
THE EMERGENCE AND EPIDEMIOLOGY OF LYME BORRELIOSIS IN EUROPE AND NORTH AMERICA

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Lyme borreliosis, also known as Lyme disease, is an infectious disease caused by the spirochete *Borrelia burgdorferi*. Several genospecies are included within *B. burgdorferi* sensu lato (“in a broad sense”). In North America all the well-characterized isolates of *Borrelia* obtained from patients with Lyme borreliosis are *B. burgdorferi* sensu stricto (“in a strict sense”). At least two other genospecies not present in the United States, *B. afzelii* and *B. garinii*, are additional proven agents of Lyme borreliosis in Europe, causing more than 70% of European-acquired infections (Wang et al., 1999). Probably owing to differences in tissue tropism, there are variations in the spectrum of clinical manifestations of the disease between genospecies, resulting also in some differences in clinical presentation between the continents. There are also some differences in serologic immune responses, which has implications for diagnostic testing (Chapter 10) and for vaccine development.

There is evidence, based on multilocus sequence typing of bacterial housekeeping genes, that *B. burgdorferi* existed in North America thousands of years before its discovery, and that it originated in Europe (Qiu et al., 2008, Hoen et al., 2009, Margos et al., 2009, Margos et al., 2008). The organism probably was carried across the Atlantic Ocean to the northeast by migratory birds, and then spread westward (Comstedt et al., 2006; Ginsberg et al., 2005).

HISTORICAL OVERVIEW

Lyme disease appeared in contemporary medical textbooks in the early 1980s, but most manifestations of the infection were known in Europe in the latter part of the nineteenth century and the early twentieth century. Undoubtedly the modern North American description spearheaded efforts to identify the etiology, leading to identification of the causative spirochete in 1983. It also led, in stages, to retroactive correlations of the “old” European manifestations. The story of that ground-breaking description has by now been told repeatedly, often with much flourish, and an engaging account directed at a broad audience was published recently (Edlow, 2004). For more detailed accounts of the discoveries pertaining to this spirochetal syndrome in Europe, see the well-researched chapters by Klaus Weber and by Willy Burgdorfer, in Weber’s *Aspects of Lyme Borreliosis* (Weber and Burgdorfer, 1993). Brief historical perspectives on various manifestations of the disease are presented in individual chapters in this book. A brief recounting of the chronology and seminal events that gave us the complete clinical picture is presented here.

Acrodermatitis Chronica Atrophicans

In 1883 the German physician Buchwald presented the case of a 36-year-old man with a “diffuse idiopathic skin atrophy” of 16 years duration (Buchwald, 1883). This is now recognized as the first description of acrodermatitis chronica atrophicans (ACA), a late manifestation of Lyme borreliosis. Herxheimer and Hartmann introduced the term “acrodermatitis chronica atrophicans” in 1902, with a review of about 12 cases published in the interim, and 12 of their own cases. (Herxheimer and Hartmann, 1902) A few years later 202 patients with “atrophy cutis” were reviewed; two-thirds of those likely had ACA (Weber and Pfister, 1993). Patients with arthralgia, bone atrophy, arthropathy, and synovial thickening were described during the next few decades (Weber and Burgdorfer, 1993). Other dermatologists in Germany noted that the “pallida reaction” (*Treponema pallidum*) was positive in several ACA patients’ serum.

An infectious etiology was proposed in 1946 by Svarts, who described improvement of ACA on penicillin (Svarts, 1946). At the University of Munich, between 1954 and 1955, dermatologist Hans Götz transplanted affected skin from an ACA patient into himself and three other physician volunteers (Götz, 1954; 1955). He documented ACA-like chronic inflammation that lasted up to 312 days that resolved with penicillin but speculated that a “large virus” was the cause.

In 1949 Thyresson reported on the partial or complete success of penicillin therapy in 40 of 57 patients with ACA (Thyresson, 1949). Finally, Asbrink confirmed the common spirochetal etiology of acrodermatitis chronica atrophicans and erythema migrans in 1984 after spirochetes were isolated on culture from an ACA lesion (Asbrink et al., 1984). In the United States ACA had been seen as far back as 1895, although it appears to have occurred mostly in immigrants from Europe (Sweitzer and Laymon, 1935).

Erythema (Chronicum) Migrans

An “erythema migrans” rash occurring after a tick bite was first mentioned at a meeting of the Swedish Dermatological Society by Swedish physician Arvid Afzelius in 1909 as a

ring-like expanding lesion with central clearing (Afzelius, 1910). It was described in more detail, as “erythema chronicum migrans” (ECM), by the Austrian dermatologist Lipschütz and by Riehl in 1913 (Lipschütz, 1913). The relationship between tick bite and erythema migrans was further specified by Afzelius in 1921 (Afzelius, 1921). Lipschütz in 1923 had astutely suggested that the tick intestinal tract and salivary glands be studied microscopically to look for bacteria as the cause of ECM, but this had to wait until Burgdorfer’s discovery, 59 years later, in the United States (Lipschütz, 1923). In fact the Swedish dermatologist Sven Hellerström is credited with the first formal proposal of a tick-borne spirochetal etiology of ECM in 1930 (Scrimenti and Scrimenti, 1993; Hellerstrom, 1930). Meanwhile, in a report entitled *Spirochetes in Aetiologically Obscure Diseases* (1948), the dermatologist Carl Lennhoff performed mercury chloride staining of biopsies in a variety of skin diseases, including erythema migrans. In retrospect, his visualization of spirochetes in so many different disorders was deemed nonspecific (Burgdorfer, 1993).

Better evidence of an infectious cause came from the success in clearing ECM with penicillin. (Hellerstrom, 1951; Hollstrom, 1951) The experiments of Binder et al. in 1955 in Würzburg yielded the first persuasive evidence of the infectious etiology of ECM (Binder et al., 1955). They reproduced ECM in three volunteers by transplanting skin from the edge of a patient with the typical rash, and re-transmitting it between volunteers. The incubation period was one to three weeks, and penicillin treatment successfully cleared the rash in each case.

The first recorded case of ECM in North America was diagnosed in 1969, by Rudolph Scrimenti, a physician in Wisconsin. He published the histopathology, consisting of lymphocytic and plasma cell periadenexal infiltrate, and treated the patient successfully with penicillin, based on his knowledge of the European disease and the description by Hellerström (Scrimenti, 1970; Scrimenti and Scrimenti, 1993). His patient also had symptoms of radiculitis, headache, and fever, and he associated the extremely large ring-like rash with the bite of what Scrimenti generically called a wood tick. Then in 1975 Mast and Burrows described a cluster of four cases in southeastern Connecticut (Mast and Burrows, 1976). (An earlier case series, published in 1962, described erythema migrans in American servicemen, but they had become infected while stationed in the Air Force base in Wiesbaden, Germany; Flanagan, 1962).

Weber summarized the knowledge obtained by 1986, stating that the term “erythema migrans” (used by Afzelius originally) should be the generally used designation because it is “shorter, self-explanatory, and older” and is more accurate, and the rash is not prolonged more than a few weeks in most cases (Weber, 1986). Erythema migrans (EM) has become the generally accepted term.

Borrelial Lymphocytoma

In 1911 the Swiss pathologist Burckhardt described a patient with a solitary cutaneous pseudolymphoma, which could have been the first documented example of a borrelial lymphocytoma (Burckhardt, 1911). However, “pseudolymphomas” described by Spiegler and Fendt in 1894 and 1900 could also have been borrelial lymphocytoma (see Chapter 7). The designation “lymphocytoma” was first used by Kaufmann-Wolf in 1921 and Biberstein in 1923 (Kaufmann-Wolf, 1921; Biberstein, 1923). These terms included both borrelial and nonborrelial types of benign hyperplasias of the skin. When it became clear through clinical observations and positive serological tests that a certain kind of benign cutaneous

hyperplasia was of borrelial origin, the designation borrelial lymphocytoma was introduced for this cutaneous sign of Lyme borreliosis (Weber et al., 1985). Another term, “lymphadenosis benigna cutis,” had been coined by Bäfverstedt from Sweden in 1943, when he presented a large series of patients with benign cutaneous hyperplasia associated in a few cases with erythema migrans (Bäfverstedt, 1943).

Paschoud in 1954 may have made the initial association of borrelial lymphocytoma with neuroborreliosis in describing a large plaque that followed a tick bite in a patient with meningoradiculitis (Paschoud, 1954). He also performed a transmission experiment in Lausanne wherein the recipients developed borrelial lymphocytoma in about six weeks (Paschoud, 1957).

Neuroborreliosis

In 1922, the French physicians, Charles Garin and A. Bujadoux reported on a case of facial palsy and meningoradiculoneuritis after a tick bite (Garin and Bujadoux, 1922). The Swedish dermatologist Hellerström read a paper to the Southern Medical Association in Cincinnati in 1929, describing meningitis associated with ECM Afzelius in European patients (Hellerstrom, 1930, 1951). In 1941 in Munich, the German neurologist Alfred Bannwarth presented three groups of patients who had either intensely painful meningoradiculoneuritis, facial palsy (four of six were children), or “chronic lymphocytic meningitis with cerebral symptoms” (Bannwarth, 1941). All had a lymphocytic pleocytosis, albeit much less prominent in the first group. Headache, vomiting, and neck stiffness were prominent only in the third group. It is interesting that papilledema, abducens paralysis, and prolonged CNS inflammation were noted in one patient each in the last group, complications that have been described recently in Lyme neuroborreliosis, notably in children (Dayan et al., 2004; Rothermel et al., 2001). Thus Bannwarth provided a description of the natural course of what we now call early neurological manifestations of Lyme borreliosis, or neuroborreliosis. In 1949 and 1962, Georg Schaltenbrand made an important postulation regarding the association of CNS manifestations and tick bites, and recorded improvement after treatment with corticosteroids and tetracycline (Schaltenbrand, 1949; 1962). Hellerström’s patient treated with penicillin in 1951 had ECM and meningitis.

Note that the following additional designations have been used in Europe: “paralysie par les tiques” (Garin and Bujadoux, 1922), “meningitis after erythema migrans and tick bite” (Hellerström 1930), “chronische lymphozytäre meningitis” (Bannwarth, 1941), “meningomyeloradiculitis after tick bite with erythema” (Bammer and Schenk, 1965), “peripheral neuropathy in ACA” (Hopf, 1966), “meningopolyneuritis Garin-Bujadoux-Bannwarth” (Horstrup and Ackermann, 1973), “erythema chronicum migrans meningitis” (Weber, 1974), “Bannwarth’s syndrome” (Weber and Burgdorfer, 1993), “progressive borrelien-enzephalomyelitis” (Ackermann et al., 1985), “cerebral vasculitis” (Weber and Burgdorfer, 1993), “neuroborreliosis” (Stiernstedt et al., 1988), “chronic peripheral neuropathy” (Kristoferitsch, 1993), and “Lyme neuroborreliosis” (Dotevall et al., 1988; Pfister et al., 1991). These terms span the spectrum of the neurological manifestations of Lyme borreliosis.

Setting the Stage for the Unified Etiology of Diverse Clinical Presentations

In Würzburg, Germany, W. Hauser concluded that the three cutaneous manifestations were related to each other and to the distribution of *I. ricinus* ticks (Hauser, 1965). Hopf described

92 patients that established the relationship between ACA and a chronic neuropathy, as well as a few cases of concomitant arthritis, facial palsy, and other neurologic symptoms that he thought were coincidental (Hopf, 1966).

In 1976 Weber systematically ruled out other possible causes in a case of meningitis that followed erythema migrans and responded to intravenous penicillin. He proposed a bacterial species, unlikely to be *Borrelia*, as a possible etiologic agent, given the knowledge that relapsing fever borreliae were transmitted by soft (argasid) and not hard (ixodid) ticks (Weber, 1974). Thus the connection between cutaneous and neurological manifestations was recognized in Europe, but the knowledge remained mainly restricted to the fields of dermatology and neurology.

Lyme Arthritis

In 1975 phone calls by members of the public to the Connecticut Health Department and the CDC led to a retrospective investigation into a remarkable cluster of new arthritis cases. Two very perceptive mothers, Polly Murray and Judith Mensch, were concerned that the majority of cases were children diagnosed as juvenile rheumatoid arthritis (JRA), often in the same family, dating at least back to 1972 (Steere et al., 1986). Their insistence on an investigation was heeded by David Snyderman at the health department, who invited Allen Steere, a rheumatology fellow at Yale University, Stephen Malawista, the Chief of Rheumatology, and their colleagues to jointly conduct an epidemiologic investigation. Snyderman began by plotting all the reported cases on a map of the towns of Lyme and Old Lyme (Edlow, 2004). All patients with arthritis were examined and a distinct geographic and seasonal (summer–fall) pattern was noted, which was inconsistent with JRA and instead pointed to an infectious etiology.

Their findings were published in stages, leading up to the complete picture of this emerging infection that closely clustered in and around the towns of Lyme, Old Lyme, and East Haddam in southeastern Connecticut. Of 39 children and 12 adults, 13 had a history of an expanding red rash that resembled European ECM descriptions. As arthritis had never been formally linked to ECM, Steere and co-investigators considered the arthritis to be a novel disease, and named it after the town of Lyme, where the first case was thought to have occurred (Steere et al., 1977). (The term “Lyme arthritis” first appeared in the literature in letters to JAMA in 1976 when the physicians referred to the ongoing investigation at Yale, before the seminal 1977 report was published; Mast and Burrows, 1976; Hazard et al., 1976). Epidemiologic evidence suggested an arthropod vector, and the investigators suspected transmission of a viral agent. Extensive viral testing yielded no putative agent. Through their continued observations on old and new patients, they were able to tie in the skin lesion with migratory joint pains, monoarticular or oligoarticular arthritis, neurologic abnormalities, myocardial conduction abnormalities, nonspecific systemic symptoms, and nonspecific serum inflammatory markers in 32 patients with onset in 1976, whom they followed prospectively (Steere et al., 1977). They specifically proposed the ixodid tick *I. scapularis* as the vector because some patients with ECM and Lyme arthritis reported tick bites (one identified as *I. scapularis*), and tick survey results were consistent with a dramatically raised risk of acquiring Lyme disease in communities on one bank of the river (Steere et al., 1978).

Further data on the incidence of cases and the distribution of *Ixodes* ticks expanded our understanding of the epidemiology of Lyme borreliosis, with recognition of distinct foci along the northeastern coast, in Wisconsin, and on the west coast in California and Oregon

(Steere and Malawista, 1979). Other locations on the continent, notably Minnesota and Ontario, were added as endemic areas for the “tick-borne dermatitis-encephalitis-arthritis syndrome” that was now firmly established in medical nomenclature as Lyme disease (Schrock, 1982, 1981; Steere and Malawista, 1979). Based on the prior European experience and anecdotal American experience, Steere and colleagues conducted an open-label randomized trial of three antibiotics for ECM. They demonstrated shortened duration of rashes as well as a decreased rate of later stage manifestations compared to untreated controls (Steere et al., 1980).

Discovery of the Spirochetal Etiology of Lyme Borreliosis

In 1981 Willy Burgdorfer of the NIH Rocky Mountain Laboratories in Montana, who was collaborating with Jorge Benach at Stony Brook, was dissecting *Ixodes* ticks collected on Shelter Island, New York, off the east end of Long Island, partly in pursuit of an additional vector of *R. rickettsii*, when he made a discovery. Burgdorfer himself called it serendipitous, although his prior expertise in tick microscopy and knowledge of historical observations undoubtedly fostered his discovery (Burgdorfer, 1993a,b).

As a graduate student in Switzerland, Burgdorfer had dissected thousands of *Ornithodoros* ticks, looking for infection with *B. duttoni*, the spirochetal agent of relapsing fever in Africa. He had moved to the Montana laboratory in 1951. In 1978, a few months after speaking with Steere about methods to identify pathogens in ticks, he spent a sabbatical in Switzerland, where he discovered certain deer parasite microfilariae in *I. ricinus*, the European sheep tick. The hemolymph from Benach’s Shelter Island ticks was negative for rickettsiae, but he noticed large microfilariae-like organisms. In order to determine how the microfilariae in ticks had entered the hemolymph, he dissected the midgut, but they were negative. Instead, he noted faintly stained spirochete-like structures. They were better visualized on Giemsa stains and on wet preparations by dark field microscopy.

Burgdorfer recalled the European literature and Hellerström’s presentation on ECM to the Southern Medical Association in Cincinnati, and decided to follow his hunch. The spirochetes reacted positively with sera from Lyme disease patients in indirect immunofluorescence tests. Burgdorfer stained some slides that he had brought back from the Swiss Plateau and found morphologically and antigenically similar organisms in smears from *Ixodes ricinus* ticks, and subsequently in the guts of a few *Ixodes pacificus* from the western United States (Burgdorfer, 1984; Burgdorfer et al., 1985). Alan Barbour, his colleague at the Rocky Mountain Laboratories, successfully cultured the spirochete in modified Kelly’s medium in November 1981 (Burgdorfer et al., 1982). One clone, the original “Shelter Island isolate,” was given the designation B31 and continues to be a standard lab research strain (ATCC 35210; Barbour, 1984). The investigators were able to reproduce erythema migrans lesions in rabbits by allowing infected *I. dammini* to feed on them. The lesions were mostly at distant sites from the tick attachment site, indicating a bacteremic spread.

In twin papers published in 1983, Steere et al. and Benach et al. established the bacterial cause of human Lyme disease with their isolation of the “*I. dammini* spirochete” from the blood, ECM skin lesions, or CSF of five patients from Connecticut and Long Island (Steere et al., 1983; Benach et al., 1983). The organism was also linked to European ECM in a paper published the same month, following studies on live ticks from Europe supplied to Burgdorfer by André Aeschlimann, his Swiss collaborator from his 1978 sabbatical (Burgdorfer et al., 1983). With these studies the etiology of Lyme disease was established beyond doubt, and in 1984 the borrelial species was named for Willy Burgdorfer (Johnson

RC, 1984). Culture evidence of *B. burgdorferi* sensu lato in ACA from Asbrink followed in 1984 and from Hovmark in borrelial lymphocytoma in 1986 (Asbrink et al., 1984; Hovmark et al., 1986). The unified etiology and clinical picture of Lyme borreliosis on either side of the Atlantic were finally established.

Because the global distribution of this disease was now recognized, the First International Symposium on Lyme disease was organized at Yale University where scientists and physicians from Europe and North America shared their experience (Schmid, 1984). In that symposium, and at a dermatology meeting in Germany, one of the authors (KW) and coworkers announced their finding of elevated antibody titers against *B. burgdorferi* in 100% of ACA and borrelial lymphocytoma patients and in some patients with erythema migrans. Steere's group as well as other European investigators reported similar findings in their patients with erythema migrans or Lyme arthritis. This was followed by similar reports in patients with meningoradiculoneuritis. Today Lyme borreliosis is regarded as the most important human tick-borne illness in the Northern Hemisphere, and experts in numerous disciplines continue to elucidate the biology of an organism that has probably been with us for millennia (Marshall et al., 1994; Dennis, 2002).

Meticulous observations—at times using human inoculation experiments that would be unthinkable today—allowed investigators over a 100-year period to describe and begin to treat this spirochetal infection, without the benefit of modern microbiologic tools. This fascinating, multifaceted, and bicontinental story is valuable to all students and practitioners of science, most important because it proves the Pasteurian principle of how chance favors the prepared mind.

EPIDEMIOLOGY OF LYME BORRELIOSIS IN NORTH AMERICA

Lyme borreliosis in North America occurs in the temperate climate zone. Although the North American vectors, black-legged ticks of the genus *Ixodes*, are widely distributed, only a few regions are considered endemic for Lyme disease. Lyme borreliosis has been called a disease of place (Brown et al., 2005). The incidence of reported cases ranges from 0 in several states to 111.2/100,000 in Delaware (CDC, 2011). The highest incidence states, all in the northeastern and northern midwestern regions, had an average annual rate of 29.2 cases per 100,000 population in the three-year period 2003 to 2005 and for 1992 to 2006 accounting for more than 90% of cases on the continent (Bacon et al., 2008). From 2005 to 2009, incidence areas leveled off or declined in several high incidence states, but rose sharply in Delaware and in New England (CDC, 2011). During 2003 to 2005, 64,382 Lyme disease cases were reported to the CDC, for an average of over 20,000 cases/year, and this number was unchanged for 2006 (Figure 1.1). A doubling of the reported cases from 1992 to 2006 is ascribed to multiple reasons: enhanced surveillance, true increase in infections, increased diagnosis, misdiagnosis, and reporting errors (Bacon et al., 2008). In 2009, there were 29,959 confirmed and 8509 probable cases, for an incidence of 13.4 confirmed cases per 100,000 population. Underreporting to the extent of 10 to 12-fold has been shown in some studies. One estimate of the actual number is 60 to 100,000 per year, which would result in overall national case rates of about 20–30/100,000 (Steere, 2006; CDC, 2007).

Further the prevalence is focal within most endemic regions, correlating with local ecologic factors (see below). Even within townships and counties, the prevalence can vary

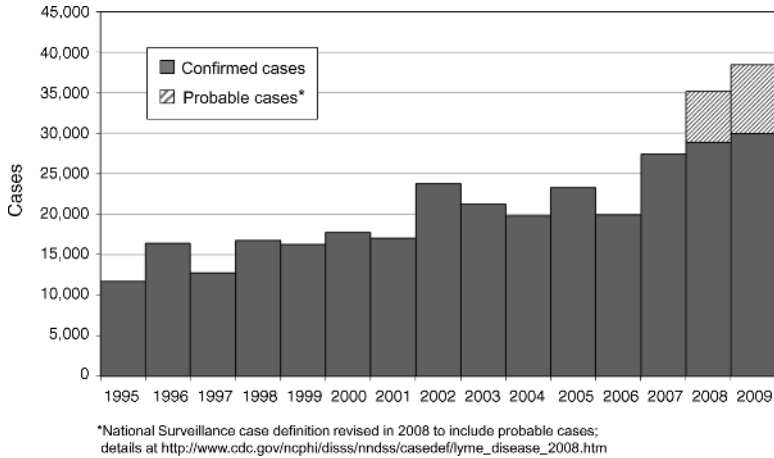
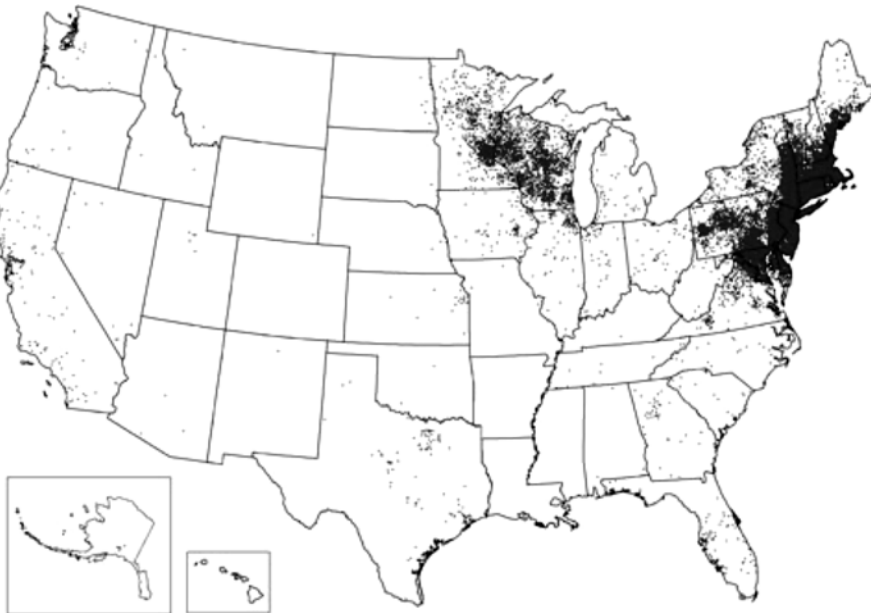


Figure 1.1 Reported cases of Lyme disease by year, United States, 1991–2009. Source: CDC. http://www.cdc.gov/ncidod/dvbid/lyme/ld_upclimblymedis.htm.

greatly. In fact 90% of cases in 1999 occurred in 109 counties, out of 3143 counties in the United States (CDC, 2001). During 2002 to 2006 the annual rate per 100,000 ranged from 219 to 962 in the 10 highest rate counties (Bacon et al., 2008). A dot plot map of reported cases is generated by the CDC (Figure 1.2). It is useful to look at this map when considering



1 dot placed randomly within county of residence for each confirmed case

Figure 1.2 Reported cases of Lyme disease, United States, 2009. Source: CDC. http://www.cdc.gov/ncidod/dvbid/lyme/ld_incidence.htm.

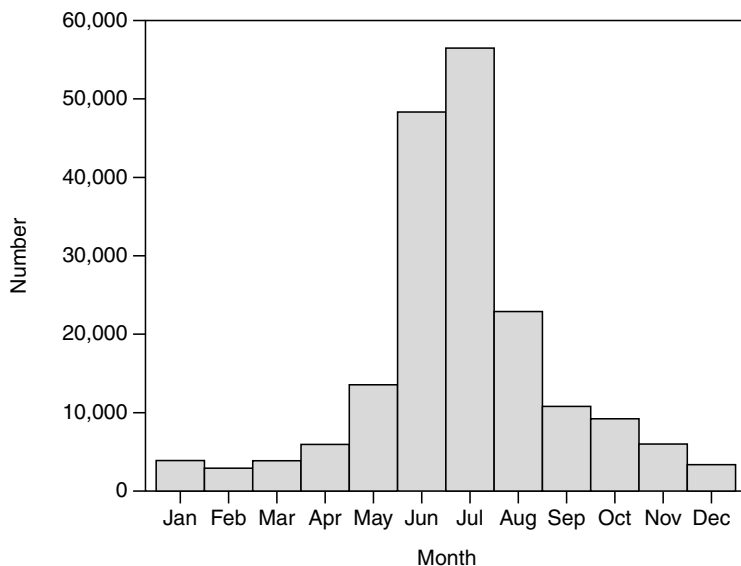


Figure 1.3 Reported cases of Lyme disease by month of illness onset United States, 1992–2006. Source: Bacon et al., 2008.

the diagnosis of Lyme borreliosis, as the epidemiologic setting is key to predictive values of both symptoms and laboratory tests (CDC, 2007). In addition to this overview map, physicians should consult a county-by-county map (see Figure 10.4, Chapter 10). The infection is uncommon in Canadian provinces, and very rare instances in Mexico have been reported in the literature (see below).

Human Factors

During 1992 to 2006 about 70% of cases occurred from June to August, consistent with the months during which nymph and adult *Ixodes* ticks are actively seeking hosts, and people are exposed more often because of recreational and occupational activities (Bacon et al., 2008) (Figure 1.3). A bimodal age distribution of Lyme borreliosis has been established in United States surveillance (Figure 1.4). The first peak (5–9 years) can be explained by outdoor activity habits of the post-toddler-years age group. The second peak starts after the fifth decade of life. The intervening trough probably reflects the relative preoccupation with study and employment in the third and fourth decades. There is a slight male predominance (53%), and an unexplained disproportionate increase in the males aged 5 to 19 years occurred from 1992 to 2006 (Bacon et al., 2008).

Tick Vectors and Environmental Aspects

The maintenance of *B. burgdorferi* in nature in North America is sustained by the multi-host life-cycle of *I. scapularis* (Figure 1.5). The reason that Lyme borreliosis is highly endemic in the northeastern and north-central regions is that high populations of white-tailed deer and rodents, particularly white-footed mice and chipmunks, sustain

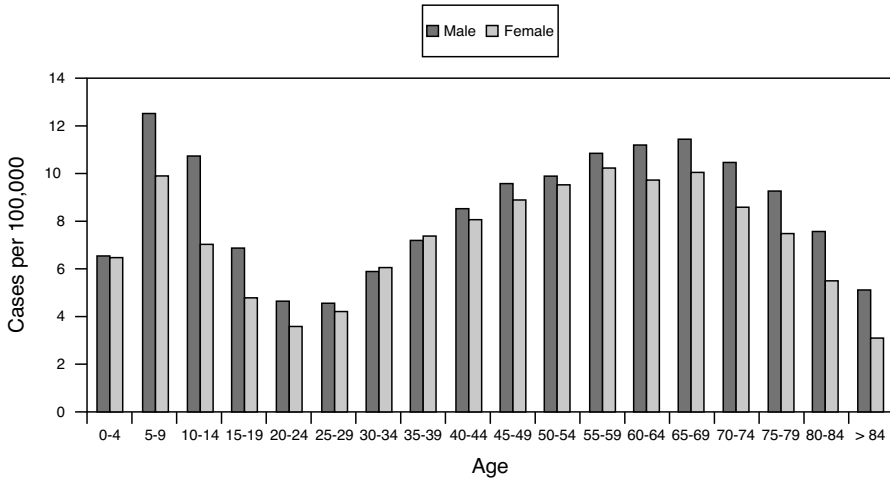


Figure 1.4 Average annual incidence of reported cases of Lyme disease by age group and sex, United States, 1992–2004. Source: CDC. http://cdc.gov/ncidod/dvbid/lyme/ld_MeanAnnualIncidence.htm.

transmission of the spirochete in nature, resulting in a higher infection rate in the deer tick variety of *Ixodes* (LoGiudice et al., 2003) (see Chapter 2). The rodents harbor the spirochete, serving as a ready reservoir that allows for tick infection, while deer appear to serve primarily as a mating ground. In contrast, the most important host for *I. pacificus* ticks in the western United States and British Columbia is the western fence lizard (*Sceloporus occidentalis*), which is not a competent reservoir of *B. burgdorferi*

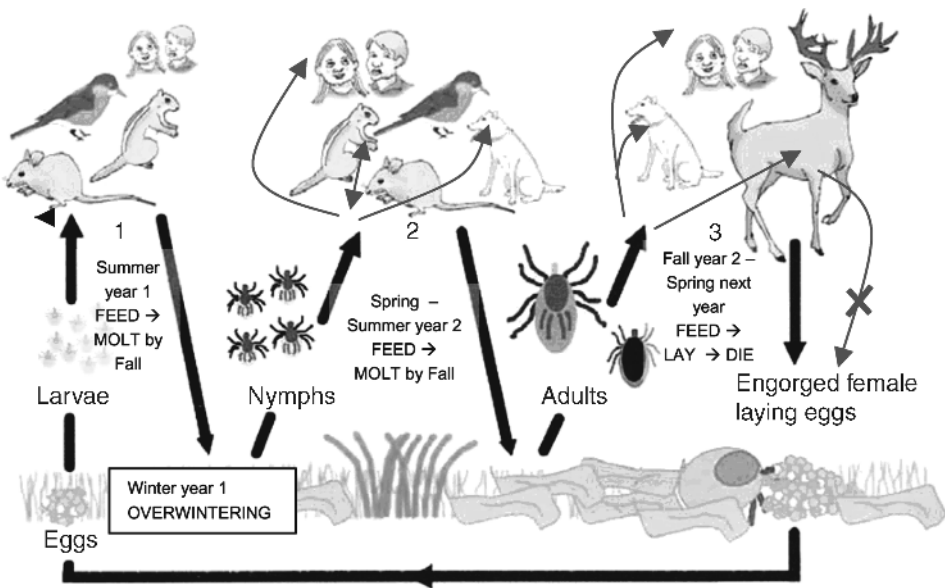


Figure 1.5 Life cycle of the blacklegged tick or “deer” tick, *Ixodes scapularis*. 1, 2, 3 = Feeding on new host. During feeding, infection is transmitted as shown by direction of red arrow. X = transmission blocked. Source: Modified from Kirby Stafford. (See insert for color representation).

(Brown et al., 2005). Its blood is borreliacidal, so the role of lizards is termed zooprophylactic, that is lowering the efficiency of transmission to other hosts (Lane and Quistad, 1998). The enzootic cycle of infection is maintained because *I. pacificus* can also feed on western grey squirrels (*Sciurus griseus*) which were recently identified as competent reservoir hosts for *B. burgdorferi*. It is also maintained by interactions with secondary hosts such as woodrats, and another tick, *I. spinipalpis* (Eisen, 2004). The zooprophylactic effect of lizards may also be a factor in explaining the lack of endemic Lyme borreliosis in the southern United States.

In the northeast and north-central endemic regions the distribution of cases mirrors the presence of *Ixodes* ticks, competent reservoir rodents, and white-tailed deer in the same environment. The two major ecological factors that make for a prime habitat for *Ixodes* are high humidity and frequenting by small rodents and deer. Wooded areas or brush near water (coastal or inland, especially along rivers and estuaries), as well as unmaintained areas around gardens and domestic properties, especially where there is a lot of leaf litter, harbor such conditions. Rodents can carry ticks into areas from which deer can be kept out, giving rise to risk to small children and pets even if they do not play in woods or brush, the so-called peridomestic or periresidential risk (Dennis, 1998). Seropositivity rates among dogs have in fact been used to predict the distribution of *B. burgdorferi* (Lindenmayer et al., 1991). In the Pacific coast endemic areas the ecological components are oak and bay tree woodlands where *I. pacificus* ticks feed mainly on lizards (Lane and Loye, 1991).

It is important to understand the distinction between endemicity of Lyme disease and presence of enzootic *B. burgdorferi*. The latter refers to established maintenance of the spirochete in animal reservoirs but does not equate with spread to humans, as environmental conditions may not be conducive to tick-borne transmission to people. For example, it is likely that borreliae do not survive long enough in the mid-gut of *Ixodes* ticks in the warmer southern climate of the United States to threaten transmission at the ticks' subsequent feeds. So, although *B. burgdorferi* is enzootic contiguous to Lyme borreliosis endemic areas (chiefly along the Atlantic coast south to Florida, and in areas of Minnesota, Illinois, and northern Michigan), cases of human Lyme borreliosis occur only sporadically in these enzootic areas. Given the frequent reporting of cases from "nonendemic" states and counties, it is important for clinicians to carefully consider whether suitable ecologic and zoonotic factors are present locally when considering the diagnosis in a patient with compatible symptoms and signs. Of course, presence of the right conditions can presage the emergence of human cases in an area.

A classic illustration of such a diagnostic dilemma is seen in the well-documented cases of tick-bite associated erythematous rash illnesses in Missouri that were initially labeled erythema migrans (Masters and Donnell, 1995). Investigative evidence to date indicates that erythema migrans-like rash illnesses in the southern United States are not caused by *B. burgdorferi* (Wormser et al., 2005; Philipp et al., 2006). It is postulated that an organism provisionally named *B. lonestari* may be transmitted by *Amblyomma americanum*, the lone-star tick, which is highly prevalent across the southern United States (Varela et al., 2004). PCR evidence of *B. lonestari* or a closely related bacterium has been demonstrated in a single case of erythema migrans-like rash lesion in a patient who was probably exposed in Maryland and in an *A. americanum* tick that was found to be attached at the center of the rash (Dennis, 2005). It is also conceivable that one or more other, as yet undiscovered, tick- or insect-borne pathogens are the etiology of erythema migrans-like rashes.

US Clinical Case Definitions

The CDC developed and has recently revised the case definition for national reporting of Lyme disease under the National Notifiable Diseases Surveillance System (CDC, 2008, 2009). It is unequivocally stated that this definition is for surveillance and is not intended for use in clinical diagnosis. The definition encompasses erythema migrans, and three late manifestations—musculoskeletal system, nervous system, and cardiovascular system Lyme disease. Laboratory evidence (a positive culture, positive two-tier testing interpreted using established criteria, or positive IgG immunoblot interpreted using established criteria) is an essential criterion for the case definition, with the exception of erythema migrans after a known exposure. Cases reported with laboratory evidence alone are considered *Suspected*. Approximately two-thirds of cases reported have erythema migrans, a third have arthritis, about 12% have neurologic symptoms, and fewer than 1% have second- or third-degree atrioventricular block. During 1992 to 2006 of the reported cases 13% had more than one clinical manifestation (Bacon et al., 2008). This spectrum of clinical manifestations based on passive reporting differs from that found in a prospective study of newly diagnosed Lyme disease in children in Connecticut (Gerber et al., 1996). Only 6% presented with arthritis. Cases of arthritis are more likely to be reported because serologic confirmation is available, whereas there is an underreporting of erythema migrans due to a failure of physicians to report an established and treatable disease.

Lyme Borrelia—An Emerging Infection

Lyme borrelia remains an emerging disease, designated by the Institute of Medicine as one of the most rapidly emerging in the United States. There is a small body of evidence for its gradual geographic spread. After its initial identification in one county in Connecticut, cases were identified in all counties by 1985 (Petersen et al., 1989). Increases in incidence and of cumulative prevalence of human cases have been documented in longitudinal studies on Fire Island, NY, and Great Island, MA (Handrahan et al., 1984; Steere et al., 1986). In three high-incidence states there was a documented increase in the number of counties reporting Lyme disease between 1992 and 2006 (Bacon et al., 2008). Several factors are at play in the emergence of Lyme borrelia in North America. The distribution of the tick vector *Ixodes scapularis* advanced inland up major river valleys from the coast in Maine and New York State, resulting in more counties being counted as endemic in these states as well (Rand et al., 2007; White et al., 1991). It has been proposed that the propensity of *B. burgdorferi* to be transmitted among several mammalian species has allowed Lyme borrelia to spread rapidly in the northeast (Hanincova et al., 2006). Focal outbreaks can rapidly emerge, chiefly attributable to proliferation of deer in the local area (Lastavica et al., 1989; Steere et al., 1986). Deer are important feeding hosts for the adult (reproductive) stage of *I. scapularis*. In fact the emergence and increase of Lyme borrelia in the United States is primarily due to ecological conditions that have allowed increases in deer populations as colonial farmlands gave way to forested areas during the past few decades (Steere, 1994). Presently the US northeast, despite having some of the largest metropolitan areas in the country, is forested for about 60% of its area, with approximately 80% forest cover in New England and Maine (Mac et al., 1998). The outward expansion of suburban developments into wooded areas, and the trend toward building new homes in rural settings in parts of the north and northeast are also major factors in the increased number of human cases (Dennis, 2002).

It appears more than coincidental that the main endemic areas on three continents are situated along the terminal moraine of glaciers that retreated after the last ice age, since emergence of new forests in these locations was conducive to increases in white-tailed deer populations (Steere et al., 2004; Mac et al., 1998).

It is important that primary care clinicians continue to report new cases to local health authorities so that accurate statistics can be maintained. These can be used to plan environmental measures and future immunization strategies.

Canada

Lyme borreliosis is rare in Canada (Anon., 1991, 2006). Both tick vectors of *B. burgdorferi* are present in the country (*I. scapularis* in eastern and central Canada and *I. pacificus* in British Columbia). Range expansions for *I. scapularis* have been observed in parts of southern Manitoba, eastern Ontario, and Nova Scotia in recent years. Ongoing surveillance efforts include preparation of risk maps for potential expansion of the range of *I. scapularis* in eastern and central Canada. Current and projected climate change models have been used to make predictions because data on rising mean temperatures in northern climes are biologically consistent with increases in vector populations (Ogden et al., 2006, 2008a,b). However, data are relatively sparse, and systematic sampling and ecological risk modeling will be key to developing more accurate predictive risk maps. Most reported cases are from Ontario, but reporting from Quebec, the other large eastern province, is considered incomplete. The infection rate of *I. pacificus* ticks in British Columbia is very low, and only two human cases of Lyme borreliosis were reported from British Columbia in 2006. A National Lyme Disease Meeting was held in March 2006 under the auspices of the Public Health Agency of Canada (Anon, 2006). Data sources included the Canadian Institute for Health Information, a hospital morbidity database, Statistics Canada's Morbidity and Mortality Database, and cases reported by the provinces to the Public Health Agency's Notifiable Disease Reporting System (NDRS). Of the 345 cases reported to the NDRS between 1994 and 2004, 193 (56%) were related to travel outside Canada. As in the United States the age distribution is bimodal (data from 1992–2002) with peaks in children 5 to 15 years and adults 50 to 60 years.

Mexico

Borrelial lymphocytoma and erythema migrans were reported from Mexico in four patients who had never traveled outside the country (Gordillo-Perez et al., 2007). In two patients with clinical features of borrelial lymphocytoma, the histopathological findings, although not pathognomonic, were consistent with those seen in European borrelial lymphocytoma. Sera from both lymphocytoma patients and from one erythema migrans patients were positive for antibodies to *B. burgdorferi* by immunoblot, and skin biopsies from all four patients were positive by PCR for *B. burgdorferi* DNA, using primers for the *B. burgdorferi* sensu lato flagellin (*fla*) gene. All lesions disappeared after antibiotic treatment. A report of seropositivity (only 9 of almost 3000 samples tested) is much less convincing (Gordillo et al., 1999). Sera were from individuals from all states of Mexico, without any indication that a history of tick exposure or of illness compatible with Lyme borreliosis was elicited. Only three bands were required for a positive by immunoblot, which is not standard in the United States. Nevertheless, and despite the

lack of culture evidence of *B. burgdorferi* to date (Johnson, 2008), it is possible that rare cases of human *B. burgdorferi* infections occur in Mexico.

Congenital Lyme Disease: Lack of Evidence from Epidemiological Studies

Case reports of suspected congenital infection with *B. burgdorferi*, all in the 1980s, were based upon “morphologically compatible” spirochetes on histology at autopsy, including brain and cardiac tissue, without accompanying evidence of inflammation (Weber et al., 1988; Schlesinger et al., 1985). Specific immunohistochemical stains for *B. burgdorferi* were not used in these cases, and microscopic visualization and silver staining are prone to false-positives. Large epidemiologic investigations, one of them a study of 2000 pregnancies, have yielded no association between the mothers’ prenatal or in-pregnancy exposure and fetal death, prematurity, or congenital malformations (Strobino et al., 1993; Gerber and Zalneraitis, 1994; Strobino et al., 1999). The authors of a recent comprehensive literature review concluded that despite evidence that *B. burgdorferi* can cross the placenta, a teratogenic effect or congenital Lyme borreliosis in infants has not been found. There is a theoretical potential for fetal loss, based on animal studies, but recommendations for the treatment of Lyme borreliosis or of tick bites in pregnancy are not modified, with the exception of avoidance of tetracyclines (Elliott et al., 2001).

EPIDEMIOLOGY OF LYME BORRELIOSIS IN EUROPE

In Europe, as in the United States, Lyme borreliosis is the most common arthropod-borne infection of human beings (Anon, 2004). European Lyme borreliosis is similar in many respects to American-acquired infection, but there are some significant variations in clinical presentations and epidemiology, related to the greater diversity of borrelial genospecies found in Europe and to environmental factors (Stanek and Strle, 2003; Stanek et al., 2011, in press).

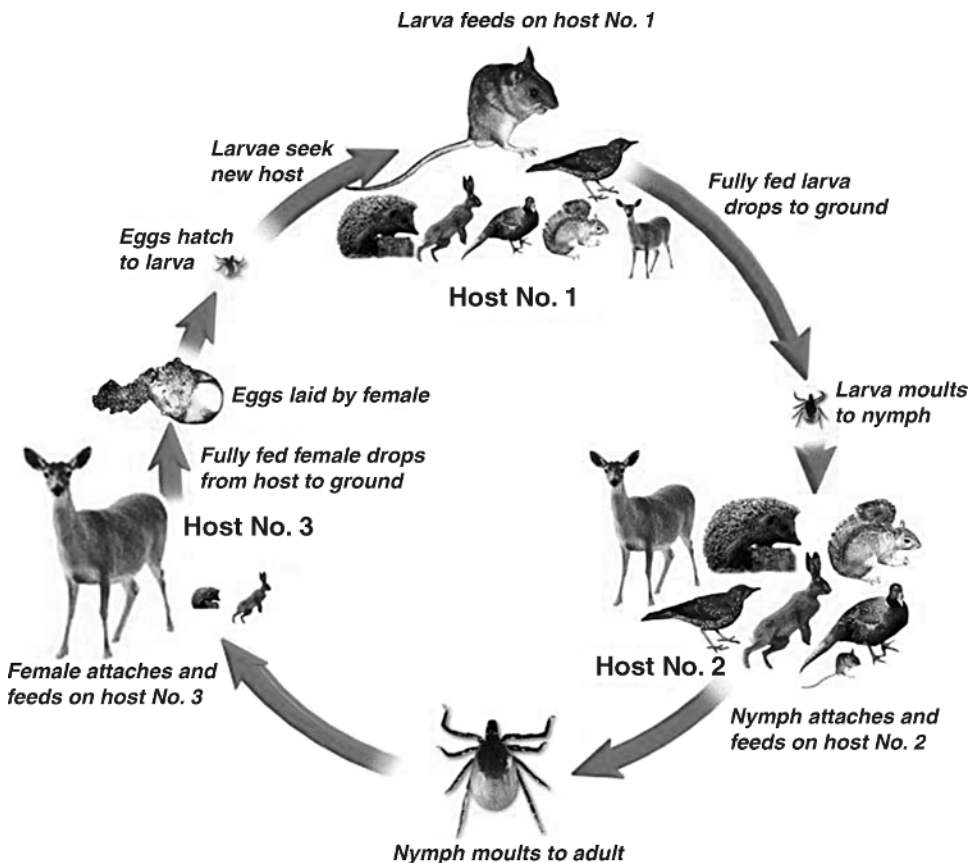
Tick Vectors and Environmental Aspects

The main European vectors of *Borrelia burgdorferi* are *Ixodes ricinus* and *Ixodes persulcatus*. *Ixodes ricinus*, the sheep tick or castor-bean tick, is widely distributed throughout Europe, extending south from Scandinavia to Mediterranean countries, and from the western edge of the continent to Russia. In Russia and the Baltic republics the eastern range of *I. ricinus* overlaps with that of *I. persulcatus*, the taiga tick, which is widely distributed throughout temperate Asia to the Far East (Gern and Humair, 2002).

The vector ticks occur in areas of deciduous woodlands and mixed forests throughout Europe. They require high humidity levels and are protected from desiccation in leaf litter. They can also be found in heathlands and pasturelands of regions that have mild, damp climates, such as the British Isles, but arid areas of southern Europe are not suitable habitats (Gray et al., 1998). Altitude is also a limiting factor for tick survival, with fewer ticks found above 1000 meters, although several recent studies have shown that their ranges are gradually extending into higher altitudes and higher latitudes, which may be related to climate change (Daniel et al., 2005; Lindgren and Jaenson, 2006; Gern et al., 2008).

The potential for human Lyme borreliosis acquisition is more focal, as it depends on interactions of numerous factors, including the presence of ticks, borreliosis reservoir-competent hosts, and human activities within the tick habitats, particularly at times of peak tick feeding activity in spring, early summer, and autumn. An optimal habitat for Lyme borreliosis acquisition is mixed deciduous woodland with a rich variety of the small mammals and birds that are potential borreliosis reservoir hosts, and larger mammals—particularly deer—which are feeding hosts for adult ticks and thus help maintain tick populations at their reproductive stage (Gray et al., 1998). Expansions of deer populations and ranges have contributed significantly to increased tick abundance in many parts of Europe. There is greater variety of borreliosis reservoir hosts in Europe than in North America, with many species of small- and medium-sized mammals and birds involved as reservoir hosts, depending on the *B. burgdorferi* genospecies (Gern and Humair, 2002; EUCALB, 2011) (Figure 1.6).

Changes in land utilization, including forestry practice, influence the suitability of habitats for Lyme borreliosis endemicity. In many parts of Europe reinstatement of



The relative size of the animals approximates their significance as hosts for the different tick life cycle stages in a typical woodland habitat.

Figure 1.6 Life cycle of *Ixodes* in Europe, to illustrate diversity of hosts. Source: Jeremy Gray, University of Dublin.

deciduous and mixed woodland in areas previously used as pine forest monoculture is likely to increase tick population densities (Anon, 2004). Many other changes in agricultural practices, land management, and residential housing can also increase or decrease tick populations, borrelial reservoir hosts, and human access, thus affecting Lyme borreliosis risk (Linard et al., 2007). Local risk assessments can be helpful in indicating need for disease awareness and for reinforcing the importance of primary prevention measures, particularly tick awareness and the early removal of attached ticks (Linard et al., 2007; EUCALB, 2011).

Borrelial Genospecies

Several pathogenic genospecies of *Borrelia burgdorferi* are present in Europe, and there are some differences in their geographic distribution. There are also variations in clinical presentations associated with the different genospecies, which are related to organotropisms and other factors. The major pathogenic genospecies are *B. garinii* and the closely related *B. bavariensis* (formerly *B. garinii* serotype 4), *B. afzelii* and *B. burgdorferi* sensu stricto. *Borrelia garinii* and *B. afzelii* are widely distributed in Europe and Eurasia. *Borrelia burgdorferi* sensu stricto has been found more focally, and can cause disease presentations similar to those seen in North America. It does not appear to be present in *I. persulcatus* ticks.

All four major pathogenic genospecies can cause erythema migrans and meningitis. *Borrelia garinii* is particularly neurotropic and is strongly associated with radiculopathic and encephalomyelitic presentations (Rupprecht et al., 2008). *Borrelia afzelii* can also cause neurological complications but is less frequently identified in CSF than *B. garinii*. It can cause the late skin manifestation, acrodermatitis chronica atrophicans (ACA), and patients with this condition may also have vasculitic peripheral neuropathy (Wang et al., 1999; Kristoferitsch, 1993). Borrelial lymphocytoma, an uncommon early skin manifestation, is caused predominantly by *B. afzelii* (Maraspin et al., 2002). Lyme arthritis is associated with all three genospecies, but mainly with *B. burgdorferi* sensu stricto and *B. garinii* (Lunemann et al., 2001). There have been a few case reports associating other genospecies, including *B. spielmanii*, *B. valaisiana*, and *B. lusitaniae*, with significant clinical manifestations, mainly erythema migrans. The most convincing evidence so far for pathogenicity in these genospecies has come from the isolation of *B. spielmanii* from a few patients with erythema migrans (Fingerle et al., 2008).

There are relatively high prevalences of *B. valaisiana* in tick populations in some of the most westerly countries of Europe, including Ireland and the United Kingdom (Kirstein et al., 1997). The significant presence in tick populations of this essentially nonpathogenic organism may be a contributing factor to the lower prevalence of clinically significant reported disease in these countries than in regions where pathogenic genospecies are predominant. Similarly *B. lusitaniae* is the most prevalent genospecies identified in ticks in Portugal, a very low disease-prevalence country, where fewer than 40 serologically confirmed cases of Lyme borreliosis were reported annually between 2000 and 2004 (0.4/100,000), following the introduction of mandatory reporting in 1999 (Lopes de Carvalho and Nuncio, 2006).

There are also intraspecies variations that may correlate with some variations in disease presentations and severity, and also with reservoir host competency. For example, most *B. garinii* serotypes are associated with avian reservoirs, particularly ground-feeding birds



Figure 1.7 Yellow-necked field mouse (*Apodemus flavicollis*). Source: Jiri Bohdal.

such as blackbirds and pheasants, but *B. bavariensis* (formerly *B. garinii* serotype 4) is maintained in nature by *Apodemus* mice (Huegli et al., 2002) (Figure 1.7).

Human Factors

Risk of Lyme borreliosis can result from people's residential, occupational, or recreational activities, and in some cases it can be difficult to assign infection acquisition definitively to a single risk category. Many people living in Lyme disease endemic areas also take part in outdoor leisure activities there, away from their immediate home environments. Some are also employed in jobs such as forestry work or deer management that place them at significantly higher risk of tick bites than other local residents. In many high prevalence areas throughout Europe, traditional woodland activities such as wild berry-picking and mushroom gathering remain very popular, and for some people, especially in eastern countries, they may be important sources of food and income. Other activities such as professional or recreational deer-hunting also increase risk of infection acquisition. Housing developments in previously rural Lyme disease-endemic areas also expose new populations to risk of infection

A population-based prospective study of nearly 5000 people in southeastern Bavaria in 1996 and 1997 showed an incidence of Lyme disease of 7.1/1000 patient observation years (33 cases), and asymptomatic infection occurring in 7.6/1000 patient observation years (35 seroconversions, Reimer et al., 2002). Tick bites were reported by 1072 participants. The overall seroprevalence at the start of the study was 11%, which rose with increasing age and outdoor activity or employment. Residential risk is significant, especially for people who have gardens backing on to woodlands, but residential risk alone seems less marked in Europe than in the United States (Linard et al., 2007; Fitzner et al., 2002).

Recreational risk both at home and abroad seems to be very significant, and has risen in the past 15 years, correlating with the rising popularity of outdoor activities and holidays such as hiking, mountain-biking, and wildlife observation. There have been major developments in activity holiday travel to many regions within the expanded European Union and neighboring countries that have areas of high endemicity for Lyme borreliosis. The most popular times for outdoor recreation and vacations coincide with peak periods for tick feeding activities.

Epidemiological data, reviewed below, indicate that in most European countries there is a bimodal age effect for disease acquisition, with peaks occurring in the 5 to 15 and 40 to 60 age groups. Peak periods for the diagnosis of erythema migrans are late spring and summer, following the peak tick feeding periods. Acute neuroborreliosis cases peak 1 to 2 months later but may be diagnosed at any time through the year.

The age at which a person acquires infection may have some bearing on the clinical features of disseminated disease. The most common complication of Lyme borreliosis reported in children is facial palsy. Painful radiculopathy is more commonly diagnosed in older adults and is rare in children. Acrodermatitis chronica atrophicans is principally seen in older people and is reported more frequently in women than in men (Stanek and Strle, 2003).

European Clinical Case Definitions

European clinical case definitions, initially published in 1996 and updated in 2010, were developed as part of the EUCALB (European Union Concerted Action on Lyme Borreliosis) initiative <http://meduni09.edis.at/eucalb/cms/index.php?lang=en> (Stanek et al., 2010). They encompass the more common clinical presentations occurring in Europe, and have been helpful both in clinical practice and toward the standardization of epidemiological data (Stanek et al., 2010). Guidelines for the diagnosis and treatment of neuroborreliosis in Europe were published by the European Federation of Neurological Societies in 2010 (Mygland et al., 2009). Several other evidence-based diagnostic and treatment guidelines are also available from national authorities or specialist groups in Europe (HPA, 2011).

European Epidemiological Data

There is no standardized or centralized method of collecting epidemiological data on Lyme borreliosis in Europe (Hubálek, 2009), and there are currently no plans to add Lyme borreliosis to the listed diseases covered the coordinated disease surveillance by the European Community (Smith and Takkinen, 2006). The European Centre for Disease Control has commissioned an epidemiologic situation analysis, reporting in 2011 (<http://www.ecdc.europa.eu/en/Pages/home.aspx>). Epidemiological evidence is obtained piecemeal from numerous sources, including national or regional mandatory notification schemes in a few countries, surveillance schemes in some endemic regions, primary care surveys, seroprevalence studies, and reporting systems based on laboratory-confirmed cases. Some examples are given below and in Table 1.1.

Overall national figures have only limited value, especially in the larger, more industrialized countries where most of the population is urban dwelling, as they do not indicate regional and subregional variations in risk, which can be very marked. Regional and local

TABLE 1.1 Reported incidence of Lyme borreliosis/100,000 population in selected European countries

| Country (Approx pop 10 ⁶) | 2001 | 2005 | 2008 | Other Data | Comments |
|---------------------------------------|----------|-----------|-----------|---|--|
| Portugal (10) (MN) | 0.03 | 0.04 | ND | | See text and Lopes de Carvalho and Nuncio (2006) |
| Spain (40) | ND | ND | ND | 9.8 in La Rioja (Lindgren and Jaenson, 2006) | Focal areas mainly in northern Spain |
| Ireland (3.8) | ND | ND | ND | 1997 seroprevalence: park rangers: 3.4%; gen pop: 0.6% (Robertson et al., 1998) | Focal areas include Connemara, West Cork and Kerry |
| England and Wales (53.3) (LC) | 0.5 | 1.1 | 1.72 (LD) | 17% of confirmed cases acquired abroad | Estimated 15 in one focal area in 2007 (HPA, 2011) |
| Scotland (5.1) (MN) | 0.6 | 1.7 | 5.5 (LD) | 285 cases in 2008 (LD) | High prevalences in parts of highlands (HPA, 2011) |
| France (60) | ND | ND | ND | 1999–2000: 9.4 national PC survey (LeTrilliart et al., 2005) 2001–2003: 180–232 | Information on other endemic areas also available from InVS |
| Belgium (10.3) | 9.7 | 16 | ND | Alsace (prospective) (InVS, 2008) <3.65 – >31.78 in municipalities (Linard et al., 2007) | Additional data available in Linard et al. (2007) |
| Netherlands (16) | 74 (est) | 103 (est) | ND | 43 (est) in 1995 (De Mik et al., 1997); 103 (est) in 2005 (Hofhuis et al., 2006) | PC estimates for EM |
| Germany (82) | ND | ND | ND | 60,000 cases/year estimated in 2002 (Mehmert and Krause, 2005) | See text and Reimer et al. (2002), Fitzner et al. (2002), Smith and Takkinen, (2006), Rath et al. (1996), Huppertz et al. (1999) |
| Switzerland (7.2) | ND | ND | ND | 30.4 (Lindgren and Jaenson, 2006) | 95 in Neuchatel canton 1996–1997 (Nahimana et al., 2000) |

(continued)

TABLE 1.1 (Continued)

| Country (Approx pop 10 ⁶) | 2001 | 2005 | 2008 | Other Data | Comments |
|---|------|------|-------|---|--|
| Italy (57.5) | 0.02 | ND | ND | | |
| Denmark (5.3) (MN of NB) | ND | 1.7- | 1.1 | 1994-2004: 471 NB cases (EpiNorth, 2010) | 82 NB cases in 2004 (Christiansen and Molbak, 2005) |
| Norway (4.5) (MN of late stage disease) | 2.7 | 6.2 | 7.2 | Marked increase in NB cases in 2004; reasons unclear (Nygard et al., 2005) | Prevalent in southern and central coastal counties (Nygard et al., 2005) |
| Sweden (8.8) | ND | ND | ND | 1992-3: 69 in southern Sweden (Berglund et al., 1995) 1997-1998: 464 EM in PC (Bennet et al., 2006) | Prospective study 1992-1993 (Berglund et al., 1995). Retrospective case search 1997-2003 (Bennet et al., 2006) |
| Finland (5.2) (LC) | 13 | 24 | 24.1 | 1999 est. 200 in southern archipelago (Junttila et al., 1999) | Seroprevalence in Aland islands adults 19.7% in 1996 (Carlsson et al., 1998) |
| Lithuania (3.7) (LC) | 33 | 34 | 106.6 | | |
| Latvia (2.4) (LC + C) | 16 | 21 | 31.8 | | |
| Estonia (1.4) (LC) | 25 | 21 | 133.3 | | |
| Poland (38.6) (MN) | 6.4 | 12 | 21.6 | > 11.55 nationally in 2005 (Stefanoff et al., 2006) | Highest risk around Finnish Gulf coast 100 focally in northeastern region (Flisiak and Prokopowicz, 1999) |
| Czech Republic (10.3) (MN) | 35 | 36 | — | | |
| Slovakia (5.4) | 13 | 16 | — | 2001 seroprevalence: gen pop: 5.4%; risk pop.: 16.8% (Stefancikova et al., 2001) | |
| Hungary (9.9) | 13 | 12 | — | | |

| | | | | | |
|-------------------|-----|-----------|---|--|---------------------------------------|
| Austria (8.1) | ND | 135 (est) | — | 1997 seroprevalence: gen pop: 7.7% (Santino et al., 1997) | National data from physician estimate |
| Slovenia (2) (MN) | 163 | 206 | — | | See text |
| Croatia (4.7) | ND | ND | — | 3317 cases reported 1987–2003 (Mulic et al., 2006) | Northwest has highest incidence |
| Greece (10.6) | ND | ND | — | 0.27% seroprevalence in naval recruits (Stamouli et al., 2000) | |
| Romania (22.4) | ND | ND | — | 1999 seroprevalence: gen pop: 4–8%; risk pop. 9.3–31.7% (Hristea et al., 2001) | |
| Bulgaria (7.9) | 4.5 | 13 | — | | |

Note: Data for 2001 and 2005 from Eurosurveillance (Smith and Takkinen, 2006), for 2008 from EpiNorth (EpiNorth, 2010) and local reports; additional data from other sources. Approx pop 10⁶: approximate population in millions (Source: *Times Atlas of the World Mini Edition*, 2003, Times Books, Ltd.)

Abbreviations

Est: estimated

Gen pop: general population

LC: laboratory-confirmed cases

LC + C: laboratory confirmed cases and clinical cases

LD: local data

MN: mandatory notification

NB: neuroborreliosis

ND: no data given in reference.

PC: primary care

Risk pop: risk population (forestry workers, etc.)

data analysis is important for the appropriate targeting of public health and clinical interventions.

About 85,000 cases are reported annually in Europe, but this is a considerable underestimate, both because of inconsistent case reporting mechanisms and underrecognition of disease manifestations, particularly erythema migrans (Lindgren and Jaenson, 2006). In 2002 it was estimated that at least 60,000 cases are likely to occur annually in Germany alone, giving an approximate incidence rate of 75/100,000 in that country (Mehnert and Krause, 2005).

Mandatory Reporting Schemes

Erythema migrans and other manifestations of the disease are mandatorily reportable nationally in Slovenia. Data for 2005 indicated an incidence rate of 206/100,000 (Smith and Takkinen, 2006). Despite the high degree of local awareness of Lyme borreliosis, it is recognized that notifications there are incomplete, especially with regard to erythema migrans (Strle, 1999). Slovenian data related to disseminated and late complications are likely to be more accurate because most patients with these presentations are managed within a few research-orientated medical institutions.

Neuroborreliosis has been notifiable in Denmark since 1994. Case notifications ranged from 41 in 2002 to 104 in 2006; the annual average is 83 (1.5/100,000) (Christiansen and Molbak, 2005). Cases of disseminated and late borreliosis have been notifiable in Norway since 1995 (Nygard et al., 2005). Annual incidence of neuroborreliosis varied from 75 to 200 cases in the 10 years 1995 to 2004 (average 3/100,000). There was a marked increase of nearly 100 cases between 2003 and 2004. As neurological complications are the most significant manifestations of disseminated and late Lyme borreliosis in Europe, data on neuroborreliosis obtained from the Slovenian, Danish, and Norwegian notification schemes give useful information on epidemiological trends in widely geographically separated areas of Europe.

Regional Clinical Surveillance and Prospective Studies

In some other countries case surveillance is regionally focused on areas of known high endemicity, such as Alsace and Limousin in France and in six eastern states of Germany (Mehnert and Krause, 2005, 2008; InVS, 2010). A French national primary care-based prospective study estimated an overall national incidence rate of 9.4/100,000 (Letrillart et al., 2005), whereas data from the Alsace study suggested a regional rate of 180-232/100,000, which varied from 30 to 511/100,000 between individual cantons in the region. Erythema migrans was the only manifestation of disease in 90% of the cases; a further 5% had evidence of neuroborreliosis. Similar detailed and useful study reports are available for several other regions of France from L'Institut Veille Sanitaire (<http://www.invs.sante.fr/surveillance/lyme/index.htm>).

The extended notification scheme in the six eastern German states (Berlin, Brandenburg, Mecklenburg-Vorpommern, Sachsen, Sachsen-Anholt, and Thuringen) reported 3019 cases (17.8/100,000) in 2002 and 3968 (23.3/100,000) in 2003 (Mehnert and Krause, 2005). Most cases were erythema migrans (89.3% and 86.7%), with neuroborreliosis accounting for less than 4% of cases in either year. Two states, Brandenburg and Sachsen, accounted for 81% of the case reports. The incidence in Brandenburg was 74/100,000 in

2003, and it ranged from 10 to 237/100,000 in districts within the state. A prevalence study of forestry workers in Brandenburg performed in 1992 had shown a seroprevalence of 8% (Rath et al., 1996). Another German prospective study, which was performed in the Wurzburg region in 1996 following an extensive awareness campaign, reported an incidence rate of 111/100,000 (313 cases). Erythema migrans was the only manifestation in 89% of cases (Huppertz et al., 1999).

A primary care-based prospective study was performed in southern Sweden in 1992 to 1993 (Berglund et al., 1995). The overall annual incidence was 69/100,000 (1471 cases) and ranged focally from 26 to 160/100,000. Erythema migrans was the presenting feature in 77%; 16% had neuroborreliosis and 7% had arthritis. A later primary care-based retrospective study in southeastern Sweden estimated a mean annual incidence rate for erythema migrans of 464/100,000 between 1997 and 2003 (Bennet et al., 2006).

In addition to their epidemiological value, prospective community-based studies can provide longer term benefits to study populations, as they raise awareness of the condition, its clinical features, management, and prevention within primary and secondary care health care providers and the general community.

Laboratory-Based Surveillance

The great majority of specialized diagnostic tests for Lyme borreliosis in England and Wales are performed in a single laboratory, enabling development of an enhanced surveillance system based on detailed clinical and epidemiological information from laboratory-confirmed cases. Annual incidence of laboratory-confirmed cases rose from 268 (0.5/100,000) in 2001 to 973 (1.79/100,000) in 2009 (HPA, 2011). Erythema migrans cases are certainly underreported. Neuroborreliosis is the most common complication (10–20% of reported cases annually). Between 15% and 20% of infections annually were acquired abroad, mainly through recreational activities in mainland Europe or the United States. Most patients acquiring infection abroad are UK residents, but migrants from other European countries, including Poland, Hungary, and the Baltic republics, have featured significantly in this subgroup in recent years, following changes in employment opportunities in the enlarged European Union. About 70% of indigenously acquired cases occurred in southern counties and were associated mainly with recreational or residential risks. In the southwest, which includes several well-known endemic areas, the overall annual rate for 2007 was 4.7/100,000 and was estimated at 15/100,000 in one focal area. Few occupationally acquired symptomatic infections are identified annually. A small prevalence study of forestry workers showed an overall seroprevalence of 25% by two-tier testing, which increased with tick exposure risk and duration of employment, but no study participant had current disease and only 10% had Lyme disease-related symptoms in the past (Guy et al., 1989). A national study of agricultural workers showed a seroprevalence of only 0.2% (Thomas et al., 1999).

Some other countries also use laboratory-based surveillance, mainly as an adjunct to clinically-based studies or notification schemes (Mehnert and Krause, 2005). Variability in test requesting patterns and diagnostic methods limit the validity of direct comparisons of laboratory-based surveillance findings between countries. Nevertheless, some useful demographic, geographic, and seasonality data may be obtained, and referring clinicians and patients are approached for additional clinical and tick exposure risk information in some schemes.

Summary

Some general conclusions can be drawn from the available data, despite the difficulties posed by lack of a standardized European reporting system. It is also possible to assess trends from year to year in countries or regions that have stable reporting systems, be they based on mandatory notification, regional clinical reporting, or laboratory surveillance. Data on neuroborreliosis incidence could be particularly valuable as a sentinel for monitoring trends and for informing public health activities, as neurological manifestations are the most common and potentially most serious complications of European-acquired Lyme disease, and are likely to have a high degree of diagnostic specificity.

Lyme borreliosis is by far the most common vector-borne infection in Europe, with a widespread distribution, correlating overall with the distribution of *Ixodes ricinus* and *Ixodes persulcatus* ticks. Disease incidence increases from the west to the east of the continent, and decreases from south to north in Scandinavia and from north to south in Italy, Spain, Portugal, and Greece. The highest prevalence regions are included in the area extending eastward from a longitude of about 5° east and southward between latitudes 62° and 42° north, but there focal areas of high prevalence elsewhere. It is interesting that the early descriptions of various clinical presentations of Lyme borreliosis originated from physicians working in the areas of Europe where recent ecological, epidemiological, and clinical studies have indicated high infection prevalence.

Reported incidence has increased very significantly throughout Europe in recent years for several reasons. Public health efforts and media attention have led to far greater awareness of the disease, leading to diagnoses of cases that might otherwise have been unrecognized. There has also been a significant genuine increase in incidence, related to expansion in density and range of tick populations and increased recreational and residential human activities in tick habitats.

Asymptomatic or minimally symptomatic infections are common in many endemic areas. Seroprevalence in high-risk occupational groups is significant in many parts of Europe, and increases with age and years of exposure risk, but incidence of occupationally acquired clinical disease seems to be low.

***Borrelia burgdorferi*, A VERSATILE ZONOTIC BACTERIUM**

Borrelia burgdorferi is a eubacterial species (phylum Spirochaetes) that uses a variety of small mammals as its reservoir, and thus is a vector-borne, accidental, zoonotic infection of humans. A review of the voluminous literature on the biology of the organism is beyond the scope of this book. The reader is referred to the books *Lyme Borreliosis: Biology, Epidemiology and Control* and *Borrelia: Molecular Biology, Host Interaction and Pathogenesis* (Gray et al., 2002; Samuels and Radolf, 2010). Among many others, scientists at the Rocky Mountain laboratories of the NIH in Hamilton, Montana, continue to build on the knowledge spawned by isolation of the organism at their institute in 1981. The following brief summary is based in part on a current review by members of this group (Tilly et al., 2008).

Published genomes of the three major *Borrelia burgdorferi* genospecies reveal that all have a linear chromosome and several linear or circular plasmids (Fraser et al., 1997; Casjens, 2000; Casjens et al., 2000). The linear structure of the chromosome and plasmids is

unusual in bacteria, which raises the question of whether it confers an evolutionary advantage for *Borrelia* species. It is clear that in several species certain plasmid-encoded genes are essential for infectivity or persistence in different hosts. In contrast with other disease-causing eubacteria, it has been difficult to define specific virulence factors in this species, leading to the concept of *B. burgdorferi* as an obligate parasite rather than a primary pathogen of mammals. A relatively small genome also distinguishes this spirochete from most free-living bacteria. *Borrelia burgdorferi* lacks many metabolic synthesis pathways and presumably derives essential nutrients and cofactors from the host. This also necessitates the use of highly enriched media for culture in the laboratory, most often containing rabbit serum (see Chapter 9).

Surface lipoproteins are perhaps the best fit for virulence factors in *B. burgdorferi*, as antibody responses to these are predictably present in disease. Certain lipoproteins, notably outer surface protein A (OspA), have a role in protecting against re-infection. The bacterium regulates the differential expression of these outer-surface proteins to cycle between its mammalian and tick hosts because it must adapt to different temperature and pH conditions in each host's internal environment. For example OspA is expressed while in the gut of the unfed tick, but OspC expression is detectable only after the tick has attached to a vertebrate host. Later during the course of mammalian infection the spirochete expresses other putative virulence factors, including antigenic variants from the variable major protein-like sequence expression locus (VlsE), which may help it evade the host immune system. Transcription of genes for differential lipoprotein expression such as OspC has been shown to be regulated by the rpoS and rpoN encoded alternative sigma factors, which modulate gene expression in response to certain environmental stresses. Studies done on plasmid retention and clonal variation of *B. burgdorferi* strain B31 passaged in mice showed that loss of two linear plasmids in particular, lp25 and lp28-1, abrogates the ability of the organism to invade multiple tissue sites. This indicates an essential role in virulence for their best-characterized gene products, nicotinamidase and VlsE proteins (Purser and Norris, 2000).

Several animal models have yielded valuable information on the pathogenesis of *B. burgdorferi* infection from either needle-injection or tick bite infection studies (Philipp and Johnson, 1994). Erythema migrans, bacteremia, and meningitis has been investigated in the rabbit model. Mice have been used to study facets of the immune response, protection from OspA vaccine, persistence of infection, carditis, and arthritis (Steere et al., 2005). Hamsters were an early model to study disseminated infection and arthritis. Dogs develop arthritis and CNS infection and have yielded data on response to antibiotic treatment. The rhesus macaque has been valuable in studying the course of neurologic infection, treatment studies, and development of diagnostic assays.

Mammalian toll-like receptor 2 [TLR2] is key to innate immune recognition of *B. burgdorferi* lipoprotein antigens components by phagocytic and antigen-presenting cells, which serve to limit the load of invading spirochetes. *Borrelia burgdorferi* genospecies also vary in their susceptibility to complement-mediated killing. Whereas *B. garinii* is highly susceptible, *B. afzelii* and *B. burgdorferi* ss express surface proteins, notably Erp proteins (Stevenson et al., 2002). These proteins confer resistance to complement-mediated killing by binding with host "H factors" that coat the bacterial surface. An argument has been made that complement factors play a role in the global ecology of Lyme borreliosis, being a key determinant of host specificity for particular genospecies (Kurtenbach et al., 1998, 2006). Neutralizing antibodies also mediate mammalian immunity, whereas transfer of T cells in a mouse model does not confer protection against

Borrelia. Neither the innate or adaptive immune responses alone are always sufficient to clear *Borrelia* infection. The concept of bacterial persistence in tissue sanctuaries, postulated to occur even after treated infection, has been cause for extensive investigation, but it is debatable whether interaction with extracellular matrix protects the bacteria from neutralizing antibodies (Cabello et al., 2007; Coburn et al., 2005). Recent findings contradict the concept that decorin and fibronectin binding proteins have a significant role in interaction with host extracellular matrix (Shi et al., 2006; Tilly et al., 2008). In tissue culture, spirochetes have been visualized inside a variety of cell types, but intracellular persistence of organisms has not been demonstrable in infected tissue from Lyme borreliosis patients or in animal models (Duray, 1987; Barthold et al., 1993; Steere et al., 2005).

The study of animal and human pathogenesis of *B. burgdorferi* infections is a burgeoning and vibrant area of research that spans genomics, immunology, molecular microbiology, entomology, veterinary biology, and field biology. *Borrelia* and tick researchers share and develop their research at a variety of scientific forums worldwide, and they have not only contributed greatly to better understanding of human infection but have also advanced the field of tick-borne diseases. Moreover elucidating the fundamental processes employed by the organism to infect and survive in such varied hosts has implications for other disease processes. Just one example is how the study of Lyme arthritis can provide clues to possible infectious triggers of other forms of arthritis.

The chapters that follow explain the biologic basis for the current approaches to diagnosis and management of Lyme borreliosis.

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