Preface and thesis outline
During the late 1970s and early 1980s, Lyme disease, or Lyme borreliosis (LB), was recognized as a tick-borne disease caused by infection of spirochetes belonging to the *Borrelia burgdorferi* group (from here on referred to as *B. burgdorferi*) [32, 260]. Since then, researchers in the United States and Europe have extensively investigated the clinical manifestations and pathogenesis of LB as well as strategies to prevent *B. burgdorferi* transmission. During forty years of research, diagnostic tests to detect *B. burgdorferi* infections in humans have been developed. Furthermore, the pathogenesis of LB with its intricate tick-pathogen-host interactions is becoming increasingly understood. The Holy Grail in LB research remains a vaccine that prevents transmission of *B. burgdorferi* to humans. Despite the remarkable progress in the past decades, there is currently no vaccine available to prevent LB in humans. The aim of this thesis is to ‘dissect’ three different aspects of LB; namely I) challenging clinical aspects of LB in contemporary medicine, II)
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**Part I - Clinical aspects**

LB manifestations can be divided into three stages, namely early localized LB, disseminated LB and late LB, which are discussed in more detail in chapter 1. Briefly, early localized LB is predominantly characterized by an expanding skin lesion at the tick bite site designated as erythema migrans (EM). Disseminated LB and late LB can manifest in the nervous system, joints and skin, of which some can be affected months or even years after a tick bite [256]. Since 1994, the incidence of LB in the Netherlands has increased from 6,500 to 25,000 cases in 2014 [104, 106]. Forty years after the discovery of LB, several challenging clinical aspects of LB remain. The idea that a small spider-like creature can cause a severe chronic disease is an important reason for patients to seek medical help after a (presumed) tick bite in combination with unexplained symptoms. However when specific objective clinical findings for LB such as skin lesions, neurological findings or joint inflammation are absent, the a priori chance of having LB is considered to be ‘very low’ according to national and international guidelines [1, 298]. Serological tests that detect antibodies against *B. burgdorferi* in serum are predominantly used to confirm LB, but these tests require careful interpretation in relation to duration of disease and the presence of objective findings. The specificity and sensitivity of serological tests that can be used to support the diagnosis of LB as well as other available tests are discussed in chapter 1. Guidelines recommend physicians to be cautious in requesting serological tests because approximately 4-8 % of the Dutch population has antibodies against *B. burgdorferi* due to previous (asymptomatic) infections [255]. Because of this, serological tests for patients with a low a priori chance for LB have a low positive predicting value. When guidelines are not followed, e.g. because physicians are not aware of the guidelines or because serological tests are specifically requested by the patient, false positive test results could lead to overdiagnosis of LB. On the contrary, when diagnostic tests are not applied when they should this could lead to a delay in the diagnosis and subsequent treatment. In chapter 2 we investigate the value of serological tests for LB in clinical practice by analyzing the population screened for LB in a laboratory
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serving both general practitioners (GPs) and regional hospitals in the Netherlands. We analyze clinical symptoms of 488 individuals for whom serological testing was requested to illustrate the difference between guideline recommendations and daily clinical practice.

A second challenging clinical aspect of LB is the persistence of symptoms after antibiotic treatment in a subset of LB patients. Based on human studies there is evidence that these persisting symptoms are most likely not due to an ongoing *B. burgdorferi* infection. In addition, reports of antibiotic failure are infrequent and are associated with typical LB manifestations [37]. Nonspecific symptoms are more commonly reported to persist and these are rather considered to be part of a post-infectious disease syndrome called Post-Treatment Lyme borreliosis syndrome (PTLBS), which is discussed in more detail in chapter 1. However, patient advocacy groups have expressed their concerns that persisting LB - i.e. an ongoing *B. burgdorferi* infection despite recommended antibiotic treatment - is underdiagnosed and have called for improved tests and specialized LB clinics. A citizens initiative raised by Dutch patient advocacy groups was supported by 70,000 signatures and lead in 2010 to a parliamentary debate on LB treatment and care. The Dutch parliament passed a resolution to improve care for LB patients and the Dutch ministry of Health called for concerted action in research and healthcare.

The majority of LB patients presents with an EM and is adequately diagnosed and treated by GPs. However there are several reasons why patients suspected of LB require additional medical evaluation. For example in the case of atypical skin lesions and doubt whether these could be EM, or in case of patients presenting with atypical symptoms and signs suggestive of disseminated LB. In addition, when serological test results are inconclusive, or when symptoms persist after antibiotic treatment and there is doubt whether there is an ongoing *B. burgdorferi* infection, patients should be further evaluated. Because LB involves several organ systems, patients are often referred to various specialists. To improve care for LB in the Netherlands, the Amsterdam Multidisciplinary Lyme Center (AMLC) was established for a multidisciplinary evaluation of patients suspected of LB in a tertiary center. We describe in chapter 3 in a retrospective case series the first 200 patients that were referred to the AMLC. Based on objective findings, medical history, tick bite history and diagnostic tests, the likelihood of a causal relationship between complaints and a *B. burgdorferi* infection was assessed in a multidisciplinary approach. Our analysis of the AMLC’s patient cohort aims to
provide insight in patients suspected of LB at a tertiary center, in current clinical challenges of diagnosing LB as well as to provide future recommendations to improve care for patients suspected of LB.

**Part II - Pathogenesis**

In the second part of this thesis, we ‘dissect’ tick-pathogen-host interactions at a molecular level. *Ixodes* tick feeding in the host’s skin can last for several days until they have taken up sufficient amounts of blood [6]. To counter host immune responses that could impair tick feeding, ticks, in their saliva, actively ‘spit’ immunosuppressive salivary gland proteins into the skin [110]. We have become aware that these immunosuppressive tick proteins are also instrumental for successful *B. burgdorferi* transmission. For example, we and others have recently shown that *B. burgdorferi* can bind immunosuppressive tick proteins during its transmission to the host to increase its chances of survival [108]. In chapter 1 we provide more information on the lifecycle of ticks, transmission of *B. burgdorferi* and important tick-pathogen-host interactions.

Further understanding of the mechanisms by which tick proteins facilitate pathogen transmission could increase our knowledge of LB pathogenesis and provide insight in host factors associated with increased susceptibility for LB. In 2001, a phage display immunoscreening performed by Das *et al* identified several tick salivary gland proteins with immunosuppressive capabilities that have significantly contributed to our understanding of LB pathogenesis [55]. Since the initial screening in 2001, improved screening techniques and the complete genome sequence of *Ixodes scapularis* have become available which has expanded the possibilities for research on tick-host-pathogen interactions. Schuijt *et al* recently performed a new screening for tick salivary proteins that facilitate tick feeding using Yeast Surface Display technology and identified a tick protein with anti-complement activity [234]. In chapter 4 we unravel the mechanism by which this tick protein inhibits the complement system and show that it is able to specifically inhibit the lectin complement pathway, one of the three pathways that can activate the complement system [215]. Based on its function, we named this protein Tick Salivary Lectin Pathway Inhibitor (TSLPI). We also describe the role of TSLPI during *B. burgdorferi* transmission from the tick to the host and *B. burgdorferi* acquisition form the host to the tick in chapter 4. Subsequently, to increase our understanding of the role TSLPI in *Ixodes* tick feeding we describe in chapter 5 the
identification and functionality of a TSLPI ortholog in the European tick *I. ricinus*. Based on our observations in chapter 4 and 5 which show that inhibition of the lectin pathway increases *B. burgdorferi* survival in the vertebrate host, we hypothesize that reduced lectin pathway activity by deficiency of Mannose Binding Lectin (MBL) - one of the activators of the lectin pathway - in the host is associated with increased susceptibility for LB. In chapter 6 we therefore study the role of MBL in the innate immune response against *B. burgdorferi* using a genetically modified mouse deficient of MBL and indeed show that reduced MBL activity is associated with increased *B. burgdorferi* loads early in murine infection.

**Part III - Prevention**

In the third part of this thesis, we explore vaccine-based strategies to prevent LB. Novel strategies are needed as the incidence of tick bites and LB is on the rise [105]. A safe and cost-effective alternative to antibiotic treatment could be a vaccine that prevents transmission of *B. burgdorferi* to humans. A vaccine named Lymerix® was commercially available in the US in the 1990s, but it was withdrawn from the market in 2002 due to low sales as the result of - unproven - claims that Lymerix® could cause arthritis [205]. Twenty years later, there is no vaccine available which has been considered a ‘public health fiasco’ [204]. A more elaborate introduction to vaccines and other preventive strategies to prevent LB is discussed in chapter 1. We explore new anti-tick vaccine strategies in chapter 7, 8 and 9 targeting *Ixodes* gut proteins. The gut is an important site for both tick feeding and *B. burgdorferi* transmission, and targeting proteins involved in one or both of these could potentially prevent *B. burgdorferi* transmission to humans [59, 207].

The first strategy in chapter 7 was to test gut proteins in *Ixodes ricinus* with homology to a protein successful used as a vaccine against another tick species. The anti-tick vaccines Gavac™ and TickGARD Plus™ effectively target *Rhipicephalus* (*Boophilus*) *microplus* and are based on the *R. microplus* gut glycoprotein Bm86 [58]. Nijhof *et al* recently identified two Bm86 homologues in *Ixodes* ticks [184], which we have tested in chapter 7 as anti-tick vaccines to impair successful feeding of adult *I. ricinus* ticks.

Secondly, we explored an anti-tick vaccine strategy that could prevent *B. burgdorferi* infection by interfering with the early phase of *B. burgdorferi* transmission in the gut of *Ixodes* ticks. The rationale behind this strategy is based on the fact that during tick feeding spirochete growth and exit from the tick gut
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involves close interactions between the spirochete and the gut epithelium [69]. Spirochetes migrate from the tick gut through the hemolymph into the tick salivary glands, from where they can be transmitted to the host. Only a few *Ixodes* gut proteins that facilitate *B. burgdorferi* migration have so far been identified [194, 307]. Therefore we screened for unknown tick proteins that interact with *B. burgdorferi* and thereby potentially facilitate spirochete transmission. In **chapter 8** we describe our screening for *B. burgdorferi*-interacting tick gut proteins. Two *B. burgdorferi* interaction tick gut proteins were identified, namely *Ixodes scapularis* fibronectin III domain (Ixfi3D) and *Ixodes scapularis* dystroglycan-like protein (ISDLP), which are discussed in respectively **chapter 8** and **chapter 9**. We characterize their role during *B. burgdorferi* transmission and we explore whether a vaccine based on either of these proteins can prevent *B. burgdorferi* transmission to the host. Finally, in **chapter 10** we provide a summary and general discussion of the data presented in this thesis with recommendations for future research.