MINI-REVIEW

Novel targets and strategies to combat borreliosis

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Abstract

Lyme borreliosis is a bacterial infection that can be spread to humans by infected ticks and may severely affect many organs and tissues. Nearly four decades have elapsed since the discovery of the disease agent called *Borrelia burgdorferi*. Although there is a plethora of knowledge on the infectious agent and thousands of scientific publications, an effective way on how to combat and prevent Lyme borreliosis has not been found yet. There is no vaccine for humans available, and only one active vaccine program in clinical development is currently running. A spirited search for possible disease interventions is of high public interest as surveillance data indicates that the number of cases of Lyme borreliosis is steadily increasing in Europe and North America. This review provides a condensed digest of the history of vaccine development up to new promising vaccine candidates and strategies that are targeted against Lyme borreliosis, including elements of the tick vector, the reservoir hosts, and the *Borrelia* pathogen itself.

Keywords Lyme borreliosis · Vaccine candidates · Anti-tick strategies · Human pathogen · Public health

Introduction

Lyme borreliosis, or Lyme disease, is the most common tick-transmitted disease worldwide and is known to infect humans as well as domestic animals including cattle, cats, and dogs (Krupka and Straubinger 2010). Since the discovery of Lyme borreliosis in 1975 (Steere et al. 1977), a great deal of effort has been dedicated to the goal of preventing the detrimental effects of this disease. Despite improvements in diagnostic tests and public awareness of Lyme borreliosis, up to 300,000 cases in the USA (Kuehn 2013) and 65,000 cases in Europe (Hubálek 2009) are reported. However, the number of infections in Europe is likely to be an underestimation, as not all countries have made Lyme borreliosis a mandatorily notifiable disease (Smith and Takkinen 2006). The agents responsible for Lyme borreliosis are a diverse group of spirochetal bacteria within the *Borrelia* genus. *Borrelia burgdorferi*

sensu lato complex comprises at least 20 named species (Margos et al. 2019), with most human Lyme cases being caused by *B. burgdorferi* sensu stricto, *B. afzelii, B. garinii,* and *B. bavariensis* (Stanek et al. 2012).

B. burgdorferi is an extracellular pathogen (Strnad et al. 2015) that can infect the skin, heart, and nervous system (Cadavid et al. 2000; Stanek and Strle 2018). Ticks of the genus *Ixodes* transmit *B. burgdorferi* between reservoir hosts such as small mammals, lizards, and birds and are the only natural agents through which humans have been shown to be infected (Steere 2001). The principal vectors are *Ixodes ricinus* in Europe, *Ixodes persulcatus* in Asia, and *Ixodes scapularis* in North America. The overall prevalence of infected ticks and *Borrelia* genospecies distribution are highly variable across geographic locations (Strnad et al. 2017).

Ticks most often acquire *Borrelia* from infected rodents during their larval feeding. After molting to the next developmental stage, the tick has to find a new host. Upon ingestion of new blood, the spirochetes migrate from the midgut to the salivary glands of an infected tick followed by entering the mammal through the bite site (Ribeiro et al. 1987). The pathogen then disseminates throughout the mammalian host to establish an infection (Moriarty et al. 2008; Norman et al. 2008). All these concerted movements in the two environments are assumed or known to involve the process of motility and adhesion to cells involving a high number of protein and carbohydrate-based interactions (Ebady et al. 2016; Vechtova

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et al. 2018). Infected nymphal ticks occasionally feed on humans and most likely transmit the spirochete and cause human Lyme borreliosis as they are abundant in the spring and early summer and are small and difficult to detect. Unlike *B. miyamotoi*, *B. burgdorferi* sensu lato is considered not to be transmitted transovarially from female ticks to their offspring. However, several recent studies have shown that field-collected *I. ricinus* larvae may contain borrelial DNA (Kalmár et al. 2013; Tappe et al. 2014) and are able to transmit *B. afzelii* to laboratory rodents (van Duijvendijk et al. 2016), suggesting the potential role of larvae in spreading the Lyme borreliosis agent.

In the absence of antibiotic therapy, disseminated *B. burgdorferi* can persist in an individual for months or years even in the face of strong immune response. Currently, antibiotic treatment is the only effective tool to clear the infection and fight against Lyme borreliosis as no vaccine for humans is available. There are a number of new promising vaccine candidates being currently developed and tested by the research community, representing new hopes for future victims of the disease. These direct anti-*Borrelia* strategies can be complemented with anti-tick vaccines to bring a whole new level of human protection (Fig. 1), as will be discussed in this review.

On-demand treatment strategies

Most cases of Lyme borreliosis can be easily managed if treated early using antibiotics. Post-infection treatment is usually managed with antimicrobial agents for 2 to 4 weeks. Doxycycline, amoxicillin, penicillin V, and cefuroxime are highly effective and are the preferred antibiotics for the treatment of early localized infection (Stanek and Strle 2018). Early disseminated infection is usually treated with intravenous ceftriaxone or penicillin (Stanek and Strle 2018) or oral doxycycline (Ljøstad et al. 2008). The most common routes of antibiotic administration are oral administration and intravenous injection. As an alternative approach, the topical application of 4% azithromycin cream was tested (Piesman et al. 2014). The result of the study showed that azithromycin was highly efficient when applied topically at the sites of tick bites in mice (Piesman et al. 2014). The outcomes of the study were however not fully confirmed in human studies (Schwameis et al. 2017; Shapiro and Wormser 2017). Azithromycin is an attractive possibility because of its good safety profile, long half-life in tissues, and potency against various *B. burgdorferi* species (Lee and Wormser 2008). Topical application of antimicrobial agents is a very attractive delivery method for a number of reasons and could be potentially used to stop the progression of the disease in the early localized stage of infection. The advantages include smaller amount of drug to be used, avoidance of the metabolic processing of the drug in the liver, ease of administration especially for young children, higher concentration of the drug to the affected area, and fairly diminished effects on nontargeted body locations such as intestinal florae. The transdermal route apart from the abovementioned advantages may be more convenient also for patients who cannot use normal oral intake because of swallowing problems such as intubation, deep sedation, or concurrent diseases (Tanner and Marks 2008). However, as a very motile organism, *B. burgdorferi* spread readily and very fast all over the body from site of skin entrance. Therefore, the
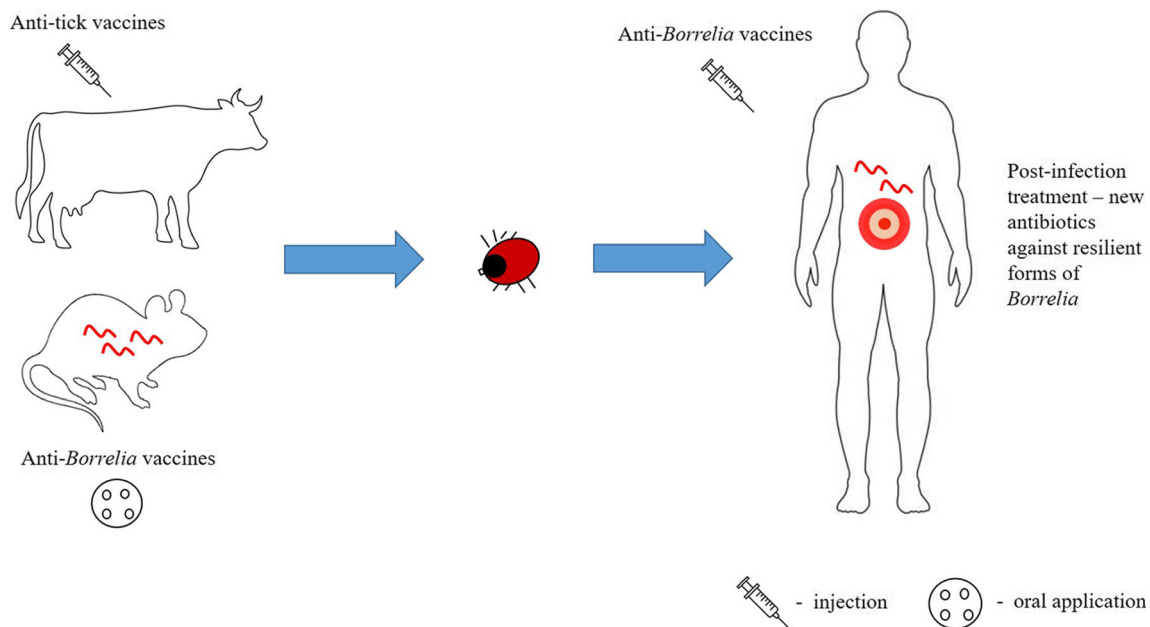
Fig. 1 The intensive research areas of current anti-*Borrelia* strategies. The focus is set on direct prophylactic anti-*Borrelia* strategies as well as on anti-tick vaccines

risk of Lyme borreliosis is substantially increased when tick bites are unrecognized, and the topical treatment of tick bite site is not managed soon enough after onset of tick feeding.

Antibiotic prophylactic treatment, or chemoprophylaxis, can be loosely defined as the administration of drug/antibiotics to prevent the development of a disease at the very beginning before the symptoms arise. This treatment strategy can also potentially play an important role as a method to prevent *B. burgdorferi* dissemination (Lascher and Goldmann 2016). Chemoprophylaxis with a single high dose of doxycycline after removal of tick from the patients within 72 h was found beneficial in the USA. During a 6-week follow-up period, 1 of 235 treated patients developed erythema migrans, whereas 8 of 247 in the placebo group developed this skin condition, showing that antibiotic prophylaxis may significantly reduce the chances of developing Lyme borreliosis (Nadelman et al. 2001; Warshafsky et al. 2010). However, the unnecessary (over)use of antibiotics may lead to accelerated antibiotic resistance and is not generally recommended.

New antibiotics against Lyme borreliosis

While still a matter of dispute (Auwaerter and Melia 2012; Baker and Wormser 2017; Wormser et al. 2017), there are several reports of antibiotic treatment unable to fully eradicate *B. burgdorferi* from blood and tissues (Rudenko et al. 2016). The phenomenon of tolerance to otherwise lethal doses of antibiotics and the antibiotic resistance is often attributed to so-called *B. burgdorferi* persisters. At least three morphological forms of persistent *B. burgdorferi* were described based on observations from experimental studies. They are capable of forming round bodies, L-forms, and biofilm-like structures. Persisters may remain viable despite antibiotic therapy and are able to reversibly convert into motile spirochetal forms under favorable conditions (Timmaraju et al. 2015; Vancová et al. 2017; Rudenko et al. 2019). The mechanism that makes the bacterium less susceptible to killing by therapeutic doses of antimicrobials is not known; however, human neutrophil calprotectin was shown to make *B. burgdorferi* more tolerant to penicillin (Montgomery et al. 2006). *B. burgdorferi* in culture can also become tolerant to antibiotics used in treating Lyme borreliosis such as ceftriaxone, doxycycline, and amoxicillin (Feng et al. 2014; Sharma et al. 2015).

If the still limited but slowly growing number of evidence supporting the existence of chronic/persistent Lyme borreliosis will end up being accepted by the Lyme community, new antibiotics or targeted designer drugs to treat the Lyme spirochetes will be needed to cope more efficiently with the outcomes of the disease (Stricker and Middelveen 2018). Some studies suggest that treatment of a borrelial infection with the currently available antimicrobial agents may suppress but not eradicate the infection (Middelveen et al. 2018). Therefore, new drug candidates have been investigated and found using high-throughput screening of existing FDA-approved drugs in the USA (Feng et al. 2014; Feng et al. 2015; Pothineni et al. 2016), with the ultimate goal of complete microbial eradication and clinical cure. The usefulness and potential application in human medicine will have to be evaluated.

Prophylactic treatment strategies

Anti-*Borrelia* strategies: human-targeted approaches

Research efforts have long focused on ameliorating the symptoms and consequences of disease through treatment, commonly using various kinds of broad-spectrum antibiotics. However, prevention of the disease is far preferable to treating the short- and long-term often debilitating consequences of the disease. Human vaccination has greatly reduced the burden of infectious diseases and prevented more suffering than any other form of medical activity (Andre et al. 2008). Prophylactic vaccination of humans was the first choice for companies and health organizations for prevention of Lyme borreliosis. Despite a plethora of highly antigenic and immunologically accessible borrelial surface-exposed proteins (Gilmore et al. 1996; Hanson et al. 1998; Probert and Johnson 1998; Fikrig et al. 2004), the first and only licensed vaccine developed to prevent Lyme borreliosis was LYMERix, with efficacy of nearly 80% after all three doses had been administered (Nigrovic and Thompson 2007). It stayed on the market for only 4 years, and in 2002 it was withdrawn from the US market due to several reported cases of diversely serious side effects. The most common adverse events noted after receiving at least one dose included pain or reaction at the injection site, joint pain, muscle pain, and headache. It was hypothesized that the vaccine antigen, outer surface protein A (OspA), behaves as an autoantigen and therefore was arthritogenic. The adverse side effects were however never fully confirmed (Shaffer 2019).

VLA15 is the only vaccination program against Lyme borreliosis currently under clinical development. To follow in the footsteps of LYMERix, VLA15 vaccine candidate developed by Valneva is also OspA-based compound. However, it seeks to improve on efficacy and global applicability of the vaccine. LYMERix was a monovalent recombinant vaccine based on bacterial OspA serotype 1 derived from *B. burgdorferi* sensu stricto. This monovalent OspA serotype 1 vaccine had barely any potential to protect against the disease outside of North America based on the fact that OspA is antigenically very heterogeneous and at least six OspA serotypes are connected with *B. burgdorferi* sensu lato species present in Europe (Wilske et al. 1993). OspA serotype and *Borrelia* species exhibit a clear connection; *B. burgdorferi* sensu stricto represents OspA serotype 1; *B. afzelii* OspA serotype 2; *B. garinii* OspA serotypes 3, 5, and 6; and *B. bavariensis* OspA serotype 4. Since OspA protective

function is to great degree type-specific, a candidate vaccine designed to confer protection against the majority of Lyme borreliosis species has to contain at least two or three antigenic variants of OspA. VLA15 targets the six most common types of *Borrelia* (Comstedt et al. 2017).

An alternative approach is called pre-exposure prophylaxis, which is based on OspA-specific human monoclonal antibodies that are borreliacidal against a broad range of *Borrelia* genospecies. Unlike a vaccine, pre-exposure prophylaxis delivers a single defensive antibody and can prevent the transmission of the spirochetes from ticks to mice (Wang et al. 2016). Unless the vaccine is available, the passive administration of a protective human antibody could be an effective approach for borreliosis prophylaxis. The feasibility of using human monoclonal antibodies for pre-exposure prophylaxis has been already shown to be effective against respiratory virus infection (Wang et al. 2011).

OspA, expressed during the tick stage of the pathogen life cycle, is proposed to be an adhesin that binds the spirochetes to the midgut cells using the tick receptor molecule, TROSPA (Pal et al. 2004). The primary mode of action of OspA-based vaccines is to block the migration of the spirochetes from the midgut to the salivary glands of the tick. It is a rather unusual mechanism as it occurs within the tick vector rather than in the vaccinated entity. Several other surface-exposed molecules were identified during this time, and their potential suitability as vaccine candidates have been examined. These, most often lipoproteins, play important roles in various aspects of tick colonization (Fikrig et al. 2004), mammalian infection, and host immune evasion and persistent infection (Lawrenz et al. 2004). This is either through direct binding to the target tissues or by interacting with host factors to create favorable conditions for *Borrelia* survival. Decorin-binding protein A (DbpA) is an outer surface molecule that is expressed on mammalian host-adapted *B. burgdorferi*. This lipoprotein has exhibited vaccine efficacy against experimental infection in the mammalian model (Hanson et al. 1998; Cassatt et al. 1998) and was shown to be immunogenic during human Lyme borreliosis (Cinco et al. 2000). Fibronectin-binding protein BBK32 plays an important role in the attachment to the extracellular matrix. BBK32 is highly immunogenic and is present in sera of Lyme disease-infected patients (Heikkilä et al. 2002; Lahdenne et al. 2006). BBK32 antisera can interfere with borrelial transmission at various stages of the vector-host life cycle (Fikrig et al. 2000). Outer surface protein C (OspC) is required for early mammalian infection (Grimm et al. 2004) and has been tested as vaccine candidate against *B. burgdorferi* infection with varying results (Zhong et al. 1999; Earnhart and Marconi 2007).

Vaccination trials testing various lipoprotein candidates have yielded mixed results despite the generation of robust antibody titers. In order to improve on the efficacy, vaccine cocktails containing multiple immunogens have been formulated and tested. A DbpA/OspA combination vaccine protected against 100-fold-higher challenge doses than did either single antigen vaccine or and conferred protection against various *Borrelia* genospecies (Hanson et al. 2000). Similarly, an OspA/OspC combination showed increased vaccine efficacy compared to single component immunizations (Wallich et al. 2001). A triple combination vaccine of DbpA, BBK32, and OspC was shown to be more effective than a single or double antigen vaccine in mice. Interestingly, the ratio of each component has an impact on the overall vaccine efficacy (Brown et al. 2005). The situation is further complicated by the existence of at least five species of *B. burgdorferi* sensu lato as causes of human Lyme borreliosis: *B. burgdorferi* sensu stricto, *B. afzelii*, *B. bavariensis*, *B. mayonii* and *B. garinii*. To deal with the high degree of heterogeneity of many proteins between the *Borrelia* strains and species, some research groups have focused on the development of a combination vaccine containing multivalent chimeric vaccinogens with protective effects against diverse Lyme species. As a result, multivalent OspC-based (Earnhart et al. 2007; Earnhart and Marconi 2007) and OspA-based (Wressnigg et al. 2013) chimeric vaccines targeting a broad spectrum of *Borrelia* species have been developed for prevention of Lyme borreliosis in Europe and the USA, and possibly worldwide. Notably, certain surface proteins that fail to evoke detectable antibody response in the mammalian host during the experimental infection are still able to elicit high-titer and long-term antibody response when applied in a recombinant form (Kung et al. 2016).

Not only the borrelial (lipo)proteins but also glycolipids (Schröder et al. 2003) and polymers consisting of sugars and peptides (Jutras et al. 2019) have shown to be antigenic and could be potential vaccine candidates, or at least serve as adjuvants. The glycolipid, acylated cholesteryl galactoside (ACGal), acts as a strong immunogen as specific antibodies against this compound are frequently found during late stage of the disease (Stübs et al. 2009). Notably, all major Lyme species possess this antigen (Stübs et al. 2009). The strategy to exploit this or somewhat similar molecules is considerably appealing for the development of a single universal "pan-vaccine" to control multiple infectious agents concomitantly (Cabezas-Cruz and de la Fuente 2017). For example, galactose-alpha-1,3-galactose (α-Gal) epitope is highly immunogenic in humans and is present on the surface of a number of deadly pathogens causing diseases such as malaria, sleeping sickness, or Chagas disease. The practical benefits of immunization with α-Gal against pathogens with α-Gal on their surface have been already demonstrated (Cabezas-Cruz and de la Fuente 2017; de la Fuente et al. 2019).

Anti-tick strategies

Ticks are obligate hematophagous arthropods and are considered to be second only to mosquitoes as vectors of human

infectious diseases worldwide. Predominantly due to climate change, ticks have spread to altitudes and latitudes where they were not present earlier (Jore et al. 2011; Jore et al. 2014), and the pathogens transmitted by ticks represent a new health threat in these areas. At least 15 tick-borne bacterial pathogens including *Rickettsia*, *Ehrlichia*, *Francisella,* and several species of the *Borrelia burgdorferi* sensu lato complex are known to be transmitted by ticks. Viral pathogens that can cause fatal diseases in human such as tick-borne encephalitis virus (TBEV) and Powassan virus are found in ticks as are protozoan parasites of the genus *Babesia* (Parola and Raoult 2001; Shi et al. 2018). Finding an efficient way to prevent ticks from feeding and therefore transmitting multiple human pathogens would kill more than two birds with one stone and would facilitate the management of many emerging infectious diseases.

Potential vaccine candidates

The supreme solution to inhibit transmission of multiple pathogens from the tick vector to humans would be development of single universal vaccine. An alternate approach to targeting antigens that are common to many tick-borne pathogens (as discussed above) is to use tick antigens as targets of immune intervention. Recently, the identification and development of such an anti-tick vaccine has been the subject of intensive research (Sprong et al. 2014; Rego et al. 2019). Multiple factors influence the efficacy of an anti-tick vaccine. As any other vaccine candidate, the vaccinogen should firstly be highly immunogenic, be able to provide long-lasting immunity, be associated with a vital function of the tick, and preferably be able to produce cross-protective immune responses against different tick species. Additionally, the immunogen should be expressed during different stages of the tick's life cycle, allowing different tick stages to be targeted. Ideally, future anti-tick vaccine should be applicable to wildlife and domestic animals and eventually also for humans.

There are three families of ticks. The family *Ixodidae*, or "hard ticks" and the *Argasidae*, or "soft ticks" are known to transmit pathogens causing diseases to humans. The family *Nuttalliellidae*, represented by a single species confined to Southern Africa, is not known as a pathogen-associated vector (Keirans et al. 1976). Ticks from *Ixodes* genus normally take 3 to 7 days to feed, allowing the host to mount an immune response against exposed tick antigens. The pathogens exploit saliva-induced modulation of immune defense exerted by the host to promote their transmission and infection, so-called saliva-assisted transmission (SAT) (Nuttall and Labuda 2004). Tick saliva, introduced into host skin during the feeding process, contains a wide range of proteins with anti-inflammatory, anti-complement, and anti-hemostatic activity (Chmelar et al. 2011; Perner et al. 2018).

The composition of tick saliva changes during the course of tick feeding as the tick counters the dynamic response of the host and appears to differ for different pathogens and tick vector species and possibly can even depend on the mammalian host species (Nuttall 2019). At least a few tick salivary gland proteins seem to facilitate *B. burgdorferi* transmission. Tick histamine release factor (tHRF) is upregulated in *B. burgdorferi*-infected *Ixodes scapularis* ticks. Silencing tHRF by RNA interference significantly impairs tick feeding and reduces spirochete burden in mice. Active immunization with recombinant tHRF or passive injection of tHRF antiserum decreases the efficiency of tick feeding and *B. burgdorferi* burden in mice (Dai et al. 2010). Additionally, a 15-kDa tick salivary gland protein Salp15 protects the spirochete directly from host immune responses by binding to the OspC of *B. burgdorferi* spirochetes (Ramamoorthi et al. 2005). Salp15 is also known to be able to suppress host immunity by binding to CD4 coreceptor to inhibit CD4^{+} T-cell activation, inhibiting subsequent receptor ligand-induced cell signaling, and altering the expression levels of cytokines (Anguita et al. 2002; Hovius et al. 2008). Tick salivary lectin pathway inhibitor (TSLPI) is a feeding-induced salivary protein present in *I. scapularis* (Schuijt et al. 2011) and *I. ricinus* (Wagemakers et al. 2016). Unlike Salp15, TSPLI does not adhere to *B. burgdorferi* but instead interacts with the lectin complement cascade. TSLPI-silenced ticks or ticks feeding on mice immunized with TSLPI are impaired in *B. burgdorferi* transmission. Moreover, *B. burgdorferi* acquisition and persistence in tick midguts are reduced in ticks feeding on TSLPI-immunized mice, signifying a crucial role in both *B. burgdorferi* transmission to the mammalian host as well as *B. burgdorferi* acquisition and persistence in ticks (Schuijt et al. 2011).

The design of transcriptomic and proteomic studies for conserved tick proteins involved in pathogen transmission is quite cumbersome due to the variation in transmission times for different pathogens during the tick feeding process. These complications could be avoided by targeting the niche organ where the spirochete resides before being transmitted – the tick midgut. The tick midgut protein Bm86 from *Rhipicephalus microplus* has been used as an immunogen in two licensed tick vaccines TickGARD (now discontinued) and Gavac since the 1990s (de la Fuente et al. 2007). Bm86-based vaccines have diverse efficacies reported worldwide (45–100%), with the greatest effect on the reduction of larval infestations in subsequent generations (Tabor 2018). Nevertheless, it has limited efficacy against other tick species (de la Fuente et al. 2007). Recently, it has been shown that *I. scapularis* secretes a protein, PIXR, that modulates the tick gut microbiome and interfere with the ability of *B. burgdorferi* to colonize the tick midgut (Narasimhan et al. 2017). This approach exploits the principle of rendering competent vectors incompetent and targets tick antigens that the parasites encounter during their life cycle. The tick microbiome could possibly be an additional target for the preventive strategies against the tick-borne pathogens (Rego et al. 2019).

Non-vaccine anti-tick strategies

Information campaigns are the most common policy measures to reduce the risk of tick-borne diseases. Common recommendations and prevention measures against Lyme borreliosis include avoiding tick-endemic areas, staying on trails while in high-risk areas, the usage of protective clothing, using tick repellents, and checking the body for ticks and removing them before or as soon as possible after they attach (Connally et al. 2009; Slunge and Boman 2018). Risk of Lyme borreliosis from an ecological perspective is measured in terms of density of infected nymphal ticks (Diuk-Wasser et al. 2012). Common methods for killing ticks include the application of acaricides to tick-rich areas, rodents hosts, or to the host animals (Hinckley et al. 2016). The acaricidal treatment of livestock remains the most effective way to prevent ticks from biting and feeding; however, the adverse effects for environmental and public health are more than self-evident (Walker 2014; De Meneghi et al. 2016).

Small mammals are considered the primary hosts of tick larvae and therefore form a key determinant for the abundance of questing nymphs (Perez et al. 2016). Hence, a possible but more difficult strategy to achieve could include landscape modifications and vegetation management strategies in urban and suburban areas in a way that it favors the small mammal species that can build resistance to ticks, a phenomenon in which ticks are unable to feed successfully after several tick infestations (Perez et al. 2016). As an example, repeated infestation of bank voles by larval ticks reduced the feeding success, whereas feeding on wood mice did not negatively affect the successive feeding of ticks (Humair et al. 1999). Changes and growth of agricultural landscapes also significantly affect the communities of small mammals. Wooded habitats are considered favorable for ticks because of temperature and humidity they constantly provide (Perez et al. 2016). Deforestation would definitely be negatively related to tick density because woodland areas act as habitats for ticks and their hosts but the detrimental effects of this action are obvious. Consequently, highly unconventional strategies such as building artificial wood ant nests would be elegant and eco-friendly solutions to reduce the tick density (Zingg et al. 2018), in comparison to rather drastic solutions such as eradication of some mammalian hosts of ticks (Rand et al. 2004).

Reservoir-based approaches

Oral vaccinations

The sources of the microbes that cause infectious diseases and where the pathogens can multiply or merely survive until they are transmitted are known as reservoirs. Vector ticks must acquire *B. burgdorferi* from wildlife reservoirs as there is no clear evidence of a transovarial transmission route (Rollend

et al. 2013). Disrupting *B. burgdorferi* transmission between the tick vector and reservoir hosts is regarded as a promising strategy to reduce human exposure to Lyme borreliosis (Melo et al. 2016). Rodents are a major reservoir for Lyme borreliosis and as such a very promising target to prevent them from getting infected with *B. burgdorferi*. The development of a specific, easily distributable, thermostable, and economically viable oral vaccine for wildlife reservoirs surrounding human communities could significantly reduce the incidence of Lyme borreliosis (Gomes-Solecki et al. 2006).

Oral vaccination is of high interest as a tool to prevent the spread of Lyme borreliosis as it can be used to deliver the vaccine to humans, domestic animals, and wildlife reservoirs of *B. burgdorferi*. The immunogen can be administered as a purified antigen (Luke et al. 1997) or as a genetically altered *Escherichia coli* (Fikrig et al. 1991). A number of oral vaccines based in *E. coli* expressing recombinant OspC, OspB, BBK32 from *B. burgdorferi*, and Salp25 and Salp15 from *Ixodes scapularis* were developed. Of the five immunogenic candidates, only OspC induced significant antibody response in mice when they were immunized by intragastric inoculation. Nevertheless, the antibodies did not prevent dissemination of *B. burgdorferi* as determined by the presence of spirochetes in the ear, heart, and bladder (Melo et al. 2016). Again, OspA seems to be a more promising oral vaccine candidate as oral vaccination of wild white-footed mice resulted in reductions of 23% and 76% in the nymphal infection prevalence (Richer et al. 2014). Significant decreases in tick infection were observed within 2–3 years after oral vaccine deployment. The usage of reservoir-based vaccines as part of a strategy to fight the expansion of Lyme borreliosis is also vastly dependent on the development of effective strategies for delivery of the immunogen. One of the promising approaches is the usage of *Lactobacillus plantarum* as a live vaccine delivery vehicle. These bacteria are naturally associated with the gastrointestinal tract and generally regarded as safe by the FDA. Oral administration of live *L. plantarum* expressing OspA was shown to be effective in blocking transmission of *B. burgdorferi* (del Rio et al. 2008). Not only is the deployment of borrelial immunogens in oral vaccines achievable but also the tick antigens can be used to inhibit the transmission of *B. burgdorferi*. Using the recombinant vaccinia virus, a single dose of the subolesin vaccine resulted in strong immune system response and partial protection from *B. burgdorferi* infection among vaccinated mice (Bensaci et al. 2012).

Immunization by genome editing

A novel theoretical model for prevention of tick-borne diseases, using CRISPR-based genome editing technology, has been recently suggested (Buchthal et al. 2019). Mice Against Ticks is a proof of principle project that aims to heritably immunize local wild white-footed mouse populations against

Lyme borreliosis and, potentially, against ticks using antibodies derived from natural adaptive immunity, with the ultimate goal to reduce the reservoir competence of a host for many decades. It is important to emphasize that the protective antibodies have not yet been identified, nor has heritable genome editing been ever achieved in white-footed mice.

Conclusion

Presently, the use of acaricides constitutes a major component of integrated tick control strategies and therefore indirectly acts as the first line of human-induced defense against a multitude of tick-borne pathogens. However, this is accompanied by the selection of acaricide-resistant ticks and severe pollution of the environment (Kunz and Kemp 1994). Lyme borreliosis is the most common disease spread by ticks in the Northern Hemisphere with an ever increasing incidence, and as such this disease is a major topic on the public health agenda. In order to effectively control the spread of Lyme borreliosis, a multifront battle should be seriously considered, involving environment management, wildlife and domestic animal vaccinations, and of course policymaking. New approaches and strategies that target against Lyme borreliosis, including elements of the tick vector, the reservoir hosts, and the *Borrelia* pathogen itself, are being developed. Human vaccination is the most effective means of prevention. However, there are a lot of hurdles to overcome, not just regulatory and scientific ones, but public acceptance as well. Let us hope that the challenges present today will be met and an efficient defense against Lyme borreliosis will not only be found but also publicly available in the very near future.

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Compliance with ethical standards

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