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INFECTIOUS DISEASE EPIDEMIOLOGY (AL REINGOLD, SECTION EDITOR)

Lyme Disease

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Abstract Lyme disease is caused by the spirochetal bacteria *Borrelia burgdorferi* and transmitted by ticks in the genus *Ixodes*. The key reservoirs for the spirochete include rodents and birds, and the primary hosts for ticks include rodents, birds, and lizards for immature stages and large mammal for the adults. Since its recognition in the USA in the 1970s, it has continued to emerge, increasing both in case numbers and geographic distribution. In the last two decades, a number of new findings have been observed, including a vast increase in disease distribution, additional *Borrelia* species causing disease in humans, and newly recognized clinical presentations of the disease. Areas of greatest need include (1) new diagnostic tests, including tests that detect *Borrelia* DNA, antigens, or metabolites, (2) a better understanding of disease pathogenesis particularly in the case of post-treatment Lyme disease syndrome, and (3) the development of safe and effective interventions.

Keywords Lyme disease · *Borrelia* · Epidemiology · *Ixodes* · Tick-borne disease

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Introduction

Lyme disease or Lyme borreliosis is a zoonotic infection caused by a group of closely related spirochetes in the genus *Borrelia*. In the USA, *Borrelia burgdorferi* is the primary agent of Lyme disease, while two additional genospecies, *Borrelia garinii* and *Borrelia afzelii,* account for the majority of cases in Europe and Asia. Lyme disease was first formally recognized in the USA in the 1970s following an outbreak of illness resembling juvenile rheumatoid arthritis among children living in Lyme, Connecticut [1]. The unique features of these cases—the rural setting, occurrence in the warmer months of the year, tight geographic clustering, and rash suggestive of an insect or arthropod bite—led to the suspicion that it could be transmitted by ticks [2, 3]. As the full clinical spectrum of the disease became known, it was recognized that similar illness had been reported in Europe half a century earlier [4]. Subsequent studies identified the causative agent and revealed that it is maintained in nature in a transmission cycle typically involving rodents, birds, deer, and ticks of the genus *Ixodes* [5–7].

This review describes the basic epidemiology, ecology, and clinical features of Lyme disease, with a focus on new observations and trends. The specific topics that will be addressed include changes in disease incidence and distribution that have occurred over the last two decades, newly identified *Borrelia* spp. that are causing disease in humans, current diagnostic algorithms and the most promising new diagnostic targets, the recent observations of Lyme carditis and sudden cardiac death, and the debate over the possible causes of persistent symptoms in patients who have received recommended antibiotic treatment regimens.

Epidemiology-Disease Ecology, Distribution, and Burden

Both the maintenance of Lyme disease in natural reservoir hosts and also the transmission to humans involve ticks in the genus *Ixodes*. While several different *Ixodes* species may be involved in reservoir maintenance, transmission to humans is mediated by *Ixodes scapularis* in the central and eastern USA and *Ixodes pacificus* in the western USA [8]. While deer serve as the primary hosts for adult *I. scapularis* and *pacificus* ticks and are generally required for long-term maintenance of vector tick populations, they are not competent hosts for *B. burgdorferi*. Small rodents serve as primary hosts for immature stages of the ticks and also as reservoirs for the spirochete [9]. In the central and eastern parts of the USA, small rodents such as the white-footed mice, chipmunks, voles, and shrews are the key reservoir hosts, with birds playing an important role not only as reservoirs but also in the geographic expansion of tick populations. In the southern and western USA, lizards often serve as hosts for immature *I. scapularis* and *I. pacificus* ticks, respectively. Because lizards are naturally refractory to *Borrelia* infection, they play a protective role by reducing infection rates in tick populations throughout this region [10, 11]. In the western USA, squirrels have an important role as reservoir hosts for *B. burgdorferi* and *I. pacificus* [11].

Many different factors contribute to the risk for humans to be exposed to infected vector ticks. Entomological risk is a term that generally refers to the abundance of questing infected nymphal *I. scapularis*, which are the principal source of human infection. Epidemiological risk, however, incorporates additional elements of human behavior and demographics.

Lyme disease has been nationally notifiable in the USA since 1991 [12]. In 2014, 33,461 cases were reported making it the 5th most common of over 60 reportable diseases and conditions in the USA [13]. As is the case with other high-incidence reportable illnesses, Lyme disease is frequently under-reported. Previous under-reporting studies have estimated the actual number of cases to be 3- to 12-fold higher than reported cases [14–16]. Two recent studies used independent methods to calculate estimates in the annual number of cases in the USA. The first was a survey of the major diagnostic companies that account for the largest numbers of Lyme disease diagnostic tests performed in the USA each year. The study reported that 3.4 million Lyme tests are performed annually on 2.9 million patients, resulting in an estimated 288,000 diagnosed Lyme cases each year (range 244,000–440,000) at a cost of over $490 million per year for testing alone [17]. The second study utilized information from a large national insurance database that incorporated billing data (ICD-9 codes) drawn from over 103 million person-years. This study calculated a similar estimation of annual Lyme disease infections at 329,000 patients treated per year with a range of 296,000 to 376,000 [18••].

Not only have Lyme disease case numbers and incidence increased in the USA over the last 20 years, but the distribution of reported cases has expanded significantly in geographic range [12]. In the northeastern USA the distribution of reported cases has spread westward across Pennsylvania and Massachusetts, northward up the Hudson River Valley in New York and through New Hampshire, Vermont and coastal Maine, and southward through Maryland, Delaware, into Virginia. Locally acquired cases have now been reported in West Virginia and in parts of North Carolina. In the upper Midwestern USA, cases have spread from a diffuse focus across central Minnesota and Wisconsin in all directions, into North Dakota, and down toward the Ohio River Valley [12]. A recent study reported that over the time period from 1993 to 2012, the numbers of high-incidence counties increased by >320 % in northeastern states and by >250 % in the north-central USA [19•]. The drivers for disease emergence are very likely multifaceted. One of the factors frequently cited is reforestation and changing land use patterns over the last century, which have led to expanding deer populations [20, 21]. A second likely contributing factor is suburban growth and design that brings both deer and also small rodents such as white-footed mice into close proximity to human dwellings [22]. As a result, people are more likely to be exposed to the bites of vector ticks. Other contributing factors that have been suggested include habitat fragmentation and the corresponding loss of biodiversity [23] and climate change [24]. A recent article on the distribution of Lyme disease vector ticks reported a 44.77 % increase from 1996 to 2015 in the numbers of counties where *I. scapularis* or *pacificus* have been identified, with these species now reported in 49.2 % of counties in 43 states in the USA [25]. The number of counties where *I. scapularis* is now established has more than doubled in the last 20 years.

Disease Etiology

Although nearly 20 distinct *B. burgdorferi* sensu lato genospecies have been described in animals, few are known to cause human infections that lead to Lyme disease [26]. In Europe, *B. garinii*, *B. afzelii*, and *B. burgdorferi* sensu stricto (a.k.a. *B. burgdorferi*) cause nearly all cases. Until recently, only *B. burgdorferi* has been documented as causing human disease in the USA. In 2016, Pritt et al. reported isolating a new pathogenic genospecies infecting patients exposed to ticks in the upper Midwest. Among the six patients described, the new genospecies (candidatus *Borrelia mayonii)*, presented with clinical manifestations that were similar to Lyme disease caused by *B. burgdorferi*. However, the documented illnesses were generally more severe with three patients exhibiting

neurologic symptoms and two hospitalized, and the concentration of spirochetes in the blood was estimated to be nearly 200-fold higher than observed with *B. burgdorferi* infections. The new genospecies was not identified in more than 90,000 clinical samples submitted to the same clinic in previous years, suggesting that this species had emerged recently [27••].

A *Borrelia bissettii*-like isolate has also been recovered recently from a single patient in Florida [28, 29]. Previous studies based on DNA detection had suggested that *B. bissettii* could infect humans, [30] but it had never been isolated in culture until recently. Further studies are required to determine whether or not this isolate represents a new *Borrelia* sensu lato genospecies and what is its importance in causing Lyme disease-like illness.

Clinical Presentations

The clinical manifestations of Lyme disease have been well described [31]. The earliest manifestation is typically the erythema migrans (EM) lesion that starts at the site of the tick bite. EM is classically described as a "bull's eye" rash; however, the majority of EM lesions do not take this form [32]. More commonly, the rash is circular or oval, and homogenously erythematous. Early infection is often accompanied by fevers, chills, headache, and other non-specific signs of infection. From the original EM lesion, the bacteria can disseminate throughout the body to other sites, often within days of the original lesion. Manifestations at this early disseminated stage of the disease include meningitis, facial palsy (and, less commonly, other cranial nerve neuropathies), radiculopathy, and carditis causing conduction abnormalities. The relative frequency of specific manifestations of infection may vary depending on the infecting strain. For example, *B. garinii* is thought to cause meningitis and radiculitis more often than *B. burgdorferi* [33].

While carditis is a known complication of Lyme disease, the potential for sudden death due to cardiac Lyme disease was largely unrecognized until the recent report of three deaths due to Lyme disease diagnosed post-mortem [34]. Two of the three patients had non-specific symptoms preceding their death. Spirochetes were detected in the myocardial tissue of all three subjects and serologic evidence of infection with *B. burgdorferi* was found post-mortem. While carditis occurs in approximately 1 % of patients with early-stage Lyme disease, sudden death due to Lyme disease remains rare [35].

The majority of the manifestations of Lyme disease will resolve even without treatment. If the diagnosis is missed during early-stage disease, patients may progress to late-stage manifestations, months after the original infection. In the USA, the most common late-stage manifestation is arthritis [36]. Lyme arthritis is typically oligoarticular and episodic. Other manifestations of late-stage Lyme disease include encephalopathy and acrodermatitis atrophicans, a cutaneous manifestation associated with the European strain *B. afzelii.*

Diagnosis

Serologic testing can be useful when evaluating patients where Lyme disease is suspected. For infections potentially acquired in the USA, CDC currently recommends a two-tiered approach to serologic testing [37]. The first tier is an immunoassay (ELISA/EIA) using whole-cell (WCS), recombinant, or synthetic peptide antigens or, rarely, an immunofluorescense assay (IFA). If the results of the first test are positive or indeterminate, supplementary Western blots for IgG or IgM anti-*B. burgdorferi* antibodies are performed to increase testing specificity. As with other serologic tests, the sensitivity and specificity of this two-tiered approach varies by stage of disease. Two-tier testing is relatively insensitive (~40–60 %) for stage I localized disease. However, in parallel with clinical duration and evidence of disseminated infection, two-tier test sensitivity increases (80−>90 %) for stage 2 and 3 infection [38]. Properly performed and interpreted, two-tier testing specificity is greater than 99 % [39].

An alternative 2 EIA algorithm, where a WCS EIA followed by the C6 EIA as the second-step test instead of the Western blot, shows promise for identification of Lyme disease caused by *B. burgdorferi* sensu stricto. In several published studies, this method has shown sensitivity and specificity equal to or greater than standardized two-tier testing for *B. burgdorferi* [38, 40]. In addition, this algorithm has the potential to provide a substantial savings compared to traditional two-tiered testing in terms of cost and time [41], with a vast improvement in ease of interpretation due to loss of subjective banding patterns. In one study, it was estimated that transitioning to this algorithm could reduce the total cost of testing in the USA ($492 million) by $57 million dollars [17]. Initial findings on the performance of standardized two-tier testing or the 2 EIA approach discussed above for a novel agent of Lyme disease in the USA *Borrelia mayonii* suggests a sensitivity similar to that for *B. burgdorferi* sensu stricto [27••]. However, the case numbers in that study were limited, and further studies are needed to better define and possibly improve test performance.

Most new Lyme disease testing alternatives aim to move away from the IgM WB due to the recognized potential for false positive test results [38]. One specific approach is to incorporate the VlsE band into the IgG WB [42] for the second-tier approach. Compared with traditional two-tiered testing using an IgM WB for early disease, the new IgG algorithm has improved sensitivity, particularly for those patients having early disseminated illness and equivalent specificity to traditional two-tiered testing performance.

Other alternatives to the traditional two-tiered serologic testing algorithm have been recently proposed to improve accuracy and reduce time and cost of Lyme disease testing [38]. One area of focus has been the development of EIAs as standalone tests that have fewer cross-reactive antigens [43]. For example, the C6 peptide EIA (mentioned above) uses a highly invariant region of the *B. burgdorferi* VlsE protein to achieve greater specificity than with most WCS EIAs [44]. However, the single EIA alternatives have not significantly improved the sensitivity of testing for early disease [45]. A combination of serologic and molecular techniques has been proposed with some very early success in animals with an immuno-PCR (iPCR) test aimed at detection of antibodies to the C6 peptide [46]. Lahey et al. (2015) has examined novel and established antigen markers to develop a multiplex panel test [47]. In a study of samples from three patient cohorts, their 10-antigen panel identified a significantly higher proportion of early Lyme disease patients compared with traditional two-tiered testing (87.5 and 67.5 %, respectively; $P < 0.05$).

Molins et al. (2015) recently described a test approach that detects patterns of serum metabolites utilizing liquid chromatography-mass spectrometry to identify early Lyme disease patients [48]. In this study, the sensitivity of testing for patients with early Lyme disease was significantly improved (88 % compared to 29–40 %) over traditional two-tiered testing. Of note, the platform for this novel method of testing is already present in many clinical laboratories where it is used for newborn screening tests.

Regardless of the method used, diagnostic tests have lower predictive value in geographic areas with a low incidence of Lyme disease. Current recommendations do not support serologic testing for patients having a low clinical pre-test probability of the disease, such as patients who lack objective findings and have only non-specific symptoms, such as fatigue [49]. In a recent study of Lyme disease in four low-incidence states, it was estimated that all patients identified as having positive IgG EIA results, and no history of travel could feasibly have represented false positive results given a specificity of 95 % for the two-tiered algorithm [50]. Similarly, in a study by Lantos et al. (2015), only 76 (1.6 %) of 4723 patients tested for Lyme disease from a low-incidence area had positive two-tiered testing results by established laboratory criteria. Among the 70 seropositive patients where medical information was available, 64 (91 %) either had a history of travel to a Lyme disease endemic area or did not have clinical presentations compatible with disseminated Lyme disease [51].

Treatment

The most commonly used antibiotics to treat Lyme disease are tetracyclines, penicillins, oral second-generation cephalosporins (cefuroxime axetil), macrolides and, when intravenous therapy is preferred, third-generation cephalosporins [31]. Oral doxycycline is the preferred agent in adults and children over 8 years of age as it is well absorbed orally, has good penetration into the central nervous system, and is active against *Anaplasma phagocytophilum*, which can be co-transmitted with *B. burgdorferi*. All of the commonly used agents are thought to be active against the recently identified pathogens, *B. mayonii* and *Borrelia miyamotoi*.

Oral therapy is recommended for most manifestations of Lyme disease including EM, cranial nerve palsies, mild cardiac disease (e.g., 1st-degree AV block) and arthritis [31]. Intravenous therapy has been recommended for meningitis, radiculopathy, more severe cardiac disease, and arthritis that did not respond to a first course of oral antibiotics; however, superiority of intravenous antibiotics has not been demonstrated in clinical trials for any of these manifestations. In fact, multiple studies in European patients have found that oral doxycycline results in equivalent outcomes to intravenous therapy in patients with neurologic Lyme disease including meningitis and radiculopathies [52–56]. Although this has not been demonstrated in the USA, there is no data to suggest the European strains of *Borrelia* are more sensitive to doxycycline than *B. burgdorferi* sensu stricto in the USA.

Persistence

While the majority of patients treated for Lyme disease recover within several months, a small percentage of patients will continue to report subjective symptoms of fatigue, arthralgias, myalgias, and difficulties with memory and concentration greater than 12 months after completion of therapy for Lyme disease [57]. This condition has been variously referred to as "chronic Lyme disease" or "post-Lyme disease syndrome" (PTLDS). The cause of these symptoms remains unknown. The major competing hypotheses include the following: (1) failure to fully eradicate bacteria, (2) lingering effects of the damage caused during the active infection, (3) development of autoimmunity due to cross-reaction to *B. burgdorferi* antigens, and (4) inflammation due to responses to remainders of the killed organism.

Regardless of the mechanism, multiple studies have now shown that additional or prolonged courses of otherwise effective antibiotics do not result in sustained improvement relative to placebo and cannot be recommended [58–61].

Recently, multiple laboratories have documented the occurrence of "persister" cells among populations of *B. burgdorferi* grown in vitro and treated with antibiotics [62–64]. This in vitro phenomenon has been described with many other bacterial species and is thought to occur because some cells within a population will invariably be in a metabolic state that makes them less susceptible to antimicrobial agents [65, 66]. The mechanism of resistance is non-genetic; regrowth from persister cells yields a population of cells with

the antimicrobial susceptibility comparable to the original population from which the persisters were derived. Compounds other than first-line antibiotics appear to be more effective at killing *B. burgdorferi* persisters in vitro, but there is currently insufficient evidence to support a clinical role for these agents [64, 67, 68].

Although *B. burgdorferi* has been shown to form persister cells in vitro, it is unknown whether this occurs in vivo. Animal studies in mice, dogs, and monkeys have shown that *B. burgdorferi* DNA can be detected long after treatment (up to 1 year) by PCR from animal tissues or by PCR from xenodiagnositic ticks that have fed on infected animals after treatment [69–72]. One laboratory has been able to show that *B. burgdorferi* RNA can be detected up to a year after treatment [71]. In none of the animal studies were investigators able to recover live, cultivable organisms raising the possibility that the detected nucleic acids are from dead organisms. Interestingly, ticks fed on infected, antibiotic-treated animals were able to transmit *B. burgdorferi* DNA to immunocompromised severe combined immunodeficiency (SCID) mice during a subsequent feed [70]. Given that the ticks take less than 1 ul of blood during their meal, it seems unlikely that they would be able to acquire and transmit sufficient amounts of DNA to be detected from another animal without the transfer of live, replicating organisms. However, live organisms were still not recoverable from the SCID mice. Many of the animal studies have been criticized for using doses of antibiotics that did not mimic human pharmacokinetics of the drugs [73]. While some have subsequently shown that dosing resulted in serum levels equivalent to those achieved by human dosing schedules, it is unclear whether underdosing may have affected the results.

It is important to note that neither the ability of *B. burgdorferi* to form persister cells in vitro, nor the detection of *B. burgdorferi* DNA in animals long after antibiotic treatment is evidence of involvement of live *B. burgdorferi* or immune reactions to its products as a cause for post-treatment Lyme disease. Unfortunately there are no animal models for post-treatment Lyme disease. Studies in humans have found evidence for *B. burgdorferi* or its DNA in a very small number of treated subjects, but have not proven a link between detection of *B. burgdorferi* or its products and post-treatment Lyme disease [74].

Conclusion

Since its recognition in the USA in the 1970s, Lyme disease continues to emerge, and with an estimated 300,000 or more cases each year in the USA, it remains today a highly significant public health concern. Recent observations on the changes in incidence and distribution, novel *Borrelia* species, previously unseen clinical presentations such as sudden cardiac death, and questions about the cause of persistent symptoms in patients with PTLDS emphasize the fact that there is still a great need for continued research. It was beyond the scope of this article to discuss prevention and control, but it is important to note that the Lyme disease vaccine LYMErix, which was pulled from the market in 2002, was the only intervention ever shown in a large-scale study to effectively prevent Lyme disease in humans [75]. In the absence of a vaccine, prevention recommendations focus on reducing exposure of people to vector ticks through a variety of methods including pesticide use, reservoir-targeted devices, landscape management, and personal protective products and measures, all of which are of limited efficacy in reducing human illness [76]. Taken together, these observations establish the need for progress in the following areas: (1) new diagnostic test development including new types of tests that detect *Borrelia* DNA, antigens or metabolites, (2) a better understanding of disease pathogenesis, particularly in the case of PTLDS, and (3) the development of safe and effective interventions.

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Compliance With Ethical Standards

Conflict of Interest Charles B. Beard, Alison F. Hinckley, and Paul S. Mead declare that they have no conflict of interest.

Linden T. Hu declares personal fees for acting as a consultant for Abzyme, contracts to his institution from Massbiologics and Sanofi, and royalties from Uptodate.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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