Summary

Ticks and tick-borne diseases are a growing problem affecting human and animal health worldwide. Traditional control methods, based primarily on chemical acaricides, have proven not to be sustainable because of the selection of acaricide resistant ticks. Tick vaccines appear to be a promising and effective alternative for control of tick infestations and pathogen transmission. The purpose of this review is to summarize previous tick vaccine development and performance, and to formulate critical issues and recommendations for future directions for development of improved and effective tick vaccines. Development of effective screening platforms and algorithms using omics approaches focused on relevant biological processes will allow the discovery of new tick protective antigens. Future vaccines will likely combine tick antigens with different protective mechanisms alone or in combination with pathogen-derived antigens. The application of tick vaccines as part of integrated control strategies will ultimately result in the control of tick-borne diseases.

Keywords: tick, control, vaccine, acaricide, vaccinomics

Introduction

Ticks (Acari: Ixodida) are obligate hematophagous arthropod ectoparasites that are distributed worldwide and transmit pathogens causing diseases in humans and animals [1,2]. In the last decades, the continuous human exploitation of environmental resources and increase in human outdoor activities has allowed for the contact with ticks normally present in the field, resulting in increased transmission of tick-borne pathogens (TBP) [3,4]. In addition, tick populations are expanding due to changes in climate and human interventions that affect reservoir host movement and human contact with infected ticks [3-8]. As blood-sucking ectoparasites, ticks inflict great damage to humans, domestic and wild animals in many parts of the world. This damage consists of direct damage to hides, reduction in animal production, secondary infections, and diseases caused by TBP [9,10]. Furthermore, despite efforts to implement measures to control tick infestations, ticks and the pathogens they transmit continue to be a serious problem to human and animal health [10-16].

Ticks are difficult to control because they have few natural enemies and traditional control methods, based on chemical acaricides, have been only partially successful [10,17]. Therefore, new strategies are needed for the control of ticks and TBP and tick vaccines appear to be a promising and sustainable approach towards this objective [10-15,18-22]. Recent reviews have focused on the efficacy and limitations of BM86-based vaccines and the discovery and characterization of new candidate tick protective antigens for the development of vaccines for the control of tick infestations and pathogen infection and transmission [10-15,23-28]. The purpose of this review is to summarize previous tick vaccine development and performance literature, and to formulate critical issues and

recommendations for future directions for development of improved and effective tick vaccines for control of tick infestations and pathogen transmission.

Traditional tick control methods and associated problems

Traditional tick control methods are primarily based on the use of chemical acaricides, which have had limited efficacy in reducing tick infestations [10]. Additionally, the use of acaricides is often accompanied by serious drawbacks including the selection of acaricide-resistant ticks, environmental contamination and contamination of milk and meat products with residues [17]. The selection of ticks resistant to chemical acaricides is a growing problem particularly affecting cattle industry worldwide [29-31]. These facts together with the high cost of developing new acaricides result in the lack of sustainability for continuous acaricide use for tick control [25].

Alternative control methods based on the use of botanical acaricides and repellents, entomopathogenic fungi and the education of farmers about recommended tick control practices and available options for the management of drug resistance have been proposed to reduce the effect of acaricide use on the selection of acaricide-resistant ticks [24,29-32]. Furthermore, integrated control programs that include habitat management and the genetic selection of hosts with higher resistance to ticks have been also proposed to reduce the use of acaricides for the control of tick infestations [33,34]. Nevertheless, based on the experience obtained with the commercial use of tick vaccines based on the *Rhipicephalus microplus* BM86 recombinant antigen for the control of cattle tick infestations, tick vaccines have been proposed as an effective component of the integrated programs for the control of tick infestations and TBP while reducing the use of chemical acaricides [10-15,23-28].

Tick vaccines for the control of tick infestations

As proposed by Elvin and Kemp [35], candidate tick protective antigens should fulfill certain important criteria such as (1) host antibodies should be able to gain access to the target protein in sufficient quantities, (2) the formation of the antibody–antigen complex should disrupt the function of the target protein and/or induce physiological changes that affect vector biology, and (3) the antigen should share conserved epitopes among several tick species to protect against multiple vector infestations. These criteria are still valid for the selection of candidate tick protective antigens considering that the vaccine should also reduce tick vector capacity for TBD [36].

The protective mechanism characterized so far for tick vaccines is based on the development of antigen-specific antibodies in immunized hosts that interact and affect the function of the targeted antigen in ticks feeding on immunized hosts [21,37]. As shown for BM86-based vaccines, tick vaccines reduce the number, weight and reproductive capacity of engorging female ticks, therefore reducing tick infestations in subsequent generations [10].

Some tick species parasitize several vertebrate hosts and share habitat and hosts with other tick species [38]. These facts stress the need for developing vaccines effective in different hosts and against several tick species. However, a limited number of tick vaccines have been characterized so far in different hosts and cross-protective against multiple tick species [25,28,39-45].

Due to the importance of tick infestations for the cattle industry worldwide, most of the efforts toward the development of tick vaccines are directed for the control of tick species infecting cattle, particularly *R. microplus* [10-15,23-28] (Table 1). However, recent

reports have addressed the effect of tick vaccines on alternative hosts such as sheep [45,46], camels [39], deer [40] and dogs [44].

Recent developments are directed towards the use of *R. microplus* BM86 homologs in other tick species infecting cattle [47-49]. Additionally, new candidate tick protective antigens for the control of *R. microplus* infestations include Subolesin, Metalloprotease, Aquaporin, Ribosomal protein P0, Silk and Ferritins (Table 1). Furthermore, antigens protective against multiple tick species have been also characterized [11,27,28,42,43,45,50]. These results support the possibility of developing vaccines effective in different hosts and for the control of multiple tick species. However, new antigens and especially antigen combinations are required to develop more effective vaccines against tick infestations.

The efficacy of antigen combinations on tick infestations was first demonstrated by Allen and Humphreys [51] using tick protein extracts. However, until recently the combination of tick protective antigens did not result in higher efficacy for the control of tick infestations [52,53]. Merino et al. [52] used a chimeric antigen composed of protective epitopes from tick Subolesin and mosquito Akirin with a higher efficacy when compared to tick Subolesin for the control of *R. microplus* infestations in cattle (Table 1). In the patent application by Schetters and Jansen [53], the inventors claim that the combination of the well characterized tick protective antigens BM86 and Subolesin in a single formulation results in high vaccine efficacy against cattle tick infestations due to a synergy between both antigens (Table 1). The combination of tick protective antigens is a promising direction to increase the efficacy of tick vaccines against multiple tick species. Other directions to improve tick vaccine efficacy include the use of novel formulations

based on more effective adjuvant and antigen presentation and the possibility of developing vaccines with tick knock-down effects (i.e. substantial decrease of tick numbers on animals) as exhibited by chemical acaricides [10,23] and suggested by recent results with the BM86+Subolesin combined antigen vaccine [53] (Table 1).

Tick vaccines for the control of pathogen infection and transmission

The ultimate goal of tick vaccines is the control of both ticks and TBD. Vaccination with tick protective antigens such as BM86 among others that were directed towards control of tick infestations has also shown reduction in pathogen prevalence as a result of reducing tick populations [15,55]. Other antigens such as Subolesin show a direct effect on affecting pathogen infection and/or transmission while reducing tick infestations [28,41,43,54,55] (Box 1). Furthermore, recent results have revealed the molecular interactions between ticks and transmitted pathogens with the identification of candidate tick antigens to reduce pathogen infection and transmission while also affecting tick infestations [52,56-66]. These results support the identification of tick protective antigens with the dual function of reducing tick infestations and pathogen infection and transmission to ultimately protect against TBD. However, the combination of tick-derived and pathogen-derived antigens is probably the best way of achieving high vaccine efficacy for the control of vector-borne diseases.

Antigens from TBP such as *Borrelia burgdorferi* [67], flaviviruses [68], *Ehrlichia chaffeensis* [69], *Anaplasma phagocytophilum* [69-71] and *Anaplasma marginale* [72-78] among others have been proposed as candidate protective antigens for the control of pathogen infection and transmission. The possibility of combining these pathogen-derived antigens with tick protective antigens should result in new vaccines for the

control of vector-borne diseases (Fig. 1). In fact, recent results using vaccination with the combination of tick Subolesin with *A. marginale* major surface protein 1a (MSP1a) as a membrane-exposed chimeric antigen [79-81] showed an effect on reducing tick infestations and pathogen infection under field conditions [45].

Tick vaccines and the development of vaccines against other major ectoparasites

Diseases caused by arthropod-borne pathogens account for over 20% of all emerging infectious diseases recorded between 1940 and 2004 [82]. Among ectoparasite arthropod vectors, ticks are considered to be second worldwide to mosquitoes as vectors of human diseases and the most important vectors of diseases that affect the cattle industry worldwide [2,83]. However, other ectoparasites are also relevant for human and animal health and current research efforts are directed towards developing vaccines for their control [84]. In this context, research on tick vaccine development is more advanced than that reported for other major ectoparasites. Therefore, tick vaccine research may provide models for development of vaccines against other arthropod pests [20,83,85-88]. In this direction, recent efforts using tick Subolesin or the Akirin homolog in mosquitoes have shown how vaccination with these antigens protects against multiple ectoparasites and the infection with vector-borne pathogens [42,43,89] (Box 1). These results encourage the use of similar strategies for the identification of protective antigens across different ectoparasite species and suggest the possibility of developing vaccines for the control of multiple ectoparasite infestations.

Conclusions and future directions

The control of TBD is a priority in the current context of the global burden that infectious diseases represent and the one-health approach through integration of physicians, ecologists and veterinarians to monitor and control of zoonotic diseases. To address this priority, tick vaccines have become a major component of strategies for the control of tick infestations and TBP. Despite the fact that vaccines are among the best achievements in science, past strategies for vaccine development need to be revised to increase possibilities for developing effective vaccines for the control of tick infestations and TBD. The identification of new tick protective antigens is a critical step for developing effective vaccines for the control of tick infestations and TBP and despite resent advances in the study of tick biology and tick-host-pathogen interactions, this continues to be the major hurdle toward conducting vaccine animal trials [26]. In this direction, recent developments in last generation omics technologies including reverse vaccinology and vaccinomics will play a key role [26,52,59,61,73-75,90,91]. The integration of omics data sets must overcome important challenges such as development of algorithms that will allow for analysis and validation of data produced by the systems biology approach to tick research and development of effective screening platforms for the selection of candidate protective antigens [26]. Systems biology studies for to the selection of candidate protective antigens should focus on the characterization of physiological processes such as suppression of host immune responses, blood digestion, embryogenesis, innate immunity and tick-pathogen interactions that are critical for tick feeding, reproduction and vector capacity [26,56,66,92-105].

Recently, Guerrero et al. [23] proposed the selection of tick antigens from unique or low copy number genes encoding membrane-associated or membrane-bound antigens that are

expressed in gut, ovary, and salivary gland tissues or in the saliva. In this regard, they proposed to select molecules with low redundancy and combining properties of "exposed antigens" (antigens that are in contact with the host immune system during tick infestation and thus hosts immunized with these antigens are boosted by continuous tick exposure) and "concealed antigens" (antigens that are not exposed to the host immune system and thus ticks are unlikely to have evolved mechanisms to effectively counteract the effect of the host immune system as had occurred with exposed antigens but requiring repeated immunizations to maintain elevated antibody titers) [11,24,106-108]. However, although these concepts are valid for the selection of candidate tick protective antigens, recent results using tick Subolesin have challenged the a priori criteria for selecting membrane exposed antigens (Box 1).

Along with the problems associated with selection of candidate tick protective antigens to reduce the need for animal trials, vaccination experiments require standardization to optimize results and make them comparable across different controlled pen and field trials (Box 2). Additionally, the development of validated models for tick life cycle under relevant field conditions will provide a valuable tool for the modeling of vaccine efficacy and impact of tick control [108].

In addition to tick vaccines, future directions for the control of tick infestations and vector-borne pathogens could also include tick autocidal control [37], transgenic or paratransgenic ticks resistant to pathogen infection as recently shown in mosquitoes [109], vertebrate hosts genetically modified to confer resistance to tick infestation and/or pathogen infection as proposed using transgenic plants [110], glyco-conjugate vaccines based on tick protein glycosylation [111] and the manipulation of the tick microbiota to

reduce pathogen infection and transmission rates [112]. Finally, cocktails of tick-derived antigens alone or in combination with pathogen-derived antigens should result in more effective vaccines that could be used in combination with other control methods for the integrated control of tick infestations and TBD (Fig. 1).

Expert commentary

In the future, TBD are expected to increase, thus having greater impact on human and animal health worldwide. Ticks are difficult to control because they have few natural enemies and traditional control methods based on chemical acaricides have been only partially successful with some implicit drawbacks such as selection of ticks resistant to acaricides. New strategies are needed for the control of ticks and TBP and tick vaccines appear to be a promising and sustainable approach towards this objective. The use of BM86-based commercial vaccines for the control of cattle tick infestations demonstrated the possibilities for tick vaccines and encouraged research for the development of improved vaccines. Currently, various candidate tick protective antigens have been identified and tested in controlled pen trials. However, the identification of new tick protective antigens is the critical step for developing effective vaccines for the control of tick infestations and TBP.

The integration of last generation omics datasets is improving the possibilities for identifying candidate tick protective antigens. However, this approach faces important challenges such as the development of algorithms that allow the analysis and validation of data produced by systems biology and effective screening platforms for the selection of candidate protective antigens. Nevertheless, focusing on the study of physiological processes that are critical for tick feeding, reproduction and vector capacity using a

systems biology approach offers great possibilities for the identification of new tick protective antigens for the development of improved vaccines for the control of tick infestations and pathogen infection and transmission.

Five-year view

In the coming years, TBD are expected to continue expansion affecting human and animal health. As part of integrated control programs, tick vaccines are a promising and effective intervention for the control of tick infestations and the infection and transmission of TBP. Research on tick vaccines will continue to focus on cattle ticks and pathogens due to the impact of TBD on the cattle industry worldwide. However, due to the fact that some tick species parasitize several vertebrate hosts and share habitat and hosts with other tick species, the development of vaccines effective in different hosts and against several tick species is a growing area of research. Additionally, TBD affecting humans, pets and other domestic and wild animals also encourage research into tick vaccines. The application of omics technologies to tick vaccine research will result in effective screening platforms and algorithms for the discovery of new tick protective antigens. Vaccinomics and reverse vaccinology approaches will be used to identify and fully characterize candidate protective antigens and validate vaccine formulations. New candidate protective antigens will most likely be identified by focusing on abundant proteins with relevant biological function in tick feeding, reproduction, development, immune response, subversion of host immunity and pathogen infection and transmission. Consequently, tick protective antigens will be discovered with multiple impacts when used in a vaccine including reductions in (a) tick infestations and fertility, (b) tick pathogen infection, (c) tick vector capacity for pathogen transmission and (d) tick

response to pathogen infection. These new vaccines will likely combine tick antigens associated with different protective mechanisms alone or in combination with pathogen-derived antigens to have an effect on reducing tick infestations while affecting pathogen infection and transmission to ultimately result in the control of TBD. Finally, the most economical integrated tick control strategies will be those combining tick vaccines with other control methods while reducing acaricide applications to reduce risks for humans, animals and the environment. These integrated tick control strategies should overcome difficulties in the commercialization of tick vaccines due to its new approach for tick control.

Key issues

- Ticks are obligate hematophagous arthropod ectoparasites that vector pathogens causing diseases in humans and animals.
- TBD are an increasing problem affecting human and animal health worldwide.
- Ticks are difficult to control and traditional control methods based primarily on chemical acaricides have been only partially successful.
- Tick vaccines appear to be a promising and sustainable approach for the control of tick infestations and pathogen transmission.
- Effective screening platforms and algorithms will be required for discovery of new tick protective antigens.
- Vaccinomics and reverse vaccinology approaches will be used to identify and fully characterize candidate protective antigens and validate vaccine formulations.
- Focusing on abundant proteins with relevant biological function will most likely identify new candidate tick protective antigens.

- New tick vaccines will likely combine tick antigens with different protective mechanisms alone or in combination with pathogen-derived antigens.
- Integrated tick control strategies combining tick vaccines with other control methods should be developed.
- The application of tick vaccines will ultimately result in reducing tick infestations while affecting pathogen infection and transmission to control TBD.

Declaration of Interest

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Figure legend

Figure 1. Future directions in tick vaccine development. New tick vaccines will likely combine tick antigens with different protective mechanisms alone or in combination with pathogen-derived antigens to ultimately result in the reduction of tick infestations while affecting pathogen infection and transmission to control TBD. A similar strategy could be applied to develop vaccines for the control of other vector-borne diseases.

Table 1. Recently evaluated candidate tick protective antigens for the control of *R. microplus* infestations in cattle.

N[a]	Recombinant tick antigen	Vaccination conditions	Vaccine efficacy[b]	References
3-4	Metalloprotease	Dose: 100 µg (doses 1 & 2), 200 µg (doses 3 & 4) Scheme: 4 doses Route: subcutaneous	60%	[113]
4	Ribosomal protein P0	Dose: 250 µg Scheme: 4 doses Route: intramuscular	96%	[114]
4	Ferritin 2	Dose: 100 µg Scheme: 3 doses Route: intramuscular	64%	[102]
5-6	Aquaporin	Dose: 100 µg Scheme: 3 doses Route: intramuscular	68-75%	[115]
4-6	Subolesin	Dose: 100 µg Scheme: 3 doses Route: intramuscular	37-44%	[116]
3	Q38[c] Silk Subolesin	Dose: 100 µg Scheme: 3 doses Route: intramuscular	75% 62% 60%	[52]
4	BM95-MSP1a Subolesin-MSP1a	Dose: 120 µg Scheme: 3 doses Route: intramuscular	64% 81%	[81]
5	BM86 BM86+Subolesin	Dose: 100 µg Scheme: 3 doses Route: subcutaneous	79% 97%	[53]

[a] Number of animals per group.

[b] Vaccine efficacy was calculated considering the effect on the reduction of tick infestations, oviposition and fertility (Box 2) but in certain experiments only the effect on some of these parameters was considered.

[c] Q38 is a tick Subolesin and mosquito Akirin chimera.

Box 1. Subolesin: A challenging candidate tick protective antigen

Tick Subolesin, the ortholog of insect and vertebrate Akirin, was discovered as a tick protective antigen in *Ixodes scapularis* by expression library immunization in a mouse model of tick infestations [50].

Subolesin/Akirin constitute a recently renamed group of evolutionarily conserved proteins in arthropods and vertebrates [42,43]. Only one *subolesin/akirin* gene has been identified in ticks and insects, which is evolutionarily and functionally related to mammalian *akirin2* [42,43].

Tick Subolesin functions as a transcription factor required for NF-kB-dependent and independent gene expression and regulation of the innate immune response to pathogen infection [42,43,54]. The broad function of Subolesin as a transcription factor explains the profound effect of gene knockdown on tick physiology and reproduction [42,43] and as a protective antigen against infestation with multiple tick species and infection with TBP [41-43,55]. Vaccination with Subolesin/Akirin has shown an effect on the reduction of infestations by soft and hard ticks (*I. scapularis, I. ricinus, R. microplus, R. annulatus, R. sanguineus, Amblyomma americanum, Dermacentor variabilis, Ornithodoros erraticus. O. moubata*), mosquitoes (*Aedes albopictus*), poultry red mites (*Dermanyssus gallinae*), sand flies (*Phlebotomus perniciosus*) and sea lice (*Caligus rogercresseyi*) [42,43]. Recently, vaccination with the membrane-exposed Subolesin-MSP1a chimeric antigen resulted in the reduction of tick infestations and pathogen infection under filed conditions [45]. Furthermore, the combination of Subolesin with BM86 was recently patented as a new and more effective vaccine formulation for the control of cattle tick infestations [53].

Subolesin knockdown by RNA interference (RNAi) produces sterile female and male ticks [37,42,43]. Therefore, a sterile acarine technique for autocidal control of tick populations by release of *subolesin*-knockdown ticks was proposed and proven effective for the control of *R. microplus* in combination with Subolesin-based vaccination in cattle [37].

Vaccination with tick Subolesin reduces tick infection with *Anaplasma marginale*, *Anaplasma phagocytophilum*, *Babesia bigemina* and *Borrelia burgdorferi* [55] and mosquito infection with malaria parasite, *Plasmodium berghei* [89]. However,

vaccination did not affect infection with tick-borne encephalitis virus [55]. Because of Subolesin role in tick innate immune response to pathogen infection [42,43], targeting Subolesin by vaccination or RNAi reduces tick immunity, thereby increasing pathogen infection levels. However, lower pathogen infection levels result from the effect on tissue structure and function and the expression of genes that are important for pathogen infection and multiplication. Both direct and indirect effects of targeting Subolesin result in lower tick infestations, feeding and fertility [55].

These results challenge the paradigm that intracellular proteins are not capable of inducing a protective response against ectoparasite infestations [42]. Host antibodies may interact with arthropod intracellular proteins through a process that has not been fully characterized but results suggests that antibodies may be specifically transported across the midgut barrier into the hemolymph, and then enter into cells to interact with these intracellular proteins [42,55]. Nevertheless, other possibilities should be considered to explain the effect of the vaccination with Subolesin including the effect of a host cell-mediated immune response and antibody responses that are cross-reactive with other proteins [55,94,106].

Box 2. Tick vaccine trials: General considerations and guidelines

Vaccination trials. To compare different vaccination trials it is important to standardize reporting guidelines. Reports should describe among other factors:

(a) Animal race, sex, age, health status considering major diseases and body condition, previous exposure to ticks and TBP, and previous or ongoing treatments with vaccines, pharmaceuticals and acaricides.

(b) Tick species properly verified by independent taxonomists and/or molecular tools, developmental stage(s), origin (laboratory colony or field collected), infection with TBP.

(c) Antigen preparation (sequence, expression system, purification protocol and purity, adjuvant, formulation) and controls applied to the final vaccine preparation.

(d) Vaccination (schedule, dose, route) and tick challenge (number of ticks, infestation model).

(e) Monitoring, collection and processing of collected ticks, including approaches for determining tick weight, oviposition and egg fertility with the corresponding statistical analyses.

Tick vaccine efficacy (E). The current standard test for E against cattle ticks was established by Canales et al. [117] and recently updated by Aguirre Ade et al. [118]. E is calculated considering the effect on the reduction of tick infestations, oviposition and fertility as 100 [l-(CRTxCR0xCRF)], where CRT, CRO and CRF are the reduction in the number of adult female ticks, oviposition and egg fertility as compared to the control group, respectively [117,118]. Despite the validity of this formula to calculate E, it may be important to make the calculation not only considering all parameters but also with the values showing significant differences between vaccinated and control animals (see for example [52]). In this way the results will reduce the impact of animal-to-animal variations on E.

Correlation between vaccination and tick phenotype. After the vaccination trial, a positive correlation between reduction in tick infestations, weight, ovisposition and/or fertility and antibody titers obtained in vaccinated animals will provide additional support to the result obtained with the vaccine (see for example [52]). Depending on the vaccine antigen and predicted protective mechanisms, additional analyses could be conducted

using different immunological parameters and molecular tools to determine gene expression and protein content in vaccinated hosts and/or ticks.

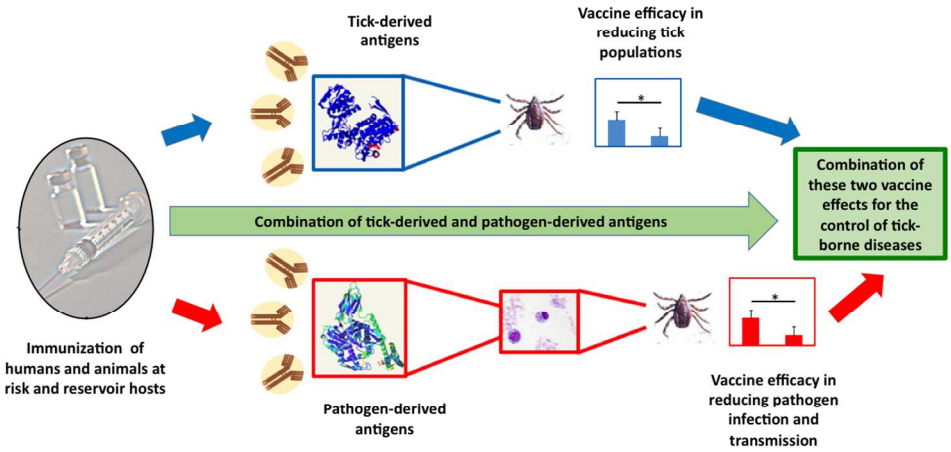

Figure 1. Future directions in tick vaccine development. New tick vaccines will likely combine tick antigens with different protective mechanisms alone or in combination with pathogen-derived antigens to ultimately result in the reduction of tick infestations while affecting pathogen infection and transmission to control TBD. A similar strategy could be applied to develop vaccines for the control of other vector-borne diseases.
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Summary

Ticks and tick-borne diseases are a growing problem affecting human and animal health worldwide. Traditional tick control methods, based primarily on chemical acaricides, have proven not to be a sustainable control method because of the selection of acaricide resistant ticks. Tick vaccines appear to be a promising and effective alternative approach for control of tick infestations and also prevention of pathogen transmission. The purpose of this review is to summarize previous tick vaccine development and performance, and to formulate critical issues and recommendations for future directions for development of improved and effective tick vaccines. Tick antigens such as BM86, Subolesin, Ferritin 2 and Aquaporin targeting relevant biological functions are likely to result in new effective vaccine formulations. Development of effective screening platforms and algorithms using omics approaches such as genomics, transcriptomics and proteomics focused on relevant biological processes will allow the discovery of new tick protective antigens. Future vaccines will most likely combine tick antigens with different protective mechanisms resulting in reduction of tick infestations and fertility, tick pathogen infection, tick vector capacity for pathogen transmission and tick response to pathogen infection alone or in combination with pathogen-derived antigens. The application of tick vaccines as part of integrated control strategies will ultimately result in the control of tick-borne diseases.

Keywords: tick, control, vaccine, acaricide, vaccinomics

Introduction

Ticks (Acari: Ixodida) are obligate hematophagous arthropod ectoparasites that are distributed worldwide and transmit pathogens causing diseases in humans and animals [1,2]. In the last decades, the continuous human exploitation of environmental resources and increase in human outdoor activities has allowed for the contact with ticks normally present in the field, resulting in increased transmission of tick-borne pathogens (TBP) [3,4]. In addition, tick populations are expanding due to changes in climate and human interventions that affect reservoir host movement and human contact with infected ticks [3-8]. As blood-sucking ectoparasites, ticks inflict great damage to humans, domestic and wild animals in many parts of the world. This damage consists of direct damage to hides, reduction in animal production, secondary infections, and diseases caused by TBP [9,10]. Furthermore, despite efforts to implement measures to control tick infestations, ticks and the pathogens they transmit continue to be a serious problem to human and animal health [10-16].

Ticks are difficult to control because they have few natural enemies and traditional control methods, based on chemical acaricides, have been only partially successful [10,17]. Therefore, new strategies are needed for the control of ticks and TBP and tick vaccines appear to be a promising and sustainable approach towards this objective [10-15,18-22]. Recent reviews have focused on the efficacy and limitations of BM86-based vaccines and the discovery and characterization of new candidate tick protective antigens for the development of vaccines for the control of tick infestations and pathogen infection and transmission [10-15,23-28]. The purpose of this review is to summarize previous tick vaccine development and performance literature, and to formulate critical issues and

recommendations for future directions for development of improved and effective tick vaccines for control of tick infestations and pathogen transmission.

Traditional tick control methods and associated problems

Traditional tick control methods are primarily based on the use of chemical acaricides, which have had limited efficacy in reducing tick infestations [10]. Additionally, the use of acaricides is often accompanied by serious drawbacks including the selection of acaricide-resistant ticks, environmental contamination and contamination of milk and meat products with residues [17]. The selection of ticks resistant to chemical acaricides is a growing problem particularly affecting cattle industry worldwide [29-31]. These facts together with the high cost of developing new acaricides result in the lack of sustainability for continuous acaricide use for tick control [25].

Alternative control methods based on the use of botanical acaricides and repellents, entomopathogenic fungi and the education of farmers about recommended tick control practices and available options for the management of drug resistance have been proposed to reduce the effect of acaricide use on the selection of acaricide-resistant ticks [24,29-32]. Furthermore, integrated control programs that include habitat management and the genetic selection of hosts with higher resistance to ticks have been also proposed to reduce the use of acaricides for the control of tick infestations [33,34]. Nevertheless, based on the experience obtained with the commercial use of tick vaccines based on the *Rhipicephalus microplus* BM86 recombinant antigen for the control of cattle tick infestations, tick vaccines have been proposed as an effective component of the integrated programs for the control of tick infestations and TBP while reducing the use of chemical acaricides [10-15,23-28].

Tick vaccines for the control of tick infestations

As proposed by Elvin and Kemp [35], candidate tick protective antigens should fulfill certain important criteria such as (1) host antibodies should be able to gain access to the target protein in sufficient quantities, (2) the formation of the antibody–antigen complex should disrupt the function of the target protein and/or induce physiological changes that affect vector biology, and (3) the antigen should share conserved epitopes among several tick species to protect against multiple vector infestations. These criteria are still valid for the selection of candidate tick protective antigens considering that the vaccine should also reduce tick vector capacity for TBD [36].

The protective mechanism characterized so far for tick vaccines is based on the development of antigen-specific antibodies in immunized hosts that interact and affect the function of the targeted antigen in ticks feeding on immunized hosts [21,37]. As shown for BM86-based vaccines, tick vaccines reduce the number, weight and reproductive capacity of engorging female ticks, therefore reducing tick infestations in subsequent generations [10].

Some tick species parasitize several vertebrate hosts and share habitat and hosts with other tick species [38]. These facts stress the need for developing vaccines effective in different hosts and against several tick species. However, a limited number of tick vaccines have been characterized so far in different hosts and cross-protective against multiple tick species [25,28,39-45].

Due to the importance of tick infestations for the cattle industry worldwide, most of the efforts toward the development of tick vaccines are directed for the control of tick species infecting cattle, particularly *R. microplus* [10-15,23-28] (Table 1). However, recent

reports have addressed the effect of tick vaccines on alternative hosts such as sheep [45,46], camels [39], deer [40] and dogs [44].

Recent developments are directed towards the use of *R. microplus* BM86 homologs in other tick species infecting cattle [47-49]. Additionally, new candidate tick protective antigens for the control of *R. microplus* infestations include Subolesin, Metalloprotease, Aquaporin, Ribosomal protein P0, Silk and Ferritins (Table 1). Furthermore, antigens protective against multiple tick species have been also characterized [11,27,28,42,43,45,50]. These results support the possibility of developing vaccines effective in different hosts and for the control of multiple tick species. However, new antigens and especially antigen combinations are required to develop more effective vaccines against tick infestations.

The efficacy of antigen combinations on tick infestations was first demonstrated by Allen and Humphreys [51] using tick protein extracts. However, until recently the combination of tick protective antigens did not result in higher efficacy for the control of tick infestations [52,53]. Merino et al. [52] used a chimeric antigen composed of protective epitopes from tick Subolesin and mosquito Akirin with a higher efficacy when compared to tick Subolesin for the control of *R. microplus* infestations in cattle (Table 1). In the patent application by Schetters and Jansen [53], the inventors claim that the combination of the well characterized tick protective antigens BM86 and Subolesin in a single formulation results in high vaccine efficacy against cattle tick infestations due to a synergy between both antigens (Table 1). The combination of tick protective antigens is a promising direction to increase the efficacy of tick vaccines against multiple tick species. Other directions to improve tick vaccine efficacy include the use of novel formulations

based on more effective adjuvant and antigen presentation and the possibility of developing vaccines with tick knock-down effects (i.e. substantial decrease of tick numbers on animals) as exhibited by chemical acaricides [10,23] and suggested by recent results with the BM86+Subolesin combined antigen vaccine [53] (Table 1).

Tick vaccines for the control of pathogen infection and transmission

The ultimate goal of tick vaccines is the control of both ticks and TBD. Vaccination with tick protective antigens such as BM86 among others that were directed towards control of tick infestations has also shown reduction in pathogen prevalence as a result of reducing tick populations [15,55]. Other antigens such as Subolesin show a direct effect on affecting pathogen infection and/or transmission while reducing tick infestations [28,41,43,54,55] (Box 1). Furthermore, recent results have revealed the molecular interactions between ticks and transmitted pathogens with the identification of candidate tick antigens to reduce pathogen infection and transmission while also affecting tick infestations [52,56-66]. These results support the identification of tick protective antigens with the dual function of reducing tick infestations and pathogen infection and transmission to ultimately protect against TBD. However, the combination of tick-derived and pathogen-derived antigens is probably the best way of achieving high vaccine efficacy for the control of vector-borne diseases.

Antigens from TBP such as *Borrelia burgdorferi* [67], flaviviruses [68], *Ehrlichia chaffeensis* [69], *Anaplasma phagocytophilum* [69-71] and *Anaplasma marginale* [72-78] among others have been proposed as candidate protective antigens for the control of pathogen infection and transmission. The possibility of combining these pathogen-derived antigens with tick protective antigens should result in new vaccines for the

control of vector-borne diseases (Fig. 1). In fact, recent results using vaccination with the combination of tick Subolesin with *A. marginale* major surface protein 1a (MSP1a) as a membrane-exposed chimeric antigen [79-81] showed an effect on reducing tick infestations and pathogen infection under field conditions [45].

Tick vaccines and the development of vaccines against other major ectoparasites

Diseases caused by arthropod-borne pathogens account for over 20% of all emerging infectious diseases recorded between 1940 and 2004 [82]. Among ectoparasite arthropod vectors, ticks are considered to be second worldwide to mosquitoes as vectors of human diseases and the most important vectors of diseases that affect the cattle industry worldwide [2,83]. However, other ectoparasites are also relevant for human and animal health and current research efforts are directed towards developing vaccines for their control [84]. In this context, research on tick vaccine development is more advanced than that reported for other major ectoparasites. Therefore, tick vaccine research may provide models for development of vaccines against other arthropod pests [20,83,85-88]. In this direction, recent efforts using tick Subolesin or the Akirin homolog in mosquitoes have shown how vaccination with these antigens protects against multiple ectoparasites and the infection with vector-borne pathogens [42,43,89] (Box 1). These results encourage the use of similar strategies for the identification of protective antigens across different ectoparasite species and suggest the possibility of developing vaccines for the control of multiple ectoparasite infestations.

Conclusions and future directions

The control of TBD is a priority in the current context of the global burden that infectious diseases represent and the one-health approach through integration of physicians, ecologists and veterinarians to monitor and control of zoonotic diseases. To address this priority, tick vaccines have become a major component of strategies for the control of tick infestations and TBP. Despite the fact that vaccines are among the best achievements in science, past strategies for vaccine development need to be revised to increase possibilities for developing effective vaccines for the control of tick infestations and TBD. The identification of new tick protective antigens is a critical step for developing effective vaccines for the control of tick infestations and TBP and despite resent advances in the study of tick biology and tick-host-pathogen interactions, this continues to be the major hurdle toward conducting vaccine animal trials [26]. In this direction, recent developments in last generation omics technologies including reverse vaccinology and vaccinomics will play a key role [26,52,59,61,73-75,90,91]. The integration of omics data sets must overcome important challenges such as development of algorithms that will allow for analysis and validation of data produced by the systems biology approach to tick research and development of effective screening platforms for the selection of candidate protective antigens [26]. Systems biology studies for to the selection of candidate protective antigens should focus on the characterization of physiological processes such as suppression of host immune responses, blood digestion, embryogenesis, innate immunity and tick-pathogen interactions that are critical for tick feeding, reproduction and vector capacity [26,56,66,92-105].

Recently, Guerrero et al. [23] proposed the selection of tick antigens from unique or low copy number genes encoding membrane-associated or membrane-bound antigens that are

expressed in gut, ovary, and salivary gland tissues or in the saliva. In this regard, they proposed to select molecules with low redundancy and combining properties of "exposed antigens" (antigens that are in contact with the host immune system during tick infestation and thus hosts immunized with these antigens are boosted by continuous tick exposure) and "concealed antigens" (antigens that are not exposed to the host immune system and thus ticks are unlikely to have evolved mechanisms to effectively counteract the effect of the host immune system as had occurred with exposed antigens but requiring repeated immunizations to maintain elevated antibody titers) [11,24,106-108]. However, although these concepts are valid for the selection of candidate tick protective antigens, recent results using tick Subolesin have challenged the a priori criteria for selecting membrane exposed antigens (Box 1).

Along with the problems associated with selection of candidate tick protective antigens to reduce the need for animal trials, vaccination experiments require standardization to optimize results and make them comparable across different controlled pen and field trials (Box 2). Additionally, the development of validated models for tick life cycle under relevant field conditions will provide a valuable tool for the modeling of vaccine efficacy and impact of tick control [108].

In addition to tick vaccines, future directions for the control of tick infestations and vector-borne pathogens could also include tick autocidal control [37], transgenic or paratransgenic ticks resistant to pathogen infection as recently shown in mosquitoes [109], vertebrate hosts genetically modified to confer resistance to tick infestation and/or pathogen infection as proposed using transgenic plants [110], glyco-conjugate vaccines based on tick protein glycosylation [111] and the manipulation of the tick microbiota to

reduce pathogen infection and transmission rates [112]. Finally, cocktails of tick-derived antigens alone or in combination with pathogen-derived antigens should result in more effective vaccines that could be used in combination with other control methods for the integrated control of tick infestations and TBD (Fig. 1).

Expert commentary

In the future, TBD are expected to increase, thus having greater impact on human and animal health worldwide. Ticks are difficult to control because they have few natural enemies and traditional control methods based on chemical acaricides have been only partially successful with some implicit drawbacks such as selection of ticks resistant to acaricides. New strategies are needed for the control of ticks and TBP and tick vaccines appear to be a promising and sustainable approach towards this objective. The use of BM86-based commercial vaccines for the control of cattle tick infestations demonstrated the possibilities for tick vaccines and encouraged research for the development of improved vaccines. Currently, various candidate tick protective antigens have been identified and tested in controlled pen trials. However, the identification of new tick protective antigens is the critical step for developing effective vaccines for the control of tick infestations and TBP.

The integration of last generation omics datasets is improving the possibilities for identifying candidate tick protective antigens. However, this approach faces important challenges such as the development of algorithms that allow the analysis and validation of data produced by systems biology and effective screening platforms for the selection of candidate protective antigens. Nevertheless, focusing on the study of physiological processes that are critical for tick feeding, reproduction and vector capacity using a

systems biology approach offers great possibilities for the identification of new tick protective antigens for the development of improved vaccines for the control of tick infestations and pathogen infection and transmission.

Five-year view

In the coming years, TBD are expected to continue expansion affecting human and animal health. As part of integrated control programs, tick vaccines are a promising and effective intervention for the control of tick infestations and the infection and transmission of TBP. Research on tick vaccines will continue to focus on cattle ticks and pathogens due to the impact of TBD on the cattle industry worldwide. However, due to the fact that some tick species parasitize several vertebrate hosts and share habitat and hosts with other tick species, the development of vaccines effective in different hosts and against several tick species is a growing area of research. Additionally, TBD affecting humans, pets and other domestic and wild animals also encourage research into tick vaccines. The application of omics technologies to tick vaccine research will result in effective screening platforms and algorithms for the discovery of new tick protective antigens. Vaccinomics and reverse vaccinology approaches will be used to identify and fully characterize candidate protective antigens and validate vaccine formulations. New candidate protective antigens will most likely be identified by focusing on abundant proteins with relevant biological function in tick feeding, reproduction, development, immune response, subversion of host immunity and pathogen infection and transmission. Consequently, tick protective antigens will be discovered with multiple impacts when used in a vaccine including reductions in (a) tick infestations and fertility, (b) tick pathogen infection, (c) tick vector capacity for pathogen transmission and (d) tick

response to pathogen infection. These new vaccines will likely combine tick antigens associated with different protective mechanisms alone or in combination with pathogen-derived antigens to have an effect on reducing tick infestations while affecting pathogen infection and transmission to ultimately result in the control of TBD. Finally, the most economical integrated tick control strategies will be those combining tick vaccines with other control methods while reducing acaricide applications to reduce risks for humans, animals and the environment. These integrated tick control strategies should overcome difficulties in the commercialization of tick vaccines due to its new approach for tick control.

Key issues

- Ticks are obligate hematophagous arthropod ectoparasites that vector pathogens causing diseases in humans and animals.
- TBD are an increasing problem affecting human and animal health worldwide.
- Ticks are difficult to control and traditional control methods based primarily on chemical acaricides have been only partially successful.
- Tick vaccines appear to be a promising and sustainable approach for the control of tick infestations and pathogen transmission.
- Effective screening platforms and algorithms will be required for discovery of new tick protective antigens.
- Vaccinomics and reverse vaccinology approaches will be used to identify and fully characterize candidate protective antigens and validate vaccine formulations.
- Focusing on abundant proteins with relevant biological function will most likely identify new candidate tick protective antigens.

- New tick vaccines will likely combine tick antigens with different protective mechanisms alone or in combination with pathogen-derived antigens.
- Integrated tick control strategies combining tick vaccines with other control methods should be developed.
- The application of tick vaccines will ultimately result in reducing tick infestations while affecting pathogen infection and transmission to control TBD.

Declaration of Interest

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Figure legend

Figure 1. Future directions in tick vaccine development. New tick vaccines will likely combine tick antigens with different protective mechanisms alone or in combination with pathogen-derived antigens to ultimately result in the reduction of tick infestations while affecting pathogen infection and transmission to control TBD. A similar strategy could be applied to develop vaccines for the control of other vector-borne diseases.

Table 1. Recently evaluated candidate tick protective antigens for the control of *R. microplus* infestations in cattle.

N[a]	Recombinant tick antigen	Vaccination conditions	Vaccine efficacy[b]	References
3-4	Metalloprotease	Dose: 100 µg (doses 1 & 2), 200 µg (doses 3 & 4) Scheme: 4 doses Route: subcutaneous	60%	[113]
4	Ribosomal protein P0	Dose: 250 µg Scheme: 4 doses Route: intramuscular	96%	[114]
4	Ferritin 2	Dose: 100 µg Scheme: 3 doses Route: intramuscular	64%	[102]
5-6	Aquaporin	Dose: 100 µg Scheme: 3 doses Route: intramuscular	68-75%	[115]
4-6	Subolesin	Dose: 100 µg Scheme: 3 doses Route: intramuscular	37-44%	[116]
3	Q38[c] Silk Subolesin	Dose: 100 µg Scheme: 3 doses Route: intramuscular	75% 62% 60%	[52]
4	BM95-MSP1a Subolesin-MSP1a	Dose: 120 µg Scheme: 3 doses Route: intramuscular	64% 81%	[81]
5	BM86 BM86+Subolesin	Dose: 100 µg Scheme: 3 doses Route: subcutaneous	79% 97%	[53]

[a] Number of animals per group.

[b] Vaccine efficacy was calculated considering the effect on the reduction of tick infestations, oviposition and fertility (Box 2) but in certain experiments only the effect on some of these parameters was considered.

[c] Q38 is a tick Subolesin and mosquito Akirin chimera.

Box 1. Subolesin: A challenging candidate tick protective antigen

Tick Subolesin, the ortholog of insect and vertebrate Akirin, was discovered as a tick protective antigen in *Ixodes scapularis* by expression library immunization in a mouse model of tick infestations [50].

Subolesin/Akirin constitute a recently renamed group of evolutionarily conserved proteins in arthropods and vertebrates [42,43]. Only one *subolesin/akirin* gene has been identified in ticks and insects, which is evolutionarily and functionally related to mammalian *akirin2* [42,43].

Tick Subolesin functions as a transcription factor required for NF-kB-dependent and independent gene expression and regulation of the innate immune response to pathogen infection [42,43,54]. The broad function of Subolesin as a transcription factor explains the profound effect of gene knockdown on tick physiology and reproduction [42,43] and as a protective antigen against infestation with multiple tick species and infection with TBP [41-43,55]. Vaccination with Subolesin/Akirin has shown an effect on the reduction of infestations by soft and hard ticks (*I. scapularis, I. ricinus, R. microplus, R. annulatus, R. sanguineus, Amblyomma americanum, Dermacentor variabilis, Ornithodoros erraticus. O. moubata*), mosquitoes (*Aedes albopictus*), poultry red mites (*Dermanyssus gallinae*), sand flies (*Phlebotomus perniciosus*) and sea lice (*Caligus rogercresseyi*) [42,43]. Recently, vaccination with the membrane-exposed Subolesin-MSP1a chimeric antigen resulted in the reduction of tick infestations and pathogen infection under filed conditions [45]. Furthermore, the combination of Subolesin with BM86 was recently patented as a new and more effective vaccine formulation for the control of cattle tick infestations [53].

Subolesin knockdown by RNA interference (RNAi) produces sterile female and male ticks [37,42,43]. Therefore, a sterile acarine technique for autocidal control of tick populations by release of *subolesin*-knockdown ticks was proposed and proven effective for the control of *R. microplus* in combination with Subolesin-based vaccination in cattle [37].

Vaccination with tick Subolesin reduces tick infection with *Anaplasma marginale*, *Anaplasma phagocytophilum*, *Babesia bigemina* and *Borrelia burgdorferi* [55] and mosquito infection with malaria parasite, *Plasmodium berghei* [89]. However,

vaccination did not affect infection with tick-borne encephalitis virus [55]. Because of Subolesin role in tick innate immune response to pathogen infection [42,43], targeting Subolesin by vaccination or RNAi reduces tick immunity, thereby increasing pathogen infection levels. However, lower pathogen infection levels result from the effect on tissue structure and function and the expression of genes that are important for pathogen infection and multiplication. Both direct and indirect effects of targeting Subolesin result in lower tick infestations, feeding and fertility [55].

These results challenge the paradigm that intracellular proteins are not capable of inducing a protective response against ectoparasite infestations [42]. Host antibodies may interact with arthropod intracellular proteins through a process that has not been fully characterized but results suggests that antibodies may be specifically transported across the midgut barrier into the hemolymph, and then enter into cells to interact with these intracellular proteins [42,55]. Nevertheless, other possibilities should be considered to explain the effect of the vaccination with Subolesin including the effect of a host cell-mediated immune response and antibody responses that are cross-reactive with other proteins [55,94,106].

Box 2. Tick vaccine trials: General considerations and guidelines

Vaccination trials. To compare different vaccination trials it is important to standardize reporting guidelines. Reports should describe among other factors:

(a) Animal race, sex, age, health status considering major diseases and body condition, previous exposure to ticks and TBP, and previous or ongoing treatments with vaccines, pharmaceuticals and acaricides.

(b) Tick species properly verified by independent taxonomists and/or molecular tools, developmental stage(s), origin (laboratory colony or field collected), infection with TBP.

(c) Antigen preparation (sequence, expression system, purification protocol and purity, adjuvant, formulation) and controls applied to the final vaccine preparation.

(d) Vaccination (schedule, dose, route) and tick challenge (number of ticks, infestation model).

(e) Monitoring, collection and processing of collected ticks, including approaches for determining tick weight, oviposition and egg fertility with the corresponding statistical analyses.

Tick vaccine efficacy (E). The current standard test for E against cattle ticks was established by Canales et al. [117] and recently updated by Aguirre Ade et al. [118]. E is calculated considering the effect on the reduction of tick infestations, oviposition and fertility as 100 [l-(CRTxCR0xCRF)], where CRT, CRO and CRF are the reduction in the number of adult female ticks, oviposition and egg fertility as compared to the control group, respectively [117,118]. Despite the validity of this formula to calculate E, it may be important to make the calculation not only considering all parameters but also with the values showing significant differences between vaccinated and control animals (see for example [52]). In this way the results will reduce the impact of animal-to-animal variations on E.

Correlation between vaccination and tick phenotype. After the vaccination trial, a positive correlation between reduction in tick infestations, weight, ovisposition and/or fertility and antibody titers obtained in vaccinated animals will provide additional support to the result obtained with the vaccine (see for example [52]). Depending on the vaccine antigen and predicted protective mechanisms, additional analyses could be conducted

using different immunological parameters and molecular tools to determine gene expression and protein content in vaccinated hosts and/or ticks.