Chapter 1

General introduction

Adapted from: Tired of Lyme borreliosis; Lyme borreliosis in the Netherlands. Jeroen Coumou, Tom van der Poll, Peter Speelman and Joppe W. Hovius.

Abstract

Lyme borreliosis (LB) has become the most common vector-borne illness in Northeastern parts of USA and Europe. It is a zoonotic disease with well-defined symptoms, caused by *B. burgdorferi* sensu lato, and transmitted by ticks. LB is endemic in the Netherlands with a yearly incidence of approximately 133 cases / 100,000 inhabitants. Similar to another spirochetal disease, syphilis, it can be divided into three stages; early, early disseminated and late disseminated manifestations of disease, of which the specific clinical presentations will be discussed in detail. The diagnosis of LB is based on a history of potential exposure to ticks and the risk of infection with *B. burgdorferi* s.l., development of specific symptoms, exclusion of other causes, and when appropriate, combined with serological and/or other diagnostic tests. The specific indications for, but also the limitations of, serology and other diagnostic tests, including the polymerase chain reaction (PCR), are detailed in this review. LB is treated with antibiotics, which are usually highly effective. Recent literature discussing the indications for antibiotic treatment, the dosage, duration and type of antibiotic, as well as indications to withhold antibiotic treatment are reviewed. This review presents recent and when available Dutch evidence-based information on the ecology, pathogenesis, clinical presentation, diagnosis, treatment and prevention of LB, argues against the many misconceptions that surround the disease, and provides a framework for the Dutch physician confronted with a patient with putative LB.
Introduction

Lyme disease, or Lyme borreliosis (LB), has become the most common tick-borne disease in the North-Eastern USA and Europe [259]. The disease is named after the town Old Lyme, Connecticut, USA (Figure 1A), where the link between a tick-borne disease and a group of children suspected of juvenile arthritis was noted in the mid-seventies [260]. Seven years later, the causative agent was discovered by Burgdorfer [32]. In Europe, syndromes, reported as early as 1883, among which Bannwarth syndrome (painful radiculitis, cranial neuritis and lymphocytic meningitis), can retrospectively be designated as manifestations of LB [16, 226]. LB is caused by spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) group (Figure 1B) [32]. In the USA, *Borrelia burgdorferi* sensu stricto, from here on referred to as *B. burgdorferi*, is the sole causative agent, whereas in Europe *Borrelia garinii* and *Borrelia afzelii* are the predominant causative agents and to a lesser extent *B. burgdorferi* and more recently also *Borrelia bavariensis* and *Borrelia spielmanii* [80, 257]. More *Borrelia* species, for example *Borrelia valaisiana* and *Borrelia lusitaniae* among others, have been identified in Europe, however for most of these species the pathogenicity to humans is not as clear [281]. In 2010, Dutch general practitioners (GPs) were estimated to have diagnosed early LB 22,000 times, corresponding with approximately 133 new cases of erythema migrans (see below) per year per 100,000 inhabitants. This number has increased from 6,500 in 1994, 13,000 in 2001 and 17,000 in 2005 [106, 107].

Ecology

In the USA *B. burgdorferi* is transmitted by the deer tick, *Ixodes scapularis*, whereas the European *Borrelia* species are transmitted by the sheep tick, *Ixodes ricinus* (Figure 1C). In general, uninfected tick larvae acquire the bacterium by feeding on infected animals. Ticks remain infected during their consecutive molting periods, enabling both nymphal and adult ticks to transmit spirochetes to other (larger) animals, including humans. After their final blood meal adult female ticks, which have already mated, lay usually uninfected eggs (Figure 2) [5]. The number of visits to Dutch GPs for tick bites rose from 371 per 100,000 in 2001, to 446 and 564 in 2005 and 2009, respectively [106]. In the Netherlands roughly 20 % of adult ticks are infected, compared to 10 % of nymphs, as shown by a European meta-analysis in 2005 [211]. In 2007, 38 % of all tick bites in the Netherlands happened in forests, 36 % in gardens and 10 % in dunes [273].
Figure 1. A. The town Old Lyme, Connecticut, USA. B. Fluorescence microscope image of the spirochete B. burgdorferi. C. Unfed, partially fed and fully engorged Ixodes ricinus ticks. We thank G.A. Oei for the picture.

Pathogenesis

Borrelia encounters different environments during its enzootic life cycle [114], for which differential expression of outer surface proteins (Osp’s) is crucial. In unfed ticks, spirochetes express OspA, which binds to the tick receptor of OspA (TROSPA), ensuring attachment of the spirochete to the tick gut [194]. In feeding ticks, approximately 24 to 48 hours after attachment, Borrelia down-regulates OspA, expresses OspC and migrates to the salivary glands [238, 306]. Here, OspC binds a tick salivary gland protein of 15 kDa (Salp15), shielding the spirochete from complement-dependent (antibody-mediated) killing when transmitted to the host [112, 209, 232]. Furthermore, we have previously shown that Salp15 exerts immunosuppressive activity [8]; inhibiting murine T-cell activation and suppressing human dendritic cell (DC) function [110], which could facilitate both tick feeding as well as Borrelia transmission. Numerous other tick proteins, that interact with other host defense mechanisms, facilitate tick feeding and/or enhance the transmission of Borrelia or other tick-borne pathogens from the tick to the host, have been identified, as we have previously reviewed [110]. Furthermore, a number of adhesins, proteins on the outer membrane of B. burgdorferi s.l. that are involved in the anchoring and interaction with host cells, have been identified, and are important for the establishment and dissemination of infection [42].

A striking feature of Borrelia is its ability to evade host immune response. One mechanism to evade host immune responses is the recombinant gene expression of the variable major protein-like sequence (vls) locus [78]. This results in altered antigenicity of the lipoprotein VlsE and thus protection against anti-VlsE antibodies [166]. Also, Borrelia can express complement regulator-acquiring surface
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Figure 1.
A. The town Old Lyme, Connecticut, USA.
B. Fluorescence microscope image of the spirochete Borrelia burgdorferi.
C. Unfed, partially fed and fully engorged Ixodes ricinus ticks. We thank G.A. Oei for the picture.

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Figure 2. Simplified diagram of the transmission cycle of B. burgdorferi sensu lato species in the Netherlands. Borrelia transmission is tightly interwoven with the tick reproductive cycle, which is estimated to take two years in the temperate climate zone. Uninfected eggs (vertical transmission, represented by the thin dashed line, seldomly occurs) hatch in summer and autumn and larvae feed in autumn before, or in spring, after winter diapause. In early spring the largest cohort of nympha ticks emerge, which may yield adult ticks in summer and autumn. In spring young hosts are infected by the emerged nymphs creating the possibility for larvae to acquire an infected blood meal which in its turn augments the abundance of infected nymphs (thick continuous lines “spring” and “summer”). On the right side of the diagram the cycle depicts the flow of Borrelia though avian populations (mainly B. garinii and B. valaisiana), while on the left side the flow of B. burgdorferi sensu stricto and B. afzelii, both with preference for mammals, is shown. However, all four species may be transferred by nympha1 and adult ticks (thin continuous lines) to large hosts such as deer, dogs and humans. We thank dr KE Hovius for the donation of the figure.
proteins (CRASPs), preventing complement mediated killing [138, 139]. Recently, another protein, Lmp1 was suggested to be important for evasion of the host adaptive immune responses. Yang et al showed that the N-terminal region of the protein increased pathogen survival [305]. Importantly, *B. afzelii* is associated with skin manifestations, *B. garinii* with neurological involvement and *B. burgdorferi* with infection of the large joints, however there is a fair amount of overlap between the tropisms of the different genospecies [267].

**Clinical manifestations**

*Tick bite*

In a Dutch study with 167 tick bite cases in a GP’s population, only one case (0.7 %) developed LB upon follow-up serology. Notably, this tick was attached longer than 24 hours [120]. In general, ticks attached shorter than 24 hours do not transmit *Borrelia* [200, 201]. Erythematous skin lesions smaller than 5 cm starting within 2 days after detachment of the tick are most likely a tick bite hypersensitivity reaction. Tick bite hypersensitivity should disappear within 1-2 days. Diagnostic tests or treatment after a possible tick bite, without symptoms of early LB (see below), are not recommended in the Netherlands [249]. A recent meta-analysis suggested, that, in highly endemic areas in the United States, one case of LB is prevented for every approximately 50 individuals who are prophylactic treated with antibiotics [291]. Importantly, asymptomatic infection is thought to be much more frequent in Europe than in the USA [115], arguing against the standard use of prophylactic antibiotics after a tick bite in Europe. However, in individual cases, when the tick was acquired in a highly endemic area of the Netherlands was attached for a longer period of time, i.e. more than 24-48 hours, and the patient presents within three days after the tick bite, prophylactic doxycycline (200 mg once) can be considered. Patient instructions to be alert for typical symptoms of (early) LB (see below) might be an equally effective alternative.

**Early LB (days to weeks)**

Typical EM is an expanding erythematous skin lesion with central clearing located at the site of tick bite starting after 3-30 days, typically after 7-14 days, which can vary from 5 to 75 cm (median 15 cm) (Figure 3A) [298]. Both systemic symptoms, such as fever, myalgias and arthralgias and local symptoms among which itching, burning, and mild pain can accompany EM. A Borrellial lymphocytoma is seldomly diagnosed, and only in Europe, and is described as a bluish red tumor-like skin
Figure 3. A. Erythema migrans B. Borrelial lymphocytoma C. Acrodermatitis Chronica Atrophicans D. Lyme arthritis E. Facialis paresis. We are grateful to Prof. Dr. A.C. Steere and Dr. D.J. Tazelaar for the pictures.

infiltrate, often located at the earlobe or nipple (Figure 3B). It is more common in children and can spontaneously resolve [257, 268]. Early LB symptoms respond well to antibiotic therapy [57]. In Europe, 77 % to 89 % of all Lyme manifestations are EM and 2 to 3% Borrelial lymphocytoma [18, 116].

**Early disseminated LB (weeks to months)**

When the infection is untreated, the spirochete can disseminate and cause early neuroborreliosis (3-16 % of Lyme manifestations), Lyme arthritis (5-7 %), and seldom a (myo)carditis with (partial) atioventricular block(<1 %) [18, 116]. Notably, since the late 1980s, increasing awareness for EM and better effective antibiotic regimes probably made these clinical manifestations become even less common [258]. The European Union Concerted Action on LB (EUCALB, www.eucalb.com) has made criteria for these manifestations for clinical purposes, which are used in the Dutch guideline developed by “Centraal Begeleidings
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Orgaan” (CBO) in 2004 and 2013 [1, 249, 254], which is currently being updated. Early neuroborreliosis can present with lymphocytic meningitis, which is more common in the United States, cranial nerve paresis, usually the facial nerve (Figure 3E), painful radiculitis, which is more common in Europe, or all of the above, which is equivalent to the Bannwarth syndrome [98, 122]. In most patients, acute neurological symptoms improve or resolve in several weeks to months, even without antibiotic treatment [258]. In the early disseminated phase of LB, infection of the joints is oligoarticular and 50% occurs in the knee (Figure 3D). This manifestation is mostly observed in the United States, where 60% of untreated patients develop arthritis [258]. Cardiac involvement in early LB in adults is rare and symptoms are usually related to atrioventricular conduction abnormalities [115].

Late LB (months to years)

One could divide late Lyme manifestations into two groups, manifestations in which persistent *Borrelia* infection is causative for the ongoing symptoms, e.g. acrodermatitis chronica atrophicans (ACA), persistent (untreated) Lyme arthritis and neuroborreliosis, and manifestations in which other mechanisms, e.g. autoimmune phenomena or irreversible tissue damage might play a role, such as antibiotic-refractory Lyme arthritis, encephalopathy (a subgroup of late neuroborreliosis) and dilated cardiomyopathy [115]. Importantly, over half of the patients with late manifestations of LB do not remember an EM [10, 98].

ACA can develop up to ten years after infection and is described as a bluish-red atrophic skin lesion, initially combined with edema, in later stages with atrophy (Figure 3C) and is predominantly located on the plantar sites of hands and feet or distal parts of the legs. Periarticular nodules, sclerotic lesions and sensory polyneuropathy can be observed [24]. ACA can be confused for vascular conditions, such as venous insufficiency, more often when the legs are affected [72]. It occurs mostly in women older than 40 years [11], but has been described incidentally in children [170].

Late neuroborreliosis is rare and includes encephalomyelitis, encephalopathy and axonal polyneuropathy, for a period of at least 6 months [258]. Encephalomyelitis can present as a slowly progressive myelopathy beginning with an atactic gait, a gradually worsening spastic para- or tetraparesis or with hearing loss and is accompanied by relatively severe cerebrospinal fluid (CSF) pleocytosis or evident
intrathecal anti-*Borrelia* antibody production [98, 192]. In contrast, symptoms of encephalopathy are mainly cognitive, in combination with nonspecific symptoms, such as fatigue, malaise and myalgia. In most of these patients, there is no evidence of inflammation due to *Borrelia* in the CNS, and therefore, an encephalopathy might actually be an indirect effect of systemic (non-CNS) infection accompanying typical clinical findings of disseminated LB [96]. Finally, an European study showed that isolated chronic polyneuropathy, without the presence of other late LB manifestation, such as ACA, is rarely caused by *B. burgdorferi* s.l. infection [173].

Joint manifestations can occur months to years after exposure, with intermittent recurrent attacks that persist for days, weeks, or months and are typically asymmetrical and pauciarticular in nature and involve one or two larger joints and almost invariably the knee [262]. Most Lyme arthritis patients respond well to conventional antibiotic treatment strategies, such as doxycycline, but a small percentage will continue to have chronic joint inflammation, not due to persistence of the spirochete. This is called antibiotic-refractory Lyme arthritis and occurs more often in the United States than in Europe and has recently been associated with polymorphisms in toll like receptor (TLR)-1 and auto-antibodies [67, 258, 270], genetic predisposition, i.e. the presence of certain HLA-DR alleles, specific *B. burgdorferi* genotypes and T cell responses, i.e. Th17 responses and low number of regulatory T cells [44, 92, 121, 242].

In dilated cardiomyopathy, a very rare manifestation of late LB, spirochetes have rarely been isolated by culture. This might indicate that symptoms could be due to past infection and myocardial scarring rather than ongoing inflammation due to the presence of the spirochete [85].

A large proportion of, treatment-naïve, individuals present with serological evidence of exposure to *B. burgdorferi* s.l. and symptoms that are nonspecific and regularly occur in the normal population, such as fatigue, myalgia, headache and joint pain. These are not considered specific symptoms or signs of late LB. However, a selection of these individuals might have an increased risk of LB, for example a history of previous EM or abundant tick infestations, or live in a highly endemic region of the Netherlands. Although these nonspecific symptoms are highly prevalent in the normal population, as are antibodies against *Borrelia* (see below), in a minority of these individuals, when other causes have been thoroughly excluded, the diagnosis LB could be considered.
Persistent nonspecific symptoms after treatment

A minority of patients, approximately 10-20 %, experience nonspecific symptoms after recommended treatment with antibiotics [162]. This complex of nonspecific symptoms might best be referred to as post-treatment LB disease syndrome (PTLBS) [76]. PTLBS, for which a definition has been postulated, has been linked to a broad array of symptoms that are highly prevalent in the normal population, similar to those described in the last paragraph of the previous section [159, 298]. This, in combination with the fact that specific antibodies against *Borrelia* occur in approximately 4-8 % of the normal Dutch population, and in even up to 20 % in highly endemic areas in other European countries, and the fact that in this group additional antibiotics after previous recommended treatment have no substantial beneficial effects compared to placebo, strongly suggest that persistent *Borrelia* infection is not the cause of the symptoms [36, 74, 93, 124, 131, 141]. Indeed, in animal models (mouse and dog), *B. burgdorferi*-infected animals readily become culture negative upon antibiotic treatment [168, 264]. In these studies, which all have major pharmacodynamic concerns, persistence of *B. burgdorferi* DNA has been reported, but has not been associated with disease [115, 301]. In humans, mostly in non-rigorous studies studies and in studies that did not use recommended courses of antibiotic treatment, treatment failure - importantly often associated with persistence or development of specific symptoms - has been described [35, 56, 151, 187]. In contrast, in well-designed studies using recommended therapies treatment failure is only seldomly reported [37, 57, 158]. Therefore, the term “chronic Lyme disease” for persistent nonspecific symptoms after recommended treatment for LB seems to be a misnomer and should be avoided [76].

Diagnostics

Considerations before diagnostic tests are performed

The diagnosis of LB is predominantly based on clinical symptoms and serological tests. The diagnosis can be readily considered in case of symptoms which have been associated with *Borrelia* infection and serological evidence for *Borrelia* infection (Table 1). However, in the absence of specific clinical symptoms, the presence of anti-*Borrelia* antibodies does not necessarily indicate the presence of an active *Borrelia* infection, since 4 to 20 % of the normal Western-European population, has detectable antibodies, most likely due to an (asymptomatic) *Borrelia*-infection in the past [36, 93, 186]. When antibodies against *Borrelia* are
detected in individuals without specific clinical symptoms, these could be considered “false positive”, since they are not predictive of disease. Therefore, international guidelines, including the CBO 2004 guideline [249, 298], recommend when there is only a small suspicion on LB not to test for antibodies against *Borrelia*. This has been recently reviewed by others and is enumerated in Table 2 [147]. Despite these recommendations, we have recently shown that of serological tests requested by Dutch physicians in the Amsterdam area (n=488), 70 % was from individuals with nonspecific symptoms (see reference [48] and chapter 2). Not surprisingly, in 5 % of these sera we demonstrated antibodies against *B. burgdorferi* s.l., which equals the seroprevalence of the Dutch population.

**General considerations on diagnostic tests**

Detection of antibodies in serum directed against *B. burgdorferi* s.l. is the most common diagnostic approach. Several guidelines recommend that (at least second generation) *B. burgdorferi* s.l. Enzyme-linked Immuno Sorbent Assays (ELISAs or EIAs) should be used as a screening test and, when reactive, should be confirmed by an immunoblot or Western blot (two-tier testing) [249, 296]. The spectrum of *Borrelia* proteins recognized on Western blot expands with the duration of symptoms [296]. A European multicenter study has indicated eight bands suitable for diagnostic purposes [217]. Compared to IgG, the specificity of IgM-components is lower, since rheumafactor, acute EBV and CMV infection and MS and other auto-immune diseases can also give a false-positive test [31, 89, 296]. Newer serological tests include an ELISA detecting antibodies against C6, a 26–amino acid peptide that reproduces the sequence of the sixth invariant region (IR6) within the central domain of the VlsE protein of *B. burgdorferi* s.l. [299]. Despite these new developments, two-tier testing is still considered to be necessary, since the immunoblot has a higher specificity than ELISA or EIA. However, in the very early stages of LB, the immunoblot can be false-negative [22, 299]. Recently, Branda *et al* proposed a new testing strategy, 2-tiered IgG testing, which avoids the use of IgM blots. Compared to the standard two-tier testing, this method had significantly better sensitivity in early disseminated Lyme borreliosis, the same sensitivity in early and late LB and a comparable specificity [22]. Other validated and widely accepted diagnostics include culture and Polymerase Chain Reaction (PCR) [249]. Clearly, a positive culture in the presence of ongoing specific symptoms indicates an active infection and should be considered as the ‘gold standard’. Unfortunately, there are limitations to culture. It is expensive, tissue samples should be incubated in special medium for weeks and there is limited availability in the Netherlands.
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[249]. Sensitivity of culture and PCR to detect Borrelia in different tissues/fluids during the different stages of LB is highly variable (Table 1). In theory, specificity for culture is 100 %. Overall specificity for PCR is 93-100 % [3, 39, 185], provided certain measures are undertaken to avoid contamination, and amplified products are specified by sequencing [296].

**Diagnostic tests for LB manifestations**

**Early LB.** EM is a clinical diagnosis and serological tests are not necessary and not recommended (Table 1). In case of atypical EM and Borrelial lymphocytoma, serological testing can be considered at least 6-8 weeks after onset of symptoms (Table 1) [249]. Alternatively, during these cutaneous manifestations, a skin biopsy at the margins of the EM could be considered for PCR or culture, for which sensitivities of respectively 60-80 % and 40-88 % have been reported [39, 216, 223, 288, 297]. Culture for Borrelial lymphocytoma has a sensitivity of 24 % [161]. Antibiotic treatment during early phases of infection cause a decrease in antibody titers against Borrelia [123].

**Early disseminated LB.** Because clinical aspects of early disseminated LB are not as visually clear as EM and have a broader differential diagnosis, laboratory evidence is necessary (Table 1). A guideline from Mygland et al in 2010 provides recommendations for the diagnosis and treatment of neuroborreliosis in Europe [172]. To demonstrate intrathecal production of anti-Borrelia antibodies, the cerebral spine fluid (CSF)/serum antibody index (AI) should be performed. Early in the course of neuroborreliosis, absent pleocytosis in CSF has been described and sensitivity of AI is around 55-80 % [20, 97]. After 6 weeks of symptoms the sensitivity of AI approximates 100 % [155]. The specificity of AI has not been the topic of extensive investigation, but ranges from 63 to 97 % [20, 154]. Several studies have shown a sensitivity of 10-50 % for the PCR on CSF during early neuroborreliosis [38, 88, 220]. PCR on CSF could be useful when there is a strong suspicion of neuroborreliosis and the AI is negative or in patients with an immunodeficiency [172]. More often, anti-Borrelia antibodies are found in CSF, but patients have other neurological diseases. This is illustrated by a study in which, of in total 123 patients with positive Borrelia serology (IgG) in CSF, 74 patients had another etiologic diagnosis [20]. Recently, the chemokine CXCL-13 in CSF has been shown to be a promising future diagnostic/treatment marker for neuroborreliosis [239, 280]. Both early Lyme arthritis and myocarditis have a broad differential diagnosis and other causes need to be excluded. This, combined with a low a priori
### Table 1. Recommended diagnostics and treatment for the different LB manifestations

<table>
<thead>
<tr>
<th>LB manifestation</th>
<th>Serum antibodies sensitivity1:</th>
<th>Other helpful diagnostics</th>
<th>Sensitivity other diagnostics2</th>
<th>First choice treatment CBO (2013) and IDSA (2006)</th>
<th>Treatment alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema migrans</td>
<td>38-88% &gt;6 weeks: 100%14</td>
<td>In case of atypical EM4</td>
<td>60-80% [39, 216, 288]</td>
<td>Doxycycline bid 100 mg for 10-14 days</td>
<td>Amoxicillin tid 500 mg for 14 days2 or Azithromycin qd 500 mg 5 days</td>
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<tr>
<td></td>
<td></td>
<td>PCR</td>
<td>40-88% [223, 297]</td>
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<td></td>
<td></td>
<td>Culture</td>
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<tr>
<td>Borreliacytoma</td>
<td>70%</td>
<td>Histopathology</td>
<td>ND</td>
<td>Doxycycline bid 100 mg for 10-14 days</td>
<td>Amoxicillin tid 500 mg for 14 days2 or Azithromycin qd 500 mg 5 days</td>
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<tr>
<td></td>
<td></td>
<td>PCR</td>
<td>24% [161]</td>
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<td></td>
<td></td>
<td>Culture</td>
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<tr>
<td>Early neuroborreliosis</td>
<td>80% &gt;6 weeks: 100%</td>
<td>Intrathecal antibodies (AI)</td>
<td>55-80% [20, 97]</td>
<td>Ceftriaxone qd 2 gram IV for 14 days</td>
<td>Penicilline-G 2-3 ME 6 times a day, for 14 days or Doxycycline bid 200 mg for 14 days172</td>
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<tr>
<td></td>
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<td>LP cell count (pleocytosis)</td>
<td>95-100% [98, 276]</td>
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<td></td>
<td></td>
<td>LP culture</td>
<td>13% [125]</td>
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<td></td>
<td>LP PCR</td>
<td>10-50% [38, 88, 229]</td>
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<tr>
<td>Late neuroborreliosis</td>
<td>100%</td>
<td>Intrathecal antibodies (AI)</td>
<td>100% [98]</td>
<td>Ceftriaxone qd 2 gram IV for 30 days</td>
<td>Doxycycline bid 100 mg for 30 days6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LP cell count (pleocytosis)</td>
<td>100% [98, 157, 249]</td>
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<tr>
<td>Lyme arthritis</td>
<td>100%2</td>
<td>Synovial fluid PCR</td>
<td>46-88% [185, 206]</td>
<td>Doxycycline bid 100 mg for 30 days</td>
<td>Ceftriaxone qd 2 gram IV for 14 days</td>
</tr>
<tr>
<td>Lyme carditis</td>
<td>80-100%3</td>
<td>ECG3</td>
<td>ND</td>
<td>Doxycycline bid 100 mg for 21 days</td>
<td>Ceftriaxone qd 2 gram IV for 14 days</td>
</tr>
<tr>
<td>Acrodermatitis chronica atrophicans (ACA)</td>
<td>100%</td>
<td>Histopathology</td>
<td>68-92% [216, 288]</td>
<td>Doxycycline bid 100 mg for 21-30 days</td>
<td>Ceftriaxone qd 2 gram IV for 14 days</td>
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<tr>
<td></td>
<td></td>
<td>PCR</td>
<td>22-60% [1]</td>
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<td></td>
<td>Culture</td>
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1 Based on the CBO guideline 2004, IDSA guideline 2006. Since 4-8% of the normal population has antibodies against Borrelia, the theoretical specificity of serology is limited to 92-96%. 2 Borrelia serology only recommended if the knee is involved. 3 Estimated to become 100% during the course of the disease based on other disseminated manifestations. 4 Serology is recommended at least 6-8 weeks after onset. 5 Only perform in case of proven early disseminated LB. 6 In theory, specificity of culture is 100%, of PCR (when appropriate measures are undertaken) 93-100%, and AI 63-97%. 7 In case of pregnancy, photosensibility or allergy for doxycycline. 8 In case of absence of pleocytosis. This table does not include treatment recommendations for children younger than 9 years. EM = erythema migrans; PCR = polymerase chain reaction; ND = no data; AI = antibody index; LP = lumbar puncture; qd = once daily; bid = twice daily, tid = three times a day; iv = intravenous.
change when other LB manifestations are absent, results in a low positive predictive value for positive antibodies. Therefore, especially for Lyme arthritis, other diagnostic tests, such as PCR on synovial fluid, should be considered (Table 1). PCR of synovial fluid has a sensitivity of 46-88 % [185, 206]. Notably, antibodies are present in 100% of Lyme arthritis and in 80% of Lyme myocarditis cases (Table 1).

Late disseminated LB. Patients with ACA have detectable antibodies in 100% of the cases, the sensitivity of PCR on ACA skin biopsy ranges from 68 to 92%, whereas culture has a lower sensitivity ranging from 22 to 60% [3, 26, 169, 216, 288]. For late neuroborreliosis, criteria include symptoms suggestive of late neuroborreliosis - no other obvious reason for the presenting symptoms - pleocytosis, and demonstration of intrathecal specific antibody synthesis (AI). In late neuroborreliosis antibodies are present in 100% of the cases, but PCR and culture have a low sensitivity and are therefore not recommended (Table 1) [125, 172]. Late Lyme arthritis also has a 100% antibody detection rate. Routine screening of patients with idiopathic dilated cardiomyopathy for antibodies against *B. burgdorferi* s.l. is of limited utility and should be reserved for patients with a clear history of antecedent LB symptoms or tick bite [203].

Invalidated or not recommended diagnostics

Over the last few years LB has attracted a lot of media attention. By some, an image is created of an insidious (almost) incurable disease, which is extremely difficult to diagnose and for which current diagnostic tests are totally useless. This has created a ground for commercial laboratories offering invalidated or not recommended diagnostic tests. By some, blood and urine are offered for the detection of *Borrelia* DNA. PCR on these body fluids is not validated and not recommended for microbiological diagnosis [296]. A meta-analysis showed a wide range in sensitivity of the urine *Borrelia* PCR of 13% to 100% [68]. A study showed that, after establishing an optimal PCR protocol with spiked urine, in only 1 of 12 patients with an acute infection (EM), *Borrelia* DNA was detected [212]. PCR on blood has a poor sensitivity of only 10-18% and there are no European studies performed with a good control group, thus the specificity remains unclear [3, 38, 87, 190]. Theoretically, the specificity should be 100%, however, as outlined before, it is of paramount importance that (commercial) laboratories avoid DNA contamination, perform the correct controls and validate their PCR amplicon. Blood microscopy should not be used for diagnosis [298].
Table 2. Testing for antibodies against B. burgdorferi in the normal population

<table>
<thead>
<tr>
<th>Test</th>
<th>LB</th>
<th>No LB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>40</td>
<td>497</td>
<td>537</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td>9,453</td>
<td>9,463</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>9,950</td>
<td>10,000</td>
</tr>
</tbody>
</table>

False positives = (497/537) * 100 = 92.5 %
False negatives = (10/9463) * 100 = 0.1 %

Sensitivity: 80 %; specificity 95 %, pre-test probability: 0.5 %. Sensitivity and specificity are based on published literature (see text). The pre-test probability is an estimation based on recent RIVM data. Note the high number of false positives when serology is performed in the normal population, or in individuals with nonspecific symptoms.

using healthy volunteers as a control group, elevated complement factors, such as C3a and C4a, have been associated with acute and chronic Lyme disease [244, 265]. These factors will be elevated in many other medical conditions and should therefore not be used to diagnose Lyme disease. Finally, diminished expression of CD57 on mononuclear cells has been claimed to be associated with PTLBS [266]. In this study HIV-infected individuals were used as a control group. In HIV infection it has been shown that CD57 expression is upregulated, making it impossible to draw conclusions from this study, and others could not confirm this finding [21, 163].

Finally, commercial laboratories offer PCR to determine if attached ticks are infected with Borrelia. This is not recommended by guidelines, since a positive PCR is not predictable for infection [248].

Therapy for LB manifestations

Antibiotics are effective in all manifestations of LB (Table 1) [258]. The difference between antibiotics and expectative policy for EM has never been studied, because the justified use of antibiotics has been shown by randomized double-masked trials in which different antibiotics were compared. However in theory, in analogy with other manifestations of LB and other spirochetal diseases such as syphilis, EM could spontaneously resolve. For early LB manifestations, such as EM and Borrelial lymphocytoma, oral treatment, i.e. 10-14 days of doxycycline, is as effective as parental antibiotics [292], but has lower risks and adverse events. Importantly, two randomized double-masked trials support a 10 day course of 2 b.i.d. 100 mg doxycycline [135, 300]. Finally, a recent European trial confirmed that oral
treatment of early LB is successful in almost 100 % of the cases. Moreover, in the unlikely event of treatment failure (0.4-0.7 %), objective symptoms of LB did occur. Importantly, not only were the newly developed nonspecific symptoms in the treated EM group comparable to those in the treated age- and sex-matched control group, also the frequency of these symptoms was identical in both groups [37].

For early disseminated LB doxycycline is also recommended, except for neuroborreliosis with CNS manifestations, for which ceftriaxone iv is first of choice (Table 1). Notably, a multicentre double-blind randomized trial compared ceftriaxone iv with oral doxycycline for adults with (early) neuroborreliosis and concluded that both are equally effective [156]. Although older open studies have suggested that longer treatment, i.e. longer than the recommended 14 to 30 days, might be justified for early (and late) disseminated LB, a multi-center placebo controlled randomized trial has shown that prolonged treatment of both early and late disseminated LB is not warranted, which is in line with most, if not all, esteemed and peer-reviewed international guidelines [191, 298].

In late manifestations of LB, the same antibiotics are recommended, but with a longer duration of treatment (Table 1) [171]. For selected individuals with nonspecific symptoms in combination with positive Lyme serology antibiotic treatment could be considered. Although, to our knowledge, evidence-based guidelines for this are non-existent, treatment could be adjusted based on the duration of symptoms, e.g short duration of symptoms (<3 months) could be treated with 10-14 days of doxycycline b.i.d. 100 mg and longer lasting symptoms with 30 days of doxycycline b.i.d. 100 mg.

In case of persistence of specific LB symptoms after treatment, persisting B. burgdorferi s.l. infection, or re-infection, should be considered and additional or prolonged therapy could be indicated. In stark contrast, patients with PTLBS, or individuals with false-positive Lyme serology and nonspecific symptoms, such as fatigue, myalgia, headache and joint pain, should not receive antibiotic treatment. However, some of these patients are occasionally treated for months to years with (multiple) intravenously administered antibiotics, for which no credible scientific evidence exists. Such approaches pose a great risk for serious adverse effects [131, 141]. As stated before, multiple placebo-controlled randomized trials showed no substantial additional effect for additional antibiotic treatment in these individuals [74, 124, 131, 141].
Prevention

The best preventive method to prevent *Borrelia* infection is attempt to avoid exposure to ticks by wearing protective clothing. In addition, a full body check within 24 hours after possible tick exposure could detect attached ticks, which should be promptly removed. This strategy is promoted by the Dutch National Institute for Public Health and Environment (RIVM) and accessible for the public at www.rivm.nl/cib/themas/teken-lyme. Calculations have indicated that a Lyme vaccine could be economically attractive when used in persons living in an area with an annual risk of more than 1 % of contracting LB [241]. Such regions are prevalent in northeastern parts of the USA, however are yet to be identified in the Netherlands [15]. The only licensed Lyme vaccine was based on recombinant OspA, which showed a 70 % efficacy in phase III human trial [2]. It became available in 1998, but was removed from the market in 2002 because of public perceptions on adverse events. We recently discussed the possibilities for vaccine strategies against LB [233], such as vaccines based on the combination of *Borrelia* and tick (saliva) proteins. Indeed, antibodies against the tick salivary gland protein Salp15 - by itself able to impair *B. burgdorferi* infection in tick-challenged mice - had synergistic effects in conjunction with a vaccine directed against *B. burgdorferi* antigens [53]. Also, combination vaccines, consisting of multiple *Borrelia* antigens, showed higher efficacy compared to vaccination based on single or double antigens, in mice [30]. Such novel approaches have yet to be tested in humans. Finally, to reduce the risk of human LB, preventive approaches include decreasing tick densities, tick *B. burgdorferi* s.l. infection rates. This could be achieved by the use of acaricides [51]. However, resistance to acaricides in ticks occurs, and acaricides are harmful for humans, animals and the environment [91]. Novel strategies comprise wildlife Lyme vaccines or prophylactic treatment of wildlife with doxycycline [53, 66].

Conclusions

LB is endemic in the Netherlands with a yearly incidence of EM of approximately 133 cases / 100,000. It is a zoonotic disease, with well-defined symptoms, caused by *B. burgdorferi* s.l. and transmitted by ticks. Diagnosis of early LB, i.e. EM, is made clinically and there is no need for serological tests. Diagnosis of later manifestations is based on the combination of specific clinical symptoms and positive serology and/or other diagnostic tests. In longer lasting manifestations of LB sensitivity of serology approaches 100 %. Specificity of serology is lower, since
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the seroprevalence of antibodies against *B. burgdorferi* s.l. is approximately 4-8 % in the general population. This includes treated LB patients, individuals who have spontaneously cleared asymptomatic *Borrelia* infection or have cross-reacting antibodies. Therefore, in individuals with nonspecific symptoms it is not recommended to test for antibodies against *Borrelia*. Antibiotics are effective in all manifestations of LB and prognosis is usually excellent. However, a minority of patients experience potentially severe, but nonspecific symptoms after previous recommended treatment for LB. In these individuals, additional antibiotics have no substantial beneficial effects compared to placebo [74, 124, 131, 141]. A challenge for the future is to develop a test to detect, or rule out, persistent active *B. burgdorferi* s.l. infection. This could reassure individuals that experience nonspecific symptoms after previous recommended therapy for LB, prevent unnecessary treatment and pave the way for research on the true etiologies of nonspecific symptoms after recommended antibiotic treatment for LB. Finally, preventing LB, by the development of novel vaccination strategies or wildlife control, remains an important challenge for the future. Thus, bearing these developments in mind, we should definitely not allow ourselves to be tired of LB.