Lyme borreliosis

Gerold Stanek, Gary P Wormser, Jeremy Gray, Franc Strle

Lyme borreliosis (Lyme disease) is caused by spirochaetes of the *Borrelia burgdorferi* sensu lato species complex, which are transmitted by ticks. The most common clinical manifestation is erythema migrans, which eventually resolves, even without antibiotic treatment. However, the infecting pathogen can spread to other tissues and organs, causing more severe manifestations that can involve a patient's skin, nervous system, joints, or heart. The incidence of this disease is increasing in many countries. Laboratory evidence of infection, mainly serology, is essential for diagnosis, except in the case of typical erythema migrans. Diagnosed cases are usually treated with antibiotics for 2–4 weeks and most patients make an uneventful recovery. No convincing evidence exists to support the use of antibiotics for longer than 4 weeks, or for the persistence of spirochaetes in adequately treated patients. Prevention is mainly accomplished by protecting against tick bites. There is no vaccine available for human beings.

Published **Online** September 7, 2011 DOI:10.1016/S0140-6736(11)60103-7

Medical University of Vienna, Institute for Hygiene and Applied Immunology, Vienna, Austria (Prof G Stanek MD); **New York Medical College, Valhalla, NY, USA** (Prof G P Wormser MD); **University College Dublin, Dublin, Ireland** (Prof J Gray PhD); **and University Medical Centre Ljubljana, Ljubljana, Slovenia** (Prof F Strle MD)

Correspondence to: Prof Gerold Stanek, Medical University of Vienna, Institute for Hygiene and Applied Immunology, Kinderspitalgasse 15, 1095 Vienna, Austria **gerold.stanek@meduniwien.ac.at**

Introduction

Lyme borreliosis, or Lyme disease, is caused by a group of related spirochaetes—*Borrelia burgdorferi* sensu lato or Lyme borrelia—that are transmitted by specific *Ixodes* spp ticks. Lyme borreliosis is the most common tick-borne infectious disease in North America and in countries with moderate climates in Eurasia. The disease is of public health importance in both regions.

The pathogens

In North America, the only species of Lyme borrelia known to cause human disease is *Borrelia burgdorferi* sensu stricto (hereafter referred to as *B burgdorferi*). In Europe, at least five species of Lyme borrelia (*Borrelia afzelii*, *Borrelia garinii*, *B burgdorferi*, *Borrelia spielmanii*, and *Borrelia bavariensis*) can cause the disease, leading to a wider variety of possible clinical manifestations in Europe than in North America. A further three species (*Borrelia bissettii*, *Borrelia lusitaniae*, and *Borrelia valaisiana*) have very occasionally been detected in patients, but are not recognised as important pathogens.[1–8] *B afzelii* and *B garinii* infections account for most Lyme borreliosis cases in Europe, whereas *B garinii* is predominant in Asia. *B afzelii* is mostly associated with skin manifestations, *B garinii* seems to be the most neurotropic, and *B burgdorferi* seems to be the most arthritogenic.[9]

B burgdorferi was the first spirochaete for which the complete genome was sequenced.[10] Genetic studies suggest a nearly complete absence of biosynthetic pathways, making the microorganism dependent on its environment for nutritional requirements. Nevertheless, Lyme borrelia can be grown in vitro in highly enriched culture media.[11,12]

Ecology of the pathogens and their vectors

The main vector of Lyme borrelia in Europe is *Ixodes ricinus*, whereas *Ixodes persulcatus* is the main vector in Asia. *Ixodes scapularis* is the main vector in northeastern and upper midwestern USA and *Ixodes pacificus* is the vector in western USA (figure 1).[13] These ticks have a four-stage life cycle—egg, larva, nymph, and adult (figure 2)—feeding only once during every active stage. Male ticks rarely feed and never engorge. Unfed (flat) ticks attach to the skin of a host animal using specialised mouthparts as the animal passes through vegetation. After feeding for a few days (about 3 days for larvae, 5 for nymphs, and 7 days for adult females), the ticks drop off their host and locate on or near the soil surface, where they need a minimum relative humidity of 80% for survival. Once there, the ticks take several months to develop into their next developmental stage, or, in the case of adult females, lay about 2000 eggs. The length of a tick's life cycle varies between 2 years and 6 years, depending on climate, host availability, and the effects of development-delaying diapause mechanisms.[14]

Transmission of Lyme borrelia occurs through injection of tick saliva during feeding. A feeding period of more than 36 h is usually needed for transmission of *B burgdorferi* by *I scapularis* or *I pacificus* ticks.[15–17] Transmission of *B afzelii* by *I ricinus*, however, can be more rapid—in experiments with gerbils,[18] transmission occurred despite removal of ticks only 17 h after they had attached. However, this study used only one isolate of *B afzelii* and few animals so its findings need to be confirmed in other studies. Because transovarial transmission is rare or non-existent, larval ticks are not important vectors of Lyme borrelia.[17] Some of the early reports of spirochaetes in larvae were probably attributable to detection of *Borrelia miyamotoi* rather than *B burgdorferi* sensu lato; *B miyamotoi* is a relapsing fever *Borrelia* species of unknown pathogenicity detected in both North America and Europe, and is transmitted transovarially.[19]

Search strategy and selection criteria

We searched Medline and Scopus from Jan 1, 2003, onwards, with the search terms "Lyme", "borreliosis", "borrelia", "erythema migrans", "borrelia lymphocytoma", "neuroborreliosis", "Lyme carditis", "acrodermatitis atrophicans", "Lyme arthritis", and "Lyme encephalopathy". In relation to clinical studies, we placed particular value on randomised controlled trials. Additionally, key reviews were consulted, particularly reference numbers 9, 13, 25, 26, 33, 44, 45, 63, and 107.

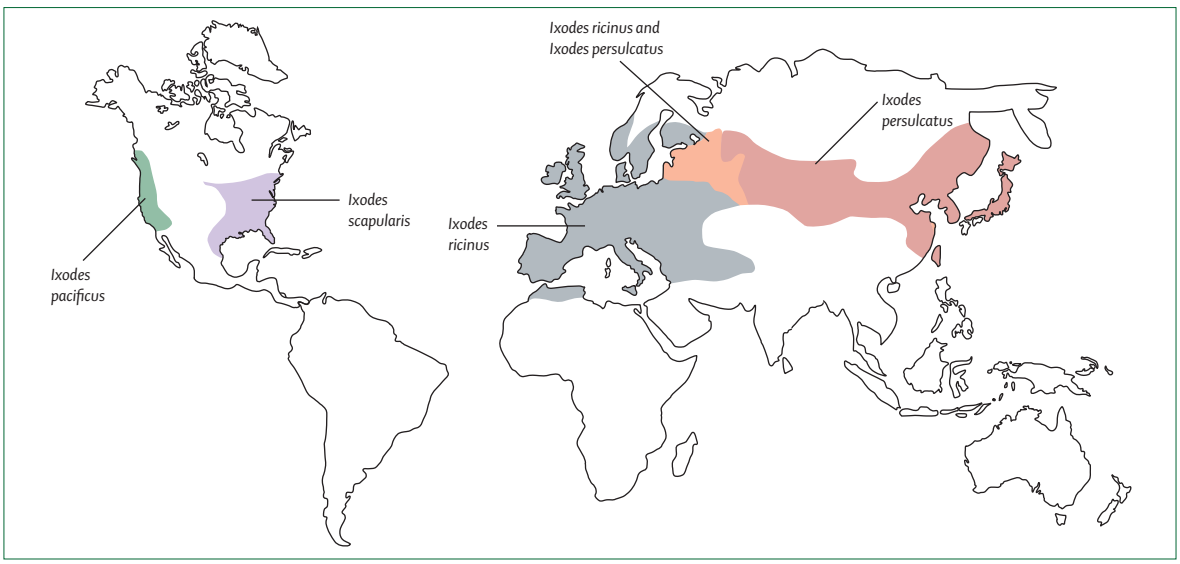

Figure 1: **Global distribution of the vectors (*Ixodes ricinus* species complex) of Lyme borrelia**
Reproduced with permission from the European Concerted Action on Lyme Borreliosis.

In endemic areas, transmission of Lyme borrelia can occur in either peri-urban areas or rural areas used for forestry and recreational activities.[20]

The lifecycle of all four tick species have distinct seasonality. In the case of *I ricinus* and *I persulcatus*, nymphs and adults become active, more or less concurrently, in early spring and continue to seek hosts until mid-summer, or until even later in the year in sheltered humid environments. With *I ricinus* a second peak of activity can occur in the autumn. *I scapularis* nymphs are active from early summer to early autumn, but the adults do not become active until autumn and remain so through winter until early spring, apart from periods when temperatures are too low for activity (<3°C). Patterns of activity of *I pacificus* seem more like those of *I ricinus* than of *I scapularis*. In all species, peak activity usually occurs slightly later in larvae than in nymphs, especially for *I scapularis* in eastern USA. The roughly 3-month difference between *I scapularis* peak nymphal and larval activity allows time for the reservoir hosts infected by nymphs to become infective for larvae and could explain the high intensity of transmission there.[17]

The main vertebrate reservoirs for Lyme borrelia are small mammals, such as mice and voles, and some species of birds. In most tick habitats, deer are essential for the maintenance of tick populations because they are one of the few wild hosts that can feed sufficient numbers of adult ticks, but they are not competent reservoirs for spirochaetes. Cattle are also incompetent hosts. Sheep also seem unlikely to be important reservoir hosts, but this issue should be studied further because little relevant data are available.[21–23] The different pathogenic genospecies of *B burgdorferi* sensu lato show a slight predilection for some vertebrates as reservoir hosts (figure 3), though this host specificity does not seem to be absolute. One factor thought to be relevant to reservoir competence is the susceptibility of the particular genospecies of Lyme borrelia to complement-mediated killing by the animal host.[24]

Small populations of deer in a tick habitat can be regarded as a good indication of Lyme borreliosis risk because an array of other hosts, including reservoir-competent animals, are also likely to be present. However, if most animals in a habitat are those which do not act as reservoirs for Lyme borrelia, such as deer or cattle, Lyme borreliosis risk decreases because ticks will feed mostly on these animals and will therefore not become infected.[25] Most transmission to human beings, manifested by cases of erythema migrans, occurs from late May to late September, coinciding with the activity of nymphs and with the increasing recreational use of tick habitats by the public. *I persulcatus* nymphs, however, bite human beings infrequently; adult female ticks are the main vectors in this species. A typical habitat for the transmission of Lyme borrelia is much the same throughout the geographical range of this disease. It usually consists of deciduous or mixed woodland, occasionally coniferous, with a substantial understory and a layer of decaying vegetation on the ground, thus providing sufficient humidity for the development and survival of ticks, and supporting a range of potential vertebrate reservoir hosts.

Pathogenesis

Lyme borrelia are carried in the midgut of unfed *Ixodes* ticks. When an infected tick takes a blood meal, the ingested spirochaetes increase in number and undergo phenotypic changes, including the expression of outer surface protein C (OspC), which allows them to invade the host tick's salivary glands. This process takes several

www.thelancet.com **Published online September 7, 2011 DOI:10.1016/S0140-6736(11)60103-7**

days and explains why transmission occurs only after a delay. Expression of OspC plays an essential part in the establishment of infection in a mammalian host, although the mechanism by which OspC promotes borrelial infectivity is unknown.[26,27]

When feeding, an infected tick deposits spirochaetes into the skin of a host animal. Later, Lyme borrelia disseminate from that site through blood or perhaps tissue planes to other locations. Evidence indicates that the risk of haematogenous dissemination by *B burgdorferi* is strain dependent.[28]

Infection of human beings or animals elicits innate and adaptive immune responses, resulting in both macrophage-mediated and antibody-mediated killing of spirochaetes. Despite a robust humoral and cellular immunological response, however, infection with Lyme borrelia can persist. Virulence factors that cause persistence include the spirochaete's ability to downregulate expression of specific immunogenic surface-exposed proteins, including OspC, and to alter rapidly and continually by recombination of the antigenic properties of a surface lipoprotein known as variable major protein-like sequence expressed (VlsE). The ability of spirochaetes to bind to various components of the extracellular matrix might also contribute to persistence.[29–31]

Lyme borrelia are not known to produce toxins. Most tissue damage seems to result from host inflammatory reactions. The intensity of the inflammatory response varies according to the *Borrelia* genospecies that causes an infection.[32] Although host genetic factors have an important role in the expression and severity of infection in animals, the only role established in man is in the development of antibiotic refractory Lyme arthritis, which is seen most often in patients with specific *HLA-DR* alleles.[30]

Clinical manifestations and epidemiological aspects

Localised infection is typically manifested by a erythema migrans skin lesion. Early disseminated disease is usually characterised by two or more erythema migrans skin lesions or as an objective manifestation of Lyme neuroborreliosis or Lyme carditis. Late Lyme borreliosis usually manifests as arthritis or the skin disorder known as acrodermatitis chronica atrophicans, but can also include specific rare neurological manifestations. The often used division of the disease into stages is somewhat theoretical and sometimes not in agreement with clinical findings.[33] For example, in some studies, most patients who present with Lyme arthritis have no recollection of having had an earlier clinical manifestation of Lyme borreliosis.[9]

Of the various objective clinical presentations of Lyme borreliosis in Europe, erythema migrans is the most common.[34–36] In one case series of patients with Lyme borreliosis,[35] 89% had erythema migrans by itself, 5% had arthritis, 3% had early neurological manifestations, 2% had borrelial lymphocytoma, 1% had acrodermatitis chronica atrophicans, and less than 1% had cardiac manifestations. None of the patients had late neurological Lyme borreliosis. A similar distribution of cases has been seen in a case series in the USA,[37–39] but no patients had borrelial lymphocytoma or acrodermatitis chronica atrophicans. Yearly incidence rates in Europe seem to increase from northern Europe to the southern parts of central Europe, and range from 69 cases per 100 000 population in Sweden to 111 cases per 100 000 in

Figure 2*: Developmental stages of *Ixodes ricinus
From left to right: larva, nymph, adult female, adult male. Reproduced with permission from the European Concerted Action on Lyme Borreliosis.

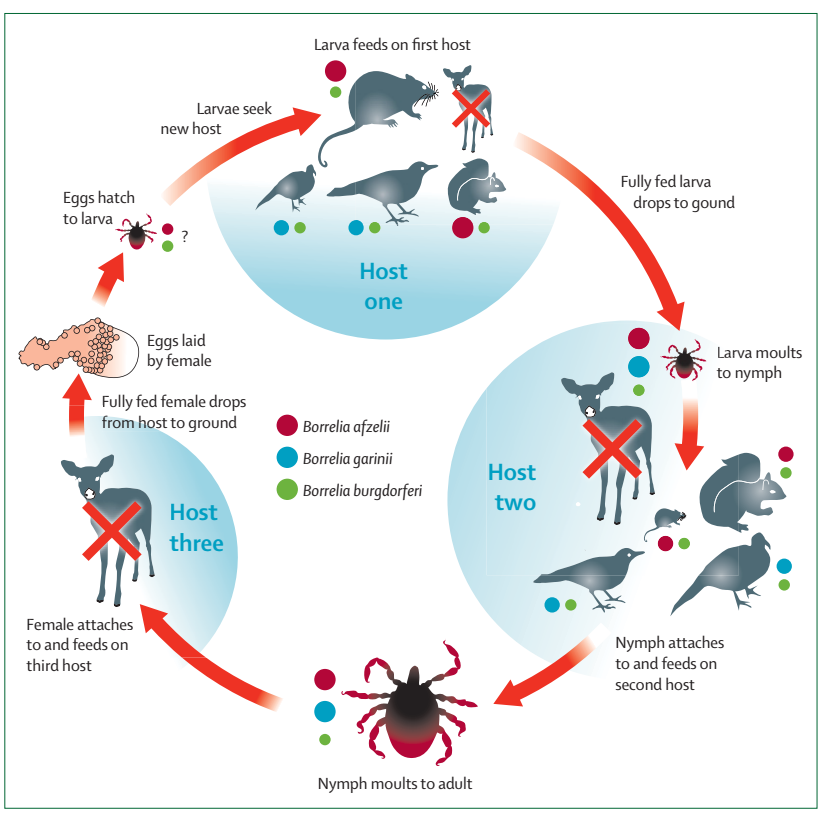

***Figure 3*: Infectious cycle of the European *Borrelia burgdorferi* sensu lato genospecies**
The size of the coloured closed circles indicates the relative involvement of the the different vertebrate reservoirs for the different genospecies. *B burgdorferi* sensu stricto is the only pathogenic genospecies present in the USA and, as in Europe, both rodents and birds are reservoirs. Reproduced with permission from the European Concerted Action on Lyme Borreliosis. A red cross indicates a non-reservoir host.

	Primary diagnostic testing	Supporting diagnostic testing	Supporting clinical findings
Erythema migrans			
Expanding red or bluish-red patch (≥5 cm in diameter),* with or without central clearing Advancing edge is typically distinct, often intensely coloured, and not noticeably raised	Diagnosis on the basis of history and visual inspection of the skin lesion Laboratory testing not needed or recommended If lesion is atypical, then acute-phase and convalescent-phase serological testing† are recommended because of insensitivity of acute phase testing‡	Culture or PCR of a skin biopsy specimen useful in research studies, but not needed for routine clinical practice	Tick bite at site; regional lymphadenopathy in North American patients
Borrelial lymphocytoma (a rare manifestation)			
Painless bluish-red nodule or plaque, usually on ear lobe, ear helix, nipple, or scrotum; more frequent in children (especially on ear) than in adults	Serological testing† usually positive at time of presentation; if negative, test convalescent phase sera (2–6 weeks later)	Lesion biopsy might be necessary to rule out neoplasm Culture or PCR of a skin biopsy specimen useful in research studies, but not needed for routine clinical practice	Tick bite at site; recent or concomitant erythema migrans
Lyme neuroborreliosis			
In adults, mainly meningo-radiculitis, meningitis, and peripheral facial palsy; rarely encephalitis, myelitis; very rarely cerebral vasculitis In children, mainly meningitis and peripheral facial palsy	Pleocytosis and demonstration of synthesis of intrathecal antibodies to *Borrelia burgdorferi* sensu lato§ Serological testing† usually positive at time of presentation; if negative, test convalescent phase sera (2–6 weeks later)	Detection of Lyme borrelia by culture or PCR of cerebrospinal fluid Intrathecal synthesis of total IgM, IgG, or IgA	Recent or concomitant erythema migrans
Cardiac Lyme borreliosis (a rare manifestation)			
Acute onset of atrioventricular (I–III) conduction disturbances, rhythm disturbances, and sometimes myocarditis or pericarditis Alternative explanations should be excluded	Serological testing† usually positive, but if negative and clinical suspicion strong, test convalescent phase sera (2–6 weeks later)	None recommended (Detection of Lyme borrelia by culture or PCR from endomyocardial biopsy restricted to research studies)	Recent or concomitant erythema migrans, neurological disorders, or both
Ocular manifestations (rare)			
Conjunctivitis, uveitis, papillitis, episcleritis, keratitis	Serological testing†	Detection of *B burgdorferi* sensu lato by culture or PCR from ocular fluid	Concomitant or previous other well-defined Lyme borreliosis manifestations
Lyme arthritis			
Recurrent attacks or persisting objective joint swelling in one or more large joints Alternative explanations should be excluded	Serological testing† As a rule, high concentrations of specific serum IgG antibodies present	Synovial fluid analysis Detection of *B burgdorferi* sensu lato by PCR of synovial fluid or tissue	Previous other well-defined Lyme borreliosis manifestations
Acrodermatitis chronica atrophicans			
Long-standing red or bluish-red lesions, usually on the extensor surfaces of extremities Initial doughy swelling Lesions eventually become atrophic Possible skin induration and fibroid nodules over bony prominences	Serological testing† As a rule, high concentrations of specific serum IgG antibodies present	Histology Detection of *B burgdorferi* sensu lato by culture or PCR from skin biopsy useful in research studies, but not for routine clinical practice	Previous other well-defined Lyme borreliosis manifestations

Data from reference 45. *If less than 5 cm in diameter, a history of tick bite, a delay in appearance after the tick bite of at least 2 days, and an expanding rash at the site of the tick bite is needed. †Two-tier serological testing is recommended, but newer first tier and immunoblot assays are increasingly incorporating the same peptides or recombinant immunodominant antigens of *Borrelia burgdorferi* sensu lato—whether doing the second tier immunoblot still increases overall specificity of serological testing is less clear. ‡As a rule, initial and follow up samples have to be tested in parallel to avoid misinterpreting changes caused by inter-assay variation. §In early cases, intrathecally produced specific antibodies might still be absent.

***Table 1*: Manifestations, brief clinical case definitions, and recommended diagnostic approach for the diagnosis of Lyme borreliosis in routine clinical practice**

Germany.[25] In many European countries, the incidence of the disease has increased in the past few years—eg, in Slovenia, the incidence was 258 cases per 100 000 in 2008, rising to 315 cases per 100 000 in 2009.[40,41] In the USA, the number of confirmed cases increased to nearly 30 000 in 2009 (13·4 cases per 100 000), although nearly 95% of these cases were reported from only 12 states located in the northeastern, middle and south Atlantic, and north central regions of the country.[42]

Most cases of erythema migrans occur between June and August. The seasonal distribution of extracutaneous manifestations is less pronounced, because the time from infection until disease onset is variable and usually longer than it is for erythema migrans. Like most tick-borne infections, slightly more men are infected than are women. In the USA, there is a bimodal age distribution with the highest incidences in children 5–9 years old and in adults 45–59 years old, but patients of all ages are at risk.[43] Generally, clinical manifestations are much the same in children and in adults, except that meningopolyradiculoneuritis and acrodermatitis chronica atrophicans are typically not seen in children; the frequency of particular clinical manifestations is more variable across different age groups in children than in adults.[9,43] Clinical manifestations of Lyme borreliosis are described in detail elsewhere,[9,33,44,45] and

 Published online September 7, 2011 DOI:10.1016/S0140-6736(11)60103-7

are summarised in table 1 (photographic examples of clinical manifestations are given in figure 4 and figure 5). Differential diagnoses are summarised in table 2.

Uncommon skin manifestations such as localised scleroderma (morphea) and lichen sclerosus et atrophicus might be caused by borrelia infection, but this association is controversial.[46–48] Sclerotic lesions that are clinically and histologically indistinguishable from localised scleroderma or lichen sclerosus et atrophicus develop in about 10% of patients with typical acrodermatitis chronica atrophicans.[49,50] Another manifestation suspected to be associated with Lyme borrelia infection is cutaneous B-cell lymphoma because of positive serological and PCR results and isolation of Lyme borrelia from skin lesions in European patients.[51–53] However, this association has not been seen in cases from Asia or the USA.[54,55] Prospective clinical studies are necessary to ascertain whether, or how often, these dermatological disorders are caused by infection with Lyme borrelia.

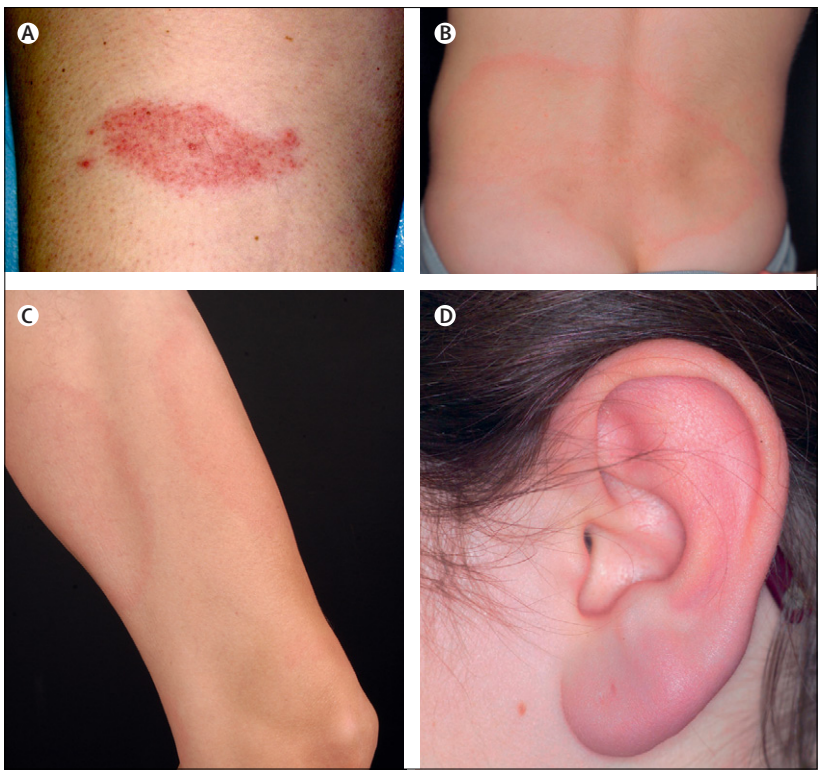

Figure 4: **Examples of erythema migrans**
(A) Erythema migrans on a patient's right thigh; time from tick bite to onset of erythema migrans is 9 days—duration of erythema migrans is 5 days. (B) Widely expanded erythema migrans with central clearing on a patient's back. (C) Erythema migrans on a patient's left thigh. (D) Borrelial lymphocytoma: reddish-blue nodule on a patient's left ear lobe.

Late Lyme neuroborreliosis

Late Lyme neuroborreliosis is uncommon.[33,45,56–61] Monophasic, slowly progressive encephalomyelitis is the most severe neurological manifestation—it mainly involves white matter and is more common in Europe than in the USA.[60,61] Examination of cerebrospinal fluid typically shows a lymphocytic pleocytosis, a slightly raised protein concentration, and a normal glucose concentration, with evidence of intrathecal production of antibodies to Lyme borrelia. MRI of the affected part of the neuraxis can show areas of inflammation, typically with increased signal on T2 and FLAIR imaging and enhancement after addition of contrast. A mild axonal neuropathy and an imprecisely defined subtle encephalopathy have been reported, mostly by researchers from the USA.[62] Peripheral neuropathy of the involved limb occurs in more than half of patients with a long-lasting acrodermatitis chronica atrophicans skin lesion.[60]

Laboratory testing in Lyme borreliosis

White blood cell count, packed cell volume and haemoglobin concentrations, and platelet counts of patients with Lyme borreliosis are usually no different from those of healthy individuals, unless co-infected with *Anaplasma phagocytophilum* or *Babesia microti*, or tick-borne encephalitis virus is present. In early localised and early disseminated Lyme borreliosis, especially in patients with erythema migrans, slightly raised liver function test results (particularly aspartate and alanine aminotransferase concentrations) can be seen in about 35% of patients in the USA and in up to 20% of patients in Europe. Erythrocyte sedimentation rates can be slightly raised in all stages of Lyme borreliosis, but values greater than 80 mm/h are very uncommon. Cerebrospinal fluid examination in Lyme neuroborreliosis typically shows a pleocytosis with more than 90% lymphocytes, a slightly raised protein concentration, and a normal glucose concentration. Synovial fluid examination in Lyme arthritis typically shows about 25 000 white cells/mm³, ranging from 500 white cells per mm³ to 110 000 white cells per mm³, with a polymorphonuclear predominance.[33,63]

Laboratory diagnosis by serological testing

Typical erythema migrans is usually sufficiently distinctive to allow a clinical diagnosis in the absence of a supporting laboratory test. Serological assays for antibodies to Lyme borrelia are positive infrequently at this stage, and thus should be obtained only in atypical cases, and then in conjunction with convalescent phase serological testing 2–6 weeks after obtaining the acute sample (table 1).

For non-erythema migrans presentations of Lyme borreliosis, the mainstay of laboratory diagnosis is two-tier serological testing in which the first tier is usually a sensitive enzyme linked immunosorbent assay (EIA).[33,64–68] If the EIA is positive or equivocal, then separate IgM and IgG immunoblots are done on the same serum sample. If symptoms have persisted for at least 4 weeks, then the IgG immunoblot should be

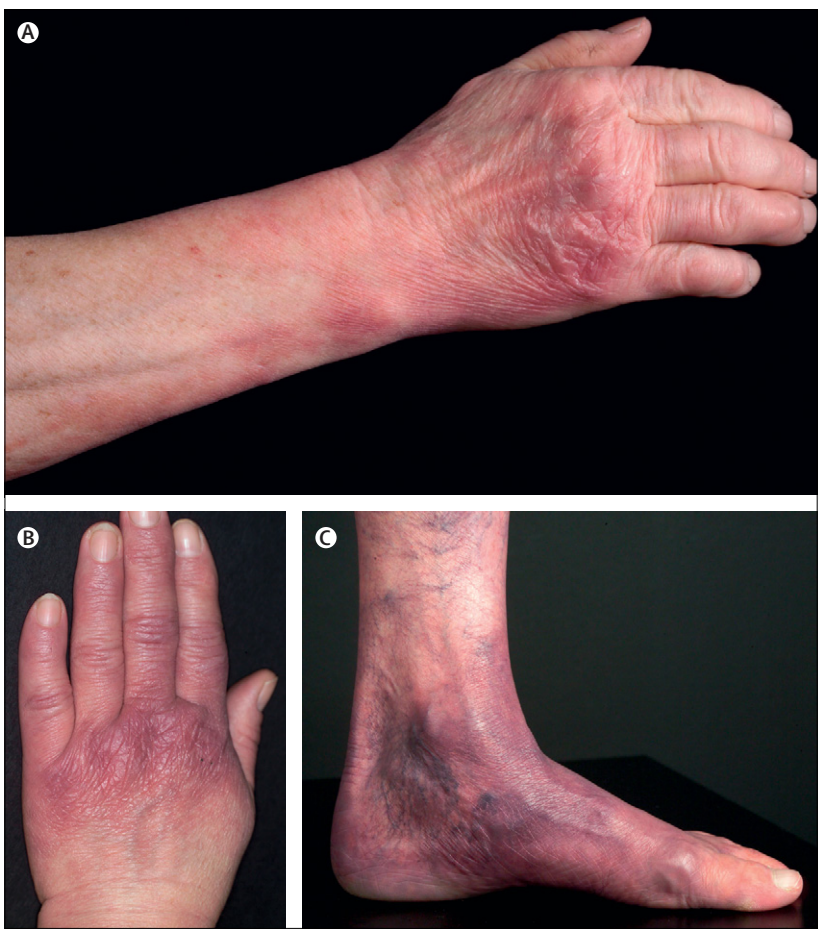

Figure 5: **Examples of acrodermatitis chronic atrophicans**
Acrodermatitis chronic atrophicans (ACA) is typically located on the extensor sites of extremities: (A) ulnar and hand lesions, (B) bluish-red lesion on the back of a patient's hand and waxy appearance of the skin of fingers, (C) lesions on a patient's left foot and lower leg.

positive.[33,65,68–70] Untreated patients who remain seronegative despite symptoms persisting for more than 6 weeks are unlikely to have Lyme borreliosis and other potential diagnoses should be actively pursued.

Omission of the first tier EIA, or interpretation of the immunoblot with criteria that are not evidence-based, will potentially decrease the specificity of testing and are contributing factors to misdiagnosis. A particular concern with the IgM immunoblot in clinical practice has been the many false positive results caused by the over-reading of non-specific weak bands.[66]

Background rates of seropositivity, which can exceed 4% in highly endemic areas of the USA,[70] with even higher rates in Europe, can also confound the interpretation of seroreactivity. Indeed, seropositivity rates of more than 50% have been reported for Austrian hunters older than 50 years.[71] In such populations, additional testing, such as tests for intrathecal antibody production in patients with suspected Lyme neuroborreliosis, PCR testing of joint fluid for suspected Lyme arthritis, or skin biopsies for suspected acrodermatitis chronica atrophicans or borrelial lymphocytoma, might increase diagnostic accuracy. Clearly, a positive serological test does not mean that a patient necessarily has active Lyme borreliosis. The positive predictive value is usually most informative when the pretest probability based on the clinical features is at least 20%. Serological testing is not indicated in routine follow-up of patients after treatment, because either IgM or IgG borrelial antibodies can persist for many years in successfully treated patients.[33,45,61]

Testing for borrelial antibodies that are produced locally in the CNS (ie, intrathecal synthesis of specific antibodies) is a mainstay of the diagnosis of Lyme neuroborreliosis in Europe, and detection of antibody in cerebrospinal fluid has been reported to precede that of serum antibody in some European patients.[44,69,72,73] However, intrathecal synthesis of antibodies can persist for several months to several years after successful antibiotic treatment.[44,69,72,73]

Other diagnostic modalities

Culture for Lyme borrelia is not routinely done or available to diagnose Lyme borreliosis because it is unnecessary for patients with erythema migrans and too insensitive for patients with extracutaneous manifestations of Lyme borreliosis. However, PCR for detection of borrelial DNA in synovial fluid specimens is positive in up to about 80% of untreated patients with Lyme arthritis, and a positive result lends support to this diagnosis in a patient with a positive IgG immunoblot.[33,69,74] The positivity rate of PCR in cerebrospinal fluid tends to be much lower than it is in synovial fluid,[68] however, and was only about 5% in a study of children from the USA with early neurological Lyme borreliosis.[75] A negative PCR result on either cerebrospinal or synovial fluid does not exclude Lyme borreliosis. PCR on blood or urine samples, tests for urine antigen detection, tests for T-lymphocyte recognition of borrelial antigens (as a measure of a cellular immune response), measurement of the number of CD57 natural killer cells, and use of live microscopy on blood to search for spirochaetes, have not been shown to be reliable and are not recommended for clinical use.[33,69,76]

Treatment

In-vitro studies have shown that Lyme borrelia are susceptible to tetracyclines, most penicillins, many second-generation and third-generation cephalosporins, and macrolides.[33,77,78] Lyme borrelia are resistant to specific fluoroquinolones, rifampicin, and first-generation cephalosporins.[33,77,78]

Although erythema migrans will eventually resolve without antibiotic treatment, oral antibiotic treatment is recommended to prevent dissemination and development of later sequelae (table 3). Doxycycline, amoxicillin, phenoxymethylpenicillin, and cefuroxime axetil are

 Published online September 7, 2011 DOI:10.1016/S0140-6736(11)60103-7

	Differential diagnosis
Erythema migrans	Tick-bite or insect-bite hypersensitivity reaction, bacterial cellulitis, erysipelas, erythema multiforme, southern tick-associated rash illness (STARI),* tinea, nummular eczema, granuloma annulare, contact dermatitis, urticaria, fixed drug eruption, pityriasis rosea, parvovirus B19 infection in children
Borrelial lymphocytoma	Breast cancer (when lymphocytoma occurs on the breast), B-cell lymphoma, pseudolymphoma
Lyme neuroborreliosis	Other causes of facial palsy, viral meningitis, mechanical radiculopathy, first episode of relapsing-remitting multiple sclerosis, primary progressive multiple sclerosis
Lyme carditis	Other infectious and non-infectious causes of conduction disturbances or myopericarditis
Lyme arthritis	Gout, pseudo-gout, septic arthritis, viral arthritis, psoriatic arthritis, HLA B27-positive juvenile oligoarthritis, reactive arthritis in adults, sarcoid arthritis, early rheumatoid arthritis, seronegative spondyloarthropathies
Acrodermatitis chronica atrophicans	Consequence of old age (old skin), chilblains, vascular insufficiency (chronic venous insufficiency), superficial thrombophlebitis, hypostatic eczema, arterial obliterative disease, acrocyanosis, livedo reticularis, lymphoedema, erythromelalgia, scleroderma lesions, rheumatoid nodules, gout (tophi), erythema nodosum

*An illness associated with an erythema migrans-like skin lesion—the cause of STARI is unknown, but it occurs in southern USA and is associated with the bite of the *Amblyomma americanum* tick, a tick species that is not a competent vector for *Borrelia burgdorferi*

***Table 2*: Considerations for differential diagnosis of Lyme borreliosis**

highly effective and are the preferred agents for this manifestation. Macrolides such as azithromycin are somewhat less effective than other oral antibiotics and are consequently used as second-line treatment.[33]

Doxycycline is the only drug for which both a prospective and a large retrospective clinical trial have shown that only 10 days of treatment is effective.[79,80] Doxycycline, however, can cause photosensitivity and is contraindicated in children younger than 8 years and in women who are pregnant or breastfeeding.[33]

Within 24 h of initiation of antimicrobial treatment, some patients treated for erythema migrans can have an increase in the size or intensity of their erythema migrans skin lesion, and more intense viral infection-like systemic symptoms. Fever, if present, should resolve within 48 h and the skin lesion usually resolves within 7–14 days. Other symptoms, such as fatigue or arthralgia, tend to improve but do not always resolve within this timeframe, lasting for more than 3 months in one-quarter of patients from the USA[79]—in Europe this proportion is about 10%.[81] Extension of the initial course of treatment does not result in faster relief of symptoms.[33,79,80,82–85] Oral antibiotic treatment is also used as first-line treatment for the other cutaneous manifestations of Lyme borreliosis, and as initial treatment for patients with Lyme arthritis.[33]

The preferred parenteral drug for Lyme borreliosis is ceftriaxone because it is highly active against Lyme borrelia in vitro, crosses the blood–brain barrier well, and has a long serum half-life, which means it can be taken only once a day. Alternative choices for parenterally given antibiotics are cefotaxime and intravenous penicillin. Parenteral antibiotic treatment is recommended for treatment of patients with late Lyme neuroborreliosis and as an initial treatment for those with cardiac Lyme borreliosis who are admitted to hospital for monitoring (table 3).

In Europe and the USA, parenteral treatment has been the preferred management strategy for Lyme neuroborreliosis, especially for meningitis and radiculitis; oral treatment is reserved for patients with uncomplicated facial palsy. Studies done in Europe, however, have provided convincing evidence that oral doxycycline is as effective as ceftriaxone for any of the primary manifestations of early Lyme neuroborreliosis.[72,86] The same might be true in the USA, but no systematic studies have been done. Other oral antibiotics such as amoxicillin have been used successfully to treat patients with uncomplicated seventh nerve palsy, but efficacy data for such drugs are restricted. Because seventh nerve palsy will resolve at the same rate with or without antibiotic treatment, the main reason to treat such patients is to prevent the development of later complications, especially Lyme arthritis, which occurred in more that 80% of patients in the USA who were untreated.[87]

Symptomatic patients with cardiac Lyme borreliosis and those with high-grade first-degree atrioventricular block (PR interval of ≥300 ms), and second-degree or third-degree atrioventricular block, should be admitted to hospital and closely monitored. Temporary cardiac pacing might be necessary. In treated patients, complete heart block generally resolves within 1 week and lesser conduction disturbances within 6 weeks.[33]

Lyme arthritis typically responds to antibiotic treatment. On the basis of clinical experience, patients whose arthritis is improved but not resolved after an initial course of oral treatment can be re-treated with a second course of oral antibiotics, reserving parenteral antibiotic treatment for those without any substantial clinical response.[33] About 10% of patients in the USA, however, do not respond clinically to antibiotic treatment and are said to have antibiotic-refractory Lyme arthritis—this disorder has been defined as persistent synovitis for at least 2 months after completion of a course of intravenous ceftriaxone (or 1 month after completion of two 4-week courses of an oral antibiotic), in conjunction with negative PCR testing on synovial fluid and on synovial tissue if available. Because these patients are no longer believed to be actively infected, they are usually treated with

	Treatment regimen	Duration	Comment
Early-localised and early-disseminated Lyme borreliosis			
Erythema migrans	Oral†‡	14 days	A 10-day course of treatment with doxycycline is effective in the USA, but the efficacy of 10-day courses of the other first-line oral antibiotics is less well substantiated No studies on this have been done in Europe Doxycycline is also active against *Anaplasma phagocytophilum*
Meningitis or radiculopathy	Parenteral§ or doxycycline‡	14 days	Evidence from European studies shows that oral doxycycline treatment is as effective as parenteral treatment, although this finding has not been systematically tested in North America
Cranial nerve involvement	Oral†‡	14 days	Although any of the first-line oral antibiotics seem to be effective in patients with cranial neuropathy, there is restricted evidence for its effectiveness in patients with a cranial neuropathy other than facial palsy or with drugs other than doxycycline
Cardiac disease	Oral†‡ or parenteral §	14 days	Information on treatment is restricted A parenteral regimen is preferential in patients who are being monitored in hospital or are in hospital for placement of a temporary pacemaker When heart block has improved and the patient is ready to be discharged, an oral antibiotic treatment regimen can be given Those who are managed as outpatients can be treated with an oral antibiotic
Borrelial lymphocytoma	Oral†‡	14 days	Little information is available on treatment The same approaches are used as for the treatment of erythema migrans Not recorded in North America
Late Lyme borreliosis			
Arthritis without neurological disease	Oral†‡	28 days	Patients are usually concomitantly treated with NSAIDs
Recurrent arthritis after one course of oral treatment	Oral‡ or parenteral§	28 days (oral) 14–28 days (parenteral)	Parenteral treatment is usually reserved for patients without even a partial response to oral treatment
Antibiotic-refractory arthritis	Symptomatic treatment¶	As required	Antibiotic refractory Lyme arthritis is defined as persistent synovitis for at least 2 months after completion of a course of intravenous ceftriaxone (or 1 month after completion of two 4-week courses of an oral antibiotic regimen); additionally, PCR on synovial fluid (and synovial tissue if available) is negative for borrelial nucleic acids
Central or peripheral nervous system disease	Parenteral§	14–28 days	No studies have compared 14-day treatment with 28-day treatment, partly because of the rarity of cases
Acrodermatitis chronica atrophicans	Oral†‡	21–28 days	No studies have compared 21-day treatment with 28-day treatment, nor treatment with different antibiotics Rarely seen in North America
Post-Lyme borreliosis syndrome			
Post-Lyme borreliosis syndrome	Symptomatic treatment	As required	Consider and assess other potential causes of symptoms

Data from reference 33. *Irrespective of the clinical manifestations of Lyme borreliosis, complete response to antibiotic treatment can be delayed beyond the treatment duration—relapse can occur with any of these regimens; patients with objective signs of relapse might need another course of treatment. †Preferred oral regimens: doxycycline (adults—100 mg two times a day; children aged ≥8 years—4 mg/kg per day divided into two doses a day [maximum dose=100 mg two times a day]); amoxicillin (adults—500 mg three times a day; children—50 mg/kg per day divided into three doses a day [maximum dose=500 mg three times a day]); phenoxymethylpenicillin (adults—500–1000 mg three times a day; children—100 mg/kg per day divided into three daily doses [maximum dose=1000 mg three times a day]); cefuroxime axetil (adults—500 mg two times a day; children—30 mg/kg per day divided into two daily doses [maximum dose 500 mg two times a day]). Alternative oral regimen (for patients intolerant of doxycycline, amoxicillin, phenoxymethylpenicillin, and cefuroxime axetil): azithromycin (adults—500 mg once daily; children—10 mg/kg per day [maximum dose=500 mg once daily])—because of the long tissue half-life of this drug, the duration of treatment is shorter than with preferred oral regimens (eg, 5–10 days rather than 14 days for erythema migrans). ‡Doxycycline is not recommended for children aged younger than 8 years, or for women who are pregnant or breastfeeding. §Preferred parenteral regimen: ceftriaxone (adults—2 g intravenously once daily; children—50–70 mg/kg per day intravenously [maximum dose=2 g intravenously once daily]). Alternative parenteral regimens: cefotaxime: (adults 2 g every 8 h intravenously for patients with normal renal function; children—150–200 mg/kg per day divided into three–four daily doses intravenously [maximum dose=6 g per day] for patients with normal renal function); penicillin G (adults—18–24 million units intravenously per day divided into six daily doses for patients with normal renal function; children—250 000–400 000 U/kg per day divided in six daily doses intravenously [maximum dose=18–24 million units per day]). ¶Symptomatic treatment might consist of non-steroidal anti-inflammatory agents (NSAIDs), intra-articular injections of corticosteroids, or other drugs including disease-modifying antirheumatic drugs; expert consultation with a rheumatologist is recommended. If persistent synovitis is associated with substantial pain or if it limits function, arthroscopic synovectomy can reduce the period of joint inflammation.

Table 3*: Recommended treatment for patients with Lyme borreliosis

non-steroidal anti-inflammatory agents, intra-articular injections of corticosteroids, or disease-modifying anti-rheumatic drugs.[88] Arthroscopic synovectomy has also been used successfully for patients with this disorder.[33,63]

Treatment for pregnant women with Lyme borreliosis is much the same as treatment for women who are not pregnant, except that doxycycline should be avoided because of the potential for adverse effects to both the fetus and the mother. No data reliably lend support to congenital Lyme borreliosis syndrome.[33,89,90]

Post-treatment symptoms and post-Lyme borreliosis syndrome

The objective manifestations of Lyme borreliosis, such as erythema migrans, meningitis, or arthritis, typically resolve during or after completion of a course of antibiotic treatment. Any accompanying subjective symptoms also usually resolve, but some patients (median of 11·5% in eight treatment trials of patients with erythema migrans in the USA and 15·4% in five treatment trials in Europe) report long-term (≥6 months) persistence of fatigue, musculoskeletal pain, or difficulties with concentration

and memory.[81] Patients with long-term post-treatment symptoms that are severe enough to be disabling are thought to have post-Lyme borreliosis syndrome.[33,91–94]

In some studies,[93,95,96] the frequency of symptoms at 6 or more months after treatment has exceeded that of control groups without Lyme borreliosis but not in others. Only one study,[81] however, has prospectively assessed both patients with Lyme borreliosis and controls in a similar manner. In that study, done in Slovenia, the frequency of subjective complaints in patients treated for erythema migrans did not exceed that of a demographically similar control group at both 6 months and 12 months of follow-up. Furthermore, the persistent complaints in those who had them were mild and did not interfere with daily activities. Some evidence suggests that the frequency of post-treatment symptoms is more common and perhaps also more severe in adult patients treated for Lyme neuroborreliosis compared with other presenting manifestations of Lyme borreliosis.[86,97,98] Additional prospective assessments of patients with Lyme borreliosis and appropriate control groups should be a research priority to identify the circumstances, if any, in which the frequency of such symptoms exceeds that seen in people without Lyme borreliosis.

The cause of post-Lyme disease syndrome has not been established. Microbiological studies have not shown convincing evidence for persistence of borrelia in such patients, and, consistent with those findings, four NIH-sponsored, randomised, placebo-controlled trials have not shown convincing evidence that the putative benefit of retreatment with antibiotics exceeds the risk from the drugs themselves or from the intravenous catheters used to deliver some of them.[33,91,92,99,100] Therefore, attention has turned to the study of other potential explanations of post-treatment symptoms[101–103] and to the search for alternative therapeutic approaches for management.[104] Definitive answers for this and other post-infection syndromes[105] are still awaited, causing much frustration for both patients and health-care providers.

Chronic Lyme disease

The term chronic Lyme disease is poorly defined but widely used. In Europe the term is sometimes used to refer to objective manifestations such as acrodermatitis chronica atrophicans, which most authorities prefer to call late Lyme borreliosis. Others have used the term to refer to patients with post-treatment subjective complaints.

Often, the term chronic Lyme disease is used as a diagnosis for patients with persistent pain, fatigue, or neurocognitive complaints, without clinical evidence of previous acute Lyme borreliosis and even without serological identification of borrelial infection.[33,94,102,106,107] This viewpoint can be traced to the belief, contrary to scientific evidence, that Lyme borreliosis often causes disabling subjective symptoms even in the absence of objective signs of disease, that diagnostic tests for extracutaneous manifestations of Lyme borreliosis are often falsely negative, and that treatment with antibiotics for months or years is necessary to suppress the symptoms of the disease, which often recur despite long-term antibiotic treatment.[107,108] Such misinformation about Lyme borreliosis is widespread on the internet.[109,110] Consequently, patients with medically unexplained symptoms,[102,108] and others with more well-defined disorders,[107,111] are increasingly being diagnosed with chronic Lyme disease. The net result is that most patients receiving treatment for chronic Lyme disease have no convincing evidence, by history (sometimes including even absence of tick exposure), physical examination, or laboratory test results, of ever having had *B burgdorferi* sensu lato infection.[102,106,107,111] The untenable position of proponents of the chronic Lyme disease theory has been highlighted elsewhere in much detail.[100,108]

The effect of health-care providers, although few,[112] who believe in chronic Lyme disease should not be underestimated. Their unorthodox views and resulting practices have contributed to injury and even deaths of patients.[113,114] At a time when wasteful health-care expenditures are being scrutinised and widespread bacterial resistance has been linked to overuse of antibiotics, the avoidance of unsubstantiated treatments is important.

Co-infections

Ixodes spp ticks can be co-infected with and transmit Lyme borrelia along with other pathogens such as *Anaplasma phagocytophilum*, *Babesia* spp, and tick-borne encephalitis virus.[115,116] Transmission of *Bartonella* by *Ixodes* spp ticks has not been recorded.[117] Co-infection should be considered in patients from geographical areas endemic for these pathogens who present with more severe initial symptoms than are commonly seen with Lyme borreliosis alone, especially for those who have a high-grade fever for more than 48 h despite antibiotic treatment appropriate for Lyme borreliosis, those who develop recurrent fever, and those who have unexplained leucopenia, thrombocytopenia, or anaemia.[33]

Reinfection

Patients treated for early Lyme borreliosis do not seem to develop an immunological response that is adequate to protect against reinfection. Reinfection has been well documented only in patients who were treated for early infection (mostly erythema migrans) and not after late manifestations of Lyme borreliosis such as Lyme arthritis. Clinical manifestations of reinfection seem to be similar to those of primary infection; whether the serological responses differ needs more investigation.[118–121]

Prevention

Lyme borreliosis can be prevented by avoidance of tick-infested environments, and, when in such environments, covering bare skin and use of tick repellents on skin or clothing. The density of tick populations around

residences can be reduced by the removal of leaf litter, the placing of wood chips where lawns are adjacent to forests, application of acaricides, and the construction of fences to keep out deer.[122] Bathing within 2 h of tick exposure decreases the risk of Lyme borreliosis.[123] Daily inspections of the entire skin surface (including scalp) to remove attached ticks is recommended because of the delay between the time of tick attachment and transmission of Lyme borrelia. Removal is done by grasping the tick as close to the mouthparts as possible with forceps (or tweezers) and then gently pulling it out. Clinical studies have shown that more than 96% of patients who find and remove an attached *I scapularis* tick will not contract Lyme borreliosis, without any other intervention, even in highly endemic geographical regions.[124] If the tick is not found or removed, the probability of infection approaches the infection rate in the regional tick population (typically 25% of nymphal stage *I scapularis* ticks are infected in highly endemic areas of the northeastern and midwestern USA, and 10% of nymphal *I ricinus* ticks in Europe).[125,126]

Doxycycline chemoprophylaxis can reduce the chance of developing Lyme borreliosis after removal of an *I scapularis* or an *I persulcatus* tick.[124,127] In a study from the USA, one 200 mg dose of doxycycline was 87% effective in the prevention of erythema migrans at the tick bite site. Use of one dose of doxycycline within 72 h of tick removal should be considered for individuals in highly endemic areas of the USA who are known to have been bitten by a nymphal or adult *I scapularis* tick that was attached for at least an estimated 36 h. In view of the uncertain efficacy of a short course of amoxicillin (compared with a 10-day course, which might be effective[128]) in this situation, observation rather than chemoprophylaxis has been recommended for individuals for whom doxycycline is contraindicated.[33] Similarly in Europe, observation is recommended for *I ricinus* tick bites, because the infection rate of ticks is lower than in the USA, and studies have not been done on the efficacy of antibiotic prophylaxis.[129] No vaccine is available to prevent Lyme borreliosis in man.

Prognosis

Most patients with Lyme borreliosis have an excellent prognosis. Although most manifestations of Lyme borreliosis will resolve spontaneously without treatment, antibiotic treatment might speed the resolution of symptoms and signs, and will prevent the development of objective late complications. Precautions to prevent future tick bites should be taken to prevent re-infections.

Contributors

GS, GPW, JG, and FS searched the published work and contributed to the scientific and technical content of the review. JG provided figure 1, figure 2, and figure 3. GS sourced figure 4 and figure 5.

Conflicts of interest

GS studied specific diagnostic tests and assays as part of his work at the Medical University of Vienna. GPW is a board member of the American Lyme Disease Foundation; has served as an expert witness in malpractice cases involving Lyme disease; has received research grants to study diagnostic tests for Lyme disease from the National Institutes of Health, Immunetics Inc, BioRad, DiaSorin Inc, and BioMerieux; and has equity in Abbott, a company not known to have any approved product for Lyme disease. JG and FS declare that they have no conflicts of interest.

Acknowledgments

We thank Bernard Kaye (School of Biology and Environmental Science, University College Dublin, Ireland) for creating figure 1, figure 2, and figure 3, and Franc Strle (University Medical Centre Ljubljana, Slovenia) and Elisabeth Aberer (Department of Dermatology, Medical University of Graz) for permission to publish the images in figure 4 and figure 5.

References

1 Ružić-Sabljić E, Maraspin V, Lotrič-Furlan S, et al. Characterization of *Borrelia burgdorferi* sensu lato strains isolated from human material in Slovenia. *Wien Klin Wochenschr* 2002; **114:** 544–50.
2 Foldvari G, Farkas R, Lakos A. *Borrelia spielmanii* erythema migrans, Hungary. *Emerg Infect Dis* 2005; **11:** 1794–95.
3 Maraspin V, Ružić-Sabljić E, Strle F. Lyme borreliosis and *Borrelia spielmanii*. *Emerg Infect Dis* 2006; **12:** 1177.
4 Fingerle V, Schulte-Spechtel UC, Ružić-Sabljić E, et al. Epidemiological aspects and molecular characterization of *Borrelia burgdorferi* sl from southern Germany with special respect to the new species *Borrelia spielmanii* sp. *Int J Med Microbiol* 2008; **298:** 279–90.
5 Nadelman RB, Wormser GP. Lyme borreliosis. *Lancet* 1998; **352:** 557–65.
6 Strle F, Picken RN, Cheng Y, et al. Clinical findings for patients with Lyme borreliosis caused by *Borrelia burgdorferi* sensu lato with genotypic and phenotypic similarities to strain 25015. *Clin Infect Dis* 1997; **25:** 273–80.
7 Margos G, Vollmer SA, Cornet M, et al. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. *Appl Environ Microbiol* 2009; **75:** 5410–16.
8 Collares-Pereira M, Couceiro S, Franca I, et al. First isolation of *Borrelia lusitaniae* from human patient. *J Clin Microbiol* 2004; **42:** 1316–18.
9 Strle F, Stanek G. Clinical manifestations and diagnosis of Lyme borreliosis. *Curr Probl Dermatol* 2009; **37:** 51–110.
10 Fraser CM, Casjens S, Huang WM, et al. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 1997; **390:** 580–86.
11 Barbour AG. Isolation and cultivation of Lyme disease spirochetes. *Yale J Biol Med* 1984; **57:** 521–25.
12 Preac-Mursic V, Wilske B, Schierz G. European *Borrelia burgdorferi* isolated from humans and ticks culture conditions and antibiotic susceptibility. *Zentralbl Bakteriol Mikrobiol Hyg A* 1986; **263:** 112–18.
13 Gray JS. The ecology of ticks transmitting Lyme borreliosis. *Exp Appl Acarol* 1998; **22:** 249–58.
14 Piesman J, Gern L. Lyme borreliosis in Europe and North America. *Parasitology* 2004; **129:** 191–220.
15 des Vignes F, Piesman J, Heffernan R, Schulze TL, Stafford KC 3rd, Fish D. Effect of tick removal on transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by *Ixodes scapularis* nymphs. *J Infect Dis* 2001; **183:** 773–78.
16 Peavey CA, Lane RS. Transmission of *Borrelia burgdorferi* by *Ixodes pacificus* nymphs and reservoir competence of deer mice (*Peromyscus maniculatus*) infected by tick-bite. *J Parasitol* 1995; **81:** 175–78.
17 Eisen L, Lane RS. Vectors of *Borrelia burgdorferi* sensu lato. In: Gray JS, Kahl O, Lane RS, Stanek G, eds. Lyme borreliosis: biology, epidemiology and control, 1st edn. New York; CABI Publishing, 2002: 91–115.
18 Kahl O, Janetzki-Mittmann C, Gray JS, Jonas R, Stein J, de Boer R. Risk of infection with *Borrelia burgdorferi* sensu lato for a host in relation to the duration of nymphal *Ixodes ricinus* feeding and the method of tick removal. *Zentralbl Bakteriol* 1998; **287:** 41–52.
19 Scoles GA, Papero M, Beati L, Fish D. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. *Vector Borne Zoonotic Dis* 2001; **1:** 21–34.
20 Dennis DT, Hayes EB. Epidemiology of Lyme borreliosis. In: Gray JS, Kahl O, Lane RS, Stanek G, eds. Lyme borreliosis: biology, epidemiology and control (1st edn). New York; CABI Publishing, 2002: 251–80.

21 Ogden NH, Nuttall PA, Randolph SE. Natural Lyme disease cycles maintained via sheep by co-feeding ticks. *Parasitology* 1997; **115:** 591–99.

22 Gray JS, Kahl O, Janetzki C, Stein J, Guy E. The spatial distribution of *Borrelia burgdorferi*-infected *Ixodes ricinus* in the Connemara region of Co. Galway, Ireland. *Experimental Appl Acarol* 1995; **19:** 163–72.

23 Matuschka FR, Heiler M, Eiffert H, Fischer P, Lotter H, Spielman A. Diversionary role of hoofed game in the transmission of Lyme disease spirochetes. *Am J Trop Med Hyg* 1993; **48:** 693–99.

24 Bykowski T, Woodman ME, Cooley AE, et al. *Borrelia burgdorferi* complement regulator-acquiring surface proteins (BbCRASPs): Expression patterns during the mammal-tick infection cycle. *Int J Med Microbiol* 2008; **298** (suppl 1)**:** 249–56.

25 EUCALB; European Union Concerted Action on Lyme Borreliosis. URL www.eucalb.com (accessed May 15, 2010).

26 Steere AC, Coburn J, Glickstein L. The emergence of Lyme disease. *J Clin Invest* 2004; **113:** 1093–101.

27 Rosa P. Lyme disease agent borrows a practical coat. *Nat Med* 2005; **11:** 831–32.

28 Wormser GP, Brisson D, Liveris D, et al. *Borrelia burgdorferi* genotype predicts the capacity for hematogenous dissemination during early Lyme disease. *J Infect Dis* 2008; **198:** 1358–64.

29 Baranton G, De Martino SJ. *Borrelia burgdorferi* sensu lato diversity and its influence on pathogenicity in humans. *Curr Probl Dermatol* 2009; **37:** 1–17.

30 Steere AC, Klitz W, Drouin EE, et al. Antibiotic-refractory Lyme arthritis is associated with HLA-DR molecules that bind a *Borrelia burgdorferi* peptide. *J Exp Med* 2006; **203:** 961–71.

31 Cabello FC, Godfrey HP, Newman SA. Hidden in plain sight: *Borrelia burgdorferi* and the extracellular matrix. *Trends Microbiol* 2007; **15:** 350–54.

32 Strle K, Drouin EE, Shen S, et al. *Borrelia burgdorferi* stimulates macrophages to secrete higher levels of cytokines and chemokines than *Borrelia afzelii* or *Borrelia garinii*. *J Infect Dis* 2009; **200:** 1936–43.

33 Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2006; **43:** 1089–134.

34 Berglund J, Eitrem R, Ornstein K, et al. An epidemiologic study of Lyme disease in southern Sweden. *N Engl J Med* 1995; **333:** 1319–27.

35 Huppertz HI, Böhme M, Standaert SM, Karch H, Plotkin SA. Incidence of Lyme borreliosis in the Würzburg region of Germany. *Eur J Clin Microbiol Infect Dis* 1999; **18:** 697–703.

36 Strle F. Lyme borreliosis in Slovenia. *Zentralbl Bakteriol* 1999; **289:** 643–52.

37 Steere AC, Sikand VK, Meurice F, et al. Vaccination against Lyme disease with recombinant *Borrelia burgdorferi* outer-surface lipoprotein A with adjuvant. Lyme Disease Vaccine Study Group. *N Engl J Med* 1998; **339:** 209–15.

38 Sigal LH, Zahradnik JM, Lavin P, et al. A vaccine consisting of recombinant *Borrelia burgdorferi* outer-surface protein A to prevent Lyme disease. Recombinant Outer-Surface Protein A Lyme Disease Vaccine Study Consortium. *N Engl J Med* 1998; **339:** 21–22.

39 Aucott J, Morrison C, Munoz B, Rowe PC, Schwarzwalder A, West SK. Diagnostic challenges of early Lyme disease: lessons from a community case series. *BMC Infect Dis* 2009; **9:** 79.

40 Anonymous. Institute of Public Health of the Republic of Slovenia. 2008 Health Statistics Yearbook. Surveillance of communicable diseases. Ljubljana 2009.

41 Anonymous. Institute of Public Health of the Republic of Slovenia. Registered communicable diseases in Slovenia in 2009. Ljubljana 2010.

42 CDC, Division of Vector-borne Infectious Diseases. Lyme Disease. http://www.cdc.gov/ncidod/dvbid/lyme/index.htm (accessed Nov 28, 2010).

43 Bacon RM, Kugeler KJ, Mead PS. Centers for Disease Control and Prevention. Surveillance for Lyme disease—United States, 1992–2006. *MMWR Morb Mortal Wkly Rep* 2008; **57:** 1–9.

44 Stanek G, Strle F. Lyme borreliosis. *Lancet* 2003; **362:** 1639–47.

45 Stanek G, Fingerle V, Hunfeld KP, et al. Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. *Clin Microbiol Infect* 2011; **17:** 69–79.

46 Weber K, Preac-Mursic V, Reimers CD. Spirochetes isolated from two patients with morphea. *Infection* 1988; **16:** 25–26.

47 Aberer E, Stanek G, Ertl M, Neumann R. Evidence for spirochetal origin of circumscribed scleroderma (morphea). *Acta Derm Venereol (Stockh)* 1987; **67:** 225–31.

48 Zollinger T, Mertz KD, Schmid M, Schmitt A, Pfaltz M, Kempf W. *Borrelia* in granuloma annulare, morphea and lichen sclerosus: a PCR-based study and review of the literature. *J Cutan Pathol* 2010; **37:** 571–77.

49 Asbrink E, Hovmark A. Early and late cutaneous manifestations in *Ixodes*-borne borreliosis (erythema migrans borreliosis, Lyme borreliosis). *Ann NY Acad Sci* 1988; **539:** 4–15.

50 Asbrink E, Hovmark A, Weber K. Acrodermatitis chronica atrophicans. Weber K, Burgdorfer W, eds. Aspects of Lyme borreliosis. Berlin, Heidelberg, New York; Springer-Verlag, 1993: 193–204.

51 Cerroni L, Zöchling N, Pütz B, Kerl H. Infection by *Borrelia burgdorferi* and cutaneous B-cell lymphoma. *J Cutan Pathol* 1997; **24:** 457–61.

52 Kütting B, Bonsmann G, Metze D, Luger TA, Cerroni L. *Borrelia burgdorferi*-associated primary cutaneous B cell lymphoma: complete clearing of skin lesions after antibiotic pulse therapy or intralesional injection of interferon alfa-2a. *J Am Acad Dermatol* 1997; **36:** 311–14.

53 Schöllkopf C, Melbye M, Munksgaard L, et al. *Borrelia* infection and risk of non-Hodgkin lymphoma. *Blood* 2008; **111:** 5524–29.

54 Li C, Inagaki H, Kuo TT, Hu S, Okabe M, Eimoto T. Primary cutaneous marginal zone B-cell lymphoma: a molecular and clinicopathologic study of 24 Asian cases. *Am J Surg Pathol* 2003; **27:** 1061–69.

55 Wood GS, Kamath NV, Guitart J, et al. Absence of *Borrelia burgdorferi* DNA in cutaneous B-cell lymphomas from the United States. *J Cutan Pathol* 2001; **28:** 502–07.

56 Stanek G, O'Connell S, Cimmino M, et al. European Union concerted action on risk assessment in Lyme borreliosis: clinical case definitions for Lyme borreliosis. *Wien Klin Wochenschr* 1996; **108:** 741–47.

57 Halperin JJ. Nervous system Lyme disease. *Infect Dis Clin N Am* 2008; **22:** 275–88.

58 Halperin JJ, Shapiro ED, Logigian E, et al. Practice parameter: treatment of nervous system Lyme disease—a report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2007; **69:** 91–102.

59 Halperin JJ, Logigian EL, Finkel MF, Pearl RA. Practice parameters for the diagnosis of patients with nervous system Lyme borreliosis (Lyme disease)—Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 1996; **46:** 619–27.

60 Kristoferitsch W. Neurological manifestations of Lyme borreliosis: clinical definition and differential diagnosis. *Scand J Infect Dis* 1991; **77:** 64–73.

61 Hansen K, Lebech AM. The clinical and epidemiological profile of Lyme neuroborreliosis in Denmark 1985-1990. A prospective study of 187 patients with *Borrelia burgdorferi* specific intrathecal antibody production. *Brain* 1992; **115:** 399–423.

62 Logigian EL, Kaplan RF, Steere AC. Chronic neurologic manifestations of Lyme disease. *N Engl J Med* 1990; **323:** 1438–44.

63 Steere AC, Glickstein L. Elucidation of Lyme arthritis. *Nat Rev Immunol* 2004; **4:** 143–45.

64 Brouqui P, Bacellar F, Baranton G, et al. ESCMID Study Group on *Coxiella*, *Anaplasma*, *Rickettsia* and *Bartonella*; European network for surveillance of tick-borne diseases—guidelines for the diagnosis of tick-borne bacterial diseases in Europe. *Clin Microbiol Infect* 2004; **10:** 1108–32.

65 Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR Morb Mortal Wkly Rep* 1995; **44:** 590–91.

66 Branda JA, Aguero-Rosenfeld ME, Ferraro MJ, Johnson BJ, Wormser GP, Steere AC. 2-tiered antibody testing for early and late Lyme disease using only an immunoglobulin G blot with the addition of a VlsE band as the second-tier test. *Clin Infect Dis* 2010; **50:** 20–06.

67 Steere AC, McHugh G, Damle N, Sikand VK. Prospective study of serologic tests for Lyme disease. *Clin Infect Dis* 2008; **47:** 188–95.

68 Wilske B, Fingerle V, Schulte-Spechtel U. Microbiological and serological diagnosis of Lyme borreliosis. *FEMS Immunol Med Microbiol* 2007; **49:** 13–21.

69 Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. *Clin Microbiol Rev* 2005; **18:** 484–509.

70 Hilton E, DeVoti J, Benach JL, et al. Seroprevalence and seroconversion for tick-borne diseases in a high-risk population in the northeast United States. *Am J Med* 1999; **106:** 404–09.

71 Cetin E, Sotoudeh M, Auer H, Stanek G. Paradigm Burgenland: risk of *Borrelia burgdorferi* sensu lato infection indicated by variable seroprevalence rates in hunters. *Wien Klin Wochenschr* 2006; **118:** 677–81.

72 Mygland A, Ljøstad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. *Eur J Neurol* 2010; **17:** 8–16.

73 Cerar T, Ogrinc K, Strle F, Ruzić-Sabljić E. Humoral immune responses in patients with Lyme neuroborreliosis. *Clin Vaccine Immunol* 2010; **17:** 645–50.

74 Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. *N Engl J Med* 1994; **330:** 229–34.

75 Avery RA, Frank G, Eppes SC. Diagnostic utility of *Borrelia burgdorferi* cerebrospinal fluid polymerase chain reaction in children with Lyme meningitis. *Pediatr Infect Dis J* 2005; **24:** 705–08.

76 Marques A, Brown MR, Fleisher TA. Natural killer cell counts are not different between patients with post-Lyme disease syndrome and controls. *Clin Vaccine Immunol* 2009; **16:** 1249–50.

77 Hunfeld KP, Ruzic-Sabljic E, Norris DE, Kraiczy P, Strle F. In vitro susceptibility testing of *Borrelia burgdorferi* sensu lato isolates cultured from patients with erythema migrans before and after antimicrobial chemotherapy. *Antimicrob Agents Chemother* 2005; **49:** 1294–301.

78 Morgenstern K, Baljer G, Norris DE, Kraiczy P, Hanssen-Hübner C, Hunfeld KP. In vitro susceptibility of *Borrelia spielmanii* to antimicrobial agents commonly used for treatment of Lyme disease. *Antimicrob Agents Chemother* 2009; **53:** 1281–84.

79 Wormser GP, Ramanathan R, Nowakowski J, et al. Duration of antibiotic therapy for early Lyme disease. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 2003; **138:** 697–704.

80 Kowalski TJ, Tata S, Berth W, Mathiason MA, Agger WA. Antibiotic treatment duration and long-term outcomes of patients with early Lyme disease from a Lyme disease-hyperendemic area. *Clin Infect Dis* 2010; **50:** 512–20.

81 Cerar D, Cerar T, Ruzić-Sabljić E, Wormser GP, Strle F. Subjective symptoms after treatment of early Lyme disease. *Am J Med* 2010; **123:** 79–86.

82 Oksi J, Nikoskelainen J, Hiekkanen H, et al. Duration of antibiotic treatment in disseminated Lyme borreliosis: a double-blind, randomized, placebo-controlled, multicenter clinical study. *Eur J Clin Microbiol Infect Dis* 2007; **26:** 571–81.

83 Wormser GP, Nowakowski J, Nadelman RB. Duration of treatment for Lyme borreliosis: time for a critical reappraisal. *Wien Klin Wochenschr* 2002; **114:** 613–15.

84 Weber K, Preac-Mursic V, Wilske B, Thurmays R, Neubert U, Scherwitz C. A randomized trial of ceftriaxone versus oral penicillin for the treatment of early European Lyme borreliosis. *Infection* 1990; **18:** 91–96.

85 Dattwyler RJ, Wormser GP, Rush TJ, et al. A comparison of two treatment regimens of ceftriaxone in late Lyme disease. *Wien Klin Wochenschr* 2005; **117:** 393–97.

86 Ljøstad U, Skogvoll E, Eikeland R, et al. Oral doxycycline versus intravenous ceftriaxone for European Lyme neuroborreliosis: a multicentre, non-inferiority, double-blind, randomised trial. *Lancet Neurol* 2008; **7:** 690–95.

87 Kalish RA, Kaplan RF, Taylor E, Jones-Woodward L, Workman K, Steere AC. Evaluation of study patients with Lyme disease, 10-20 year follow-up. *J Infect Dis* 2001; **183:** 453–60.

88 Steere AC, Angelis SM. Therapy for Lyme arthritis: strategies for the treatment of antibiotic-refractory arthritis. *Arthritis Rheum* 2006; **54:** 3079–86.

89 Lakos A, Solymosi N. Maternal Lyme borreliosis and pregnancy outcome. *Int J Infect Dis* 2010; **14:** 494–98.

90 Maraspin V, Cimperman J, Lotric-Furlan S, Pleterski-Rigler D, Strle F. Treatment of erythema migrans in pregnancy. *Clin Infect Dis* 1996; **22:** 788–93.

91 Klempner MS, Hu LT, Evans J, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. *N Engl J Med* 2001; **345:** 85–92.

92 Krupp LB, Hyman LG, Grimson R, et al. Study and treatment of post Lyme disease (Stop-LD). A randomized double-masked clinical trial. *Neurology* 2003; **60**: 1923–30.

93 Cairns V, Godwin J. Post-Lyme borreliosis syndrome: a meta-analysis of reported symptoms. *Int J Epidemiol* 2005; **34:** 1340–45.

94 Marques A. Chronic Lyme disease: a review. *Infect Dis Clin North Am* 2008; **22:** 341–60.

95 Skogman BH, Croner S, Nordwall M, Eknefelt M, Ernerudh J, Forsberg P. Lyme neuroborreliosis in children: a prospective study of clinical features, prognosis, and outcome. *Pediatr Infect Dis J* 2008; **27:** 1089–94.

96 Seltzer EG, Gerber MA, Cartter ML, Freudigman K, Shapiro ED. Long-term outcomes of persons with Lyme disease. *JAMA* 2000; **283:** 609–16.

97 Vrethem M, Hellblom L, Widlund M, et al. Chronic symptoms are common in patients with neuroborreliosis—a questionnaire follow-up study. *Acta Neurol Scand* 2002; **106:** 205–08.

98 Berglund J, Stjernberg L, Ornstein K, Tykesson-Joelsson K, Walter H. 5-y follow-up study of patients with neuroborreliosis. *Scand J Infect Dis* 2002; **34:** 421–25.

99 Fallon BA, Keilp JG, Corbera KM, et al. A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy. *Neurology* 2008; **70:** 992–1003.

100 Lantos PM, Charini WA, Medoff G, et al. Final report of the Lyme disease review panel of the Infectious Diseases Society of America. *Clin Infect Dis* 2010; **51:** 1–5.

101 Solomon SP, Hilton E, Weinschel BS, Pollack S, Grolnick E. Psychological factors in the prediction of Lyme disease course. *Arthritis Care Res* 1998; **11:** 419–26.

102 Hassett AL, Radvanski DC, Buyske S, et al. Role of psychiatric co-morbidity in chronic Lyme disease. *Arthritis Rheum* 2008; **59:** 1742–49.

103 Chandra A, Wormser GP, Klempner MS, et al. Anti-neural antibody reactivity in patients with a history of Lyme borreliosis and persistent symptoms. *Brain Behav Immune* 2010; **24:** 1018–24.

104 Weissenbacher S, Ring J, Hofmann H. Gabapentin for the symptomatic treatment of chronic neuropathic pain in patients with late-stage Lyme borreliosis: a pilot study. *Dermatology* 2005; **211:** 123–27.

105 Hickie I, Davenport T, Wakefield D, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. *BMJ* 2006; **333:** 575.

106 Steere AC, Taylor E, McHugh GL, Logigian EL. The overdiagnosis of Lyme disease. *JAMA* 1993; **269:** 1812–16.

107 Feder HM Jr, Johnson BJ, O'Connell S, et al. A critical appraisal of "chronic Lyme disease". *N Engl J Med* 2007; **357:** 1422–30.

108 Auwaerter PG, Bakken JS, Dattwyler RJ, et al. Scientific evidence and best patient care practices should guide the ethics of Lyme disease activism. *J Med Ethics* 2011; **37:** 68–73.

109 Cooper JD, Feder HM Jr. Inaccurate information about Lyme disease on the internet. *Pediatr Infect Dis J* 2004; **23:** 1105–08.

110 Sood SK. Effective retrieval of Lyme disease information on the Web. *Clin Infect Dis* 2002; **35:** 451–64.

111 Reid MC, Schoen RT, Evans J, Rosenberg JC, Horwitz RI. The consequences of over diagnosis and over treatment of Lyme disease: an observational study. *Ann Intern Med* 1998; **128:** 354–62.

112 Johnson M, Feder HM Jr. Chronic Lyme disease: A survey of Connecticut primary care physicians. *J Pediatr* 2010; **157:** 1025–29.

113 Holzbauer SM, Kemperman MM, Lynfield R. Death due to community-associated *Clostridium difficile* in a woman receiving prolonged antibiotic therapy for suspected Lyme disease. *Clin Infect Dis* 2010; **51:** 369–70.

114 Patel R, Grogg KL, Edwards WD, Wright AJ, Schwenk NM. Death from inappropriate therapy for Lyme disease. *Clin Infect Dis* 2000; **31:** 1107–09.

115 Lotric-Furlan S, Petrovec M, Avsic-Zupanc T, et al. Prospective assessment of the etiology of acute febrile illness after a tick bite in Slovenia. *Clin Infect Dis* 2001; **33:** 503–10.

116 Swanson JS, Neitzel D, Reed KD, Belongia EA. Coinfections acquired from Ixodes ticks. *Clin Microbiol Rev* 2006; **19:** 708–27.

117 Telford SR 3rd, Wormser GP. *Bartonella* spp. Transmission by ticks not established. *Emerg Infect Dis* 2010; **16:** 379–84.

118 Krause PJ, Foley DT, Burke GS, Christianson D, Closter L, Spielman A. Tick-Borne Disease Study Group. Reinfection and relapse in early Lyme disease. *Am J Trop Med Hyg* 2006; **75:** 1090–94.

119 Nadelman RB, Wormser GP. Reinfection in patients with Lyme disease. *Clin Infect Dis* 2007; **45:** 1032–38.

120 Nowakowski J, Schwartz I, Nadelman RB, Liveris D, Aguero-Rosenfeld M, Wormser GP. Culture-confirmed infection and reinfection with *Borrelia burgdorferi*. *Ann Intern Med* 1997; **127:** 130–32.

121 Golde WT, Robinson-Dunn B, Stobierski MG, et al. Culture-confirmed reinfection of a person with different strains of *Borrelia burgdorferi* sensu stricto. *J Clin Microbiol* 1998; **36:** 1015–19.

122 Stafford KI, Kitron U. Environmental management of Lyme borreliosis control. In: Gray JS, Kahl O, Lane RS, Stanek G, eds. Lyme borreliosis: biology, epidemiology and control". New York; CABI Publishing, 2002: 301–34.

123 Connally NP, Durante AJ, Yousey-Hindes KM, Meek JI, Nelson RS, Heimer R. Peridomestic Lyme disease prevention: results of a population-based case-control study. *Am J Prev Med* 2009; **37:** 201–06.

124 Warshafsky S, Lee DH, Francois LK, Nowakowski J, Nadelman RB, Wormser GP. Efficacy of antibiotic prophylaxis for the prevention of Lyme disease: an updated systematic review and meta-analysis. *J Antimicrob Chemother* 2010; **65:** 1137–44.

125 Piesman J. Lyme borreliosis in North America. In: Gray JS, Kahl O, Lane RS, Stanek G, eds. Lyme borreliosis: biology, epidemiology and control. New York; CABI Publishing, 2002: 223–49.

126 Gern L, Humair PF. Lyme borreliosis in Europe. In: Gray JS, Kahl O, Lane RS, Stanek G, eds. Lyme borreliosis: biology, epidemiology and control. New York; CABI Publishing, 2002: 149–74.

127 Korenberg EI, Vorobyeva NN, Moskvitina HG, Gorban' LYa. Prevention of borreliosis in persons bitten by infected ticks. *Infection* 1996; **24:** 187–89.

128 Shapiro ED, Gerber MA, Holabird NB, et al. A controlled trial of antimicrobial prophylaxis for Lyme disease after deer-tick bites. *N Engl J Med* 1992; **327:** 1769–73.

129 Stanek G, Kahl O. Chemoprophylaxis for Lyme borreliosis? *Zentralbl Bakteriol* 1996; **289:** 655–65.