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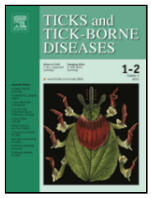

Review Article

Vaccine approaches applied to controlling dog ticks

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ABSTRACT

Ticks are considered the most important vectors in veterinary medicine with a profound impact on animal health worldwide, as well as being key vectors of diseases affecting household pets. The leading strategy applied to dog tick control is the continued use of acaricides. However, this approach is not sustainable due to surging tick resistance, growing public concern over pesticide residues in food and in the environment, and the rising costs associated with their development. In contrast, tick vaccines are a cost-effective and environmentally friendly alternative against tick-borne diseases by controlling vector infestations and reducing pathogen transmission. These premises have encouraged researchers to develop an effective vaccine against ticks, with several proteins having been characterized and used in native, synthetic, and recombinant forms as antigens in immunizations. The growing interaction between domestic pets and people underscores the importance of developing new tick control measures that require effective screening platforms applied to vaccine development. However, as reviewed in this paper, very little progress has been made in controlling ectoparasite infestations in pets using the vaccine approach. The control of tick infestations and pathogen transmission could be obtained through immunization programs aimed at reducing the tick population and interfering in the pathogenic transmission that affects human and animal health on a global scale.

1. Introduction

Globally, ticks are the most important arthropod vectors, transmitting a wider variety of pathogens than any other group of vectors (Jongejan and Uilenberg, 2004; Anderson et al., 2017). Tick infestation remains a serious impediment to profitable livestock production (Grisi et al., 2014; Laing et al., 2018; Rodríguez-Hidalgo et al., 2017) while, at the same time, presenting an opportunity for the domestic pet market (Coles and Dryden, 2014).

The development and application of tick control vaccines should be seen as a sustainable measure, reducing the use of acaricides. These premises emphasize the need to develop improved vaccines using different strategies (de la Fuente and Merino, 2013). The primary mechanism behind the vaccine action is the fact that ectoparasites feeding on immunized hosts ingest specific antibodies that attach to essential proteins, thus inducing impaired feeding, interfering in both the life and reproductive cycles, and resulting in lower vector numbers (de la Fuente et al., 2011; Moreno-Cid et al., 2013; Bensaci et al., 2012; Merino et al., 2011). The discovery of new vaccine antigen candidates for the control of tick infestations requires the development of screening platforms that allow for effective trials of suitable candidates (de la Fuente and Merino, 2013). Ideally, vaccination could confer cross-protection against a number of tick species considering the existence of protein antigens that are largely conserved across tick genera (Perez-Perez et al., 2010).

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The most common approach to tick control is through the use of acaricides that combine good efficacy with low cost, yet they frequently lead to: (i) selection of acaricide-resistant tick populations (Heath and Levot, 2014; Rodríguez-Hidalgo et al., 2017; Webster et al., 2015), (ii) health risks to animals and humans (Banumathi et al., 2017; Kumar et al., 2005), and (iii) chemical contamination of the environment (George et al., 2004; Graf et al., 2004). Taken together, the downside of using acaricides highlights the need to develop new control alternatives (Rodríguez-Mallon et al., 2012; Sabadin et al., 2017). A vaccine-based control is a promising approach presented as a cost effective and environmentally friendly alternative (de la Fuente et al., 2007; Sonenshine, 2006). Although vaccination is considered a rational strategy for controlling ticks, the veterinary market lacks available products (White and Gaff, 2018). With that in mind, the pipeline of universal vaccine development could be based on inducing cross-reactive immunity against different tick species by devising a formulation with conserved, antigenic proteins. Over the past three decades, a growing number of tick proteins have been evaluated as vaccine candidates. As such, the aim of this review is to summarize the leading candidates for tick vaccines, plus identify and describe new improved vaccines for vector and pathogen control.

2. Pathogens transmitted by *Rhipicephalus sanguineus* sensu lato

There is growing concern over emerging and reemerging tick species in many parts of the world (Kean and Irvine, 2013; Mlera and Bloom, 2018; Ocampo et al., 2003). Tick infestation in dogs can be an occasional nuisance or even a continuous infestation covering a broad clinical spectrum, ranging from adverse effects on health to fatal diseases (García-García et al., 2010; Weinberger et al., 2010). The increased spread of arthropod vectors and their diseases can be explained by ecological and climatic factors, as well as by the mobility of human and animal populations (Anderson et al., 2017). In this context, *R. sanguineus* s.l. has been implicated as a vector of human pathogens in Europe, Asia, and Africa. This species complex serves as a vector of the human pathogen *Rickettsia conorii*, the causative agent of Mediterranean spotted fever, and in North America as a vector of *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever (Stafford et al., 2017; Parola et al., 2005; Wikswo et al., 2007). Brown dog ticks infected with *R. rickettsii* have been recovered in Arizona where an outbreak of the disease had occurred (Stafford et al., 2017). Rocky Mountain spotted fever is among the most lethal diseases in Mexico (Álvarez-Hernández et al., 2017), Brazil (Campos et al., 2020), and other countries (Warner and Marsh, 2002). Importantly, *Dermacentor andersoni* and *Dermacentor variabilis* are the main vectors of *R. rickettsii* in North America (Burfdorfer 1975; (McDade and Newhouse, 1986)), which has since spread to Argentina (Paddock et al., 2008). In addition, ticks belonging to the *Amblyomma* and *Rhipicephalus* genera have been reported as vectors of this pathogen (Demma et al., 2005, Labruna et al., , 2008, Parola et al., 2013).

The disease was first described in the early 1940s by scientists who carefully documented specific environmental determinants that were responsible for devastating outbreaks in several communities. These researchers discussed the basic role of domestic dogs and *R. sanguineus* s.l. as the principal driving force of the epidemic. Years later, under the same environmental conditions, the disease reappeared early this century (Álvarez-Hernández et al., 2017). Specific ecological and epidemiological circumstances can trigger and perpetuate epidemic levels of disease, as exemplified by recent outbreaks in the southern and eastern regions of the state of Arizona, caused by an increase in the numbers of free-roaming dogs and host-seeking *R. sanguineus* s.l. in low-income communities (Regan et al., 2015; Drexler et al., 2015; Demma et al., 2005).

Hepatozoon canis is an important tick-borne infection in dogs (Baneth, 2011). In North America, another species of this parasite, *Hepatozoon americanum*, also causes disease in dogs (Vincent-Johnson et al., 1997). Moreover, the bacterial family Anaplasmataceae contains several species that infect horses, ruminants, humans, and dogs, in addition to wild animals such as *Odocoileus virginianus* and coyotes (Dumler et al., 2001). In Europe, the main causative agent is *Anaplasma phagocytophilum* (Nováková and Víchová, 2010). Other species belonging to Anaplasmataceae have also been found in Romanian dogs, such as *A. platys* (Andersson et al., 2013) and *Ehrlichia canis* (Mircean et al., 2012). The tick-borne bacterium *Candidatus* Neoehrlichia mikurensis has been detected in several mammalian species, including humans (Francischetti et al., 2011; Grankvist et al., 2014; Welinder-Olsson et al., 2010). *Babesia canis* and *Babesia vogeli*, in Brazil and Cuba, are the main pathogens transmitted by *R. sanguineus* s.l. to dogs, and *E. canis*, causing the so-called "Tick disease" (Castro et al., 2020; Navarrete et al., 2016; Silveira et al., 2009).

3. Tick control strategies based on acaricide products

Tick control is primarily based on chemical means, with topical treatments having become the standard accepted method of application (Dryden and Payne, 2004; Rust and Dryden, 1997). The chemicals used in the treatment of ectoparasites act systemically through direct contact with the target parasites following external application (Rodríguez-Vivas et al., 2014). Spot-on formulations and oral medication provide ease of use and a longer dosing interval (usually monthly), which may aid in preventing pathogen transmission to dogs and their owners (Davoust et al., 2003; Welinder-Olsson et al., 2010). Chemical classes include a variety of products such as chlorinated hydrocarbons (*e.g.*, DDT and lindane), organophosphorus compounds (*e.g.*, coumaphos), carbamates (*e.g.*, carbaryl), formamidines (*e.g.*, amitraz), pyrethroids (*e.g.*, permethrin, flumethrin), formamidines (*e.g.*, amitraz), macrocyclic lactones (*e.g.*, ivermectin), phenylpyrazoles (*e.g.*, fipronil), insect growth regulators (*e.g.*, fluazuron), and isoxazolines (*e.g.*, afoxalaner, fluralaner, sarolaner), which are neurotoxins and, therefore, directly affect the ectoparasite's nervous system (Benavides et al., 2006; Fernández-Salas et al., 2019; Heath and Levot, 2014; Sharma et al., 2012; Webster et al., 2015).

Traditional control methods, such as acaricides and repellents and educational campaigns on recommended practices to reduce exposure to ticks, have been partially successful, with drug resistance and contamination constituting important limitations (Kunz and Kemp, 1994; Stutzer et al., 2018). Despite the variety of acaricide products on the market, ticks remain an ongoing problem for pet owners (McTier et al., 2016) because of their resistance and high number of generations per year, resulting in a great number of offspring.

The resistance process is usually slow as resistant tick specimens appear in small numbers in the population (Nath et al., 2018). However, continued use of acaricides eliminated sensitive individuals and the resistant population appears (Guerrero et al., 2014; Wang et al., 2015). In addition, the susceptibility of populations may diminish with prolonged exposure to individual products (de la Fuente et al., 2017). When these products used at the recommended product concentration and in accordance with all other recommendations over a long period of time, a selection of resistant genes initially present in low numbers in populations can dominate, resulting in a high resistance profile (Mansfield et al., 2017; de la Fuente and Kocan, 2014).

Unfortunately, such resistance has developed in most countries that are endemic for vector-borne diseases (Banumathi et al., 2017). The arachnid's genetic background and the indiscriminate use of chemical products have largely contributed to the selection of resistant arthropods, thereby threatening the success of disease control programs (David et al., 2016; Nkya et al., 2013). Due to increased resistance and the spread of infectious ticks to new areas (Djouaka et al., 2008; White and Gaff, 2018), new control strategies are required.

Since then, leading research on tick vaccines has discovered new protective antigens using different methodological approaches in various tick species (Contreras et al., 2019) and in other ectoparasite vectors (Bartley et al., 2017). It has always been considered that the combination of vaccination with other control measures, such as insecticides/acaricides and repellents, is needed for the effective control of ectoparasite vectors (de la Fuente, 2018), including dog ticks. Therefore, preventive measures could be adopted, such as restricting dog contact with ticks in endemic areas, applying long-acting tick products to dogs, and tick treatments in the environment (kennel and adjacent facilities). These products should be reapplied according to the recommended effectiveness periods.

4. General aspects for tick vaccine

Globally, most of the vaccines available to treat tick-borne diseases are live (attenuated or blood-derived) (Marcelino et al., 2012). Although these vaccines can be effective, little is known about their mechanism of action (dos Santos et al., 2014). Thus, additional research is needed for the development of safe, more cost-effective, and better defined alternative formulations (Guerrero et al., 2014; Marcelino et al., 2012).

The feasibility of controlling tick infestations by immunizing the hosts with selected tick antigens has already been achieved, resulting in reduced infestations (de la Fuente et al., 2017) and allowing the inclusion of multiple antigens. This approach could target a broad range of tick species and prevent tick-borne pathogens (de la Fuente et al., 2007). Another challenge is the maintenance of high antibody titers in vaccinated individuals due to the lack of natural immunological boosting, given that most of the proposed antigens are normally found in proteins that are not in contact with the host during the vector blood meal (Coutinho-Abreu and Ramalho-Ortigao, 2010; Neelakanta and Sultana, 2015). According to Elvin and Kemp (1994), candidate antigens need to present important criteria in order to develop an effective vaccine, such as host-induced antibodies capable of acceding sufficient target proteins, an antibody-antigen complex formation capable of disrupting the function of the target protein, and/or inducing physiological modifications affecting the vector.

Advances made in the characterization of tick genomes, along with the use of bioinformatics, ribonucleic acid interference (RNAi), mutagenesis, immunomapping, transcriptomics, proteomics, expression library immunization, and other technologies have allowed a rapid, systematic, and comprehensive approach to a tick vaccine discovery (de la Fuente and Kocan, 2003; Graf et al., 2004; Mctier et al., 2016). Computational approaches and tick-borne pathogen genomics have furthered our understanding of the genetic factors and molecular pathways involved at the host-vector-pathogen interface that could be used to identify signaling pathways and protein interaction networks, resulting in a better comprehension of molecular and biochemical processes at the tick-host-pathogen interface (Busby et al., 2012; de la Fuente et al., 2017).

Recent developments in omics analyses of ticks and tick-borne pathogens, coupled with the application of systems biology to the study of tick-host-pathogen molecular interactions, have advanced our understanding of the genetic factors and molecular pathways involved at the tick-host, tick-pathogen, and host-pathogen interface (Galindo and de la Fuente, 2012; de la Fuente et al., 2017). de la Fuente and Merino (2013), and Galindo and de la Fuente et al. (2012) described a vaccinomics approach based on transcriptomics and proteomics data. These authors studied a combination with vaccination trials for the discovery of tick protective antigens for the control of *Ixodes ricinus* and *Dermacentor reticulatus* infestations of companion animals (dogs and rabbits) with adult salivary glands. The experiments resulted in the identification of new antigens that exhibited a protective efficacy of vaccination against *I. ricinus* and *D. reticulatus* infestations. However, the information available in tick genomic, transcriptomic, and proteomic databases, together with the fact that most of the annotations are based on sequence identification and not on functional studies, requires validation of the results after data integration and analysis (de la Fuente and Merino, 2013). An important consideration for arthropod vector vaccines in general is the need to find antigens capable of protecting against different vector species and interfering with pathogen infection and transmission.

One effective antigen against ticks is the protein Bm86, specifically targeting the cattle tick *Rhipicephalus microplus*, which is the basis of two commercial vaccines: TickGARD® (Queensland Dairyfarmes Oraganization, Australia) (Jonsson et al., 2000), Gavac® (Heber Biotec S.A.) (de la Fuente et al., 2007) and the recently released Ixovac® (Lapisa S.A). The most significant effect was the reduction of larval infestations in subsequent generations by reducing the number of engorged female ticks, their weight, and reproductive capacity (de la Fuente et al., 2011; Marcelino et al., 2012; Sonenshine, 2006). Although it has proven to be a cost-effective alternative for the control of cattle tick infestations and pathogen infection (de la Fuente et al., 2007), there are some limitations associated with the Bm86 vaccine, such as its limited efficacy against *Rhipicephalus* species, hence reinforcing the need for new vaccines (de la Fuente and Kocan, 2003; Sonenshine, 2006).

5. Vaccine trials to analyze the efficacy against *Rhipicephalus* ticks

Szabó and Bechara (1995) used the principle described by Allen and Humphreys (1979) (Fig. 1) in order to control *R. sanguineus* s.l. infestations in dogs vaccinated with an extract gut proteins from *R. sanguineus* s.l. It was observed in dogs immunized with gut extract that some ticks did not oviposit and, after death, they turned black and acquired a hard consistency (Szabó and Bechara, 1995). Notably, the reduction in tick oviposition is one of the most important benefits obtained with tick vaccines that lead to a reduction of tick populations in the field (de la Fuente and Kocan, 2003; Moreno-Cid et al., 2013; Sonenshine, 2006).

The efficacy of Bm86 antigen against *R. sanguineus* s.l. has been investigated by (Perez-Perez et al., 2010) (Fig. 1). After Bm86 immunization in dogs, a reduction in the recovery rates of larvae (38 %), nymphs (29 %), and adult females (31 %) was observed after tick blood meal by Perez-Perez et al. (2010). The efficiency rate analysis of conversion to eggs revealed distinct mean values for the control (56.2 %) and vaccinated (46.4 %) groups. In addition, a preliminary experiment with *R. sanguineus* s.l. demonstrated a synergistic effect of subolesin and Bm86 knockdown (de la Fuente et al., 2006), suggesting the possibility of combining these antigens to improve the control of dog tick infestations.

Novel proteins that are structurally homologous to Bm86 were reported (Nijhof et al., 2010) as the synthetic peptide from the ATAQ protein, which is present in the gut and Malpighi tubes of *R. microplus* (Aguirre et al., 2016). The ATAQ proteins were isolated, characterized, and sequenced from several species of the *Rhipicephalus* genus, presenting 93.3 % similarity among the ATAQ DNA. These data supported new vaccine trials using mice, rabbits, and cattle to evaluate the humoral immune response and efficacy against *R. sanguineus* s.l. and *R. microplus*. Furthermore, a 35 % reduction in overall life cycle parameters was reported for *R. microplus* and 47 % for *R. sanguineus* s.l. (Aguirre et al., 2016).

The efficacy of a 20 amino acid synthetic peptide from ribosomal protein P0 from *Rhipicephalus* sp. ticks was assayed as a vaccine against *R. sanguineus* s.l. in rabbits (Rodríguez-Mallon et al., 2012) (Fig. 1). The overall efficacy was 90 %, demonstrating that immunization with the tick peptide from P0 protein reduced tick survival, suggesting its application as an effective tick control approach.

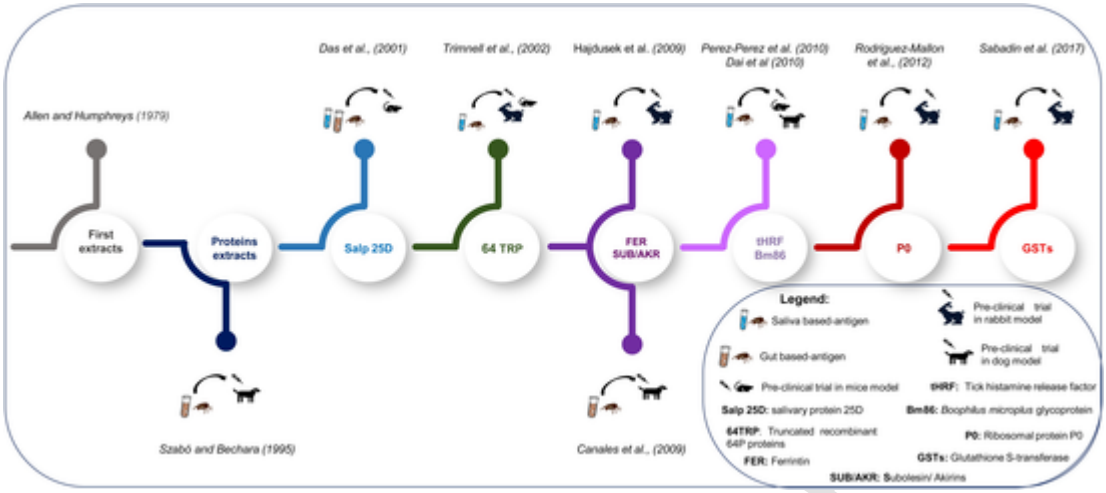

Fig. 1. Timeline in the tick vaccine development.

Glutathione S-transferases (GSTs) can be found in most animals (Agianian et al., 2003), and several tick GSTs have also been characterized (He et al., 1999; de Lima et al., 2002; Junior et al., 2004) as being involved in the metabolic detoxification of xenobiotics and other endogenous compounds (Agianian et al., 2003). Sabadin et al. (2017) investigated the effect of recombinant glutathione S-transferase (Fig. 1) from *Haemaphysalis longicornis* tick (rGST-HI) vaccine against *Rhipicephalus appendiculatus* and *R. sanguineus* s.l. infestation in rabbits. The rGST-HI antigens triggered high antibody production, leading to a reduction in the number, weight, and fertility of engorged *R. appendiculatus* adults and an overall vaccine efficacy of 67 %. Interestingly, histological analysis of organ morphology showed damage to salivary glands and ovaries in adult female *R. appendiculatus* that fed on vaccinated animals.

The protein 64TRP (recombinant forms of the *R. appendiculatus* tick cement antigen 64 P) is expressed in tick salivary glands (Havlíková et al., 2009) and seems to form part of the cement cone that anchors tick mouth parts to the host skin, preventing the leakage of fluids and allowing ticks to remain firmly attached to the host for several days (Fig. 1). A protective immune response was induced in guinea pigs immunized with recombinant 64TRP and challenged with nymphs and adults *R. sanguineus* s.l. and *I. ricinus* (Trimnell et al., 2005). Importantly, seven of the twenty-two surviving female ticks died after two days post-detachment from vaccinated animals, presenting distention damage or rupture in the midgut (Trimnell et al., 2005).

Ticks are exposed to large amounts of iron present in the blood meal during feeding. Excessive amounts of iron can react with H_2O_2, generating hydroxyl radicals, a potent biological oxidant (Kumar, 2016). Therefore, a balanced regulation of iron levels is essential for tick and pathogen survival (Galay et al., 2014). The ferritin (FER) FER1 and FER2 proteins act as the primary iron storage and transporter, respectively, to produce reactive oxygen species (ROS) from iron (Ferrolho et al., 2017; Galay et al., 2014). Ferritins may also contribute to nutritional immunity by depriving pathogens of their essential iron supply. Interestingly, FER2 is expressed in all tick stages. The FER2 silencing by RNAi affects tick physiological processes, such as blood acquisition and reproduction (Hajdusek et al., 2009), in addition to larval hatching, indicating the importance of FER2 as a promising vaccine strategy (Ferrolho et al., 2017). In fact, the vaccine formulation using recombinant ferritin 2 from *I. ricinus* (IrFER2) against *I. ricinus*, *R. microplus,* and *R. annulatus* elicit an overall efficacy of 98 %, which was attributed to a reduction in the number and weight of engorged female ticks and egg fertility (Hajdusek et al., 2010).

Moreover, the tick subolesin (SUB) has been considered another promising antigen in vaccine formulation (Fig. 1). SUB is an ortholog of insect and vertebrate akirins (AKR) protein, which is involved in gene-expression regulation (Canales et al., 2009; Goto et al., 2004; Vincent-Johnson et al., 1997), and a highly conserved protein involved in modulating feeding and reproduction with a protective effect against all tick developmental stages when used in recombinant protein immunization (Domingos et al., 2013). This protein was silenced by de la Fuente et al. (2006) through RNAi in *Dermacentor variabilis,* leading to the degeneration of certain tick tissues, such as the guts, salivary glands, reproductive tissues, and embryos. In different experiments, vaccination with SUB provided control for hard (*Ixodes* spp., *Rhipicephalus* spp., *Amblyomma americanum*, *D. variabilis*) and soft (*Ornithodoros* spp.) ticks, mosquitoes (*Aedes albopictus*), sand flies (*Caligus rogercresseyi*), and tick infections with *A. phagocytophilum*, *A. marginale*, *Babesia bigemina*, and *Borrelia burgdorferi* sensu lato (Moreno-Cid et al., 2013; Merino et al., 2011).

These findings suggest that vaccination reduces protein levels in feeding ticks through an unknown mechanism, yet likely mediated by antibody-antigen interactions in the cell cytoplasm (Merino et al., 2011). SUB translocation to the nucleus was discovered where it functions as a transcriptional regulator of its own expression and of genes involved in various biological processes, playing an important role in tick feeding, reproduction, and pathogen infection (de la Fuente et al., 2011; de la Fuente and Merino, 2013). Moreover, the results of the vaccine trial with recombinant *A. albopictus* AKR failed to demonstrate a significant effect against *R. sanguineus* s.l. infestations in dogs (Canales et al., 2009).

6. Future trial trends involving new vaccine antigens against dog ticks

The discovery driven approach to identifying tick protective antigens results in a large number of candidate antigens that require further screening using platforms capable of finding the best vaccine candidates (de la Fuente and Merino, 2013; Kotsyfakis et al., 2015). Such screening platforms include protein fractionation and testing in vaccinated hosts (Sonenshine, 2006), expression library immunization (Almazán et al., 2005), suppression subtractive hybridization (de la Fuente et al., 2007, 2017), microarray hybridization (Maritz-Olivier et al., 2012), *in vitro* tick feeding systems (Almazán et al., 2005; Gonsioroski et al., 2012), and RNAi (Almazán et al., 2010; Barnard et al., 2012; Mulenga et al., 2013). However, this methodological approach to protective antigen screening is onerous, costly, and time consuming and does not take advantage of recently developed

omics technologies (de la Fuente and Merino, 2013). A systems biology approach, using omics datasets, has revealed that tick-borne pathogen infection induces transcriptional reprogramming that affects a number of metabolic pathways in ticks, facilitating infection, multiplication, and transmission (de la Fuente and Merino, 2013). These results suggest that the response of tick cells to tick-borne pathogens is associated with tolerance to infection (Cabezas-Cruz et al., 2019).

Expression library immunization was used for the discovery of the candidate tick protective antigen Subolesin in a mouse model of *I. scapularis* infestations (Merino et al., 2011), but it requires the use of animal models, such as mice, in which DNA immunization is effective, and supports tick infestations in a reproducible way. Suppression subtractive hybridization has been used for the study of tick–pathogen interactions and the selection of candidate tick protective antigens (Antunes et al., 2012; de la Fuente et al., 2007; Kocan et al., 2009).

RNAi may also allow for the characterization of antigens that interfere with pathogen development and transmission (Antunes et al., 2012; de la Fuente et al., 2007; Kocan et al., 2009). However, recent studies have shown that RNAi screening alone may not result in the selection of good tick protective antigens (Almazán et al., 2010; Prudencio et al., 2010). One possibility that has been tested is the combination of RNAi and *in vitro* tick feeding as an algorithm to improve the identification of tick protective antigens (de la Fuente and Merino, 2013). RNAi allows screening of a relatively large number of genes involved in tick–pathogen or tick–host interactions, whereas *in vitro* feeding with antibodies against selected candidate antigens should provide results resembling closer vaccine protective capacity. Nonetheless, limitations of *in vitro* tick feeding systems, such as differences in pathogen infection between *in vivo* and *in vitro* tick feeding, should be considered (Kocan et al., 2007).

Even after effective selection of candidate protective antigens, some antigens could fail to protect hosts because they may be expressed at low levels or be inaccessible to host antibodies. Additionally, ticks have evolved mechanisms to counteract the effect of host immune response, particularly for exposed antigens. These mechanisms may include the evolution of immunosuppressive molecules secreted during tick feeding and multigene families with redundant biological functions (Menten-Dedoyart et al., 2011; Ribeiro and Francischetti, 2003). Taken together, these results stress the need to conduct vaccination trials in the target host species to validate candidate tick protective antigens. These studies are important to improving vaccine efficacy using formulations and immunization schemes that accurately demonstrate protective responses and through the combination of antigens with synergistic activity. Vaccine efficacy of the Bm86 (commercially available vaccines) antigen correlates with antibody titers in cattle (de la Fuente et al., 2007). Several candidate tick protective antigens, such as 64TRP, Subolesin, and Ferritin 2, have been included in vaccination trials and their function investigated (Hajdusek et al., 2009; Havlíková et al., 2009; Merino et al., 2011; Trimnell et al., 2005). Although the protection mechanisms elicited by these antigens are likely mediated by vaccine-induced antibodies (Hajdusek et al., 2010; Merino et al., 2011), additional analysis is required to fully understand how they protect against tick infestations and pathogen infection (de la Fuente and Merino, 2013; Hajdusek et al., 2010; Trimnell et al., 2005).

However, the specificity of tick-pathogen interactions resulting in productive infection is not yet completely understood. The presence of pathogen-specific tick receptors affects vector competence for these pathogens, but other mechanisms could be involved in this process. Identifying the molecular drivers that facilitate tick survival, spread, and pathogen transmission provides an opportunity to disrupt these processes and could lead to a reduction in tick burden and prevalence of tick-borne diseases (de la Fuente et al., 2017).

Overall, the combination of distinct approaches, such as vaccine antigens and acaricides interventions, could result in a more effective and environmentally friendly control of tick populations (de la Fuente et al., 2016).

7. Perspectives and conclusions

Tick infestation remains a serious impediment with problems associated with the widespread use of chemical acaricides, calling for alternative interventions, particularly vaccines. Whereas the rate of tick antigens identification has accelerated over the past 20 years, an effective anti-tick vaccine is far from reaching the veterinary market.

The global market for insecticidal/acaricidal and repellent compounds is immense and growing. For example, the repellent market in 2016 had an estimated value of US$ 3.2 billion and was projected to reach US$ 5 billion by 2022 (Statista, 2019). In fact, the stimulus provided by industry to sustain the acaricide manufacturers obstructs vaccine funding for controlling ticks. Moreover, there is a consensus that an effective approach to developing effective tick control is associated with combined control measures, including immunization and acaricides (de la Fuente and Estrada-Peña, 2019). Taking into account that the identification of high performance vaccinal antigens against dog ticks is not available, the vaccinal approach should be considered as an alternative and complementary intervention, ultimately reducing the use of insecticides/acaricides while raising the demand for vaccines. In addition, cost-effective vaccines and safety are important factors as they are often expensive in the veterinary market. To address these issues, research should be focused on developing effective formulations with new adjuvants for antigens-based vaccine delivery (Dar et al., 2019; Contreras et al., 2019). Furthermore, having dog owners worldwide insist on having access to products with a low environmental impact could provide the veterinary medicine industry with the stimulus it needs to develop new control measures against ticks using fewer acaricide products.

The number and categories of antigens with a potential for protecting against tick infestation is rising, with a good number of these molecules demonstrating strong reactivity (Fatmi et al., 2017). Developing safe, affordable vaccine alternatives will improve the chances of reaching a point of broad distribution and effective vaccine coverage.

Although significant advances have been made, further research in tick vaccinology is needed to prove the efficacy of different antigen candidates in dogs. The studies described here have suggested that vaccines possess various biological actions at different stages of the life cycle and may have a therapeutic potential to prevent many diseases. Meanwhile, integrated control of disease vectors using anti-tick vaccines to reduce the volume and frequency of acaricide application remains a practical and sustainable approach to tick-borne disease management as we inch towards a fully effective vaccine.

Although vaccines are among the crowning achievements in medicine, the limiting step in the development of vector vaccines has been the identification of new antigens that induce protective immune responses while preventing pathogen transmission (de la Fuente and Kocan, 2003). Furthermore, when developing new and effective vaccines against ticks, the antigen combinations may target multiple ectoparasite species in different hosts. However, the antigen structure and/or immunological interactions may interfere with and reduce vaccine immunogenicity and efficacy. Therefore, new formulations should consider these factors and the possibility of combining protective epitopes from different proteins into a single antigen to produce a multi-antigenic chimeric protein (*e.g.*, Subolesin/Akirin chimeras). Additionally, antigens effective against multiple ectoparasitic species in different hosts ought to be considered, alone or in combination with other antigens.

The combined use of several technologies may be the most effective way of identifying vaccine candidates by focusing on biochemical pathways that are functionally important for tick feeding, development and reproduction, and pathogen infection and transmission.

Moreover, tick vaccine development has proven to be feasible; recent studies have reported important vector control results based on the induction of host immune responses (Pereira-Filho et al., 2020; Rodrigues-Alves et al., 2020; Graciano et al., 2019; Gonçalves et al., 2019), which probably will be the next frontier in vaccinology for vector-borne diseases controls in human and veterinary medicine.

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Uncited references

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2020.101631.

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