



VACCINES FOR LYME BORRELIOSIS: FACTS AND CHALLENGES

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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 transovariously, 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

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

Stage		Clinical features	
Stage I (days-weeks)	Early and localized infection	Erythema migrans	Headache, fatigue, myalgia, arthralgia, malaise, fever
		Borrelial lymphocytoma	Skin manifestations occurring on the earlobe in children or on the nipple in adults (rare)
Stage II (weeks-months)	Systemic symptoms, disseminated infection	Lyme neuroborreliosis	Lymphocytic meningitis, headache, mild stiff neck, facial paralysis and radiculoneuritis (motor or sensory) Rarely cerebellar ataxia or encephalomyelitis
		Lyme carditis	Atrioventricular nodal blockade Pancarditis or cardiomegaly Acute myopericarditis or mild left ventricular dysfunction (unusual)
Stage III (months- years)	No systemic syndromes, localized infection	Lyme arthritis	Pain and swelling of the weight-bearing joints (knee), Persistent synovitis sometimes occurs
		Acrodermatitis chronica atroficans	Slowly progressive lesion located on the acral parts of the limbs relatively rare and especially in older women.

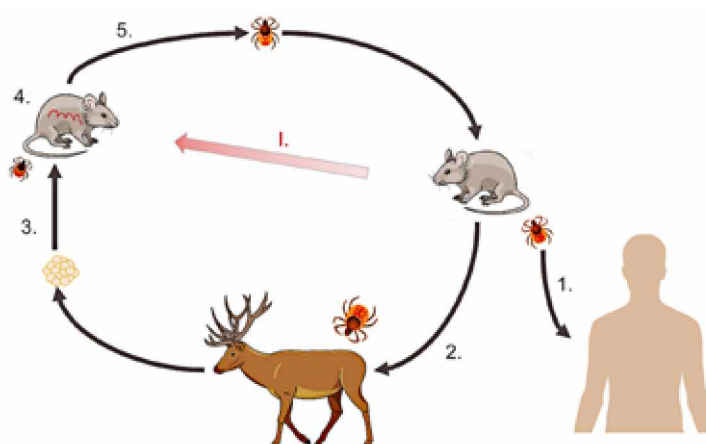


Fig. 1. The Enzootic cycle of *B. burgdorferi*

I—*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 Mérieux 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. ZS7. The vaccine was administered intramuscularly at a dose of 0.5 ml in three doses (0, 1, and 12 months). The third phase of the clinical study involved more than 10,000 individuals from endemic areas, aged 15—70 years, the study was placebo-controlled. The efficacy of the vaccine was set at 76% (after three doses), with no significant side effects observed at this stage [56]. However, the LYMERix™ had several disadvantages:

Its effectiveness was less than 80%, which meant that the remaining 20% of fully vaccinated people could still be infected with LB [42].

- Three doses were needed to gain full protection.

- There was a lack of vaccine testing in young children as a population at high risk of developing LB [59].
- The vaccine was only effective against a North American *Borrelia* strain (*B. burgdorferi*).
- It was not clear what the final length of vaccine-induced immunity would be whether the vaccine will not need to be re-administered each year [42].

The second vaccine developed was ImuLyme™. The vaccine itself consisted of recombinant OspA from *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. bava-riensis* (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 chimeritope 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-chimeritope of OspC. Combining the OspA and chimeritope 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 (1×10^5 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 borreliacidal 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]. Benčurová 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[®]cr-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 2. Comparison of human and available canine vaccines against Lyme borreliosis

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

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REFERENCES

1. Adeolu, M., Gupta, R. S., 2014: A phylogenomic and molecular marker based proposal for the division of the genus *Borrelia* into two genera: the emended genus *Borrelia* containing only the members of the relapsing fever *Borrelia*, and the genus *Borrelia* gen. nov. containing the members of the Lyme disease *Borrelia* (*Borrelia burgdorferi* sensu lato complex). *Antonie Van Leeuwenhoek*, 105, 6, 1049—1072. DOI: 10.1007/s10482-014-0164-x.
2. Aronowitz, R. A., 2012: The rise and fall of the lyme disease vaccines: a cautionary tale for risk interventions in American medicine and public health. *Milbank Q*, 90, 2, 250—277. DOI: 10.1111/j.1468-0009.2012.00663.x.
3. Bencurova, E., Gupta, S. K., Oskoueian, E., Bhide, M., Dandekar, T., 2018: Omics and bioinformatics applied to vaccine development against *Borrelia*. *Mol. Omics*, 14, 5, 330—340. DOI: 10.1039/c8mo00130h.
4. Bensaci, M., Bhattacharya, D., Clark, R., Linden, T. Hu., 2012: Oral vaccination with vaccinia virus expressing the tick antigen subolesin inhibits tick feeding and transmission of *Borrelia burgdorferi* vaccination. *Vaccine*, 30, 42, 6040—6046. DOI: 10.1016/j.vaccine.2012.07.053.
5. Bins, A. D., Jorritsma, A., Wolkers, M. C., Hung, Ch. Fu., Wu, T. C., Schumacher, T. N. M., Haanen, J. B. A. G., 2005: A rapid and potent DNA vaccination strategy defined by *in vivo* monitoring of antigen expression. *Nat. Med.*, 11, 8, 899—904. DOI: 10.1038/nm1264.
6. Bunikis, J., Tsao, J., Luke, C. J., Luna, A. G., Fish, D., Barbour, A. G., 2004: *Borrelia burgdorferi* infection in a natural population of *Peromyscus leucopus* mice: a longitudinal study in an area where Lyme Borreliosis is highly endemic. *J. Infect. Dis.*, 189, 8, 1515—1523. DOI: 10.1086/382594.
7. CDC 24/7: Saving Lives, Protecting People™: Notice to Readers Availability of Lyme Disease Vaccine. <https://www.cdc.gov/mmwr/preview/mmwrhtml/00056299.htm>. Updated January 22, 1999. Accessed February 19, 2021.
8. Comstedt, P., Hanner, M., Schüler, W., Meinke, A., Schlegel, R., Lundberg, U., 2015: Characterization and optimization of a novel vaccine for protection against Lyme borreliosis. *Vaccine*, 33, 44, 5982—5988. DOI: 10.1016/j.vaccine.2015.07.095.
9. Earnhart, C. G., Buckles, E. L., Marconi, R. T., 2006: Development of an OspC-based tetravalent, recombinant, chimeric vaccinogen that elicits bactericidal antibody against diverse Lyme disease spirochete strains. *Vaccine*, 25, 3, 466—480. DOI: 10.1016/j.vaccine.2006.07.052.
10. Earnhart, C. G., Marconi, R. T., 2007: Construction and analysis of variants of a polyvalent Lyme disease vaccine: approaches for improving the immune response to chimeric vaccinogens. *Vaccine*, 25, 17, 3419—3427. DOI: 10.1016/j.vaccine.2006.12.051.
11. Embers, M. E., Narasimhan, S., 2013: Vaccination against Lyme disease: past, present, and future. *Front. Cell Infect. Microbiol.*, 3, 6. DOI: 10.3389/fcimb.2013.00006.
12. Federizon, J., Frye, A., Huang, W.-C., Hart, T. M., He, X., Beltran, C., et al., 2020: Immunogenicity of the Lyme disease antigen OspA, particleized by cobalt porphyrin-phospholipid liposomes. *Vaccine*, 38, 4, 942—950. DOI: 10.1016/j.vaccine.2019.10.073.
13. Fingerle, V., Schulte-Spechtel, U. C., Ruzic-Sabljic, E., Leonhard, S., Hofmann, H., Weber, K., et al., 2008: Epidemiological aspects and molecular characterization of *Borrelia burgdorferi* s. l. from southern Germany with special respect to the new species *Borrelia spielmanii* sp. nov. *Int. J. Med. Microbiol.*, 2, 279—290. DOI: 10.1016/j.ijmm.2007.05.002.
14. Gerritzen, M. J. H., Martens, D. E., Wijffels, R. H., Van der Pol, L., Stork, M., 2017: Bioengineering bacterial outer membrane vesicles as vaccine platform. *Biotechnol. Adv.*, 35, 5, 565—574. DOI: 10.1016/j.biotechadv.2017.05.003.
15. Gomes-Solecki, M., Arnaboldi, P. M., Backenson, P. B., Benach, J. L., Cooper, C. L., Dattwyler, R. J., et al., 2020: Protective immunity and new vaccines for Lyme disease. *Clin. Infect. Dis.*, 70, 8, 1768—1773. DOI: 10.1093/cid/ciz872.
16. Gomes-Solecki, M. J. C., Brisson, D. R., Dattwyler, R. J., 2006: Oral vaccine that breaks the transmission cycle of the Lyme disease spirochete can be delivered via bait. *Vaccine*, 24, 20, 4440—4449. DOI: 10.1016/j.vaccine.2005.08.089.
17. Hassan, W. S., Giaretta, P. R., Rech, R., Ollivault- Shiflett, M., Esteve-Gasent, M., 2019: Enhanced protective efficacy of *Borrelia burgdorferi* BB0172 derived-peptide based vaccine to control Lyme disease. *Vaccine*, 37, 5596—5606. DOI: 10.1016/j.vaccine.2019.07.092.
18. Izac, J. R., O'Bier, N. S., Oliver, L. D., Camire, A. C., Earnhart, C. G., LeBlanc Rhodes, D. L. V., et al., 2020:

- Development and optimization of OspC chimeritope vaccinogens for Lyme disease. *Vaccine*, 38, 8, 1915—1924. DOI: 10.1016/j.vaccine.2020.01.027.
19. Jan, A. T., 2017: Outer membrane vesicles (OMVs) of Gram-negative bacteria: A perspective update. *Front. Microbiol.*, 8, 1053. DOI: 10.3389/fmicb.2017.01053.
 20. Kamp, H. D., Swanson, K. A., Wei, R. R., Pradeep, K. D., Dharanipragada, R., Kern, A., Sharma, B., et al., 2020: Design of a broadly reactive Lyme disease vaccine. *Npj. Vaccines*, 5, 1, 1—10. DOI: 10.1038/s41541-020-0183-8.
 21. Khatchikian, C. E., Nadelman, R. B., Nowakowski, J., Schwartz, I., Wormser, G. P., Brisson, D., 2014: Evidence for strain-specific immunity in patients treated for early Lyme disease. *Infect. Immun.*, 82, 4, 1408—1411. DOI: 10.1128/IAI.01451-13.
 22. Klouwens, M. J., Salverda, M. L. M., Trentelman, J. J., Ersoz, J. I., Wagemakers, A., Gerritzen, M. J., et al., 2021: Vaccination with meningococcal outer membrane vesicles carrying *Borrelia* OspA protects against experimental Lyme borreliosis. *Vaccine*, 39, 18, 2561—2567. DOI: 10.1016/j.vaccine.2021.03.059.
 23. Klouwens, M. J., Trentelman, J. J., Ersoz, J. I., Nieves Marques Porto, F., Sima, R., Hajdusek, O., et al., 2021: Investigating BB0405 as a novel *Borrelia afzelii* vaccination candidate in Lyme borreliosis. *Sci. Rep.*, 11, 1, 4775. DOI: 10.1038/s41598-021-84130-y.
 24. Klouwens, M. J., Trentelman, J. J. A., Wagemakers, A., Ersoz, J. I., Bins, A. D., Hovius, J. W., 2021: Tick-tattoo: DNA vaccination against *B. burgdorferi* or *Ixodes scapularis* tick proteins. *Front. Immunol.*, 12, 615011. DOI: 10.3389/fimmu.2021.615011.
 25. Kuleš, J., Horvatić, A., Guillemin, N., Galan, A., Mrljak, V., Bhide, M., 2016: New approaches and omics tools for mining of vaccine candidates against vector-borne diseases. *Mol. Biosyst.*, 12, 9, 2680—2694. DOI: 10.1039/C6MB00268D.
 26. Kumar, M., Kaur, S., Kariu, T., Yang, X., Bossis, I., Anderson, J. F., et al., 2011: *Borrelia burgdorferi* BBA52 is a potential target for transmission blocking Lyme disease vaccine. *Vaccine*, 29, 48, 9012—9019. DOI: 10.1016/j.vaccine.2011.09.035.
 27. Kung, F., Kaur, S., Smith, A. A., Yang, X., Wilder, C. N., Sharma, K., et al., 2016: A *Borrelia burgdorferi* surface-exposed transmembrane protein lacking detectable immune responses supports pathogen persistence and constitutes a vaccine target. *J. Infect. Dis.*, 213, 11, 1786—1795. DOI: 10.1093/infdis/jiw013.
 28. Kutzler, M. A., Weiner, D. B., 2008: DNA vaccines: ready for prime time? *Nat. Rev. Genet.*, 9, 10, 776—788. DOI: 10.1038/nrg2432.
 29. LaFleur, R. L., Callister, S. M., Dant, J. C., Jobe, D. A., Lovrich, S. D., Warner, T. F., et al., 2010: One-year duration of immunity induced by vaccination with a canine Lyme disease bacterin. *Clin. Vaccine Immunol.*, 17, 5, 870—874. DOI: 10.1128/CVI.00524-09.
 30. LaFleur, R. L., Dant, J. C., Wasmoe, T. L., Callister, S. M., Jobe, D. A., Lovrich, S. D., et al., 2009: Bacterin that induces anti-OspA and anti-OspC borreliacidal antibodies provides a high level of protection against canine Lyme disease. *Clin. Vaccine Immunol.*, 16, 2, 253—259. DOI: 10.1128/CVI.00373-08.
 31. Levy, S. A., 2002: Use of a C6 ELISA test to evaluate the efficacy of a whole-cell bacterin for the prevention of naturally transmitted canine *Borrelia burgdorferi* infection. *Vet. Ther. Res. Appl. Vet. Med.*, 3, 4, 420—424.32.
 32. Levy, S. A., Millership, J., Glover, S., Parker, D., Hogan, J., Heldorfer, M., et al., 2010: Confirmation of presence of *Borrelia burgdorferi* outer surface protein C antigen and production of antibodies to *Borrelia burgdorferi* outer surface protein C in dogs vaccinated with a whole-cell *Borrelia burgdorferi* bacterin. *Intern. J. Appl. Vet. Med.*, 8, 3, 123—128.
 33. Little, S. E., Heise, S. R., Blagburn, B. L., Callister, S. M., Mead, P. S., 2010: Lyme borreliosis in dogs and humans in the USA. *Trends Parasitol.*, 26, 4, 213—218. DOI: 10.1016/j.pt.2010.01.006.
 34. Mancini, F., Rossi, O., Necchi, F., Micholi, F., 2020: OMV vaccines and the role of TLR agonists in immune response. *Int. J. Mol. Sci.*, 21, 12, 4416. DOI: 10.3390/ijms21124416.
 35. Marconi, R. T., Garcia-Tapia, D., Hoevers, J., Honsberger, N., King, V. L., Ritter, D., et al., 2020: VANGUARD[®]-cr-Lyme: A next generation Lyme disease vaccine that prevents *B. burgdorferi* infection in dogs. *Vaccine X*, 6, 11, 100079. DOI: 10.1016/j.jvacx.2020.100079.
 36. Meirelles Richer, L., Aroso, M., Contente-Cuomo, T., Ivanova, L., Gomes-Solecki, M., 2011: Reservoir targeted vaccine for Lyme borreliosis induces a yearlong, neutralizing antibody response to OspA in white-footed mice. *Clin. Vaccine Immunol.*, CVI, 11, 18, 1809—1816. DOI: 10.1128/CVI.05226-11.
 37. Murfin, K. E., Fikrig, E., 2017: Tick bioactive molecules as novel therapeutics: Beyond vaccine targets. *Front. Cell Infect. Microbiol.*, 7, 222. DOI: 10.3389/fcimb.2017.00222.
 38. Nadelman, R. B., Wormser G. P., 1998: Lyme borreliosis.

- Lancet*, 352, 9127, 557—565. DOI: 10.1016/S0140-6736(98)01146-5.
39. Nardelli, D. T., Munson, E. L., Callister, S. M., Schell, R., 2009: Human Lyme disease vaccines: past and future concerns. *Future Microbiol.*, 4, 4, 457—469. DOI: 10.2217/fmb.09.17.
 40. Nayak, A., Schöler, W., Seidel, S., Gomez, I., Meinke, A., Comstedt, P., et al., 2020: Broadly protective multivalent OspA vaccine against Lyme borreliosis, developed based on surface shaping of the C-terminal fragment. *Infect Immun.*, 88, 4. DOI: 10.1128/IAI.00917-19.
 41. Nelson, C. A., Saha, S., Kugeler, K. J., Delorey, M. J., Shankar, M. B., Hinckley, A. F., et al., 2015: Incidence of clinician-diagnosed Lyme disease, United States, 2005—2010. *Emerg. Infect. Dis.*, 21, 9, 1625—1631. DOI: 10.3201/eid2109.150417.
 42. Nigrovic, L. E., Thompson, K. M., 2007: The Lyme vaccine: a cautionary tale. *Epidemiol. Infect.*, 135, 1, 1—8. DOI: 10.1017/S0950268806007096.
 43. Pereira, V. B., Zurita-Turk, M., Saraiva, T. D. L., De Castro, C. P., Souza, B. M., Agresti, P. M., et al., 2014: DNA vaccines approach: From concepts to applications. *World J. Vaccines*, 4, 2, 50—71. DOI: 10.4236/wjv.2014.42008.
 44. Plotkin, S. A., 2016: Need for a new Lyme disease vaccine. *N. Engl. J. Med.*, 375, 10, 911—913. DOI: 10.1056/NEJMp1607146.
 45. Pol van der, L., Stork, M., Ley van der, P., 2015: Outer membrane vesicles as platform vaccine technology. *Biotechnol. J.*, 10, 11, 1689—1706. DOI: 10.1002/biot.201400395.
 46. Poland, G. A., 2011: Vaccines against Lyme disease: What happened and what lessons can we learn? *Clin. Infect. Dis.*, 52, 253—258. DOI: 10.1093/cid/ciq116.
 47. Radolf, J. D., Caimano, M. J., Stevenson, B., Hu, L. T., 2012: Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nat. Rev. Microbiol.*, 10, 2, 87—99. DOI: 10.1038/nrmicro2714.
 48. Rizzoli, A., Hauffe, H. C., Carpi, G., Vourc'h, G. I., Netele, M., Rosa, R., 2011: Lyme borreliosis in Europe. *Euro-surveillance*, 16, 27, 19906, 1—8. DOI: 10.2807/ese.16.27.19906-en.
 49. RxList, 2016: *Side Effects of Lymerix* (Lipoprotein Outer Surface A Vaccine), Warnings, Uses. RxList. <https://www.rxlist.com/lymerix-side-effects-drug-center.htm>. Last reviewed on RxList April 29, 2016. Accessed February 19, 2021.
 50. Scheckelhoff, M. R., Telford, S. R., Hu, L. T., 2006: Protective efficacy of an oral vaccine to reduce carriage of *Borrelia burgdorferi* (strain N40) in mouse and tick reservoirs. *Vaccine*, 24, 11, 1949—1957. DOI: 10.1016/j.vaccine.2005.10.044.
 51. Schuijt, T. J., Hovius, J. W., Poll van der, T., Dam van, A. P., Fikrig, E., 2011: Lyme borreliosis vaccination: the facts, the challenge, the future. *Trends Parasitol.*, 27, 1, 40—47. DOI: 10.1016/j.pt.2010.06.006.
 52. Seinost, G., Golde, W. T., Berger, B. W., Dunn, J. J., Qiu, D., Dunkin, D. S., et al., 1999: Infection with multiple strains of *Borrelia burgdorferi* sensu stricto in patients with Lyme disease. *Arch. Dermatol.*, 135, 11, 1329—1333. DOI: 10.1001/archderm.135.11.1329.
 53. Sigal, L. H., Zahradnik, J. M., Lavin, P., Patella, S. J., Bryant, G., Haselby, R., et al., 1998: A vaccine consisting of recombinant *Borrelia burgdorferi* outer-surface protein A to prevent Lyme disease. Recombinant outer-surface protein A Lyme disease. *N. Engl. J. Med.*, 339, 4, 216—222. DOI: 10.1056/NEJM199807233390402.
 54. Šmit, R., Postma, M. J., 2015: Lyme borreliosis: reviewing potential vaccines, clinical aspects and health economics. *Expert. Rev. Vaccines*, 14, 12, 1549—1561. DOI: 10.1586/14760584.2015.1091313.
 55. Stanek, G., Wormser, G., Gray, J., Strle, F., 2012: Lyme borreliosis. *Lancet*, 379, 9814, 461—473. DOI: 10.1016/S0140-6736(11)60103-7.
 56. Steere, A. C., Sikand, V. K., Meurice, F. Parenti, D. L., Fikrig, E., Schoen, R. T., et al., 1998: Vaccination against Lyme disease with recombinant *Borrelia burgdorferi* outer-surface lipoprotein A with adjuvant. Lyme Disease Vaccine Study Group. *N. Engl. J. Med.*, 339, 4, 209—215. DOI: 10.1056/NEJM199807233390401.
 57. Steere, A. C., Strle, F., Wormser, G. P., Hu, L. T., Branda, J. A., Hovius, J. W. R., et al., 2016: Lyme borreliosis. *Nat. Rev. Dis. Primer*, 2, 16090. DOI: 10.1038/nrdp.2016.90.
 58. Sykes, R. A., Makiello, P., 2017: An estimate of Lyme borreliosis incidence in western Europe. *J. Public Health Oxf. Engl.*, 39, 1, 74—81. DOI: 10.1093/pubmed/fdw017.
 59. Todaro, W. T., Schoen, R. T., 2000: The Lyme disease vaccine: Conception, development, and implementation. *Ann. Intern. Med.*, 132, 8, 661—668.
 60. Töpfer, K. H., Straubinger, R. K., 2007: Characterization of the humoral immune response in dogs after vaccination against the Lyme borreliosis agent: A study with five commercial vaccines using two different vaccination schedules. *Vaccine*, 25, 2, 314—326. DOI: 10.1016/j.vaccine.2006.07.031.
 61. Valneva, 2020: *Valneva Announces Acceleration of Pediatric*

Development for Lyme Disease Vaccine Candidate—Valneva. <https://valneva.com/press-release/valneva-announces-acceleration-of-pediatric-development-for-lyme-disease-vaccine-candidate/>. Updated December 2, 2020. Accessed February 4, 2021

62. Valneva, 2021: *Valneva Announces Positive Initial Results for Phase 2 Study of Lyme Disease Vaccine Candidate—Valneva*. <https://valneva.com/press-release/valneva-announces-positive-initial-results-for-phase-2-study-of-lyme-disease-vaccine-candidate/>. Updated July 22, 2020. Accessed February 4, 2021.

63. Valneva, 2021: *Valneva Announces Positive Initial Results for Second Phase 2 Study of Lyme Disease Vaccine Candidate*

VL415—Valneva. <https://valneva.com/press-release/valneva-announces-positive-initial-results-for-second-phase-2-study-of-lyme-disease-vaccine-candidate-vla15/>. Updated October 20, 2020. Accessed February 4, 2021.

64. Wagemakers, A., Mason, L. M. K., Oei, A., Wever de, B., Poll van der, T., Bins, A. D., et al., 2014: Rapid outer-surface protein C DNA tattoo vaccination protects against *Borrelia afzelii* infection. *Gene Ther.*, 21, 1051—1057. DOI: 10.1038/gt.2014.87.

65. We Respect Animals—Bioveta, 2015: *Borrelym 3—new unique vaccine*. <https://www.bioveta.eu/en/news/news-in-assortment/borrelym-3-new-unique-vaccine.html>: Updated March 3, 2015. Accessed March 15, 2020.

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