Dissecting Lyme borreliosis; Clinical aspects, pathogenesis and prevention
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Link to publication

Citation for published version (APA):
Coumou, J. (2016). Dissecting Lyme borreliosis; Clinical aspects, pathogenesis and prevention ‘s-Hertogenbosch: Boxpress

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Chapter 10

Summary and general discussion
Part I – Clinical aspects of Lyme borreliosis

General aspects of LB
Lyme borreliosis (LB) is the most prevalent vector-borne disease in Western Europe and Northeastern parts of the USA. The causative agents of LB are spirochetes belonging to the *Borrelia burgdorferi* s.l. group (from here on referred to as *B. burgdorferi*), which are transmitted by *Ixodes* ticks. In chapter 1 we have provided a general introduction on ticks, *B. burgdorferi* and the pathogenesis of LB as well as clinical manifestations of LB, diagnostic tests for LB and treatment of LB.

Value of serological tests for LB in clinical practice
The diagnosis of LB is based on the development of specific clinical findings combined with positive serological and/or other diagnostic tests, as described in more detail in chapter 1 [1]. Despite these criteria the diagnosis of LB in clinical practice is in some cases difficult to establish or to exclude. Mostly due to previous (asymptomatic) infections, around 4-20 % of the Western-European population has antibodies against *B. burgdorferi*, which can remain detectable even years after the (treated) initial infection [36, 73, 93]. The seroprevalence can be even higher in certain risk groups, such as hunters and forestry workers [142]. Thus *B. burgdorferi* antibodies do not equal disease and guidelines therefore recommend to estimate the a priori chance of LB in a patient based on the presenting clinical findings before requesting serological tests [1]. Although estimating the a priori chance for a disease before requesting diagnostic tests is a general requirement for tests, guidelines for LB specifically address this issue because of concerns present in society that a broad array of symptoms such as fatigue, myalgia or cognitive impairment can be caused by a *B. burgdorferi* infection. Although these symptoms can indeed accompany LB manifestations, it should be noted that they are nonspecific, can occur with many other diseases and are highly prevalent in the general population [1, 146, 147]. Because the value of serological tests for LB is mainly determined by the pre-test probability in the tested population, guidelines recommend not to perform serological tests in patients with a very low a priori chance, as the chance for a false positive test result is calculated to be 90 % in such a population. In chapter 2 we have examined the value of serological tests for LB in clinical practice. We have analyzed clinical information of 488 patients for whom serological testing was requested by GPs or regional hospitals in the Netherlands. Serological testing consisted of a screening C6 Enzyme Immunoassay (EIA) and a subsequent confirmatory immunoblot. We found that 82 % of the requests were
Summary and general discussion

not in accordance with guideline recommendations and 70% of the requests were from patients with a very low a priori chance for LB. Approximately 45% of all positive test results in our cohort belonged to patients with a very low a priori chance for an active B. burgdorferi infection. These patients propose a challenge for physicians, as they need to decide whether to prescribe antibiotic therapy, which has a risk of complications or can result in delayed treatment for the actual underlying disease. In contrast, only in a minority of cases did serological testing contribute to the diagnosis of LB, i.e. in case of symptoms compatible with LB or atypical skin lesions suspected for EM. In conclusion, the current use of serological tests in clinical practice could lead to overdiagnosis of LB. To prevent unnecessary antibiotic treatment and lower costs we recommend better implementation of current guidelines and more education on the low positive predicting value of current serologic tests for LB when not cautiously used. It should be mentioned that estimating the pre-test probability of LB can be difficult in daily practice. We therefore hope that the above and our findings in chapter 2 can support a physician in his or her decision-making whether to request a LB diagnostic test and to explain his or her considerations to the patient.

A tertiary multidisciplinary center for patients suspected of LB

When the diagnosis of LB has been established most patients are adequately diagnosed or treated by their GPs. However in some cases a referral to a medical specialist is needed. Because LB is a multisystem infectious disease, patients can be referred to several medical specialist for evaluation of LB associated symptoms. Motives for referral can be suspicion of disseminated diseases, inconclusive serological test results, atypical manifestations of LB, a second opinion by the patient or when there are persisting symptoms after (recommended) antibiotic treatment. The latter seems to occur rather often due to misdiagnosis of LB as described in chapter 2 and by others [245, 263]. In addition, a subset of LB patients may experience long-lasting and debilitating subjective symptoms despite recommended antibiotic treatment [115]. Although some have referred to this phenomena as “chronic Lyme”, this is considered a misnomer as it is can refer to different entities, namely late disseminated LB, persisting B. burgdorferi infection as well as to post-infectious disease symptoms, also known as ‘post-Treatment Lyme borreliosis syndrome’ (PTLBS). Criteria for PTLBS have been described in international guidelines and PTLBS is discussed in more detail in chapter 1 [37, 298]. A recent study by the Dutch National Institute for Public Health estimated there are a total of 1000-2500 patients in the Netherlands with persisting symptoms.
attributed to LB [105]. These patients were found to have a far more substantial disease burden compared to EM or disseminated LB [284]. PTLBS does not seem to be caused by an active B. burgdorferi infection, supported by several studies that have failed to detect active B. burgdorferi infection shown in these patients and the fact that patients with PTLBS do not benefit from additional and prolonged antibiotic therapy [124, 130, 131, 141]. However, the reported symptoms, disabilities and decreased quality of life in these patients do call for a thorough evaluation to exclude other underlying diseases and/or the possibility that antibiotic treatment for LB has been inadequate.

The Amsterdam Multidisciplinary Lyme Center (AMLC) at the Academic Medical Center Amsterdam was established in 2011 to evaluate patients suspected of LB in a multidisciplinary setting. The AMLC is a tertiary referral center and entails a collaboration between the outpatient clinics of infectious diseases, neurology, rheumatology, dermatology, psychiatrics and pediatrics and is supported by the department of medical microbiology. Patients that are referred by GPs or medical specialist are evaluated for all stages of LB. In addition, medical specialists at the AMLC evaluated in patients with persisting symptoms that were attributed to LB – either by the patient or by the referring physician - whether these symptoms were the result of a persisting B. burgdorferi infection. In chapter 3 we describe in a retrospective case series the characteristics and LB classification of 200 adult patients suspected of LB referred to the AMLC. Most patients presented with symptoms that were chronic (lasting more than a year) and were nonspecific for LB (e.g. fatigue, arthralgia or myalgia). Furthermore, more than half had already received antibiotic therapy prior to their visit at the AMLC. The majority of patients had B. burgdorferi antibodies present in serum, but only a minority had objective clinical findings compatible with LB (e.g. skin lesions, neuroborreliosis or arthritis). Patients at the AMLC were classified based on established criteria as having LB, PTLBS, persistent B. burgdorferi infection or no LB [256, 298]. In addition, cases were classified as ‘definite,’ ‘probable’ or ‘questionable’ by our own criteria to address the certainty or lack of certainty when considering LB as the cause of the presenting symptoms. On the basis of our classifications - which can be found in chapter 4 - that include medical history, clinical findings, exclusion of other diseases and extensive diagnostic tests, only a minority of patients suspected of LB (31; 16 %) had an EM or a form of disseminated LB. In the majority of patients (120; 60 %) LB could be excluded and in a significant proportion of those patients (43/120; 36 %), an alternative diagnosis could be established. The
remaining patients were classified as having either PTLBS (34; 17%) or persistent 
*B. burgdorferi* s.l. infection (15; 8%). Of importance, none of the patients classified 
as persistent *B. burgdorferi* s.l. infection were considered ‘definite’, in only three 
patients a persistent infection was considered ‘probable’ and in the remaining 12 
patients this was classified as ‘questionable’.

In total, in almost half of patients suspected of (persisting) LB (24/46; 52%), the 
certainty of a causal relation between an active *B. burgdorferi* infection and 
symptoms was considered to be ‘questionable’. In these patients it remained 
challenging to either rule out or demonstrate an association with a *B. burgdorferi* 
infection. However, addressing the uncertainty was useful for discussing treatment 
options with these patients, including antibiotic (re)treatment or a referral to a 
specialized center for Medically Unexplained Symptoms (MUS) at the VU Medical 
Center in Amsterdam after careful exclusion of other causes.

**Future directions to improve care for patients suspected of LB**

By our retrospective analysis of serological tests for LB in chapter 2 and the 
patients suspected of LB at a tertiary multidisciplinary center in chapter 3 we hope 
to contribute to improve care for patients suspected of LB. Both studies show that 
development of well-validated tests that can differentiate between an active and a 
previous *B. burgdorferi* infection would be extremely helpful for both physicians 
and patients. Research on such tests should be high on the (inter)national research 
agenda. Most tests that are currently being developed are cellular tests, of which 
some seem promising [9, 160], but these assays have as a disadvantage that they 
are expensive, logistically challenging and more difficult to standardize. Until new 
tests are available, care for suspected LB patients can be improved by education of 
patients and physicians about both the strengths and limitations of current 
diagnostics tests, as well as the lack of evidence for prolonged or repeated 
antibiotic treatment in case of PTLBS. We propose that patients suspected of LB 
who do not improve over time after antibiotic therapy should be preferably be 
evaluated in a specialized center, where also possible other causes could be 
identified. A persisting *B. burgdorferi* infection, albeit normally rare, should not be 
misunderstood, but physicians should also consider the psychopathogenesis of PTLBS 
[246]. Furthermore, patients with persisting symptoms after being treated for LB 
could be helped by counseling, education on lifestyle changes or even cognitive 
behavioral therapy. To prevent miscommunication and guide future discussions 
one should avoid terms as ‘chronic Lyme’, but instead use terms and criteria that
more precisely define the underlying cause of the complaints [47, 113, 183]. The national CBO guideline has replaced the term PTLBS with ‘Lyme related complaints’ [1], but this definition could be applied to post-infectious symptoms as well as symptoms caused by an active or persistent *B. burgdorferi* infection. We propose that, when I) nonspecific symptoms persist after recommended antibiotic treatment for a definite or probable case of LB and II) an ongoing *B. burgdorferi* infection is considered unlikely and III) other causes have been evaluated, to speak about post infectious symptoms or PTLBS. Such symptoms indeed occur after many other infectious diseases [103, 199]. We realize that PTLBS as well as our classifications for patients suspected of LB in chapter 3 are not flawless but we believe it will facilitate communication between physicians and patients and guide future research. Finally, it remains unsatisfying for patients, physicians and researchers to not have a better understanding of the phenomena of persisting symptoms after recommended antibiotic treatment for LB. Future research, for example the currently ongoing prospective trial in the Netherlands, the LymeProspect study, will hopefully provide more insight in this subject and identify causative or associated microbiological, immunological, genetic, cognitive or behavioral factors.

**Part II - Pathogenesis of Lyme borreliosis**

Care for LB patients can also be improved by a better understanding of the pathogenesis of LB. Forty years of research on *Ixodes* ticks, *B. burgdorferi* and host factors has significantly expanded our understanding of the pathogenesis of LB [114, 207]. A more detailed overview of the pathogenesis of LB is provided in chapter 1. *B. burgdorferi* transmission begins in the gut of *Ixodes* ticks from where *B. burgdorferi* migrates via the salivary glands into the skin of the host. Tick saliva that is injected into the skin during tick feeding contains proteins that impair immune and coagulation responses, thereby facilitating tick feeding for several days [114, 230]. Several immunosuppressive tick proteins have been identified that were found to interfere with innate immune responses, e.g. inhibiting the complement system and impairment of neutrophils and natural killer cells, as well as with adaptive immune responses, e.g. reduction in antibody titers and inhibition of B-cells and T-cells [62, 108, 134, 167]. Research on the tick-host-pathogen interface helps us understand the pathogenesis of LB, but can also reveal host factors that are associated with an increased risk for *B. burgdorferi* infection.
Tick salivary lectin pathway inhibitor

In chapter 4 and 5 we demonstrate the immunosuppressive mechanism of a recently identified tick salivary protein. Previously, Schuijt et al. performed a screening for I. scapularis tick proteins to which rabbits develop an antibody response after repeated tick infestation – a phenomena called ‘tick immunity’ [176, 275]. Tick immunity prevents successful tick feeding and can results in the death of ticks. By using the anti-tick antigen antibodies derived from the serum of a tick immune rabbit, tick salivary gland proteins that are crucial for tick feeding were identified. This screening identified several tick proteins that were instrumental for tick feeding [230, 234], of which one, the tick salivary gland protein P8, was found to not only facilitate tick feeding but also protect B. burgdorferi from killing by human serum [234].

Killing of B. burgdorferi by human serum is mediated by the complement system, which is a part of the innate immune response. Activation of the complement system results in direct lysis and death of the pathogen by the Membrane Attack Complex (MAC), attraction of immune cells and enabling of phagocytosis [215]. The complement system can be activated via three pathways, namely the classical pathway, the lectin pathway and the alternative pathway [215]. In chapter 4 we show that P8 impairs complement-mediated killing of B. burgdorferi by specifically inhibiting the lectin pathway of the complement system. Hence, we have named P8 “Tick Salivary Lectin Pathway Inhibitor” (TSLPI). Further analysis revealed that TSLPI inhibits the lectin pathway by interfering with Mannose Binding Lectin (MBL); a c-type lectin that activates the lectin pathway by recognizing carbohydrates on pathogens [215]. Our data indicate that TSLPI blocks MBL activity by binding to its carbohydrate recognition domains. Impaired MBL activity by TSLPI not only reduced complement-mediated killing of B. burgdorferi but also reduced phagocytosis of B. burgdorferi by human neutrophils and chemotaxis. The role of TSLPI in B. burgdorferi transmission to the vertebrate host was further assessed by in vivo studies using B. burgdorferi infected I. scapularis ticks. Borrelia transmission was impaired in ticks in which TSLPI was down-regulated by RNA-interference or in ticks that fed on TSLPI-immune mice. Moreover, B. burgdorferi acquisition – i.e. the uptake of B. burgdorferi from a B. burgdorferi infected host by the tick - was impaired in ticks feeding on TSLPI-immunized mice.
In addition, we also aimed to identify the *I. ricinus* ortholog of TSLPI. In chapter 5 we describe an *I. ricinus* ortholog with 89% homology to *I. scapularis* TSLPI that was also capable of inhibiting MBL activity. The *I. ricinus* TSLPI ortholog decreased complement mediated killing of *B. burgdorferi* and was also found to protect *B. garinii*, a more prevalent complement-sensitive *Borrelia burgdorferi* s.l. genospecies in Europe that can cause LB. In conclusion, our work demonstrates the exploitation of a vector protein by *Borrelia* to facilitate transmission and illuminates the central role of the lectin complement pathway in the eradication of *Borrelia*.

**The role of MBL during *B. burgdorferi* infection**

Based on the role of the lectin pathway in the innate immune response against *B. burgdorferi* infection we hypothesized that individuals that lack MBL are more susceptible for LB. MBL deficiency is reported to be as high as 25% in the general population and has been associated with a more severe course of disease for other infectious diseases [70, 86, 287]. Interestingly, a recent study showed that MBL deficiency was found more often in individuals with antibodies against *B. burgdorferi* compared to seronegative individuals [224]. In chapter 6 we have described the role of MBL during *B. burgdorferi* infection by using genetically modified MBL deficient mice. We observed that MBL deficient mice developed a higher *B. burgdorferi* burden in skin compared to wildtype (WT) mice. This was shown in mice infected with *B. burgdorferi* by needle inoculation and in mice that were infested with *B. burgdorferi*-infected *I. scapularis* ticks. However, in both models there was no difference in *B. burgdorferi* dissemination to other organs between MBL deficient mice and WT mice. Ex vivo experiments such as complement killing assays, peritoneal macrophage and whole blood stimulations and phagocytosis assays did not reveal the underlying mechanism.

In summary, we have identified an immunosuppressive tick salivary protein that facilitates *B. burgdorferi* infection by inhibition of the complement lectin pathway via MBL. The role of the lectin pathway in the pathogenesis of LB was further demonstrated by the protective role of MBL in the early stages of *B. burgdorferi* murine infection. Although our *in vitro* experiments using human serum in chapter 4 and 5 showed that inhibition of MBL by TSLPI reduced complement mediated *B. burgdorferi* killing and phagocytosis by human neutrophils, these findings could not be corroborated with murine serum lacking MBL in chapter 6. Limited functionality of murine serum *ex vivo* compared to human serum could have been a contributing factor to this discrepancy [145]. Also, we speculate that there could
be compensatory mechanisms, for example an upregulation of ficolins in MBL deficient mice, compensating for a decreased ability to kill complement-sensitive spirochetes by MBL deficient serum in vitro. In addition, future research should attempt to clarify the mechanism by which MBL deficiency results in increased burden of *B. burgdorferi* infection in the murine skin in vivo. We hypothesize that MBL is involved in the early clearing of spirochetes by innate immune response in local skin tissue by being involved in chemotaxis of phagocytes. This could be further investigated by assessing early migration and influx of neutrophils and macrophages upon *B. burgdorferi* infection in murine skin [109]. Furthermore, comparing pathogen transmission between normal ticks and TSLPI-silenced ticks on both WT and MBL deficient mice could help understand the interaction between TSLPI, MBL and the lectin pathway during *B. burgdorferi* infection. Finally, we will assess in the currently ongoing prospective study LymeProspect whether MBL is associated with an increased susceptibility for LB (see above in Part I).

**PART III Prevention**

*A vaccine to prevent LB*

With the increasing incidence of LB in Europe and the USA, a vaccine that could prevent LB is becoming a much-needed alternative to reduce the disease burden of LB. Although antibiotics are effective in the treatment of LB, (unobserved) tick bites can also result in the dissemination of *B. burgdorferi* to vital organs [256]. Also, rare but serious adverse effects of antibiotic therapy can occur and disabling symptoms may persist after treatment [115]. An overview of preventive measurements, including previous efforts to develop a vaccine against LB such as the Lymerix® vaccine, is described in chapter 1.

The increase in tick bites, incidence of LB and growing public awareness for LB since the 1990s have increased interests to develop a new vaccine after the withdrawal of Lymerix® in 2002 because of public perceptions on adverse events. Recent efforts have resulted in an improved multivalent OspA vaccine design to protect against all *B. burgdorferi* s.l. species known to cause LB, making it more attractive for European countries [303]. Although a randomized double-blind phase I/II trial published in 2013 showed the multivalent OspA vaccine was well-tolerated, a subsequent efficacy (Phase III) trial has yet to be performed [303]. The previous controversy surrounding Lymerix® could hamper a successful
reintroduction of an OspA-based vaccine [148] and therefore alternative vaccine strategies to prevent LB in humans have to be explored.

An anti-tick vaccine to prevent Lyme borreliosis

One alternative vaccine strategy that has been explored by us and other research groups are vaccines that target tick antigens to prevent tick feeding and/or B. burgdorferi transmission [233]. The rational to use an anti-tick vaccine to prevent LB is based on observations of acquired tick immunity in guinea pigs and rabbits after repeated exposure to tick bites which have been first described in 1939 by William Trager [275]. Ticks that feed on tick immune animals have lower post feeding weights and can even be killed by the immune response of the host. In addition, pathogen transmission to tick immune animals is reduced [176]. Vaccines based on tick salivary gland proteins previously identified to facilitate B. burgdorferi transmission, including Salp15, TSLPI and tHRF provided (partial) protection against B. burgdorferi transmission [52, 53, 231].

Targeting Ixodes gut proteins has not been an extensively explored vaccine strategy, as these proteins are more concealed from the host immune response. Tick gut proteins could be interesting targets for vaccination because the gut plays a crucial role in both tick feeding and B. burgdorferi transmission [59, 207]. Initial research on the OspA vaccine already demonstrated that antibodies from the host are effectively delivered in the Ixodes tick gut [79]. The few Ixodes gut protein vaccines that have been targeted until now were effective in reducing successful tick feeding or B. burgdorferi colonization of the gut [94, 194]. In this thesis we have explored two novel strategies for an Ixodes tick gut based vaccine. The first strategy that we explored was to target adult Ixodes ricinus tick feeding. Our hypothesis was that a vaccine that can effectively impair feeding of adult female I. ricinus ticks would result in reduced oviposition (laying eggs) and would consequently reduce Ixodes ricinus tick populations. As Ixodes ricinus is the most important reservoir host of B. burgdorferi in Europe this would consequently result in reduced exposure of humans to B. burgdorferi-infected ticks. However, tick gut proteins that are known to facilitate feeding of Ixodes ricinus are not well-studied. Therefore, we used Ixodes ricinus proteins that share homology with a protein from another tick that is used in a commercially available anti-tick vaccine. GavaC™ and TickGARD Plus™ effectively reduce feeding of R. microplus and partially reduce transmission of the parasite Babesia bovis to cattle by targeting the gut glycoprotein Bm86 [90, 294]. In chapter 7 we tested two Ixodes ricinus homologues of Bm86, designated Ir86-1 and
Ir86-2 [184], as vaccines to prevent successful *Ixodes ricinus* tick feeding [184]. The effect of Ir86-1 and Ir86-2 vaccines on tick feeding was studied by immunizing rabbits against recombinant Ir86-1, Ir86-2 or a combination of those two. After high titers against proteins were realized, we infested vaccinated rabbits with adult *I. ricinus* ticks. Tick weights were measured after feeding and the weight of eggs produced by female ticks was measured after several weeks. The three vaccines were initially tested in a pilot study in a single rabbit. The vaccine that included Ir86-1 and Ir86-2 seemed to reduce tick feeding by approximately 32%. However, a confirmatory experiment with a larger number of immunized animals did not show any effect on tick feeding; therefore we concluded that Bm86 homologues in *Ixodes ricinus* do not impair tick feeding or egg production.

**Targeting tick gut proteins that facilitate *B. burgdorferi* transmission**

The second strategy that we explored was to prevent *B. burgdorferi* migration from the gut by identifying and targeting tick midgut proteins involved in this process. Early in infection, before migration from the gut to the salivary glands occurs, *B. burgdorferi* has been shown to bind to, and congregate at, the epithelium of the gut [69]. We hypothesized that by blocking the interaction of *B. burgdorferi* with tick midgut epithelium we could prevent *B. burgdorferi* egress from the tick gut. As only a few *B. burgdorferi*-interacting tick gut proteins have so far been identified, we screened for novel tick gut proteins to which *B. burgdorferi* could bind. In chapter 8 we describe the screening method we performed, i.e. Yeast Surface Display (YSD) technology, which allows for expression of eukaryotic proteins with post-translational modifications on the membrane of modified *S. cerevisiae* yeast cells [198, 234]. For this method we transfected yeast cells with a cDNA library of *I. scapularis* gut proteins to create a large library of yeast cells that display tick gut proteins on their extracellular membrane. Subsequently, via enrichment with magnetic sorting and flow cytometry, those yeast clones that were bound by purified *B. burgdorferi* membrane extract were selected and sequenced to identify the involved *Ixodes* gut protein. The screening identified four unknown tick gut proteins with *B. burgdorferi*-binding properties. Two proteins were selected for further characterization based on their putative protein function and predicted cellular location. One protein was predicted to be an extracellular protein with a potential transmembrane domain and four fibronectin type III domains and was named *I. scapularis* fibronectin-like protein (Ixofin3D). The other protein had a potential transmembrane domain and several dystroglycan-like domains and was therefore named *I. scapularis* dystroglycan like protein (ISDLP).
The characteristics of Ixofin3D and ISDLP are described in respectively chapter 8 and chapter 9. We confirmed the presence of native Ixofin3D and ISDLP in the tick gut and showed that expression of both Ixofin3D and ISDLP was increased during tick feeding and that for both proteins expression was higher in B. burgdorferi-infected compared to non-infected ticks. In addition, in vitro experiments confirmed binding of both recombinant proteins to B. burgdorferi. To determine whether Ixofin3D and ISDLP facilitate B. burgdorferi transmission, expression of Ixofin3D or ISDLP during tick feeding was down-regulated using RNA-interference (RNAi) in ticks. Down-regulation of both Ixofin3D and ISDLP resulted in decreased migration of spirochetes to the salivary glands and consequently decreased transmission to the murine host, indicating both proteins are instrumental for B. burgdorferi transmission. We therefore immunized mice against Ixofin3D or ISDLP to test whether a vaccine based on either protein was effective in reducing B. burgdorferi transmission and found that whereas vaccination against ISDLP did not significantly reduce transmission to the murine host, vaccination against Ixofin3D resulted in lower B. burgdorferi burden in the murine skin at day 7 and day 14 post-tick feeding. Additional research showed that antibodies against ISDLP were able to bind ISDLP but were unable to prevent binding of Borrelia to ISDLP. Next, to further elucidate how Ixofin3D facilitates B. burgdorferi transmission we decreased ixofin3d expression in the I. scapularis gut by RNAi. The effect of silenced Ixofin3D protein levels on B. burgdorferi binding to the gut epithelium and migration to the salivary glands was studied by immunofluorescence microscopy. Interestingly, spirochetes in ixofin3d-silenced ticks did not attach to gut epithelial cells but remained in the gut lumen; indicating that spirochete attachment to the gut – a critical step for spirochete migration to the salivary glands and host - is Ixofin3D dependent. Future vaccine research should determine whether vaccination against Ixofin3D is sufficiently effective to prevent B. burgdorferi exit from the gut. The effect on B. burgdorferi transmission by Ixofin3D vaccination was not as effective as RNAi, which could be due to suboptimal blocking of B. burgdorferi interacting epitopes by anti-Ixofin3D antibodies. Identification of the specific Ixofin3D epitopes to which B. burgdorferi binds could enhance our understanding how B. burgdorferi migration from the gut is facilitated. This could result in an improved vaccine design that specifically targets these epitopes, thereby more effectively impairing B. burgdorferi migration from the gut.
Future directions for an anti-tick vaccine to prevent LB
Since the withdrawal of the Lymerix® vaccine several anti-tick vaccines to prevent LB have been explored [52, 53, 231]. Anti-tick vaccines have shown to be partially effective, possibly due to functional and structural paralogy of tick proteins [214]. We therefore speculate that a combination of two or more tick proteins is the key to a successful vaccine. Future research should test the efficacy of a vaccine that targets various aspects of B. burgdorferi transmission, for example a vaccine that targets Ixofin3D and TSLPI. In addition, artificial Ixodes tick feeding models, which allow for rapid assessment of the efficacy of combinations of anti-tick vaccines, can improve vaccine development. Ideally an anti-tick vaccine should not only prevent B. burgdorferi transmission, but also prevent against other tick borne diseases that can be transmitted by Ixodes ticks, such as Tick Borne Encephalitis Virus, Borrelia miyamotoi, Anaplasma phagocytophilum, Rickettsia spp. and Babesia spp. Although localization within the tick and tick-pathogen interactions differs for each of these pathogens, identifying and combining tick antigens that facilitate transmission of these pathogens would be worthwhile to explore. A large European consortium (ANTIDotE) is currently in search of an anti-tick vaccine that prevents transmission of B. burgdorferi, as well as other tick borne diseases, using proteomic- and transcriptomic-based techniques to identify tick proteins that could be used as novel vaccine targets [251].

Obviously, when applied in humans, such an anti-tick vaccine would need to be safe and effective and would need to be able to elicit high immunogenicity and long-lasting protective antibody titers. Perhaps even more important is public education on the need for a LB vaccine, as well as on the efficacy and possible side effects of a future vaccine. Furthermore, previous calculations in the USA which predict that a vaccine is economically attractive in areas with an annual risk of more than 1 % of contracting LB should also be performed in Europe [241]. Individuals or groups with a high risk for contracting LB as well as high-risk areas in Europe and the USA for contracting LB should be identified to determine the feasibility of a future LB vaccine. One of the objectives of the ANTIDotE project is to integrate the interests of public health institutes, health organizations and industrial companies in the exploitation and implementation of a future (anti-tick) vaccine. Despite the withdrawal of Lymerix®, based on the extensive vaccine research in the last decennia there is enough reason to remain optimistic for a vaccine that can prevent LB.