ABSTRACT

Lyme borreliosis (LB) is a multisystem infectious disease abundant in the northern countries of the world and is caused by Borrelia species. Vaccination against LB is an effective way to prevent and reduce the number of diseases in endemic areas. Several vaccines have been developed and tested in the past, but no human LB vaccine is currently available on the market. This review aims to uncover and delineate various strategies and diverse technological approaches related to vaccine production. Furthermore, we characterize already tested vaccines, possibilities for their future development, and reasons for their failure.

Key words: Borrelia; LB prevention; Lyme borreliosis; vaccination options

INTRODUCTION

Lyme borreliosis (LB) is one of the most common infectious diseases transmitted by arthropods in temperate regions of northern America, Asia, and Europe. LB is caused by spirochetes of the Borrelia species, in the USA it is B. burgdorferi, while in Europe and Asia it is B. afzelii, B. garinii, or B. bavariensis [20, 40]. There is currently a debate on the feasibility of separating LB into a separate genus Borrelia, including B. burgdorferi sensu stricto (s.s.) and other genospecies B. burgdorferi sensu lato (s.l.) complex [1]. The Centre for Disease Control and Prevention estimates that there are approximately 300,000 cases of LB per year in the United States, and the incidence in Europe is 230,000 cases per year [41, 44, 58].

Lyme borreliosis, one of the most common seasonal diseases transmitted by tick bites, is essentially a bacterial infection that is successfully treated with antibiotics (the fatal consequences of LB are very rare) [2]. However, the most important is the early detection of a tick bite (at the acute phase) and immediate antibiotic treatment. If the infection occurs without primary manifestations, the disease often progresses to a chronic stage, when antibiotic treatment is less effective and undesirable health complications can occur [48, 55].
Usually the symptoms of the disease range from erythema migrans (which is a typical manifestation of LB) to infections of the nervous system (neuroborreliosis), heart (Lyme carditis), joints (Lyme arthritis), or skin (acrodermatitis chronica atroficans) [40] (Table 1).

Ticks *Ixodes* spp. go through a life cycle in three stages: a larva, a nymph, and an adult. Although some species of *Borrelia* can be transmitted transvariously, in *B. burgdorferi* s.l complex this does not happen (each generation of ticks must be re-infected) [47]. The life cycle of ticks *Ixodes* spp. is shown in Fig. 1.

### VACCINES FOR LYME BORRELIOSIS

The vaccination against various infectious diseases is considered to be a highly effective means of controlling the spread of a given infection in a population [11]. Prophylactic vaccination against LB is a relatively attractive approach in preventing the risk of LB infection [12].

The development of new strategies in LB vaccination focuses on the host and its immunity, and the reservoirs of bacteria (including ticks and vertebrates themselves). There is a need to address how bacteria can be targeted via

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**Table 1. Stages and clinical features of Lyme borreliosis. Adapted from [57]**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage I</strong></td>
<td><strong>Early and localized infection</strong></td>
</tr>
<tr>
<td></td>
<td>Erythema migrans</td>
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<td></td>
<td>Borrelial lymphocytoma</td>
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<td><strong>Stage II</strong></td>
<td><strong>Systemic symptoms, disseminated infection</strong></td>
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<tr>
<td></td>
<td>Lyme neuroborreliosis</td>
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<td></td>
<td>Lyme carditis</td>
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<tr>
<td><strong>Stage III</strong></td>
<td><strong>No systemic syndromes, localized infection</strong></td>
</tr>
<tr>
<td></td>
<td>Lyme arthritis</td>
</tr>
<tr>
<td></td>
<td>Acrodermatitis chronic atrophicans</td>
</tr>
</tbody>
</table>

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**Fig. 1. The Enzootic cycle of *B. burgdorferi***

1—*B. burgdorferi* is kept in its reservoir host; 1—The infected nymphal tick can transmit *B. burgdorferi* to animals including humans, during feeding; 2—The nymphal tick moults to the adult stage, which feeds and mates on large mammals and lays eggs; 3—Eggs hatch into uninfected larval ticks; 4—*B. burgdorferi* is transmitted by larval tick feeding on an infected small mammal; 5—The larval tick moults to a nymph
various vaccine candidates for direct application in human and veterinary medicine. Importantly, it is crucial to disrupt the transmission of substances (agents) that maintain the enzootic cycle of *B. burgdorferi* and study how these indirect strategies will affect the incidence of an infection in a random host (human and domestic animals) [15].

**Vaccines directed against spirochetes in the vector: outer surface protein A**

Outer surface protein (OspA) is considered a leading vaccinogen against LB. OspA (31.5—33 kDa) is a surface protein expressed in the unfed midgut of ticks. OspA expression allows spirochete colonization and persistence in the vector by TROSPA (Tick receptor of OspA) binding [12]. Thus, antibodies directed against OspA can recognize and neutralize spirochetes in the vector in a complementary manner [8].

Intensive research into LB vaccines took place in the United States in the 1990s, when two different vaccines against LB appeared [39]. Both types of vaccines: LYMErix™ (SmithKline Beecham, now GlaxoSmithKline) and ImuLyme™ (Pasteur Mérix Connaught, now Sanofi Pasteur) used recombinant OspA as the immunogen [42]. The mechanism of action was the same for both vaccines: the OspA vaccine-induced host antibodies, neutralized the OspA expressed by the spirochetes in the vector and thus blocked the transmission of pathogens from the vector to the host [26].

The LYMErix™ vaccine was marketed in 1998 and was voluntarily withdrawn from the market in 2002. The vaccine consisted of 30 μg of recombinant OspA lipoprotein expressed in *Escherichia coli* as an adjuvant with aluminum hydroxide. The specific OspA strain used in the vaccine was *B. burgdorferi* s.s. strain B31 with no adjuvant used. ImuLyme™ has undergone the third phase of clinical trials (more than 10,000 subjects aged 18—92 years from LB endemic areas) [42]. The efficacy of the vaccine was determined to be 68% after two doses and 92% after three doses [53]. However, the vaccine manufacturer did not apply for its approval, nor did he specify exactly the reasons why he did so [42].

A second-generation OspA vaccine, VLA 15 (Valneva, France), has recently been developed and is currently in clinical trials. Clinically relevant OspA serotypes that cause most LB infections in Europe and North America have been used to develop the VLA 15 vaccine, as follows: *B. burgdorferi* (serotype 1-ST 1), *B. afzelii* (serotype 2-ST 2), *B. garinii* (serotype 3,5,6-ST 3,5), and *B. bavariensis* (serotype 4-ST4) [13, 38]. It is a multivalent protein LB subunit vaccine consisting of three OspA heterodimers (each heterodimer is a fusion of the C-terminal portion of OspA from two different serotypes with a 21 amino acid long linker) [8].

The vaccine is currently in the second phase of clinical trials, a randomized, observer-blinded, placebo-controlled study in endemic areas of the United States and Europe. The clinical trial involved 452 healthy subjects aged 18 to 65 years who received the vaccine (dose level 135 μg and 180 μg) intramuscularly in three doses on days 1, 29, and 57. Immunogenicity was determined as the number of IgG antibodies against each of the six most prevalent OspA LB serotypes at day 85 (i.e. one month after the end of the primary immunization) [62]. Preliminary results published on the vaccine manufacturer’s website are as follows:

Vaccine VLA 15 is immunogenic in all tested groups (in the group from 18—49 years, the seroconversion rate ranged from 85.6—97%). This vaccine is also safe at all doses tested and in all groups. The tolerability profile, including the occurrence of fever, is comparable to other lipid recombinant vaccines. Reactogenicity decreased af-
ter the first dose. The seroconversion rate (SCR) showed similar responses after primary vaccination and ranged from 93.8—98.8% [62]. The next step in clinical trials is a further randomized, placebo-controlled phase II under the following conditions: 600 healthy participants, aged 5—65 years (including the pediatric population aged 5—17), who will be vaccinated at a dose of 180 µg (based on the results already published). The aim is to compare two types of dose plans: initial vaccination (0—2—6 months) and reduced (0—6). The initial data from this study are expected in the second quarter of 2022 [61].

**Vaccines directed against spirochetes in the host—outer surface protein C**

There may be several strains of *B. burgdorferi* s. s. in one endemic area, and a high proportion of reservoirs may be infected by more than one strain, which means that patients with LB can be infected with another strain of *Borrelia* [6, 52] even after successful antibiotic treatment [21]. The outer surface protein—OspC (22 kDa) has long been considered a suitable candidate for an LB vaccine, because it’s a highly polymorphic surface protein that is recognized by strain-specific neutralizing antibodies in infected hosts. Anti-OspC antibodies protect against infection, but only against OspC-expressing strains [9]. However, the scope of prevention is relatively narrow due to the specific intertype variation of epitopes, but on the other hand, these specific OspC epitopes allow the development of chimeric polyvalent recombinant OspC vaccines [10, 54].

In 1999, Immuno-AG (now Baxter, Austria) produced a 5-valent adjuvanted OspC vaccine composed of a recombinant OspC strain of *B. burgdorferi* and two strains from *B. afzelii* and *B. garinii*. After three administrations of the multivalent OspC vaccine, more than 95% of the subjects showed an antibody response. It was relatively well tolerated in phase I clinical trials, but approximately half of the subjects showed erythema and swelling at the injection site. Phase II safety and preliminary efficacy data have never been disclosed due to the reactogenicity of the skin at the site of application [54].

The reason for the failure of OspC vaccines was probably the fact that there is significant heterogeneity of OspC between *Borrelia* spp. Therefore, it is necessary to develop a vaccine that contains several heterologous epitopes, which would increase its efficacy [17].

**Chimeric vaccines against LB**

Chimeric vaccines can be characterized as subunit vaccines containing chimeric proteins that consist of linear epitopes derived from different proteins, i.e., their variants. The advantage of chimerotope vaccinogens is that they can be designed to induce a robust antibody response [18]. One of the chimeric vaccines that has already come into clinical use is the canine vaccine VANGUARD®crLyme (Tab. 2). This subunit chimeric vaccine consists of the outer surface protein OspA and 14 different linear epitopes-chimerotope of OspC. Combining the OspA and chimerotope OspC into a single vaccine has led to the development of a humoral immune response that has a synergistic effect on targeting spirochetes, both in ticks and mammals [35].

A six-component vaccine has been developed containing the outer surface protein OspA, which has been combined with bacterial ferritin to form self-assembling nanoparticles. OspA-ferritin nanoparticles have elicited high antibody titres for more than six months (in mice, and rhesus macaques) against infection by American, European, or Asian strains of *Borrelia* and offer their potential to prevent the spread of LB [20].

**Vaccines based on blocking LB transmission**

Transmission-blocking vaccines are divided into LB reservoir-targeted vaccines and anti-tick vaccines. The implementation of effective bacterial transmission-blocking strategies as a tool to control the incidence of LB depends on several factors: an understanding of the eco-epidemiological determinants (informing about the risk of potential LB in a certain geographical location) and the development of suitable vaccine carriers [15].

Vaccines targeting animal reservoirs affect the natural enzootic cycle and reduce the risk of LB by reducing the number of infected vectors. This hypothesis was tested as follows: mice (*Peromyscus leucopus*) were subcutaneously administered a recombinant OspA vaccine, which resulted in a reduction in the prevalence of nymphal infection after one year. OspA-based vaccines are effective against most species and strains of *B. burgdorferi* [36]. Various potential candidates for a transmission-blocking vaccine were evaluated:

- Surface proteins of *B. burgdorferi* (BB0405, BBA52, BBI39),
• Tick antigens (subolesin, salivary proteins, tick salivary lectin pathway inhibitor, tick histamine factor) [4—27].

The introduction of vaccines targeting LB reservoirs as a part of integrated pest management depends on the development of oral carriers for immunogen delivery [16, 50].

**LB vaccines based on outer surface membrane vesicles**

Outer surface membrane vesicles (OMVs) can be characterized as bilayer, spherical and membranous nanostructures with a size of 20—250 nm, which are released during the growth of various gram-negative bacteria [19, 45]. The OMVs are composed of lipopolysaccharides, phospholipids, outer membrane proteins, and entrapped periplasmic components. Due to their natural properties such as: immunogenicity, self-adjuvant and absorption by immune cells, they are considered to be attractive for vaccines development against various pathogenic bacteria, including *B. burgdorferi* [14].

A great advantage of OMVs is the modulation of the host immune response through the transfer of antigens (including heterologous ones) from other pathogens that have been expressed on the vesicular structure. Antigens generated by exposure to the surface of OMVs are capable of forming specific binding through B cells as well as antigens without surface exposure are capable of inducing an antibody response [34].

Recently, a vaccine against LB was developed based on native OMVs (nOMVs), which were isolated by EDTA extraction from *N. meningitidis* serogroup B OMVs with OspA *B. burgdorferi* B 31. The vaccine (40 µg meningococcal nOMVs) was tested in mice (females, from six to eight weeks) to which the vaccine was administered according to the following schedule: 0, 14, and 28 days, antibody titres were determined from blood serum on day 42 of the experiment. Subsequently (two weeks after vaccination with the last dose), mice were administered subcutaneously with *B. burgdorferi* N40 (1 × 10⁶ spirochetes). Mice were sacrificed on day 62 and samples were collected from various tissues (skin, bladder, ear, heart, and ankle) that were cultured to detect spirochetes. The results of the experiment documented that the vaccine based on native OMVs induced high antibody titres. Furthermore, vaccinated mice were protected from infection revealing significantly lower amounts of spirochetes in analysed tissue [22].

**Vaccines based on the principle of DNA against LB**

The DNA vaccines against LB represent one of the innovative and alternative approaches as standard research on LB vaccines focuses on recombinant proteins [5, 23]. The DNA vaccines can be characterized as vaccines containing purified plasmid particles that contain transgenes encoding proteins or peptides that elicit an immune response (via T and B cell activation) against various diseases. The DNA vaccine can be considered immunogenic, safe (without significant side effects), and it can also be modified in a relatively short time [28]. One of the disadvantages of this vaccine is its lower efficiency in producing antibodies [43].

A codon-optimized bb0405 DNA vaccine was prepared according to the procedure published in [64] using the plasmid pVAC, which was tested as follows: The DNA vaccine was administered to mice, and the vaccine was found not to induce sufficient antibody production or protection against strain *B. afzelii* (mice were stimulated with a tick nymph infected with this spirochete). This phenomenon has been explained by the fact that *B. afzelii* spirochetes drastically reduce the expression of the BB0405 protein and thus the DNA vaccine does not provide cross-protection against *B. afzelii* in a mammalian host [23].

Recently a study was published in which the authors evaluated several DNA tattoo vaccines against borreli al OspC or tick antigens. One group of mice received an OspC DNA vaccine capable of generating a robust IgG response, with no positive culture of *B. burgdorferi* s.l. (after infection of mice with *I. scapularis* nymphs) present in the skin and bladder tissues of most mice. Tick Salivary Gland DNA vaccine (against TSLPI, Salp15, tHRF, as well as Tix-5) did not induce robust IgG responses in mice, and spirochetes were also detected in the skin and bladder tissues in *B. burgdorferi* infection in all individuals. The DNA tattoo vaccination can be considered as an effective vaccination platform aimed at evaluating new candidates of *B. burgdorferi* antigens in a relevant model [24].

**Development of new LB vaccinogens on the omics approach**

The availability of *in silico* methods and "omics" technologies such as genomics, proteomics, transcriptomics, or immunomics have simplified and accelerated the development and selection of suitable vaccinogens for various diseases (including LB) [37]. The most likely com-
ponent of the vaccine is the protein expressed during the infection of the host. Not only the identification of a suitable vaccinogen, but also the definition of immunological correlates is important for vaccine development due to the choice of a suitable carrier—vectors, adjuvants and also the vaccination schedule [25]. Bencúrová et al. [3] identified the following proteins as potential candidates for the LB vaccine by interactome and orthological reconstruction: ErpX, ErpL, ErpY, and also VLP (Variable large protein). These proteins also have a suitable antigenic profile as well as a positive compartment localization, making them good vaccine candidates. [3].

**Canine LB vaccination**

LB is a tick-borne disease often found in dogs, but the majority of dogs do not become ill after infection [33, 51]. There are currently several commercially available vaccines for dogs on the market, such as VANGUARD®-Lyme (Zoetis, USA), Nobivac® (Merck Animal Health, Germany), Borrelym (Bioveta, Czech Republic), Duramune Lyme (Elanco, USA), which are recommended for vaccination of dogs in endemic areas of America and Europe. A comparison of several commercially available OspA vaccines was performed, where it was shown that the administration of LB vaccine in three doses significantly increased antibody titres more than administration of the vaccine in two doses [60]. High protection was also found in dogs given a bivalent vaccine consisting of a strain of *B. burgdorferi* and negative strains OspA and OspC. This approach resulted in high antibody titres against OspA and OspC [30]. One year after vaccine administration, OspA antibody titres decreased and OspC antibody titres disappeared. After the addition of the infected tick, 40% of the vaccinated dogs were infected with *Borrelia*, and the infection was removed within two months and there was no dissemination of LB to other organs. Thus, vaccination of dogs with multiple Osp proteins is effective [29].

A summary of LB vaccines (for human and veterinary use) is in Table 2.

**CONCLUSIONS**

The development of an effective vaccine to prevent LB remains a major challenge, as the non- adoption of the first LYMErix™ vaccine by the public has hampered their further development.

Various potential candidates and strategies against LB are currently being discussed and researched. The leading vaccinogen against LB is considered to be the OspA expressed by bacteria of *Borrelia* species. Several vaccines have been developed on this approach: the first-generation vaccines, LYMErix™ and ImuLyme™, were based on the full-length OspA isolated from *B. burgdorferi* s.s. (serotype 1), which limited their use to the North American region. However, the effectiveness of the vaccine was at the level of 80%. The recently developed VLA 15 vaccine (Valneva, France) is a multivalent OspA vaccine containing 6 different serotypes of *Borrelia* species. This vaccine is currently in clinical trials; partial results are given in the article. Other approaches to vaccination are based on the principle of blocking spirochetes in the host (on this principle a polyvalent OspC vaccine was developed which, however, due to the reactogenicity of the skin at the injection site did not get into practice) or blocking LB transmission at the reservoir, which, however, is conditioned by the development of a suitable oral carrier. New approaches in the development of anti-LB vaccines are chimeric vaccines, such as DNA-based vaccines, and OMVs-based vaccines. These perspectives are currently at the stage of intensive research but have not yet entered clinical practice.

There are several reasons why the LB vaccine is still not available. One reason is that vaccine production is time-consuming and technically demanding, as well as the fact that anti-LB vaccine production has been a subject of discussion from the beginning (insufficient documentation of serious adverse reactions in the first vaccines). Well, there is still a great need to develop vaccines against this disease, because several thousand cases of LB are reported every year. Among other reasons why human vaccines were not very successful was the fact that the disease against which the vaccine was supposed to protect is relatively well manageable even with antibiotic treatment (the fatal consequences of LB are very rare).

The aim is to develop a vaccine with the efficiency of at least 80% for two years, which prevents the transfer of LB in Europe as well as in North America, that is tolerated (without cross-reaction epitopes and human proteins) and is also approved for the use in children.
<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Use</th>
<th>Principle</th>
<th>Vaccination schedule</th>
<th>Efficacy</th>
<th>Side effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYMErix™ (SmithKline Beecham, now GlaxoSmithKline)</td>
<td>Human</td>
<td>30 µg of lipidated recombinant OspA of B. burgdorferi s.s. strain ZS7 adsorbed onto the aluminum adjuvant</td>
<td>0 — 1—12 months (dose 0.5 ml)</td>
<td>50 % after 2 doses 78 % after 3 doses</td>
<td>• muscle and joint pain  • pain and redness at the injection site  • fatigue  • numbness and tingling  • backache and headache</td>
<td>[7] [49]</td>
</tr>
<tr>
<td>ImuLyme™ (Pasteur Mérieux Connaught, now Sanofi Pasteur)</td>
<td>Human</td>
<td>30 µg recombinant OspA of B. burgdorferi s.s. strain B31 was used in the manufacture of the vaccine, without adjuvant</td>
<td>0 — 1—12 months (dose 0.5 ml)</td>
<td>68 % after 2 doses 92 % after 3 doses</td>
<td>No significant side effects were noted (in clinical trials)</td>
<td>[46] [53] [42]</td>
</tr>
<tr>
<td>VLA 15 (Valneva France)</td>
<td>Human</td>
<td>135/180 µg C-terminal part of OspA serotypes: B. burgdorferi (ST 1) B. afzelii (ST 2) B. garinii (ST3, ST5, ST6) B. bavariensis (ST 4) with aluminium hydroxide</td>
<td>0—2—6 months or 0—6 months (dose 0.5 ml)</td>
<td>SCRs showed similar responses after primary vaccination and ranged from 93.8—98.8 %</td>
<td>No related serious adverse events</td>
<td>[63] [62]</td>
</tr>
<tr>
<td>Multivalent OspC vaccine (Immuno-Ag, now Baxter)</td>
<td>Human</td>
<td>Recombinant OspC of: B. burgdorferi B. afzelii and B. garinii adsorbed onto the aluminum adjuvant</td>
<td>No information</td>
<td>Preliminary efficacy and safety data have not been disclosed</td>
<td>Erythema and swelling on injection side (in clinical trials)</td>
<td>[54]</td>
</tr>
<tr>
<td>VANGUARD®cr-Lyme (Zoetis, USA)</td>
<td>Veterinary</td>
<td>OspA and recombinant chimeric OspC of B. burgdorferi</td>
<td>0—21 days (dose 1 ml)</td>
<td>The efficacy of the vaccine was determined as an absence of development of antibodies to peptide C6, non-manifestation of clinical symptoms of LB</td>
<td>No information</td>
<td>[35]</td>
</tr>
<tr>
<td>Nobivac® Lyme (Merck Animal Health, Germany)</td>
<td>Veterinary</td>
<td>Two inactivated isolates of B. burgdorferi</td>
<td>0—14 (28) days</td>
<td>The efficacy of the vaccine was determined as induction of borreliacidal activity (OspA and OspC)</td>
<td>Swelling of soft tissue (temporary)</td>
<td>[29] [30]</td>
</tr>
<tr>
<td>Borrelym (Bioveta, Czech Republic)</td>
<td>Veterinary</td>
<td>Inactivated B. burgdorferi sensu lato (B. garinii, B. afzelii) and B. burgdorferi s. s.</td>
<td>0—14 (21) days</td>
<td>The efficacy of the vaccine was determined as induction anti-OspA antibodies against Borrelia spp.</td>
<td>Transient swelling (rare) Temporary increase of body temperature Allergic reaction (very rare cases)</td>
<td>[65]</td>
</tr>
<tr>
<td>Duramune Lyme (Elanco, USA)</td>
<td>Veterinary</td>
<td>Multiple types OspA, OspB, and OspC of B. burgdorferi</td>
<td>0—14 (21) days</td>
<td>92.2 % against natural infection in endemic areas</td>
<td>No information</td>
<td>[31] [32]</td>
</tr>
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</table>
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