequency of fetal cardiac abnormality; severe early congenital infection with fulminant neonatal sepsis and meningoencephalitis and a high frequency of cardiac abnormality; mild early congenital infection with growth retardation and mild cardiac abnormality; and late congenital infection with growth retardation, developmental delay, and neurologic, cutaneous, dental, and skeletal involvement).

THE ORGANISM

Borrelia organisms are arthropod-borne spirochetes that infect birds, domestic and wild animals, and humans.[49, 50, 52] It is now recognized that *B. burgdorferi* is a phenotypically and genotypically heterogeneous genospecies complex, and the name has been modified to *Borrelia burgdorferi sensu lato* to reflect this. There are several genospecies of *Borrelia burgdorferi sensu lato: Borrelia burgdorferi sensu stricto, Borrelia andersonii, Borrelia garinii, Borrelia afzelii, Borrelia valaisiana, Borrelia lusitaniae, Borrelia japonica, Borrelia tanukii, Borrelia turdae,* and several genetically distinct genomic groups that have not yet achieved genospecies status.[51–71, 884] *B. burgdorferi sensu stricto, garinii,* and *afzelii* have been associated with human Lyme borreliosis[55]; *B. valaisiana* DNA has been found in EM lesions of two patients by polymerase chain reaction (PCR)[72]; and strains similar to strain 25015 in



A



B

FIGURE 11–2 The increase in the number of cases and expansion of the geographic distribution of Lyme disease in the United States from 1982 through 1998. The number of cases of Lyme disease reported to the Centers for Disease Control and Prevention (CDC) by state health departments in *(A)* 1982, *(B)* 1987, and *(C)* 1998.[4, 461] National surveillance began in 1982, and Lyme disease became a notifiable disease in 1990.[3] Cases of Lyme disease have also been reported to the Canadian Laboratory Centre for Disease Control (LCDC), mostly from southern areas that border Lyme-endemic areas of the northeastern, upper midwestern, and northwestern United States.[479]

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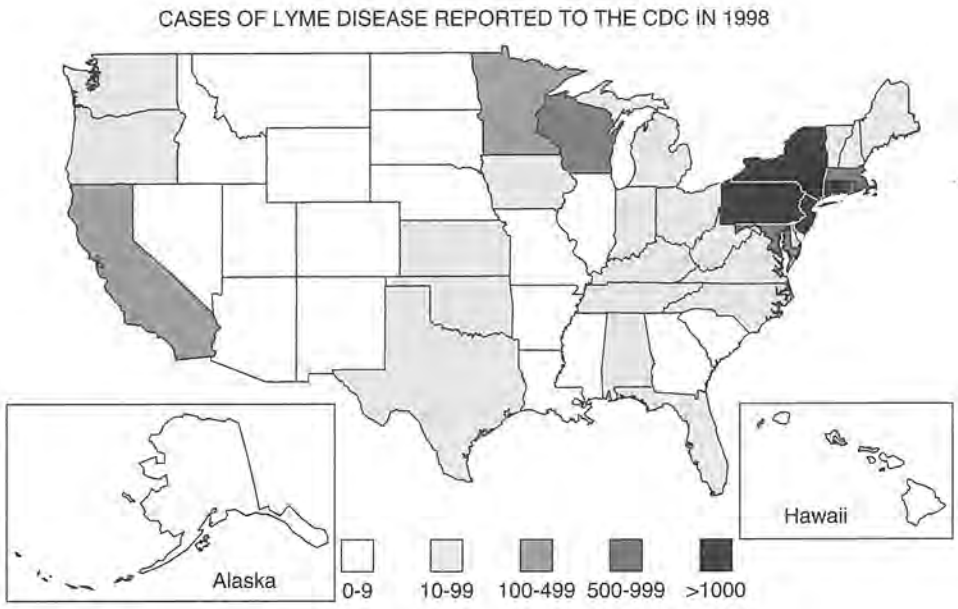

C

FIGURE 11–2 *Continued*

genomic group DN127 occasionally have been associated with Lyme disease.[83, 865] The other genospecies are involved in enzootic cycles of maintenance of *B. burgdorferi* in nature, but have not yet been isolated from patients with Lyme borreliosis.[55, 56] There is a newly described uncultivable *Borrelia* species, *Borrelia lonestarii*, which has been found in the Lone Star tick, *Amblyomma americanum*, and may be associated with Lyme-like disease in the southern United States.[73] Certain genospecies have been associated more frequently with certain clinical manifestations.[10, 74–76] *B. lonestarii* and a new species, *Borrelia miyamotoi*,[77] may be more closely related to the relapsing fever borreliae than to *B. burgdorferi sensu lato*.[73, 77]

Borrelia burgdorferi as the Etiologic Agent of Lyme Borreliosis

In 1981, Burgdorfer and associates discovered (isolated) a new species of *Borrelia* in *Ixodes dammini* (later renamed *Ixodes scapularis*[78]) ticks from a Lyme-endemic area in New York, demonstrated elevated antibody titers to this spirochete in convalescent sera of patients with Lyme disease, and proposed that this spirochete was involved in the etiology of Lyme disease.[1, 17]

In 1982, Berger and colleagues demonstrated rare spirochetes, similar to the *I. dammini (scapularis)* spirochete, by Warthin-Starry silver stain in skin biopsy specimens of untreated patients with EM skin lesions; they were able to isolate spirochetes from one specimen, thus supporting a spirochetal etiology for EM.[79] In 1985, Berger and co-workers grew the *I. dammini (scapularis)* spirochete from several skin biopsy specimens of EM lesions[79] and thus confirmed this spirochete as the etiologic agent of North American EM.

In 1983, Steere and associates isolated the new spirochete, which was subsequently named *Borrelia burgdorferi*, from blood, spinal fluid, and joint fluid of American Lyme disease patients and from *I. dammini (scapularis)* ticks in a Lyme-endemic area of Connecticut; they

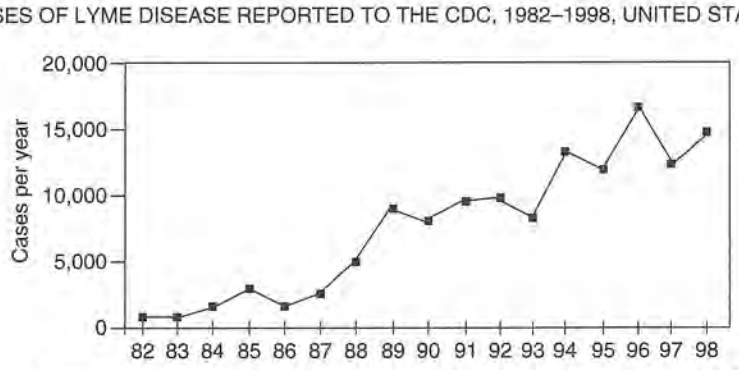

FIGURE 11–3 The number of cases of Lyme disease in the United States reported to the CDC by the individual state health departments has increased steadily from 1982 to 1998.[4, 461] Lyme disease became a reportable disease in 1990.[3]

demonstrated serum IgM and IgG antibody titer increases in these patients directed against this spirochete.[18] Simultaneously in 1983, Benach and colleagues isolated the same spirochete from the blood of patients with American Lyme disease and demonstrated similar seropositivity in these patients.[80] Both groups proposed the *I. dammini (scapularis)* spirochete as the etiologic agent of Lyme disease.[18, 80] In the same year, Barbour and co-workers, including Burgdorfer, isolated a new spirochete, similar to the *I. dammini (scapularis)* spirochete, from *Ixodes ricinus* ticks from an EM-endemic area of Switzerland.[81]

Ryberg and associates, including Burgdorfer, in 1983 demonstrated significant levels of IgM and IgG serum antibodies against the North American Lyme disease spirochete in sera of European patients with lymphocytic meningoradiculitis (Bannwarth's syndrome); they proposed the Lyme disease spirochete as the etiologic agent of Bannwarth's syndrome.[21]

In 1984 and 1985, Asbrink, Hovmark, and colleagues isolated the *I. ricinus* spirochete from skin biopsy specimens of European patients with EM,[19] acrodermatitis chronica atrophicans,[19, 20] and lymphadenosis benigna cutis[22]; antibody titer elevations against this spirochete were demonstrated in these patients, thus confirming the spirochetal etiology of these European skin diseases. In 1987, de Koning and co-workers demonstrated spirochetes, morphologically consistent with *B. burgdorferi*, in European EM and lymphadenosis benigna cutis skin lesions, in synovia of patients with European Lyme arthritis, and in spinal fluid of a patient with European Bannwarth's syndrome, and thus confirmed the spirochetal etiology of these additional European diseases.[82]

Some genospecies, such as *B. burgdorferi sensu stricto*, *garinii*, and *afzelii*, have been associated with human Lyme borreliosis, and others, such as *B. japonica*, only with tick vectors and reservoir hosts but not yet with human disease.[55, 56] *B. valaisiana* DNA has been found in EM lesions of two patients by PCR[72]; *B. burgdorferi sensu lato* isolates similar to strain 25015 of group DN127 were found in the cerebrospinal fluid (CSF) and EM of nine Slovenian patients[83, 884]; and *B. burgdorferi* genospecies DN127 was isolated from one patient with borrelial lymphocytoma.[74]

There is clustering of genospecies from patients with different clinical manifestations, such as EM, ACA, neuroborreliosis, arthritis, and carditis[55, 67, 74–76, 84–89]; this clustering suggests the possibility of differences in pathogenicity and organotropism of strains of different phenotypes and genotypes, which may be related to differences in clinical syndromes associated with these strains.[51–53]

In North America, where ACA does not occur, *B. burgdorferi sensu stricto* is the only agent of human Lyme disease, and is associated with all North American manifestations of Lyme disease, EM, neuroborreliosis, arthritis, and carditis.[5] In Europe, ACA is associated predominantly with *B. afzelii*, and occasionally with *garinii* or *sensu stricto*[67, 74–76, 85–87, 89]; EM with all three genospecies (*B. burgdorferi sensu stricto, garinii, afzelii*)[74–76, 85, 89]; neuroborreliosis predominantly but not exclusively with *B. garinii*[74–76, 85, 87, 88]; arthritis predominantly with *sensu stricto* and sometimes with *garinii*[75, 76]; and carditis with *sensu stricto* and occasionally with *garinii*.[75, 76]

Within genospecies, there may be strains that are more pathogenic than others, as may be involved in the clustering of strains isolated from European patients with disseminated Lyme borreliosis in one sub-branch of *B. garinii*,[55] the clustering of *B. garinii* strains associated with adult neuroborreliosis in Osp A serotype 4, and the clustering of *garinii* associated with pediatric neuroborreliosis in Osp A serotype 6.[88]

A large study by the EUCALB, of over 2000 patients with Lyme borreliosis in 15 European countries during 12 months in 1994, found that the incidence of Lyme borreliosis per 100,000 population increased from Western to Eastern Europe, with higher incidences east of the Netherlands, France, and Italy.[10]

Morphology

Borrelia burgdorferi[56, 81, 91–98, 218] is a long (10 to 30 micrometers in length), narrow (0.18 to 0.25 micrometer in diameter), irregularly and loosely coiled, helical, motile, flexible spirochete with tapered ends and sheathed flagellae.

It has an inner and an outer cell membrane and four to eight flagellae, located in the periplasmic space between the inner and outer trilaminar cell membranes. These membranes, which are inserted at each end and extend toward the middle of the spirochete, allow it to move efficiently through viscous solutions and presumably enhance its ability to disseminate in body tissues. The trilaminar outer membrane structure is similar to, but more fluid than, that of gram-negative bacteria, and it contains the embedded outer surface membrane lipoproteins and a lipopolysaccharide with weak endotoxin-like activity.[100] The flexible cell wall is located just outside the cytoplasmic membrane.[99] In addition to the typical *B. burgdorferi* morphology, morphologic variants have been found in tissue biopsies.[101–103]

Molecular Biology

B. burgdorferi has several major antigens that can be separated by polyacrylamide gel electrophoresis and characterized antigenically by reactivity in Western blots with *B. burgdorferi*–specific polyclonal and monoclonal antibodies.[51, 52, 92, 93, 104, 105]

The 83- to 100-kilodalton (kd) antigen p83/100 is *Borrelia* genus–specific,[51, 106, 107] cross reacts minimally with other bacteria,[104] is associated with either the flagella or the protoplasmic cylinder, and is a chromosomally encoded immunodominant antigen of *B. burgdorferi sensu lato*, which has minor homology with the muscle and cytoskeletal proteins myosin and troponin, and contains an amino acid sequence that is a common cell recognition signal of integrins and may be involved in spirochetal attachment to cells.[106] The constant-molecular-weight, major immunodominant 60-kd common antigen HSP60, and the 70-kd antigen HSP70 are heat shock proteins that function as flagellin chaperones, are encoded by chromosomal genes, and cross react broadly with other bacteria.[104, 109, 882] The 35-kd protein, a *B.*

burgdorferi sensu lato–specific lipoprotein encoded by a chromosomal gene, is expressed early in human infection and is an important immunodominant marker for early human infection.[110] There are several other significant antigens, including the 39-kd molecular weight protein, some encoded by chromosomal and some by plasmid genes.[882]

The 41-kd flagellar antigen p41 is the other major protein of the organism[51, 52]; it has a uniform molecular weight in all *B. burgdorferi* strains,[51] is encoded by a highly conserved gene (with 96–97% sequence homology between strains) located on the main chromosome,[111] and is the antigen most often recognized in Lyme borreliosis patient sera.[112] *B. burgdorferi* flagellin has an epitope that shares amino acid homology with the N-terminal amino acid sequences of human chaperonin, a 60-kd heat shock protein,[113] and has some cross-reactivity with other spirochetes.

B. burgdorferi has several major outer surface lipoproteins—Osp A,[114] Osp B,[114] Osp C,[114, 116] Osp D,[117] Osp E,[118] Osp F,[118] and pG[119]—that are encoded by plasmids.[114, 120, 882] The 18-kd EppA protein (exported plasmid protein A) is thought to be either an outer membrane or a secreted protein.[121] Osp A has the least variability and the greatest homology (77–83%) of the three major *B. burgdorferi* genospecies[108, 114, 122]; Osp B has high variability[114]; and Osp C has the highest variability and exhibits polymorphism of its amino acid sequences and Osp C–encoding gene sequences.[114, 120, 123] Osp C is expressed early in infection,[124, 125] and, despite this heterogeneity, the three major genospecies have common as well as genospecies-specific Osp C immunogenic epitopes recognized by patient sera.[108, 125] Osp A has an immunodominant epitope that shares amino acid sequence homology and encoding DNA sequence homology with human leukocyte function–associated antigen-1 (LFA-1), which is a candidate arthritogenic autoantigen that may be involved in the immunopathogenesis of Lyme arthritis.[126]

The smaller, variable-molecular-weight outer surface membrane lipoproteins of *B. burgdorferi* are species-specific, and antigenic modulation, variation in size, antigenicity, and expression of these outer surface proteins have been found.[51, 52, 92, 93, 129, 130] In 1998, Kawabata and associates reported that *B. burgdorferi sensu stricto* strain 297 has VMP-like proteins coded by VMP-like sequences (Vls) located in multiple copies on the 20 kilobase pair plasmid.[130] In 1997, Zhang and colleagues described a system in *B. burgdorferi sensu stricto* strain B31 that produces extensive antigenic variability in a surface lipoprotein.[131] *B. burgdorferi* Vls is expressed in patients with Lyme borreliosis,[130] and the system of antigenic variability may enhance evasion of the host immune response.[130, 131]

B. burgdorferi also has nonprotein antigens, composed of lipid-carbohydrate-, and phosphorus-containing compounds, which react with Lyme disease patient sera but are of unknown significance.[132]

The genome of *B. burgdorferi* has been sequenced.[133, 882] *B. burgdorferi sensu stricto* strain B31 has a large linear chromosome of 910, 725 base pairs (about 900 kbp) and at least 17 plasmids (10 linear plasmids ranging in size from 17 to 56 kbp, and 7 circular plasmids ranging from 9 to 32 kbp) with a combined total of 533,000 base pairs (about 500 kbp) of double-stranded DNA with an average G plus C content of 28.6%.[882] The linear chromosome has been sequenced and contains 853 genes that encode proteins needed for DNA replication, transcription, translation, energy metabolism, and solute transport, but not for cellular biosynthesis. Eleven of the plasmids (ranging from about 9 to 54 kbp in size), containing 430 genes, have been sequenced. The functions of most of these genes are unknown, but they may be involved in antigenic variation and immune evasion; some, such as the 53- to 58-kbp linear plasmid in *B. burgdorferi sensu stricto, garinii,* and *afzelii,* and the 90- to 105-kbp linear plasmid in *B. japonica,* encode outer surface proteins A and B. Others, such as the 26- to 27-kbp circular plasmid, encode Osp C. Fifty-nine percent of the chromosomal genes have known biologic roles, 12% match genes in other organisms with unknown roles, and 29% are new genes; these percentages for plasmid genes are 16, 26, and 58, respectively.[882] Almost all of the membrane proteins of *B. burgdorferi* are lipoproteins, and 8% of its genes encode 105 putative lipoproteins, which is a much greater percentage than occurs with most other bacteria; six percent of the genes encode proteins involved in spirochetal motility and chemotaxis.[882]

Although North American and European *B. burgdorferi sensu stricto* isolates tend to cluster into separate subbranches by DNA analysis,[55] there are genetic similarities between some isolates from the two continents, suggesting some previous interchange of strains between the two continents.[62]

Among the different genospecies,[657, 117, 134] there are differences in the number, size, and sequences of the linear and circular plasmids, as well as their presence or absence, which correlate with the expression of the outer surface proteins they encode. The Osp A– and Osp B–encoding linear plasmid is present in all *B. burgdorferi sensu lato* genospecies (although some individual isolates may lack the Osp B gene, and this plasmid may be lost in culture). Almost all North American and European strains express Osp A and it shows the least antigenic variability between genospecies[120]; Osp A serotyping has been used to divide *B. burgdorferi sensu lato* into different phenotypes,[105] which correlate with different genotypes by Osp A gene sequencing. The Osp C gene is located on a 26-kbp circular plasmid that is present in all genospecies, but its expression, both qualitatively and quantitatively, is variable; most European strains express Osp C, but Osp C has been found to be cryptic in North American strains, where it is expressed only in strains that have lost all plasmids other than the Osp C–encoding and Osp AB–encoding plasmids.[116] The Osp D gene is highly conserved and is present in 24, 50, and 90%, respectively, of isolates of *B. burgdorferi sensu stricto, afzelii,* and *garinii*; its encoding plasmid has significant size variability, ranging from 36 to 40 kbp, and contains varying numbers of copies of a 17-kbp repeating sequence bordering a variable region with evidence of homologous recombinational events.[117] The Osp E and Osp F genes are located in tandem on the

45-kbp linear plasmid.[118] The pG gene is located on a 48-kbp linear plasmid that has some sequence homology to the Osp EF gene and is detectable in most strains of *B. burgdorferi sensu stricto* and *B. afzelii*, but not in *B. garinii* or *B. japonica*.[119] There is p83/100 gene heterogeneity in *B. garinii*, but not in either *B. burgdorferi sensu stricto* or *B. afzelii*; *B. garinii* strains could be separated into two major subtypes on the basis of p83/100 gene sequence variation, one corresponding to Osp A serotype 4 and the other to serotypes 3, 5, 6, and 7.[106] The EppA protein gene is located on the 9-kbp circular plasmid, and loss of this plasmid has been associated with loss of virulence during passage of *B. burgdorferi* in culture.[121]

It has been proposed that the high level of variability of Osp C[115] and D,[117] and the existence of a VMP-like system[130, 131] may be involved in immune evasion by *B. burgdorferi*. Evasion of the immune response by a *B. burgdorferi* strain expressing a truncated Osp B also raised this as a possible immune escape mechanism.[135, 136]

Differential gene expression, which has been found in *B. burgdorferi*, has also been suspected to be involved in infectivity, invasion, and dissemination, and in evasion of the host immune response to the infection[120, 137]; it may also have a role in differential organotropism. Abundant Osp A and Osp B, and no Osp C, are expressed by *B. burgdorferi* in unfed tick midguts. The beginning of tick feeding and the arrival of the blood meal in the tick midgut trigger downregulation of Osp A and B, and upregulation of Osp C expression of *B. burgdorferi* in the engorged tick midgut.[138–140] Although Osp A and B are not expressed initially after infection, they are eventually expressed, in particular in patients with chronic Lyme arthritis. Although Osp E and Osp F are expressed by *B. burgdorferi* in ticks and in the mammalian host, it appears that the Osp E and F homologues, the Erp proteins (Osp EF–related proteins), form a gene group that is differentially expressed at different stages of the spirochete's life cycle; the Osp E homologue, p21, which has 70% amino acid homology with Osp E, and the Osp F homologues, pG, bbk2.10, and bbk2.11, are expressed only in the mammalian host and not in the spirochete in culture or in ticks.[119, 127, 141] Expression of p21 does not occur even in engorged ticks, only in the mammalian host; antibody to p21 is found in 28 to 33% of patients with early or late Lyme disease, including Lyme arthritis, indicating its expression during Lyme disease.[127] Confirmation of differential gene expression during Lyme disease was first reported in 1998, when p35 (the 35-kd protein) and p37 (the 37-kd protein) messenger RNA (mRNA), but not Osp A mRNA, was found in EM skin biopsies and Lyme arthritis synovium, consistent with upregulation of p35 and p37 and the downregulation of Osp A.[141] The protein EppA (exported plasmid protein A) is downregulated at the transcriptional level in cultured *B. burgdorferi*, is expressed only in the mammalian host, and is associated with virulent strains of *B. burgdorferi*.[121] Temperature increases, as occur with ingestion of the blood meal by the tick, and even increases in culture temperature from 23° C to 35° C, induce downregulation of Osp A expression, and upregulation of Osp C, Osp E, Osp F, and of the Osp EF homologues, the Erp proteins.[128, 140, 142] As Osp A is downregulated and disappears, the spirochete becomes resistent to antibody against Osp A; this is important in vaccine development, as is discussed in the section Prevention: Vaccine Development.

B. burgdorferi produces none of its own proteolytic enzymes. It acquires a host-derived activated proteolytic complex consisting of plasmin, plasminogen, and a urokinase-type plasminogen activator, which arrives at the tick midgut in the blood meal, binds to Osp A while it is still expressed, and coats the spirochete; this complex is presumably able to dissolve extracellular matrix, facilitate dissemination of the spirochete to the tick salivary glands for transmission to the host, and then enhance spirochete dissemination in host tissues, where the host-derived antigens cause the spirochete to be invisible immunologically to the host.[143–146] Surface antigens of *B. burgdorferi*, particularly Osp A, are also involved in binding of the spirochete to collagen fibers, vascular endothelium, and other cells,[147] including antigen-presenting cells,[148] and in triggering a variety of events in host cells, ranging from expression of adhesion molecules to production of cytokines and other factors involved in the immunopathogenesis of the infection,[149, 150] as is discussed in the section Pathology and Pathogenesis.

Some antigens of *B. burgdorferi* have epitopes that share homology and cross react with host epitopes, leading to molecular mimicry,[151] such as *B. burgdorferi* Osp A and human leukocyte function–associated antigen-1 (LFA-1),[126] and possibly p83/100 and the human muscle and cytoskeletal proteins myosin and troponin,[106] *B. burgdorferi* flagellin, and human axonal heat shock protein 60.[113] This is discussed further in the sections Pathology and Pathogenesis, and Interactions with the Immune System: Correlation of Clinical Manifestations with HLA Type.

Taxonomy

Borrelia burgdorferi,[51, 94] the etiologic agent of Lyme borreliosis, is a member of the order Spirochaetales, the family Spirochaetaceae, the genus *Borrelia*, and the species *burgdorferi*. Borreliae are more closely related genetically to *Spirochaeta* than to *Treponema*, and all borreliae are transmitted by arthropods.[99]

B. burgdorferi was initially divided into four phenotypes,[92] and later into eight serotypes[51, 52, 105, 259] on the basis of antigenic diversity of Osp A as determined by reactivity with various monoclonal antibodies and by Osp A gene sequencing.[105] It was also initially divided into three genotype subspecies, based on DNA homology and ribosomal RNA restriction endonuclease pattern analysis,[50, 53] and corresponding to phenotypes based on major protein antigenicity, with 76 to 100% DNA homology within groups, and 46 to 74% between groups.[53]

As more isolates of *B. burgdorferi* have been studied by various methods, it has become clear that *B. burgdorferi* has phenotypic and genotypic heterogeneity.* On the basis of phenotypic and genotypic differences from

*See references 13, 51–66, 68, 70, 100, 105, 115, 117, 123, and 884.

B. burgdorferi sensu stricto and from each other, further subdivision into additional subbranches was done, dendrograms of genetic relatedness were constructed,* and some of the subbranches were designated as new genospecies—*Borrelia garinii* (formerly 20047),[53] *B. afzelii* (formerly VS461),[53, 67] *B. andersonii* (includes former groups 21038 and 21123),[56, 59, 66, 70, 100] *B. valaisiana* (formerly VS116 and M19),[61] *B. lusitaniae* (formerly PotiB2),[65] *B. japonica* (formerly HO14),[66, 69] *B. tanukii* (formerly Hk501),[58, 68] *B. turdae* (formerly Ya501),[58, 68] and *B. miyamotoi* (formerly HT31).[77] There is also a genomic group DN127, which includes strain CA55 and sometimes strain 25015, and which is distinct from the other genospecies.[55, 57, 64, 123, 884]

In 1998, isolation of an unusual strain of *B. burgdorferi sensu lato* was reported from *Ixodes dentatus* and *A. americanum* in southeastern Missouri, which is similar to strains isolated from *I. dentatus* in New York and Georgia, but different from *B. burgdorferi sensu stricto*.[153] Also, an uncultivable borrelia, *Borrelia lonestarii*, was found in *A. americanum* from New York, New Jersey, Missouri, and North Carolina,[73] which may be related to the Lyme-like disease in the southern states. A borrelia identified as *B. burgdorferi* has been found in *A. americanum* in New Jersey, Missouri, Texas, Oklahoma, Virginia, North Carolina, and Alabama.[154–158]

There is clustering of *B. burgdorferi* genospecies from different geographic areas, such as North America, Europe, Asia, and the circumpolar arctic and subantarctic regions, and from different tick vectors.[10, 13, 51–57, 59, 62, 71, 74] *B. garinii, afzelii, sensu stricto, valaisiana*, and *lusitaniae* accounted for 39.7, 37.1, 15.9, 6.7, and 0.6% of *B. burgdorferi sensu lato* genospecies isolated from arthropod vectors, animal hosts, and human patients in Europe.[54] *B. burgdorferi sensu stricto* is found in *I. scapularis* and *I. pacificus* in North America.[55, 56, 59, 63, 70, 100, 123, 159] *B. andersonii* is found in *I. dentatus*,[59, 100] and *I. scapularis* in North America.[64] *B. bissettii* is found in *I. pacificus*[55–57, 59, 123] and group CA55 in *Ixodes neotomae* in the western United States,[57, 59, 884] and group 25015 in *I. scapularis* from New York.[55–57, 123, 884] *B. bissettii* represents the only strain other than *sensu stricto* to be present in both Europe and North America.[83, 884] Four genospecies—*B. burgdorferi sensu stricto, B. afzelii, B. garinii*, and *B. valaisiana*—are found in *I. ricinus* in central Europe.[61, 74] Human co-infections[74, 84] and *I. ricinus* co-infections[83, 85, 160–162] with different genospecies have been reported. *B. afzelii* and *B. garinii* have been found in *Ixodes persulcatus* in eastern Europe and in Asia, including Japan, and *B. burgdorferi sensu stricto* has not been found.[67, 74, 163, 164] *B. japonica* is found in *Ixodes ovatus* in Japan[66, 69, 163]; *B. garinii*, and no other genospecies, is found in *Ixodes uriae* and *I. ricinus* in the far northern subarctic latitudes,[152, 165, 166] and in *I. uriae* in the far southern subantarctic latitudes; genetically heterogeneous *B. burgdorferi sensu stricto, B. garinii*, and *B. afzelii* occur in migratory passerine (perching) birds in Sweden.[13]

Hypotheses about the phylogenetic origins and historical patterns of global migration of the different *B. burgdorferi* genospecies have been developed, based on genetic analysis of the different strains. Initially, it was thought that there was greater diversity of genospecies in Europe,[53] with *B. garinii, afzelii*, and *sensu stricto*, and in Asia, with *B. garinii, afzelii*, and *japonica*, than in North America, where only *B. burgdorferi sensu stricto* was thought to occur; this led to hypotheses that *B. burgdorferi* was introduced into North America from Europe, possibly by migratory birds or small mammalian hosts of infected ticks.[13, 157, 165, 167–171] The initial genetic studies were done mainly on isolates from the restricted hyperendemic areas of the Northeast and Upper Midwest; later, when isolates from the South and West were studied, more genetic heterogeneity was found,[62, 63] raising the reverse hypothesis—that introduction was from North America into Europe. The similarity in Osp A phenotype of a few west central European strains and the North American strains raises the possibility that the *B. burgdorferi* originally introduced into the United States came from west central Europe,[92] or that North American strains were introduced into Europe. The differences in DNA sequences for outer surface proteins of North American and European strains of *B. burgdorferi* suggest that these strains may have diverged long ago and may be pathogenically different.

B. burgdorferi is clonal, and widespread genetic exchange between chromosomal genes is thought not to occur.[57, 60] The order of occurrence of genes is the same across different genospecies, and there is no evidence of chromosomal rearrangements since the evolutionary divergence of the different genospecies from a common ancestor.[57, 60] Genetic exchange between plasmid genes, such as the Osp A and Osp B linear plasmid genes, has been found[80] but is thought to be rare[57, 60]; there is evidence of exchange with other plasmid genes, such as the Osp D–encoding plasmid, which suggests that *B. afzelii* and *garinii* are closely related and that *B. burgdorferi sensu stricto* only recently acquired the Osp D gene.[117]

There are differences in vector competence of *I. ricinus* and *I. scapularis* for three genospecies of *B. burgdorferi sensu lato*, which correlates with the known geographic association of these vectors and genospecies: Acquisition of infection by *I. scapularis* was 83 to 90, 87, 10, and 5% for *B. burgdorferi sensu stricto, afzelii, garinii* VS286, and *garinii* VSBP, compared with acquisition of infection by *I. ricinus* of 3, 90, 5, and 3%, respectively.[878] Other genospecies are associated with some tick species and have not been found in others.[68, 69]

There is clustering of *B. burgdorferi* genospecies from different reservoir host species[74] and some host species, which may act as biologic filters.[172, 173]

Isolation and Cultivation

B. burgdorferi lives in hosts such as vertebrates or hematophagous arthropods and is not found living free in the environment. In 1981, it was first isolated by Burgdorfer and associates from the midgut and other tissues dissected from *Ixodes scapularis (dammini)* ticks from Shelter Island, a Lyme-endemic area of New York, and was cloned to become the B31 strain of *B. burgdorferi*.[1] In 1983, Burgdorfer and colleagues also first isolated a similar spirochete from *Ixodes ricinus* ticks from the Seo-

*See references 55–57, 59–63, 65, 66, 68, 76, 77, and 117.

wald Forest, a Lyme-endemic area of Switzerland, and showed it to be morphologically and antigenically similar to the *I. dammini* spirochete.[81] Since then, it has been isolated from several species of ticks, vertebrate hosts, and humans; this is described in the section Epidemiology and Transmission.

B. burgdorferi is fastidious and microaerophilic and grows best in a liquid medium, modified Barbour-Stoenner-Kelly medium (BSK II), at 33° C to 35° C.[50, 81, 91] It has an 11- to 24-hour doubling time, which may be shortened to 11 to 12 hours under ideal conditions, but it still may take 3 weeks or longer to grow sufficiently in culture to become detectable by microscopy.[18, 50, 91, 174] However, the use of *B. burgdorferi*–specific PCR has shortened the time for detection in culture media.[175] It can also grow anaerobically, and has even been grown aerobically in the presence of 1 to 5% carbon dioxide.[99]

Unlike other spirochetes, *B. burgdorferi* can be grown in solid media.[97] It has been found to produce colonies of several types, including a compact 0.43-mm round colony at the agarose surface, and three types of colonies that penetrated into the agarose—a 1.43-mm colony with a raised center surrounded by a diffuse ring, a colony composed of many small aggregations, and a diffuse 1.8-mm colony. It was also found to cause intense hemolysis on solid BSK II medium with horse blood.[879] More recently, *B. burgdorferi* has been found to have shorter doubling times of even 7 hours, when grown in solid media under strict anaerobic conditions, and it may be considered an obligate anaerobe.[176]

B. burgdorferi can be seen in cultures by dark-field or phase-contrast microscopy. It stains with acridine orange, Giemsa, and silver stains such as Warthin-Starry or Dieterle's[25] or Bosma-Steiner stain,[82] and can be identified with immunofluorescence techniques using *B. burgdorferi*–specific polyclonal or monoclonal antibodies[177] or *B. burgdorferi*–specific PCR.[175]

Transformation of *B. burgdorferi* from typical motile spirochetes to immotile cystic spheroplast L-forms occurs when *B. burgdorferi* is grown in culture in the presence of antibiotics, *B. burgdorferi*–specific antibody, or normal CSF.[102] The conversion to spheroplast forms may be related to the ability of the spirochete to persist in tissues without elimination by the host immune response.

B. burgdorferi shows antigenic variation and loss of pathogenicity after 10 to 15 passages in culture, and becomes noninfectious; this correlates with loss of plasmids.[129, 133, 134, 178, 882] Loss of several outer surface proteins and their encoding plasmid genes, including Osp B, C, and D, with passage has been noted; there is a suggestion that linear plasmid of 24.7 kbp (1p24.7) is required for infectivity of *B. burgdorferi sensu stricto*, *garinii*, and *afzelii*, and that 1p38 (which encodes Osp D) is not required. Loss of 1p27.5 may increase infectivity, but correlation of individual plasmids with infectivity has been inconsistent.[117, 134, 178] High-passage strains of *B. burgdorferi* have also been found to decrease both invasiveness and cytopathic killing of B and T lymphocytes.[179]

B. burgdorferi is relatively easily isolated and grown from midgut and other tissues dissected from infected *Ixodes* ticks,[50, 74, 174, 180, 181] from which the isolation rate depends on the incidence of infection within the tick population (see section Epidemiology and Transmission: *B. burgdorferi* Tick Infection Rates); from blood and organ cultures of infected reservoir-competent host animals[167, 182] (see section Epidemiology and Transmission: *B. burgdorferi* Reservoir Animal Infection Rates); and from biopsy specimens of the leading edge of EM skin lesions, from which the isolation rate is usually 28 to 86% (it may be higher in disseminated infection).[183, 184] It has been isolated occasionally from blood, CSF, and ACA skin biopsy specimens, and rarely from borrelial lymphocytoma skin biopsies, synovium and synovial fluid, myocardium and heart valves, the iris, ligamentous tissue, placenta, fetal tissues, or other tissues because the organism density is low[50] (see section Diagnosis and Differential Diagnosis: Diagnostic Tests: Culture).

The *B. burgdorferi*–specific PCR[185–187] increases the sensitivity of detection of *B. burgdorferi* in body fluids and tissues by using DNA target sequences that are unique to *B. burgdorferi*, are not present in other closely related *Borrelia* species or other spirochetes, and are highly conserved among *B. burgdorferi* strains. PCR has been used to demonstrate the spirochetes in EM, ACA, and borrelial lymphocytoma skin biopsy specimens; serum, plasma, and bone marrow; CSF, brain biopsy, sural nerve biopsy, and vitreous fluid; synovial fluid and membrane; urine; breast milk; placental tissue; and various animal hosts and tick vectors (see section Diagnosis and Differential Diagnosis: Diagnostic Tests: Polymerase Chain Reaction).

Antibiotic Susceptibility

Isolates of *B. burgdorferi* from humans and ticks from different geographic areas, including the United States and Europe, generally have similar antimicrobial susceptibility patterns,[50, 174, 188–190, 192, 196–198] as is shown in Table 11–1. *B. burgdorferi* antibiotic susceptibility can be assessed in vitro by comparison of the minimal inhibitory concentrations (either mean MIC or MIC 50%) and the minimal bacteriocidal concentrations (either mean MBC or MBC 50%) for various antibiotics, and in vivo by comparison of the antibiotic dose required to cure 50% of infected animals of their infection (CD_{50}). However, there is one report[196] of lower doxycycline MIC values for cutaneous isolates than for CSF isolates.

B. burgdorferi was the most susceptible in vitro to the macrolides erythromycin, azithromycin, clarithromycin, and roxithromycin (MIC, 0.01 to 0.17 μg/ml); the penicillins penicillin, amoxicillin, ampicillin, amoxicillin–clavulanic acid, mezlocillin, azlocillin, and oxacillin (MIC, 0.02 to 1.1 μg/ml); the second- and third-generation cephalosporins ceftriaxone, cefotaxime, cefuroxime, ceftizoxime, and cefixime (MIC, 0.02 to 0.8 μg/ml); and the tetracyclines doxycycline, minocycline, and tetracycline (MIC, <0.13 to 0.79 μg/ml). Isolates were also susceptible to imipenem (MIC, 0.12 μg/ml) and chloramphenicol (MIC, 2 μg/ml). The mean MIC (or MIC 50%) value for penicillin was 0.02 to 1.1 μg/ml, but the range was wide (up to 8 μg/ml). According to MIC values, the aminoglycosides, sulfonamides, metronida-

TABLE 11–1
In Vitro and In Vivo Antimicrobial Susceptibilities of *Borrelia burgdorferi*

ANTIMICROBIAL AGENT	MEAN[a] (RANGE[b]) MIC (μg/ml)	MEAN[c] (RANGE[d]) MBC (μg/ml)	SUSCEPTIBILITY[e] IN VITRO	CD_{50}[f] (mg/kg/day)	SUSCEPTIBILITY IN VIVO
Penicillin	0.02–1.1(0.003–8)	1.08–8.7(0.1–50)	S-MS-R	>320–>1975	R
Amoxicillin	<0.03–0.25(<0.03–1)	0.06–1.9(<0.03–3.2)	S	50	S
Ampicillin	<0.25–0.47(<0.25–1)		S		
Amox/clav[g]	0.12(0.12–5)		S	25	S
Mezlocillin	0.5(0.25–1)		S		
Oxacillin	0.5(0.25–2)		S		
Cefaclor	(23–128)	(64–>256)	MS		
Cefadroxil	(11–128)	(32–>128)	MS		
Cefalexin	(16–32)	(32–>256)	MS		
Cefixime	0.8(0.8)	(0.8–1.6)	S		
Cefotaxime	<0.03–0.45(<0.03–1)	<0.03–0.17(<0.03–0.8)	S	50	S
Ceftizoxime	0.125(0.06–.5)	0.5(0.25–1)	S		
Ceftriaxone	0.02–0.06(0.006–1)	0.04–3.8(0.02–50)	S	50–240	S
Cefuroxime	(0.06–0.5)	(0.25–0.75)	S		
Doxycycline	0.125–1(0.1–2)	0.71–2(0.2–6.4)	S		
Minocycline	<0.13(<0.12–0.25)	2.3	S		
Tetracycline	0.14–0.79(0.01–2)	0.8–4(0.8–6)	S	50–287	S
Azithromycin	0.01–0.017(0.003–0.03)		S	8	S
Clarithromycin	0.01(0.003–0.06)	0.13(0.06–0.25)	S	>50	R
Erythromycin	0.03–0.15(0.007–1)	0.05–2.17(0.04–10)	S	400–2353	R
Roxithromycin	0.02–1.05(0.02–1.6)	1.1(0.02–1.6)	S	>50	R
Ciprofloxacin	1(0.25–4)	2–4(0.5–16)	MS		
Ofloxacin	2(0.5–8)	2(1–8)	MS		
Gentamicin	>16		R		
Amikacin	>32		R		
Chloramphenicol	2(1–3)		S		
Imipenem	0.12(0.06–1)		S		
Rifampin	>16		R		
Trimethoprim-sulfamethoxazole	>256		R		

[a]MIC = minimal inhibitory concentration (either mean MIC or MIC 50%).
[b]MIC Range, minimum and maximum MIC values reported.
[c]MBC = minimal bactericidal concentration (either mean MBC or MBC 50%).
[d]MBC Range, minimum and maximum MBC values reported.
[e]S = susceptible to antimicrobial agent; MS = moderately susceptible to antimicrobial agent; R = resistant to antimicrobial agent.
[f]CD_{50} = dose of antimicrobial agent required to cure 50% of infected animals in animal model.
[g]Amox/clav = amoxicillin-clavulanic acid.
Data obtained from references 50, 79, 174, 188, 189, 191, 192, 194–200, and 621.

zole, rifampin, and quinolones were not useful for *B. burgdorferi*. Although *B. burgdorferi* is resistant to co-trimoxazole in vitro, a minor synergistic decrease in the roxithromycin MIC from 0.031 to 0.015 μg/ml and a significant decrease in spirochetal motility were reported to occur in combination with co-trimoxazole.[199]

For the various antibiotics, the in vitro MIC efficacy and the in vivo CD_{50} efficacy were in agreement except for penicillin, erythromycin, clarithromycin, and roxithromycin. For erythromycin, clarithromycin, and roxithromycin, evaluation of the CD_{50} showed that despite excellent MIC values, they were poorly active in vivo in the animal models. For penicillin, the poor in vivo efficacy may be due to strains of *B. burgdorferi* with high MIC values.

B. burgdorferi is killed slowly even by antibiotics to which it is sensitive, and prolonged exposure of the spirochetes to the antibiotics is necessary to achieve adequate killing.[188, 192, 200] In one study,[188] the length of time required to kill 99% of *B. burgdorferi* exposed to twice the MIC of antibiotic ranged from 72 hours for ceftriaxone and cefuroxime to 96 hours for cefixime. In another study,[192] the length of time needed to kill 99% of *B. burgdorferi* was 72 hours for 0.1 μg/ml and 48 hours for 1.0 μg/ml of both penicillin and ceftriaxone, and 72 hours for 1.0 μg/ml of tetracycline. Low concentrations of tetracycline (0.1 and 1.0 μg/ml) allowed regrowth of organisms after prolonged incubation for 96 hours or longer, but no such regrowth occurred with low concentrations of penicillin or ceftriaxone, or higher concentrations of tetracycline (above 10 μg/ml). In one study,[200] some differences in the kinetics of killing of different *B. burgdorferi* strains by different antibiotics were found after 48 hours, but all strains were effectively killed by antibiotics to which they were susceptible after 72 hours.

Results of the animal model efficacy studies show better correlation for some antibiotics than others with

clinical human patient results. For example, Steere and colleagues reported[201] that, of the oral antibiotics, tetracycline was most effective, penicillin was next most effective, and erythromycin was least effective for treatment of early Lyme disease. Clarithromycin[202] and azithromycin[193, 203] have been found to be equally or almost equally as efficacious as amoxicillin and doxycycline in the treatment of EM. Several factors, in addition to the MIC of the antibiotic, play a role in determining whether an antibiotic will be clinically effective in the elimination of *B. burgdorferi* infection; these include the duration of adequate serum, spinal fluid, intraocular, intrasynovial, and tissue antibiotic concentrations; the efficacy of the host immune response; and the potential sequestration of organisms in protected sites.

Interactions with the Immune System

B. burgdorferi infection triggers a sequence of immunologic and other cellular events that are involved in the local and systemic dissemination of the infection, the immunopathogenesis of the various manifestations of the infection, and the host elimination of the infection, as well as in the ability of the spirochete to evade host defenses.[148, 149, 151, 204–207] A discussion of the immunopathogenesis of Lyme borreliosis is provided in the section Pathology and Pathogenesis.

T LYMPHOCYTE REACTIVITY

B. burgdorferi antigen–triggered T cell activation occurs within a few days of the tick bite, develops before the B cell antibody response, rises during infection, is directed initially against the 41-kd flagellar and the 31-kd Osp A antigens, and is directed later against additional outer surface membrane proteins.[208, 209, 211, 212] *B. burgdorferi* spirochetes, Osp A, and Osp B have been reported to induce specific proliferation in T lymphocytes from Lyme disease patients[213, 214]; the response is predominantly due to $CD4^+$ and $CD8^+$ T lymphocytes,[214] and there is also a response due to $CD56^+$ NK (natural killer) cells.[213] *B. burgdorferi*, Osp A, Osp B, and even Osp-containing membrane blebs have been found to possess nonspecific B lymphocyte proliferative activity.[215, 216] However, *B. burgdorferi*–induced nonspecific T lymphocyte or mononuclear cell proliferation has been found by some groups[217] and not by others.[213]

B. burgdorferi antigen–specific T lymphocyte reactivity, measured by the *B. burgdorferi*–specific lymphocyte proliferative assay, is long lasting, and may persist even in seronegative patients with Lyme borreliosis.[208, 214, 218, 219] The lymphoproliferative response may be greater in spinal fluid and synovial fluid than in peripheral blood in some patients with neurologic or arthritic manifestations of Lyme borreliosis.[214, 220, 221] There is *B. burgdorferi*–specific synovial fluid T lymphocyte production of Th1-type cytokines interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α).[393] There is peripheral blood and intrathecal *B. burgdorferi*–specific T lymphocyte production of the Th1-type cytokine IFN-γ, as well as specific B lymphocyte production of IgG antibody, all of which persist for several months after clinical recovery from treated neuroborreliosis.[214] After successful antibiotic therapy of Lyme disease, the reactivity may decrease somewhat but is usually still detectable if the most sensitive assay methods are used.[208–210, 212, 213, 231]

DEVELOPMENT OF SERUM ANTIBODY

The antibody response to *B. burgdorferi* infection begins to develop a few days after the tick bite, after the development of the T lymphocyte response,[211] and there are several studies of the temporal evolution of serum IgG and IgM antibody responses to the infection in North American[222, 226, 232] and European[227, 228] patients. *B. burgdorferi sensu stricto* is the only major genospecies causing Lyme disease in North America; all three of the major genospecies, *B. burgdorferi sensu stricto*, *B. garinii*, and *B. afzelii*, cause Lyme borreliosis in Europe, resulting in some differences between the antibody responses of North American and European patients. Because of these differences, distinct criteria for Western blot positivity for each of the three genospecies in European patient sera, and for *B. burgdorferi sensu stricto* in North American patient sera, have been recommended.[233, 234, 237, 238] In both North American and European patients, the initial polyvalent antibody response to *B. burgdorferi* infection is directed primarily against the 24-kd Osp C[223, 235, 239, 240] and the 41-kd flagellar antigen. The early response to the 39-kd antigen is more common in North American than European patients,[233, 237] and the late antibody response is more often directed primarily against the outer surface membrane proteins, that is, 31-kd Osp A and 34-kd Osp B, in North American than in European patients.[234, 237, 241, 242]

The *B. burgdorferi*–specific IgM response develops in 1 to 2 weeks, peaks at 2 to 8 weeks, and usually disappears after several months in uncomplicated treated patients but may persist in patients with disseminated rather than localized infection, patients with persistent infection, some with late chronic infection,[18, 222, 226, 240, 243] patients with initially delayed antibiotic therapy (even after clinical recovery),[226, 244] and some patients with promptly and successfully treated EM and neuroborreliosis.[226, 227, 233] Although comparisons of the temporal evolution of antibodies detectable by Western blots to individual *B. burgdorferi* antigens are often difficult because of lack of standardization of band and molecular weight nomenclature, a general pattern of progressive expansion of the antibody repertoire after infection emerges. There is general agreement that the initial specific IgM response is made to the 24-kd Osp C antigen and to the 41-kd flagellar antigen. Several investigators describe early development of IgM antibody and other antigens as well. After recovery, Western blot IgM antibody reactivity to several antigens declines after 1 month and usually disappears after several months. IgM reactivity to the 24-kd Osp C and 41-kd flagellin may persist,[226, 240, 247, 248] and is even still detectable in 38% of patients with successfully treated EM 1 year later[226]; IgM antibody to Osp C is detectable in 45% of patients with chronic arthritis for months to years, and in 20% of those with chronic neuroborreliosis.[240] However, in a follow-up study of resolved pediatric Lyme arthritis,

only 5% had any IgM Western blot reactivity at a mean of 10 months after treatment, and this was only to the 41-kd flagellar antigen.[229] In very early infection, in both North American and European patients, IgM antibody to Osp A may be bound in immune complexes, and may be detectable only when these are dissociated.[246] The acute IgM response during EM in North American patients who progress to severe persistent Lyme disease includes the 83-kd and 34-kd antigens, and these responses persist into chronic disease.[222] In North American patients, the IgM Osp C antibody response is greatest in patients with EM and meningitis, early in the course of infection, and decreases to low levels in those who develop chronic neuroborreliosis.[240]

In North America, the CDC criteria for a positive IgM Western immunoblot are the presence of two of the following three bands in early disease: 24-kd Osp C, 39-kd Bmp A, and 41-kd Fla.[238] In Europe, proposed criteria for IgM Western blot positivity include the following: for *B. burgdorferi sensu stricto* IgM, at least one of 39, Osp C, and 17a or a strong 41; for *B. afzelii* IgM, at least one of 39, Osp C, and 17 or a strong 41; and for *B. garinii* IgM, at least one of 39 and Osp C or a strong 41.[237]

A delay in initial antibiotic therapy appears to be associated with increased dissemination, with development of higher polyvalent enzyme-linked immunosorbent assay (ELISA) titers and greater numbers of Western blot IgM bands, and with persistence of IgM positivity even after clinically successful treatment[226, 232, 244]; however, prompt antibiotic treatment of early Lyme disease appears to be associated with disappearance of IgM positivity within several months.[226, 229, 248] Longer disease duration is associated with a higher incidence of IgM seropositivity.[124] The IgM ELISA antibody is higher in neuritis and arthritis patients with early Lyme disease than in patients with only EM.[224] In late chronic Lyme borreliosis, such as arthritis, neuroborreliosis, and sometimes even in acrodermatitis chronica atrophicans, the specific IgM is often persistently positive by immunofluorescent assay (IFA), ELISA, or Western blot assays.[18, 209, 222, 223, 241, 242, 244, 249–252]

The *B. burgdorferi*–specific IgG response develops at 2 to 8 weeks, peaks at 4 to 6 months, and in uncomplicated treated patients, usually gradually declines and sometimes eventually disappears after several months, but it may persist for years in persistent infection, sometimes even after successful antibiotic therapy.[18, 222, 226–228] This response may be aborted by early antibiotic therapy.[226, 253] Delays in initial antibiotic therapy are associated with a higher incidence of dissemination, progression to later stages of infection, strongly positive IgG responses, higher polyvalent ELISA antibody titers, and increased numbers of IgG Western blot bands.[222, 226, 234, 235, 254] In one study, the number of Western blot bands reacting with serum IgG antibody decreased after successful treatment of pediatric Lyme arthritis, and no new ones appeared.[229] As is the case for IgM Western blot bands, direct comparison of IgG bands from different studies is not always possible. However, general patterns of the temporal evolution of the IgG antibody response to infection emerge. The initial IgG response is made to the 41-kd flagellar and the 24-kd Osp C antigens; it progressively expands to include additional antigens, such as the 26-kd Osp F,[245] and eventually, within the first month after successful treatment, it includes many additional antigens.[211, 222, 225, 226, 235, 240, 245] In very early infection, in both European and North American patients, IgG antibody to Osp A is often present in immune complexes, but may be detectable only if these are dissociated.[246] In persistent infection, the IgG response expands over months to years.[211, 222, 255, 256] In late European Lyme borreliosis, IgG antibody is almost always directed toward the 41-kd flagellin, and the 58-kd and 83/100-kd antigens.[107, 108, 237, 247, 257] The progressive expansion of the IgG antibody response develops regardless of whether the late manifestations are arthritic, neurologic, or cardiac,[279] although the Western blot antibody patterns may differ with various late manifestations.[234, 237, 247, 257]

In North America, the CDC criteria for a positive IgG Western immunoblot are the presence of five of the ten most common bands after the first few weeks of disease.[238] The proposed criteria for IgG Western blot positivity in Europe can be seen in reference 237.

The development of IgM and IgG antibody to new antigens months to years after onset of infection suggests either the persistence of viable *B. burgdorferi* throughout the illness, or reinfection.[222, 226, 228] There are varying opinions regarding the significance of positive IgM antibody in Lyme disease of more than 1 month's duration, but there is agreement that a diagnosis of active Lyme disease should not be made on this basis alone.[24, 226–228, 233, 244, 254]

Patients with neuroborreliosis usually have higher polyvalent *B. burgdorferi* antibody in spinal fluid than in serum,[258, 259] and some may have spinal fluid antibody in the absence of serum antibody.[253, 260, 261] Patients with arthritis usually have higher polyvalent specific antibody in synovial fluid than in serum.[262]

Highly specific antibody capable of killing *B. burgdorferi* in culture and of passively protecting mice against experimental *B. burgdorferi* challenge develops during infection and quantitatively increases with increasing severity and duration of the infection.[230] In one study, the seroprotective and borreliacidal activity occurring in patient sera from late but not early Lyme borreliosis correlated with the presence of reactivity to Osp A and Osp B.[230] Borreliacidal antibody is seroprotective against the homologous strain, and sometimes against heterologous strains.[263]

INDUCTION OF OTHER ANTIBODIES

The *B. burgdorferi*–specific IgM antibody rise during infection is also associated with polyclonal B lymphocyte activation that peaks 3 to 6 weeks after onset of infection and corresponds to the time of maximal total and *B. burgdorferi*–specific IgM antibody.[243, 264] This B cell hyperactivity leads to the development of several antibodies that are not specific for *B. burgdorferi* and are directed against host tissues, such as rheumatoid factor,[211, 243, 264] antinuclear antibody,[211, 243] anti-cardiolipin antibody,[211, 243] antibody to fibronectin-binding protein,[255]

antibody to neuronal axons,[151, 265] antibodies to myelin basic proteins,[266] and antibody to neurofilament proteins[266] and oligoclonal bands.[258, 267, 268] False-positive Venereal Disease Research Laboratory (VDRL) antibody,[211] cryoglobulins,[211, 243] and circulating immune complexes[211, 243, 264] are also found during this time. In patients with Lyme arthritis, the circulating immune complexes disappear from serum in 3 months but increase in synovial fluid; in patients with cardiac or neurologic involvement, the immune complexes persist in the serum.[262, 269]

Induced low levels of rheumatoid factor are detectable in 32% of Lyme patients by ELISA IgM and in 4% by latex agglutination assay.[264] Serum IgM antibodies to neuronal axons were found in all patients with neuroborreliosis in one study[151]; autoantibodies were found in the spinal fluid of 20% of patients with neuroborreliosis in another study.[266] *B. burgdorferi*–specific oligoclonal bands were found in the spinal fluid of 40 to 100% of patients with neuroborreliosis.[258, 267]

Anti–tick saliva antibody (ATSA) develops after a tick bite in response to the bolus of tick saliva injected, peaks at 3 to 5 weeks, persists for weeks to months, and subsequently decreases.[270] This antibody is a good biologic marker for tick exposure and may be useful in confirming tick exposure in seronegative patients with suspected Lyme borreliosis.

FAILURE TO DEVELOP SERUM ANTIBODY

Early antibiotic therapy may attenuate or eliminate the *B. burgdorferi*–specific antibody response.[18, 208, 209, 218, 225, 253, 273] Normally, *B. burgdorferi* antigen triggers B lymphocyte as well as T lymphocyte responses, but if antigen is removed by early antibiotic therapy, the antigen-dependent T cell stimulation of B cell maturation does not occur, and the mature antibody response does not develop.[253] Thus, if antibiotic therapy is given before the development of the mature IgG antibody response, this response may be aborted even though the infection may not be fully eradicated, and the patient may be seronegative. If antibiotic therapy is given after the development of the mature IgG response, the antibody response may eventually decrease, disappear, or persist, even after successful eradication of the infection.[274–277] The longer the Lyme disease persists before antibiotic therapy is begun, the more *B. burgdorferi*–specific antibody bands develop by Western blot assay.[208, 226, 244] Persistent *B. burgdorferi* infection may also occur in sequestered sites such as the central nervous system, inducing local CSF but not systemic antibody responses. Seronegative patients usually still have detectable T lymphocyte proliferative responses.[208, 218, 219, 234, 269, 277, 278] Seropositivity or seronegativity alone is not always a reliable indicator of cure.

Steere and colleagues[279] reported the incidence of true seronegative Lyme disease to be 4% in a large study of 180 patients with confirmed North American Lyme disease; they noted that all were EM history–positive, 75% had *B. burgdorferi*–specific T lymphocyte reactivity, and manifestations were usually neurologic or musculoskeletal. In seronegative patients, clinical manifestations were attenuated compared with those in seropositive patients; in seronegative patients with symptoms of significant arthritis, the term *seronegative Lyme arthritis* is contradictory, as the *B. burgdorferi* antibody response is considered to be involved in the pathogenesis of the arthritis, and these patients are unlikely to have Lyme disease.

In some patients, apparent seronegativity is due to testing by standard ELISA and Western blot assays, which detect free antibody, and specific antibody may be detected by using methods that dissociate immune complexed antibody.[278]

Failure to develop *B. burgdorferi* serum antibody in patients with confirmed Lyme borreliosis may be due to serologic testing done very early after onset of infection, during the spirochetemic phase, before the development of even a very early antibody response. Thirty-five to 100% of early Lyme borreliosis patients with *B. burgdorferi* detectable in plasma or serum by PCR were seronegative,[280, 281] and 53% of seronegative Lyme borreliosis patients had *B. burgdorferi* DNA detectable in serum by PCR, compared with none of the seropositive patients.[281]

DEVELOPMENT OF CEREBROSPINAL FLUID ANTIBODY

B. burgdorferi invasion of the central nervous system (CNS) occurs early in two thirds of patients with disseminated infection even in the absence of neurologic symptoms; this has been reported from both North America[282, 283] and Europe.[284] Patients who develop either acute or chronic neurologic involvement may have intrathecal production of specific IgG, IgM, or IgA antibodies to *B. burgdorferi* demonstrable by IFA, ELISA (standard, antibody capture, or immune-complex ELISA), or Western blot assay.*

Intrathecal production of *B. burgdorferi*–specific antibody confirms neuroborreliosis. Patients with late neuroborreliosis may be seronegative and still have intrathecal specific antibody production, presumably because oral antibiotic therapy eradicates the majority of organisms systemically, but it may fail to achieve adequate MICs in the CSF, thus allowing persistence of the organism in this privileged site.[253] Some patients with early neuroborreliosis may also have specific intrathecal antibody production, as has been observed with *B. burgdorferi* IgM antibody, without seropositivity.[253, 260, 261] Early in CNS invasion, *B. burgdorferi*–specific CSF antibody may be located in immune complexes, which are not detected by free antibody assays.[283]

There are some differences in intrathecal *B. burgdorferi* antibody between North American and European patients.[259, 290, 293] Polyclonal intrathecal *B. burgdorferi*–specific antibody was found in almost all North American patients with early Lyme meningitis, and in almost half of those with late central nervous system borreliosis, but not in those with late peripheral nervous system borreliosis. Polyclonal intrathecal *B. burgdorferi*–specific antibody was found in almost all European patients with either early or late neuroborreliosis. In one study of North American Lyme disease,[292] there was intrathecal

*See references 211, 253, 259, 260, 265, 267, 268, 283, and 285–292.

B. burgdorferi–specific ELISA IgM in 100% and IgG in 40% of patients with meningitis, as well as ELISA IgM and IgG in 26 to 30% of patients with encephalitis; in another study of North American early and late neuroborreliosis, intrathecal free antibody detectable by ELISA was found in 48% and specific immune complex–associated IgG and IgM antibody in 43%.[283]

B. burgdorferi–specific CSF antibody was directed primarily against the 41-kd flagellar antigen, and also against the 33-kd Osp A and 17-kd antigens.[258, 260, 283, 293] CSF ELISA antibody levels were higher than serum antibody levels,[259] but IFA antibody levels were higher in serum than in CSF.

INTERACTIONS WITH COMPLEMENT

B. burgdorferi activates the alternate and classic complement pathways but is resistant to the nonspecific bactericidal activity of normal human serum. However, in the presence of *B. burgdorferi* immune serum, it is sensitive to serum and is killed via the classic pathway.[96] Host-specific differential transmission of different *B. burgdorferi sensu lato* genospecies by ticks has been found to correlate with the differential susceptibility of the genospecies to bacteriolysis by serum complement, including via the alternate pathway, of the different host species.[294]

INTERACTIONS WITH PHAGOCYTES

Peripheral blood polymorphonuclear and mononuclear phagocytes and macrophages are able to phagocytose opsonized and nonopsonized *B. burgdorferi*.[295, 296] *B. burgdorferi* binds to polymorphonuclear phagocytes via integrin $\alpha_m\beta_2$, the CR3 complement receptor, during nonimmune phagocytosis.

B. burgdorferi stimulates human endothelial cells to express the neutrophil adhesion molecule, E-selectin, and the neutrophil chemotactic agent, interleukin-8 (IL-8), both of which are probably involved in recruitment of neutrophils to sites of *B. burgdorferi*–induced inflammation, and in transmigration of neutrophils across the endothelium.[150, 297] Whole *B. burgdorferi* spirochetes were demonstrated to be strong inducers, equivalent to or more potent than lipopolysaccharide (LPS), of chemoattractant cytokine production by human monocytes, including MIP-1α (macrophage inflammatory protein-1α), MCP-1 (monocyte chemotactic protein-1), and RANTES (regulated upon activation, normal T cell expressed and secreted), which attract monocytes and lymphocytes, and IL-8 (interleukin-8) and GRO-α (melanoma growth–stimulatory activity), which attract neutrophils and contribute to tissue inflammation and damage.[298]

Recombinant lipidated, but not unlipidated, *B. burgdorferi* Osp A, even in minute amounts, is a potent human neutrophil activator that induces neutrophil responses similar to those induced by bacterial LPS. Neutrophils are the main cell type in Lyme arthritic joints; they are involved both in maintaining an inflammatory response and in the destruction of opsonized *B. burgdorferi*, presumably via a combination of reactive oxygen intermediates and lysosomal products, including the proteolytic enzyme elastase.[299] Elastase has been demonstrated to be the main borreliacidal factor in human neutrophils.[300]

A possible additional mechanism by which the spirochete might evade borreliacidal antibody and temporarily persist in a protected niche is by invasion and killing of both B and T lymphocytes.[179]

EVASION OF HOST DEFENSES AND PERSISTENCE IN TISSUE

B. burgdorferi has the unusual, but fortunately uncommon, ability to evade the host immune response and persist in tissues for months to years, sometimes even after antibiotic therapy, and sometimes even after intravenous antibiotic therapy.* When it occurs, this persistence is usually either in immunologically privileged sites inaccessible to host defenses, after local or systemic steroid therapy, after initially delayed or inadequate antibiotic therapy, or in patients with risk factors such as HLA-DR4 specificity, and may occur in the presence or absence of seropositivity.[306, 307]

B. burgdorferi has been isolated, months to several years after oral or intravenous (IV) antibiotic therapy, from CSF[284, 302]; synovial fluid[200]; EM skin lesions[83, 200, 302, 303]; mitral valve tissue[200]; ligamentous tissue[304]; and iris biopsy tissue.[284] It has also been isolated, 1 month to 10 years after onset, without preceding antibiotic therapy, from CSF[302]; synovial fluid[301]; EM skin biopsy[305–307]; ACA skin lesions[19, 20]; and myocardium.[308] Its presence has been demonstrated, months to 27 years after antibiotic therapy, by *B. burgdorferi*–specific PCR or antigen capture ELISA in CSF[269, 283, 287, 309–311]; brain[311]; synovial fluid and membrane[312–314]; ACA skin biopsy[315]; and serum, blood, plasma, and bone marrow.[281, 311] Persistence for 1 month to 10 years without antibiotic therapy has also been demonstrated by PCR or antigen-detection methods in CSF and ACA skin biopsy.[143, 316, 317] The development of *B. burgdorferi*–specific IgM antibody responses to new spirochetal antigens late in the course of Lyme disease also indicates long-term persistence of live organisms in these patients.[222]

Differential gene expression of *B. burgdorferi* antigens, which results in variation in antigenicity of the spirochete during different stages of infection, is thought to be involved in evasion of the immune response.[120, 137, 142]

It has been proposed that the spirochete may be able to evade the host immune response while still inducing the inflammatory pathology characteristic of the various manifestations of Lyme disease. Differential expression of surface lipoproteins during various stages of infection allows the spirochete to vary its antigenicity[120, 130, 131, 135–137, 141, 142] while maintaining its ability to activate cells because the lipid moiety of the lipoproteins is responsible for cell activation.[147]

The use of the host's own fibrinolytic enzymes for invasion, while eliciting minimal immunologic response by the host, is an immunologically silent method of invasion called "stealth pathogenesis,"[144] which may ex-

*See references 19, 20, 206, 269, 281, 283, 284, 287, 302–308, and 311–318.

plain the long-term persistence of *B. burgdorferi* in host tissues with only minimal mononuclear cell infiltration. *B. burgdorferi* invasion of epidermal dendritic Langerhans' cells induces downregulation of major histocompatibility class II (MHC II) molecules on this major antigen-presenting cell, and may result in inability of Langerhans' cells to eliminate the spirochete and long-term *B. burgdorferi* persistence in the skin.[148]

The immunosuppressive and immunomodulatory properties of *B. burgdorferi* may also be involved in its ability to evade the host immune response. The addition of *B. burgdorferi* to lymphocyte proliferative assays reduces the proliferative responses of human peripheral blood lymphocytes to concanavalin A and phytohemagglutinin. It has been proposed that this immunosuppressive effect may allow the spirochete to rapidly disseminate from the skin inoculation site and persist in the host; it could also explain the better efficacy of prompt antibiotic therapy in elimination of the spirochete.[217]

Another mechanism by which the spirochete might evade borreliacidal antibody is by entering a protected niche such as an intracellular or other environment that is inaccessible to either a borreliacidal immune response or antibiotic therapy. Proposed potential sites for such persistence include the central nervous system, the eye, and the joints.[179, 320, 321] Temporarily, persistence in a protected niche occurs by invasion of B and T lymphocytes. *B. burgdorferi* persistence in ligamentous tissue, the iris, synovium, and the central nervous system may also represent the use of a protected niche.

Several antigens of *B. burgdorferi* have portions that share amino acid homology with human cellular proteins; molecular mimicry may also be involved in immune evasion.[113, 126, 151, 204, 205, 256, 265, 323]

CORRELATION OF CLINICAL MANIFESTATIONS WITH HLA TYPE

Differences in HLA specificities may determine *B. burgdorferi* antigen binding and presentation to T cells and the composition of the T cell response, and may be related to susceptibility to infection.[256]

Several studies by Steere and colleagues and others reported that HLA-DR4 specificity and Osp A or Osp B IgG seropositivity are associated with chronic antibiotic-resistant Lyme arthritis but not with EM or acute or chronic neuroborreliosis.[256, 312, 324, 325] Long-duration chronic Lyme arthritis patients had high frequencies of HLA-DR4 or -DR2 positivity (89%) compared with those with short-duration Lyme arthritis (27%), and HLA-DR4 positivity but not -DR2 positivity correlated with lack of response to antibiotic therapy.

Correlation of HLA specificity with outcome of antibiotic therapy of Lyme arthritis is discussed in the section Therapy: Predictors of Antibiotic Therapy Cure.

EPIDEMIOLOGY AND TRANSMISSION

World Wide Web sites for the Centers for Disease Control and Prevention (CDC), http://www.cdc.gov/ncidod/dvbid/lymegen.htm, and the European Union Concerted Action of Risk Assessment in Lyme Borreliosis (EUCALB), http://www.dis.strath.ac.uk/vie/LymeEU,[326] have updated Lyme borreliosis epidemiologic and clinical information.

Historical Review

In 1909, Afzelius described a migrating annular skin lesion in a Swedish woman at the site of an *Ixodes ricinus* sheep tick bite, called it erythema chronicum migrans (ECM), and proposed that it was a zoonosis transmitted by a tick from an animal reservoir to humans.[14, 17] ECM became a well-recognized European disease thought initially to be caused by either a tick-associated toxin or an infectious agent.[17]

Another European disease, acrodermatitis chronica atrophicans (ACA), which had first been described by Buchwald in 1883 in Germany,[327] was noted to be preceded frequently by ECM and was named ACA Herxheimer by Herxheimer and Hartman in 1902. In 1922, Garin and Boujadoux described cutaneous lesions and paralysis after a tick bite and suspected a spirochetal etiology,[328] and in 1944, Bannwarth described chronic lymphocytic meningitis after European ECM; this became known as Garin-Boujadoux-Bannwarth syndrome, or simply Bannwarth's syndrome.[17, 329]

In 1948, Lennhoff reported spirochetes in ECM skin biopsy specimens,[17, 330] but this finding could not be confirmed by others and was essentially forgotten. By 1949, there were suggestions in Europe that penicillin therapy was beneficial in ECM,[17, 331] and between 1948 and 1957, Hollstrom found that most European ECM cleared within 2 weeks after intramuscular penicillin therapy.[79, 331] In 1949, Thyresson successfully treated patients with ACA with penicillin, and in 1952, Gruneberg considered spirochetes as possible etiologic agents.[20]

In 1955, Binder and associates, in Europe, transplanted skin biopsy specimens from the rim of an ECM lesion from a patient to three scientist-volunteers who then developed ECM lesions within 3 weeks. They established that ECM was caused by a penicillin-susceptible infectious agent transmitted by the *Ixodes ricinus* tick.[17] In 1955, Gotz transmitted ACA from patient to patient by transplantation of ACA skin biopsy specimens[20] and thus confirmed ACA as an infectious disease. Both ECM and ACA became well-known European skin diseases.

The first report in the medical literature of North American erythema migrans (EM), as ECM was eventually called, was from Wisconsin in 1970 by Scrimenti,[333] although retrospective studies have found that it existed in small foci in New England as early as 1962 and 1965.[334, 335]

The recognition of Lyme arthritis as a distinct disease came in 1975, when two mothers from the small village of Old Lyme, Connecticut, brought the existence of an epidemic of children diagnosed as having juvenile rheumatoid arthritis to the attention of the state health department and the Yale Rheumatology Clinic. Steere and colleagues investigated and recognized an outbreak

of infectious arthritis, noted that many patients had an unusual rash similar to European EM, proposed that transmission occurred via an arthropod vector, and named the disease Lyme arthritis.[15] By 1980, it became known as Lyme disease because meningoencephalitis and myocarditis were also recognized as part of the disease.

In 1980, Steere and co-workers[339] found that penicillin or tetracycline therapy shortened the duration of EM and reduced the severity and frequency of subsequent arthritis. They concluded that antibiotic therapy was useful and that the disease was caused by a penicillin-sensitive bacterium such as a spirochete.

In 1981, a new spirochete was accidentally discovered by Burgdorfer in *I. dammini* ticks (now renamed *I. scapularis*) collected for a rickettsial study from Shelter Island, New York, a highly Lyme-endemic focus.[1, 17] It induced EM lesions in rabbits, and convalescent sera from Lyme patients reacted with it.[1, 17] In 1983, two groups of investigators, Steere and associates[18] and Benach and colleagues,[80] isolated the same spirochete from patients with Lyme disease, found specific antibody titers against this spirochete in convalescent sera of Lyme disease patients, and concluded that the *I. dammini* spirochete was the etiologic agent of Lyme disease. In 1984, it was named *B. burgdorferi* when it was confirmed to be a new species.[16]

In 1983, Barbour, Burgdorfer, and co-workers isolated a spirochete similar to the *I. dammini* spirochete from *Ixodes ricinus* ticks[81]; it was indistinguishable from *B. burgdorferi* and was also confirmed to be the etiologic agent of European ECM,[19] European ACA,[19, 20] European Bannwarth's syndrome,[21, 302] and European borrelial lymphocytoma.[22]

The recent application of new molecular biologic techniques such as the polymerase chain reaction (PCR) to the historical study of *B. burgdorferi* in museum specimens of ticks and animals has made it possible to retrospectively document its presence in Europe in museum tick specimens as early as 1882 to 1897,[340] and in North America in museum mouse specimens as early as 1894. This dates the presence of the spirochete in Europe to the times of the earliest clinical descriptions of Lyme borreliosis.

The first case of congenitally transmitted Lyme borreliosis was described by Schlesinger and associates in 1985 after gestational Lyme disease acquired in Wisconsin.[25] Since then, several additional cases have been reported, and it has become clear that gestational Lyme borreliosis carries a low but serious risk of congenital infection.

Tick (and Other Arthropod) Vectors

Epidemiologic studies have indicated that Lyme borreliosis is caused by *B. burgdorferi sensu lato* transmitted from animals to humans by ixodid ticks that are members of the *Ixodes ricinus* complex,[167, 336] and that this transmission occurs during tick feeding because of either tick salivation or regurgitation of organisms.[341, 342] Ticks that are members of the *I. ricinus* complex and have been associated with human Lyme borreliosis transmission are the deer tick *Ixodes dammini/scapularis* in the northeastern and upper midwestern United States,[335, 337] the black-legged tick *Ixodes pacificus* in the western United States,[2, 180, 335, 337, 343] the sheep tick *Ixodes ricinus* in Europe,[11, 81] and the *Ixodes persulcatus* tick in Asia.[163, 344] Other ticks that are not members of the *I. ricinus* complex are also associated with enzootic *B. burgdorferi* cycles, but either are not or are rarely involved in human Lyme borreliosis transmission and may be involved in bridging between separate enzootic cycles.[13] (In this chapter, the name *I. scapularis* is used to indicate both northern and southern ticks.[346, 348])

B. burgdorferi is often found in nymphal and adult stages of *Ixodes scapularis*, *pacificus*, *ricinus*, and *persulcatus*, but rarely in unfed larvae, because infection is acquired by larvae feeding on *B. burgdorferi*–infected animal reservoirs, is passed transstadially (between stages) from larvae to nymphs to adults, and is rarely passed transovarially from infected female ticks to less than 1% of eggs and larvae.[18, 154, 167, 182, 352–356] However, because occasional female ticks may produce progeny with high infection rates, rare transovarial transmission may be important for establishment of new endemic foci of Lyme disease in instances in which an infected tick is transported by birds or other methods into a new, previously nonendemic area. Partially fed larval ticks (in which feeding on infected hosts was interrupted) are able to transmit *B. burgdorferi* during refeeding, which may explain some larval positivity.[357]

In North America, most Lyme disease transmission is due to northern *I. scapularis*[348] and *I. pacificus* tick vectors,[180, 348] which frequently bite humans, but several other species of ticks have been thought to be vectors in some geographic areas, particularly in areas where northern *I. scapularis* and *I. pacificus* are not prevalent[348]; the southern *I. scapularis* has been considered a Lyme disease vector in parts of the southern United States.[358–361] In the western United States, *I. neotomae* and *Ixodes spinipalpus* are involved in *B. burgdorferi* enzootic cycles, and *I. pacificus* serves as a bridge vector to man[361]; in the eastern United States, *I. dentatus* and *Ixodes minor* are also involved in *B. burgdorferi* enzootic cycles, and *I. scapularis* may be involved as a bridge vector.[361] Although infrequent, human bites have been documented for *I. spinipalpus*[362] and *I. dentatus*,[70, 363] and for two other ticks that are not members of the subgenus *Ixodes*—*Ixodes angustus*[364] and *Ixodes cookei*[363]; rare cases of possible EM have been reported after human bites by *I. angustus* in Washington state[364] and *I. cookei* in West Virginia.[363]

Other ticks in North America commonly biting humans are the dog tick *Dermacentor variabilis*, and the Pacific Coast tick *Dermacentor occidentalis*[348, 365]; *D. variabilis* in Kentucky[366] has been considered a possible secondary human Lyme disease vector. The Lone Star tick *Amblyomma americanum*, which is the most common tick biting humans in the southeastern and south central United States,[367] has been considered a potentially important alternate human vector in New Jersey,[155] southeastern Missouri,[153, 367, 368] North and South Carolina,[359, 360] Kentucky,[366] Alabama,[156] and Texas.[369] *B. burgdorferi sensu lato*[153] and *Borrelia lonestari*,[73] a noncultivable *Borrelia* possibly related to Lyme-like disease in

the South, have been found in *A. americanum*. *H. leporispalustri* and *Dermacentor parumapertus* rarely bite humans.[336] There have been occasional reports of suspected Lyme borreliosis transmission by other hematophagous arthropods such as mosquitoes[370] and tabanid flies (deer and horseflies) in North America and Europe.[371, 372] Figure 11–4 shows different stages of three common North American ticks: *I. scapularis*, *A. americanum*, and *D. variabilis*.

In South America, the *Ixodes affinis* and *Ixodes pararicinus* ticks from Peru are also members of the *Ixodes ricinus* complex and are considered potential vectors of *B. burgdorferi*.[373] In Asia, although the *Ixodes ovatus* tick in Japan frequently bites humans and has been found to harbor *B. japonica*, this has not been found to be associated with human Lyme borreliosis.[163, 374] The *Ixodes holocyclus* tick in Australia is the tick most often biting humans, but it has not been found to harbor *B. burgdorferi*.[375]

In addition to *Ixodes scapularis*, *pacificus*, *ricinus*, and *persulcatus* ticks,[336] *B. burgdorferi* has been isolated from ticks of other *Ixodes* species and of four additional genera (Table 11–2).

For a tick to be vector-competent for *B. burgdorferi*, it must be able to become and remain infected, pass the infection transstadially, and transmit the infection to a host. *Ixodes scapularis*, *pacificus*, *ricinus*, *persulcatus*, *dentatus*, *neotomae*, and *hexagonus* are efficient and competent *B. burgdorferi* vectors,[170, 336, 355, 357, 365, 378, 390] and *I. uriae*,[165, 166] and *I. spinipalpus*[362] are probably efficient and competent vectors.

It has been recognized that there are significant differ-

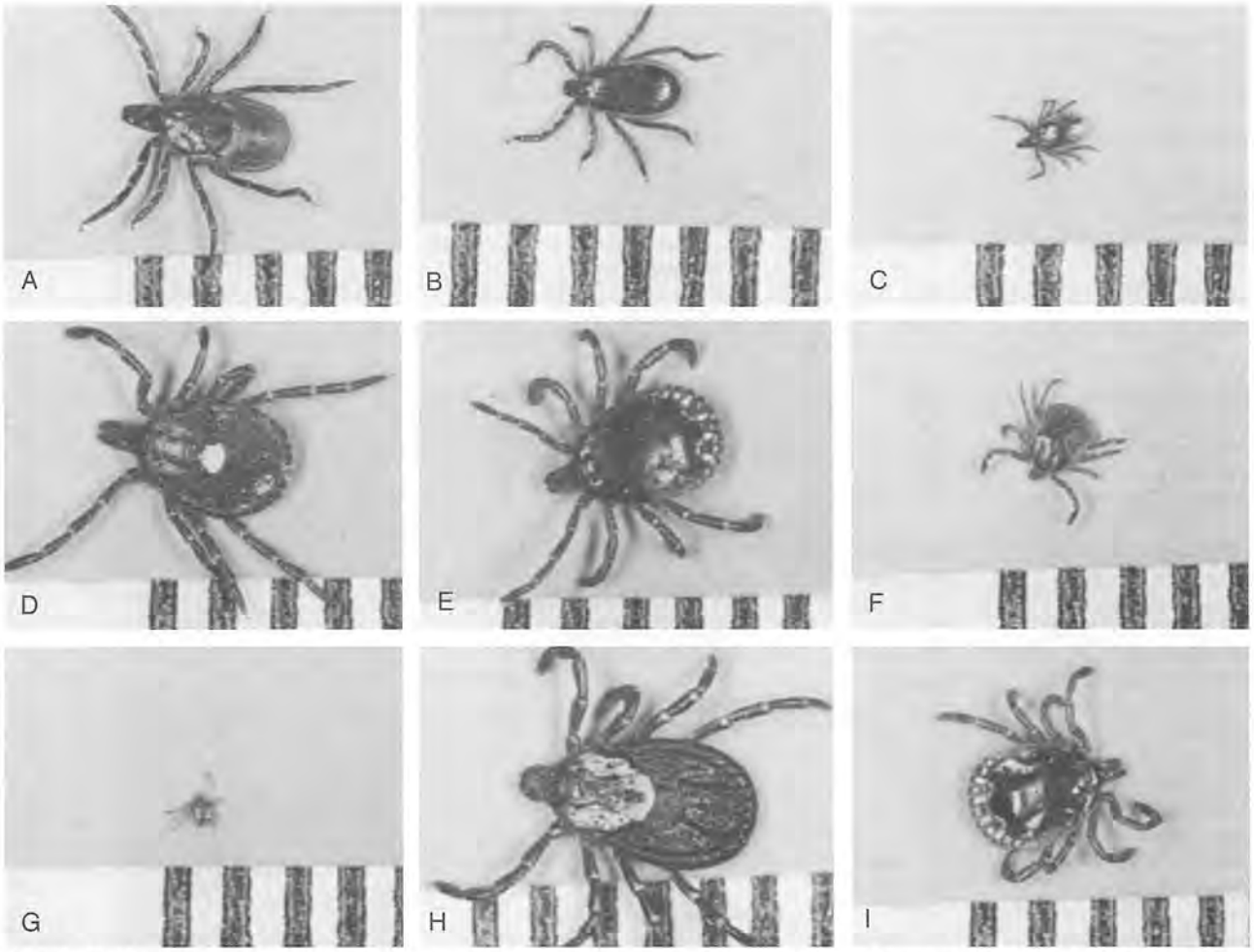

FIGURE 11–4 Common species of ticks. *Ixodes ricinus* complex ticks are vectors of transmission of the Lyme disease spirochete, *Borrelia burgdorferi*, to humans. *A, Ixodes dammini/scapularis* (northern species) adult female. *B*, Adult male. *C,* Nymph. The North American deer tick *Ixodes dammini* (the same species as the black-legged tick *Ixodes scapularis*) is the vector in the northeastern and north central, and possibly in southeastern and south central, United States. Other *Ixodes* ticks are similar in appearance, such as the western black-legged tick *Ixodes pacificus* (the vector in the northwestern United States), the European wood or sheep tick *Ixodes ricinus* (the vector in Europe), and the taiga tick *Ixodes persulcatus* (the vector in Eurasia). Some non-*Ixodes* ticks have been suspected but not proved to be associated with transmission of the Lyme disease spirochete to humans. *(D) Amblyomma americanum* adult female, *(E)* adult male, *(F)* nymph, and *(G)* larva. The Lone Star tick *A. americanum* may be a vector in the southeastern and south central United States. *H, Dermacentor variabilis* adult female. *I,* Adult male. The American dog tick *Dermacentor variabilis* and the Rocky Mountain wood tick *Dermacentor andersoni* may be occasional vectors and are similar in appearance.

TABLE 11–2
Arthropod Species in Which *Borrelia burgdorferi sensu lato* Has Been Confirmed

SPECIES	GEOGRAPHIC LOCATION	COMMON NAME	*Bb SENSU LATO* GENOSPECIES ISOLATED	VECTOR COMPETENCE FOR *B. BURGDORFERI*
Ixodes scapularis	N. America	Deer tick	*B.b.s.s., B. andersonii B. bissettii*	Efficient
Ixodes pacificus	N. America	Western black-legged tick	*B.b.s.s., B. andersonii, B. bissettii*	Efficient
Ixodes ricinus	Europe	Sheep tick	*B. garinii, B. afzelii, B.b.s.s., B. valaisiana, B. lusitaniae*	Efficient
Ixodes persulcatus	Asia	Taiga tick	*B. garinii, B. afzelii, B. valaisiana, B. lusitaniae, B. miyamotoi*	Efficient
Ixodes dentatus	N. America	Rabbit tick	*B.b.s.l., B. andersonii*	Efficient
Ixodes neotomae	N. America	Woodrat tick	*B.b. B. bissettii, B.b.s.l.*	Efficient
Ixodes angustus	N. America		*B.b.*	
Ixodes cookei	N. America		*B.b.*	Poor
Ixodes spinipalpis	N. America	Mexican woodrat tick	*B.b.*	Efficient
Ixodes hexagonus	Europe	Hedgehog and fox tick	*B.b.s.l.*	Efficient
Ixodes canisuga	Europe	Fox tick	*B.b.s.l.*	?
Ixodes frontalis	Europe	Bird tick	*B.b.s.l.*	?
Ixodes ovatus	Asia	Oriental and Palearctic tick	*B. japonica*	?
Ixodes granulatus	Asia		*B.b.*	?
Ixodes uriae	Subarctic and subantarctic islands	Seabird tick	*B. garinii*	Efficient
Ixodes rangtangensis	Asia		*B.b.*	?
Ixodes columnae	Asia		*B.b.* Am501	?
Ixodes tanuki	Asia		*B. tanukii*	
Ixodes turdus	Asia		*B. turdae*	
Amblyomma americanum	N. America	Lone Star tick	*B.b.s.s., B.b.s.l., B. lonestarii*	Poor
Amblyomma maculatum	N. America	Gulf Coast tick	*B.b.*	?
Dermacentor variabilis	N. America	Dog tick	*B.b.*	Poor
Dermacentor albipictus	N. America		*B.b.*	
Dermacentor occidentalis	N. America	Pacific Coast tick	*B.b.*	Poor
Dermacentor parumapertus	N. America	Rabbit tick	*B.b.*	?
Dermacentor marginatus	Europe		*B.b.*	?
Rhipicephalus sanguineus	N. America	Dog tick	*B.b.*	?
Haemaphysalis leporispalustris	N. America	Rabbit tick	*B.b.*	?
Haemaphysalis punctata	Europe		*B.b.*	?
Haemaphysalis concinna	Asia		*B.b.s.l.*	?
Haemaphysalis bispinosa	Asia		*B.b.s.l.*	?
Haemaphysalis longicornis	Asia		*B.b.s.l.*	?
Ctenocephalides felis	N. America	Cat flea	*B.b.*	?
Chrysops and *Hybomitra* spp.	N. America	Tabanid (Deer and horse) flies	*B.b.*	Poor
Aedes spp. and *Culex* spp.	N. America, Europe	Mosquitoes	*B.b.*	Poor

Data obtained from references 53, 59, 61, 64–70, 73, 74, 77, 100, 153–158, 163–166, 170, 180, 336, 344, 350, 351, 355, 362, 365, 376–381, 382–389, 405, 412, 420, 438, 449, 621, 878, 884, and additional references noted in text.

ences in vector competence of *I. scapularis* and *I. ricinus* for different genospecies of *B. burgdorferi*, and even for different strains within the same genospecies,[878] which may be related to differential susceptibility to bacteriolysis of various *B. burgdorferi* genospecies by complement of different host species.[294]

In North America, *I. scapularis* has also been reported to be the vector of the agent of human babesiosis, *Babesia microti*,[394] and of the agent of human granulocytic ehrlichiosis (HGE) (which is closely related to *Ehrlichia equi/phagocytophila*)[395–398]; presumably, *I. pacificus* in the western United States[398] and *I. ricinus* in Europe[398–400]

act in the same capacity. In North America, *Amblyomma americanum* is the vector of the agent of human monocytic ehrlichiosis (HME), *Ehrlichia chaffeensis* (initially incorrectly reported as *Ehrlichia canis*), and has also been considered a possible secondary vector of *B. burgdorferi*; the European vector of HME is not known. The vector of the *Babesia* species piroplasm WA1, which causes human infection in California, is not known.[401] Coinfections of ticks with *B. burgdorferi* and *Ehrlichia* or *Babesia* have been reported.

Enzootic Cycles: Tick Vector Life Cycles and Reservoir Animal Hosts

The *Ixodes ricinus* complex ticks are all three-host ticks with a 2- to 3-year life cycle, and each of the three stages of the tick feeds once (Table 11–3): Larvae feed on small rodents, reptiles, and birds; nymphs feed on small or medium-sized mammals; and adults feed on large mammals.* Eggs laid by infected adult female ticks usually hatch into uninfected larvae, as the rate of transovarial transmission of the spirochete is very low,[352–356] and larvae acquire the spirochete by feeding on *B. burgdorferi* spirochetemic–competent reservoir hosts. The infection is maintained in the larvae through the transstadial molt and is passed from the larval to the nymphal stage. The infected nymphs transmit the infection to reservoir-competent hosts by feeding, maintain the infection through the transstadial molt, and pass it to the adult stage of the tick, which then mates while feeding on a large mammalian host. The prevalence of *B. burgdorferi* infection in a tick population is determined by the frequency of feeding of larvae and nymphs on infected reservoir-competent hosts. The infection rate in adult ticks is higher than in nymphal ticks.[161, 396, 409, 410] Larval ticks have been found to acquire *B. burgdorferi* even after only partial feeding.[357]

For *B. burgdorferi* infection to be maintained in nature, there must be horizontal transmission of infection from infected nymphs to a competent reservoir host to larvae, which requires that nymphs feed before larvae on the same reservoir-competent host.[347] The white-footed mouse, *Peromyscus leucopus*, and other *Peromyscus* species mice are reservoir-competent for *B. burgdorferi*, are easily infected by a single infected tick bite, develop persistent spirochetemia, are able to infect feeding ticks, and are almost universally infected in endemic areas.[411] Humans are accidental hosts of all stages of *I. scapularis* and *I. ricinus*, and of the adult ticks of *I. pacificus*, *I. persulcatus*, and *I. ovatus*. Some animal hosts of *I. ricinus* complex ticks, such as North American catbirds, western fence lizards, and European blackbirds, have a zooprophylactic effect, and decrease the force of *B. burgdorferi* transmission by eliminating infectious spirochetes within feeding ticks, thus removing these ticks from the enzootic cycle.[173]

The life cycle of *I. scapularis* has been the most extensively studied.[138, 154, 167, 168, 182, 390, 407, 408] Eggs laid on the ground in the spring hatch into larvae in mid- to late summer. In late summer, July and August, larvae become

*See references 138, 167, 168, 182, 336, 346, 347, 390, 407, and 408.

TABLE 11–3
Preferred Hosts for Different Stages of *Ixodes ricinus* Complex Ticks That Transmit Lyme Borreliosis to Humans[a, b]

TICK	LARVAL AND NYMPHAL STAGES	ADULT STAGE	TOTAL NO. HOSTS			
			Mammal	Bird	Reptile	All
I. scapularis						
(northern U.S.)	White-footed mouse, *Peromyscus leucopus*	White-tailed deer, *Odocoileus virginianus*	31	49	0	80
(southern U.S.)	Lizards and skinks Cotton mouse, *Peromyscus gossypinus* Cotton rat, *Sigmodon hispidus*	White-tailed deer, *Odocoileus virginianus* Black-tailed deer, *Odocoileus hemionus columbianus*	39	11	6	53
I. pacificus	Fence lizard, *Sceloporus occidentalis*	Black-tailed deer, *Odocoileus hemionus columbianus*, cattle, horses, bears	54	19	7	80
I. ricinus	Woodmouse, *Apodemus sylvaticus*, and Yellow-necked mouse, *Apodemus flavicolus* Bank vole, *Clethrionomys glareolus* Black rat, *Rattus rattus*, and Norway rat, *Rattus norvegicus*	Deer, *Capreolus capreolus* Deer, canids, cattle, hares, sheep	91	132	14	237
I. persulcatus	Woodmouse, *Apodemus speciosus* Red-backed vole, *Clethrionomys rutilus* Black-faced bunting, *Emberiza spodocephala* Red-bellied thrush, *Turdus chrysolaus*	Deer, canids, cattle, hares	89	121	2	212

[a]Data from references 163, 164, 167–169, 182, 336, 348, 378, 413, and 419–421.
[b]Humans are incidental hosts of all stages of the ticks.

infected with *B. burgdorferi* by feeding for 3 to 5 days on small rodents such as the white-footed mouse, which are amplifying reservoirs for *B. burgdorferi* infection; the fed larvae then fall to the ground. The infection persists in the larvae throughout the winter and through the transstadial molt the following spring into the nymphal stage. The nymphs are voracious and feed in the spring and early summer (May, June, and early July) for 4 to 7 days on a variety of hosts, including small rodents such as the white-footed mouse, birds, wild and domestic animals, and occasionally, humans; the fed nymphs fall to the ground. Because transovarial passage of *B. burgdorferi* infection is rare, horizontal transmission is necessary to maintain the tick infection, and it occurs because infected nymphs feed earlier in the season on the same hosts as the larvae and infect the hosts, which then infect the larvae. The nymphs molt into adults by late summer or fall, and the spirochete is passed transstadially to the adult form. The adults quest for vegetation, especially at edges between lawns and forests,[336] and for medium-sized to large mammalian hosts, such as white-tailed deer, in the fall (mid-October through November), warm days in winter, and the following spring (April and early May); they mate while the females are feeding on these hosts. Questing adult field-collected infected ticks contain a median of 1500 to 1900 spirochetes per tick.[412] Because tick mating occurs on these large mammalian hosts, particularly deer, these hosts are needed for tick survival but not for maintenance of the *B. burgdorferi* infection.[411] The females then feed for 8 to 11 days, fall to the ground, lay eggs in the spring, and die; the eggs hatch in 45 to 53 days into larvae in the summer. The prevalence of *B. burgdorferi* infection increases from the nymphal to the adult stage because the ticks feed on amplifying reservoir-competent hosts.

In northeastern and upper midwestern North America, the preferred small rodent host of *I. scapularis* is the white-footed mouse, *Peromyscus leucopus*, which is also the primary reservoir of *B. burgdorferi* infection in nature,[167, 182, 346, 411] and the preferred large mammal host is the white-tailed deer, *Odocoileus virginianus*, which is the host of the reproductive stage of the tick[346, 408]; however, larvae and nymphs have been found attached to 80 different species of mammals and birds, but not reptiles, and adult ticks to 13 species of medium-sized to large mammals.[167, 168, 336, 337, 413] The mice remain chronically spirochetemic but asymptomatic. The deer are occasionally spirochetemic with *B. burgdorferi* but are also asymptomatic.[169, 407, 408, 413] The deer are responsible for the geographic expansion of Lyme-endemic areas because the infected *I. scapularis* adult females overwinter and mate on the deer, and the deer travel widely but are not considered reservoirs for *B. burgdorferi* maintenance in nature. The geographic distribution of North American Lyme disease and *I. scapularis* correlates with that of the white-tailed deer.[408]

Other reservoir-competent small mammal hosts may be involved in the maintenance of *B. burgdorferi* infection in nature[182] in certain geographic areas, or at times in which the population of the usual reservoir host, the white-footed mouse, is low or absent. The deer mouse, *Peromyscus maniculatus*, has been shown to be a competent reservoir host for *I. scapularis* on an offshore island in Maine with no resident *P. leucopus*, and may also be an important alternate reservoir host in the northern forests of Maine.[414] The eastern chipmunk, *Tamias striatus*, is an important reservoir-competent alternate host for immature *I. scapularis*, which can feed on either mice or chipmunks in hardwood forests of the Upper Midwest, including Wisconsin[416] and northwestern Illinois[417]; the meadow vole *Microtus pennsylvanicus* is a secondary, less important, small mammal reservoir host of *I. scapularis* in some areas of eastern North America.[168] A parallel cycle involving the cottontail rabbit, *Sylvilagus floridanus*, *I. scapularis*, and the rabbit tick *Ixodes dentatus*[169, 170] occurs either in areas where the enzootic *I. scapularis*–white footed mouse cycle of maintenance of *B. burgdorferi* infection is inefficient or does not occur, or in areas such as Nantucket Island, Massachusetts, New York,[100] and other parts of the northeastern United States[70] where the *I. scapularis*–mouse cycle occurs but the *I. dentatus*–rabbit cycle functions as an independent complementary cycle.[170] The *I. dentatus*–rabbit cycle is silent with respect to human Lyme disease as *I. dentatus* rarely bites humans; *I. scapularis* rarely feeds on rabbits,[170] but may be important in the spread of *B. burgdorferi* to new geographic areas because immature *I. dentatus* also feeds on birds.[157]

In some parts of North America, *B. burgdorferi* is present in areas that are not endemic for human Lyme disease because *B. burgdorferi* is maintained in nature by enzootic cycles that produce endemic foci that are silent with respect to human transmission of Lyme disease. One such cycle is the *I. spinipalpus–Mexican woodrat* cycle in Colorado; this tick has a broad host range, including rodents, rabbits, and ground-dwelling birds, but humans are rarely bitten because questing ticks are found only in woodrat nests; therefore, this cycle does not contribute to transmission of human Lyme disease.[362]

In the southern United States, the enzootic cycles that maintain *B. burgdorferi* in nature have been less fully described, are more complex and less efficient than those in the North, and result in lower *B. burgdorferi* tick infection rates.[156, 167, 361] The most common reservoir hosts for maintenance of *B. burgdorferi* infection in nature are the cotton mouse, *Peromyscus gossypinus*, and the cotton rat, *Sigmodon hispidus*; however, the life cycle of southern *I. scapularis* is less synchronized, so that nymphal feeding does not always precede larval feeding, thereby reducing the acquisition of infection by feeding larvae.[361] The southern *I. scapularis* is able to feed on 53 species of hosts, including mammals, birds, and reptiles, but the preferred hosts for immature *I. scapularis* are lizards and skinks (which are incompetent hosts incapable of maintaining and amplifying *B. burgdorferi* infection)[336, 348, 418]; the large mammal hosts for adult *I. scapularis* are white- and black-tailed deer, *Odocoileus virginianus* or *hemionus columbianus*. Reptiles exert a zooprophylactic effect on Lyme disease transmission, with a decrease in transmission in areas where reptiles predominate: South of the 38 degrees North latitude boundary, which runs from Virginia through Missouri, reptiles make up over 10% of the total *I. scapularis* hosts available (reptile index is greater than 10), and questing

ticks are significantly diverted away from reservoir-competent amplifying hosts, such as the cotton mouse and the cotton rat, leading to lower tick infection rates.[156, 348, 418] *B. burgdorferi* in Missouri, and probably in Georgia, appears to be maintained in a cottontail rabbit–*I. dentatus* enzootic cycle, and *I. scapularis* and possibly *A. americanum* have been proposed as bridge vectors from rabbits to humans.[153, 361] *I. cookei* has been reported to bite humans in West Virginia, where it is considered a possible human Lyme disease vector; its immature forms feed on small and medium-sized carnivores, and its adults only on medium-sized carnivores.[363] *I. affinis* may enhance enzootic *I. scapularis*–cotton mouse/rat cycles, and *I. minor* may be involved in parallel enzootic cycles with the eastern woodrat, *Neotoma floridana*, or birds; these cycles maintain *B. burgdorferi* infection in nature in Georgia and South Carolina.[361]

Ixodes pacificus,[167, 180, 182, 336, 419, 420] in the far western United States, has a life cycle similar to that of *I. scapularis* but with some differences in hosts, reservoirs, and seasonality of feeding. Although *I. pacificus* is able to feed on a wide variety of hosts, including 80 different species of mammals, birds, and reptiles, its immature stages feed preferentially on lizards,[421] which are not competent *B. burgdorferi* reservoirs and cannot infect feeding ticks[336, 347, 348]; its larval feeding peaks before nymphal feeding,[336, 421] leading to the relatively low tick infection rates reported for adult ticks.[420] The black-tailed deer, *Odocoileus hemionus columbianus*, is the host of the adult tick, which feeds mostly in fall and winter, and to a lesser degree of the immature stages; in one study, all three stages were present simultaneously on deer.[421] *B. burgdorferi* infection is maintained in nature by a parallel enzootic cycle involving the competent reservoir host, the dusky-footed woodrat *Neotoma fuscipes*, and a non–*I. ricinus* complex tick, *I. neotomae* (now *I. spinipalpis*),[378] which rarely bites humans. *I. pacificus* is responsible for human transmission and acts as a bridging vector between the *I. neotomae*–woodrat cycle and man. In 1995, in California, the nymphal tick infection rate was found to be 14%, compared with the adult rate of 4%, and the possibility of a borreliacidal factor in lizard hosts was raised.[173, 180]

There are some differences between the life cycles of European *I. ricinus* and North American *I. scapularis* ticks.[167–169, 182, 336] *I. ricinus* has a 2- to 3-year life cycle (occasionally, 5 to 6 years in far northern latitudes), less coherent seasonal activity, and all three tick stages have feeding activity at the same time, particularly from mid-May to early July[181]; it has a broader host range, which includes 237 to 317 species of mammals, birds, and reptiles. *I. ricinus* abundance correlates with that of deer,[181] but *I. ricinus* occurs in some geographic areas even in the absence of deer because it can use cattle as well as deer as the large mammalian host.[168] The geographic distribution of Lyme borreliosis in Europe correlates with the geographic distribution of *I. ricinus* ticks,[11] particularly the distribution of *B. burgdorferi*–infected ticks,[181, 402] and even more with the distribution of highly infected ticks[422] and of deer,[168, 181] as in North America. The hedgehog *Erinaceus europaeus*–*I. hexagonus* cycle is involved in maintenance of *B. burgdorferi* infection in nature in Europe and Asia, but *I. hexagonus* rarely bites humans and is not considered important in the transmission of human Lyme disease.[182] In some areas, such as an urban park in Magdeburg, Germany, Norway rats, *Rattus norvegicus*, and *I. ricinus* are involved in maintenance of *B. burgdorferi* in nature in a cycle that occurs in addition to the mouse cycle.[423]

I. persulcatus[167, 169, 182] is responsible for human Lyme disease transmission in Asia; it has a similar life cycle to *I. ricinus* but a greater host range, which includes 212 to 241 different species of mammals, birds, and reptiles, although deer, canids, cattle, and hares are particularly important hosts.[344] The life cycle is usually 2 to 3 years, but in extreme northern latitudes it may be 5 to 6 years. The geographic distribution of Lyme disease and the genospecies of *B. burgdorferi* isolated from human Lyme disease patients in China, Japan, and eastern Russia correlate with the geographic distribution of, and genospecies isolated from, *I. persulcatus*.[163, 164, 374] There appear to be two separate enzootic cycles involving larvae and nymphs in Japan—the *I. persulcatus*–rodent cycle involving mainly the woodmouse (and sometimes the vole), and the *I. persulcatus*–bird cycle[163]; adult ticks feed mainly on large animals.

I. holocyclus, the most common tick in Australia, is not competent for *B. burgdorferi*.[392] So far, no competent vector or reservoir host has been identified in Australia. The mammalian hosts of *B. burgdorferi* in the northern hemisphere are all placental animals, and none of these are present in Australia, where the small mammals are mostly marsupial.[375]

A migratory seabird–*I. uriae* enzootic cycle has been described in high-latitude subarctic and subantarctic circumpolar areas, in which the seabirds maintain *B. burgdorferi* (*B. garinii*) infection in nature without the involvement of mammalian hosts.[152, 165, 166] The geographic distributions of *I. uriae* and *I. ricinus* overlap on islands in the Bothnian Gulf at the northern end of the Baltic Sea, and bridging may occur between the two enzootic cycles.[13, 152] It has been proposed that the migratory seabird is the reservoir for *B. burgdorferi* in the southern hemisphere, is responsible for the transhemispheric and global spread of *B. burgdorferi*, and may be important for the spread of Lyme disease to Australia and South Africa.[165]

In addition to *I. uriae*, other ixodid ticks, including the human Lyme disease vectors, *I. ricinus*, *I. scapularis*, *I. pacificus*, and *I. persulcatus*, and the rabbit-feeding ticks, *I. dentatus*, *I. spinipalpus*, and *H. leporispalustris*, are able to feed on birds as alternate hosts in addition to mammalian hosts[157, 163, 167, 182, 362]; therefore, they presumably have an opportunity to be transported by migratory birds to new geographic areas,[425, 426] and also to acquire *B. burgdorferi* from birds that may be reservoir-competent. The potential epidemiologic impact of migratory birds as transporters of infected ticks is great because an estimated 100 million birds migrate into Sweden each spring, carrying 6.8 million new ticks, 1.8 million of which carry *B. burgdorferi*; also, 4.7 million ticks, 1.3 million of which harbor *B. burgdorferi*, are transported out of Sweden toward the South every fall.

Small mammals, including mice and rabbits, and their

ticks may be important in establishment and maintenance of new cryptic *B. burgdorferi* endemic foci in nature by providing reservoir-competent hosts for infected ticks carried to new sites by migratory birds.[170, 171]

Seasonality of Human Tick Bites/ Transmission of *Borrelia burgdorferi* Infection

Humans acquire Lyme borreliosis by being used as the incidental host of a *B. burgdorferi*–infected tick. Table 11–4 shows the seasonality of human tick bites and the time of onset of Lyme borreliosis by geographic region.

In North America, humans are incidental hosts of all stages of *I. scapularis*,[337] and in the Northeast and Upper Midwest, they are usually infected by voracious host-seeking *I. scapularis* nymphs during the spring and early summer (in May and June); the peak incidence of Lyme disease with erythema migrans occurs 1 month later during June and July.[336, 428] In mid-Atlantic states such as Maryland, the onset of most cases of Lyme disease is from May through September.[429] Epidemiologic studies have found that the tick infectivity rate increases from less than 1% of larvae, to 20 to 74% of nymphs, to 57 to 87% of adult ticks.[411] Nymphs are responsible for transmission of almost 90% of cases of Lyme disease.[347] Because the nymphs are so small, and because the tick injects saliva containing anti-inflammatory, analgesic, antihemostatic, and immunosuppressive components while feeding,[168] the bites are not painful and often go unnoticed long enough to allow *B. burgdorferi* transmission, which usually takes 2 to 3 days.[430] However, there are rare European reports of transmission after less than 24 hours[430, 431] and within 2 hours.[9] Human infection is less often caused by adult female *I. scapularis*, which feeds in late fall through early winter (from October through May), with a peak in October, even though *B. burgdorferi* infection rates among adults are higher than for nymphs, because the adults are larger and more easily detected and can be removed before transmission of *B. burgdorferi* infection occurs.[336, 430]

The *I. scapularis* tick takes a long time to feed; during a 5-day feeding period, the female tick ingests 3.5 ml of blood and injects or regurgitates 2.5 ml of fluid secretions into the host.[270] The blood meal triggers multiplication of the *B. burgdorferi* associated with the tick's gastrointestinal tract, which disseminate to the hemolymph by the third day of feeding and then spread to the host either by injection of *B. burgdorferi*–containing tick saliva or by regurgitation of *B. burgdorferi*–containing tick gut contents into the dermal feeding cavity created by the tick.[341, 342] These immunosuppressive salivary secretions and other factors related to the spirochete and its acquisition of host extracellular matrix digestive enzymes[144] result in host-specific immune evasion by the tick, which modifies the tick attachment site so that *B. burgdorferi* deposited in the skin may be in an immunologically privileged site and may be protected against attack by the host immune system.[168]

In the Pacific Northwest, along the Pacific Coast, humans are also incidental hosts of both the adult and immature stages of *I. pacificus*, which is one of the most common ticks biting humans[336, 420]; it is responsible for 59% of human tick bites[180] (66% of bites by adult ticks, and 44% by nymphs). The incidence of *B. burgdorferi* infection in nymphal ticks is much higher than in adult ticks, possibly because of the zooprophylactic effect of the reptile hosts of the immature stages.[180] The peak onset of Lyme disease with EM (March through August) corresponds to the nymphal feeding season (March through September), rather than to the adult tick feeding season (October through June, with peaks in December and March).[180] Because the incidence of *B. burgdorferi* infection of *I. pacificus* is lower than that of the northeastern *I. scapularis*, the rate of human infection following *I. pacificus* bites is also lower.[378, 420]

In Europe, humans are incidental hosts for all stages of the *I. ricinus* tick—which is the most common tick in Europe,[336] the most frequent cause of human tick bites in Central Europe, and the main vector for *B. burgdorferi* transmission to humans in Europe.[181, 336] The feeding activities of the three stages of *I. ricinus* overlap throughout Europe, especially from April through July,[181, 409, 422,

TABLE 11–4
Seasonal Risk of Human Tick Bites and Development of Lyme Borreliosis (LB[a])

GEOGRAPHIC LOCATION	*B. BURGDORFERI* TICK VECTOR	MONTHS OF TICK FEEDING ACTIVITY, BY STAGE			MOST COMMON MONTHS OF ONSET OF LB
		Larvae	Nymphs	Adults	
North America					
Northeast, Atlantic, Midwest	*I. dammini/scapularis*	July–Sept.	May–July[b]	Oct.–May	May–Sept. (peak June–July)
Pacific Northwest	*I. pacificus*	Mar.–Sept.	Mar.–Sept.[b]	Oct.–June (peaks Dec. and Mar.)	Mar.–Aug.
Europe	*I. ricinus*	Mar.–Nov.	Mar.–Nov.	Mar.–Nov.	May–Oct.
Asia	*I. persulcatus*			May–June[c]	May–June

[a]Data from references 23, 180, 243, 251, 336, 344, 347, 358, 371, 428, 434, 460, 463, 464, 466–469, 596, and 637.
[b]Nymphal ticks feeding during this time are responsible for most *B. burgdorferi* transmission to humans.
[c]Adult ticks feeding during this time are responsible for most *B. burgdorferi* transmission to humans.