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Vaccines against Cattle Ticks: Current Status

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Abstract

Rhipicephalus microplus, famous as the cattle tick, is a hematophagous ectoparasite distributed worldwide in tropical and subtropical regions. Its infestations represent a threat to the cattle industry since they generate a negative economic impact on cattle production and animal welfare. The method par excellence for tick control is based on applying chemical agents. However, their use has had limited efficacy, often accompanied by environmental contamination and the selection of tick's resistant to most chemical agents, rendering them ineffective. One of the alternative methods to combat *R. microplus* infestations is the development of vaccines. Two commercial vaccines are based on the Bm86 antigen, TickGARD®, and Gavac®. However, the efficacy is highly variable between different geographical regions. While it is true that other alternatives have been tested, the search for vaccine antigens that provide high protection against *R. microplus* remains a challenge in which genomic, transcriptomic, and proteomic studies are required. This review briefly summarizes the current situation regarding the discovery of candidate antigens for vaccines against ticks in cattle, as well as the methodologies employed for their search and development.

Keywords: Vaccine; *R. microplus*; Cattle Tick

Development

Ticks are arthropod ectoparasites that infest a wide range of animal hosts, including humans. In addition to the direct harm caused to their hosts, they are often responsible for pathogen transmission that impacts public and animal health [1]. To date, almost 900 species of ticks have been described [2]; among these species, the *Rhipicephalus microplus* is the most important tick for the cattle industry. In addition, it is responsible for significant global economic losses, especially in tropical and subtropical countries [3,4].

Globally, controlling tick populations through the recurrent use of chemical acaricides has been the primary strategy [5]. However, this method has significant disadvantages, such as weak efficacy in some regions due to the selection of acaricide-resistant ticks, environmental contamination, and drug residues in cattle products (milk

and meat) [3]. Vaccination is one of the alternatives for the control of *R. microplus*. It has several benefits compared to chemical acaricides, such as environmental safety, absence of human and animal health risks, no drug residues in meat and milk, ease of administration, and cost [4].

The feasibility of using recombinant protein vaccines as a long-term sustainable, cost-effective alternative control method against ticks has been demonstrated since the development and commercialization of the recombinant Bm86 antigen in the early 1900s: TickGARD® (Hoechst Animal Health) and Gavac® (Heber Biotec) derived from Australian and Cuban tick strains, respectively [6]. However, TickGARD® is no longer commercially available [7], and Gavac® continues to be marketed mainly in several Latin American, Asian, and Oceanic countries, showing variable efficacy against various geographic strains of *R. microplus* [8,9].

The search and discovery of new candidates for anti-tick vaccine development have two approaches: The first is the use of "exposed" antigens, proteins, or peptides secreted in the tick's saliva during the attachment and feeding of these ectoparasites on the host. Because these molecules are exposed to the host by every tick feeding, acquired immunity against exposed antigens is naturally established and stimulated by multiple ticks feeding. In contrast, "concealed" antigens are invisible to host immune mechanisms, and the natural feeding of ticks does not stimulate the acquired immunity against concealed antigens. Therefore, repeated immunizations are required to maintain adequate levels of protective antibodies [10,11]. These antigens are potential vaccine candidates if: a) they are associated with a play key role in tick biology, such as regulating physiology, modulation of host immune response, and transmission of pathogens by ticks; b) these induce long-lasting and effective immune responses in the host; and c) these induce cross-reactive immunity in the host against different tick species [1,12].

Identifying new candidate antigens for anti-tick vaccines is mainly based on reverse vaccinology (RV), a methodology that focuses on functional genomics, bioinformatics, and system biology [13]. This methodological approach begins with an analysis of the sequences present in the databases identified in genomes, transcriptomes, and proteomes, subsequently uses a bioinformatics program to recognize those sequences with immunogenic characteristics, finally tests the antigen in vaccine trials and evaluates its efficacy in the control of parasites [4]. This approach has obtained important results in developing new vaccines against *R. microplus*, such as identifying antigens (peptides) capable of triggering a specific immune response that affects tick feeding [14,15].

The exponential growth of genomic, proteomic, and transcriptomic data is the key to performing studies based on identifying genes encoding candidate antigens to develop an effective vaccine against *R. microplus*. In addition, genome sequences can provide important information for creating studies of comparative genomics studies and knowing the organization, location, and length of genes associated with parasite-host interaction, which could be important for combating tick infestation [14]. However, transcriptomes of ovaries [16], salivary glands, and larvae have been reported [17,18]. The complexity of the *R. microplus* genome has made its publication in the database difficult since it is approximately 7.1 Gpb in length, 70 % of which is repetitive DNA, which explains the difficulty of its assembly [19].

Developing an effective vaccine begins with identifying an antigen capable of introducing a long-lasting and effective immune response in the host [20]. First, it requires multiple in silico analyses of the nucleotide sequences and peptide sequence of the candidate antigen, e.g., B and T cell epitope prediction [21,22], search for families and functional domains [23], identification of transmembrane regions [24], hydrophobicity analysis [25], secondary and tertiary structure prediction [26], signal peptide prediction [27] and identification of potential glycosylation sites [28]. The discovery of new vaccine antigens should also be guided by in vivo expression data. Gene expression levels can be a good correlation point concerning protein expression and can therefore be used to infer whether a protein is potentially expressed [29].

Following the identification of an antigen candidate for the production of an anti-tick vaccine, it is necessary to produce enough antigen to obtain the scientific "proof of concept" in a cattle model to study the effects of vaccination on tick populations of interest, using biotechnology tools as recombinant proteins [1,4]. Many new antigens against ticks have been expressed as recombinant proteins and have conducted vaccination trials as candidate vaccines around the world and demonstrated to induce a certain level of protective immunity [1,4,11]. Therefore, based on the available literature, this review will focus on some antigens evaluated in cattle vaccination trials by various research groups worldwide (Tables 1 & 2).

Single-antigen				
Antigen	**Localization**	**Tick species**	**Efficacy (%)**	**References**
Boophilus microplus 95 (Bm95)	Gut	*R. microplus*	89	[8]
Boophilus yolk pro-cathepsin (BYC)	Egg	*R. microplus*	25.24	[30]
Boophilus annulatus 86 (Ba86)	Gut	*R. annulatus*	83	[31]
5′ -nucleotidase	Malpighian tubules	*R. microplus*	No efficacy	[32]
R. microplus Ferritin 2 (RmFER2)	Gut	*R. microplus*	64	[33]
Subolesin (SUB)	Intracellular	*R. microplus*	37.2-60	[34-36]
Subolesin (SUB)	Intracellular	*R. annulatus*	60	[34]
Glutathione S-transferases-*Haemaphysalis longicornis* (GSTHl)	Salivary glands	*Haemaphysalis longicornis*	57	[37]
Rhipicephalus microplus Larvae Trypsin Inhibitors (RmLTI)	Larvae	*R. microplus*	32	[38]
Hyalomma anatolicum 86 (rHaa86)	Gut	*H. anatolicum*	36.5	[39]
Boophilus microplus 91 (Bm91)	Salivary glands	*R. microplus*	6	[40]
Flagelliform Silk Protein (SILK)	Salivary gland	*R. microplus*	62	[35]
Tick receptor for outer surface protein A (TROSPA)	Gut	*R. microplus*	No efficacy	[35]
R. microplus Aquaporin 1 (RmAQP1)	Gut	*R. microplus*	68–75	[41]
Bm7462®	Gut	*R. microplus*	72.4	[42]
R. appendiculatus Subolesin (SUB)	Intracellular	*R. appendiculatus A. variegatum*	47-90 50-89	[43]
A. variegatum Subolesin (SUB)	Intracellular	*R. appendiculatus A. variegatum*	83-86 47-76	
R. microplus peptide Subolesin (pSub)	Intracellular	*R. microplus*	67	[44]

Table 1: Candidate recombinant antigens for the control of tick infestations in cattle.

Multi-antigenic				
Antigen	**Localization**	**Tick species**	**Efficacy (%)**	**References**
Rhipicephalus microplus 86 (Bm86) + *R. microplus* 91 (Bm91)	Gut Salivary glands	*R. microplus*	No reported Reduction in the number of engorged ticks and egg weight	[45]
Ubiquitin-Major surface protein 1a (UBQ-MSP1a)	Chimeric	*R. microplus*	No efficacy	[46]
Subolesin-Major surface protein-1a (SUB-MSP1a)	Chimeric	*R. microplus*	81	[46]
Elongation factor 1 alpha- Major surface protein 1a (EF1a-MSP1a)	Chimeric	*R. microplus*	38	[46]
Boophilus microplus 95- Major surface protein 1a (Bm95-MSP1a)	Chimeric	*R. microplus*	64	[46]

Haemaphysalis longicornis-Glutathione-S transferase (GST-Hl) + Rhipicephalus microplus-vitellin-degrading cysteine endopeptidase (VTDCE) + *R. microplus*-Boophilus Yolk Cathepsin (BYC)	Salivary glands Egg Egg	*R. microplus*	51.3–61.6	[47]
Q38	Chimeric	*R. microplus*	75	[35]
Subolesin (SUB) + B. microplus 86 (Bm86)	Intracellular Gut	*R. microplus*	97	[48]
Reprolysin Rhipicephalus microplus-Metalloprotease 4 (rBrRm-MP4)	Chimeric	*R. microplus*	60	[49]
R. microplus 39 (Rm39) + *R. microplus* 76 (Rm76) + *R. microplus* 180 (Rm180) + *R. microplus* 239 (Rm239)	Salivary gland	*R. microplus*	73.2	[50]
Rhipicephalus microplus Larvae Trypsin Inhibitors-Boophilus microplus Campo Grande-Heat-Labile enterotoxin B (RmLTI-BmCGLTB)	Chimeric	*R. microplus*	55.6	[51]
Subolesin + Heat inactivated Mycobacterium bovis (SUB+IV)	Intracellular Inactivated bacteria	*R. microplus*	65	[52]
R. microplus peptide Subolesin (pSub) + B. microplus 86 (Bm86)	Intracellular Gut	*R. microplus*	49	[48]

Table 2: Candidate recombinant multi-antigens for the control of tick infestations in cattle.

Since the commercialization of Bm86 almost 30 years ago, despite the efforts of multiple research groups, vaccines with other antigens and higher efficacy against *R. microplus* and other tick species are still unavailable [7]. However, the fact that Bm86 alone has been used as a reference antigen may be supported due to a positive correlation between anti-Bm86 IgG antibody titers, a decrease in tick infestations, and the prevalence of several tick-borne diseases [53,54].

Ideally, vaccine efficacy should be greater than 50% [51], and several recombinant antigens have demonstrated greater efficacy in inoculated cattle, which suggests they are potential candidates (Table 1). However, the commercial success of a new generation of vaccination against ticks cannot be guaranteed simply by having an antigen that performed well in vaccine pen trials, field trials are required to assess the vaccine's performance under real-life rearing conditions [47] (Figure 1) and few studies have been reported under field conditions in cattle [40,47,48,55]. Furthermore, meeting the requirements of safety and efficacy demonstrated under field conditions is only part of the equation for taking a new antigen as a product to market [10], and most of the protocols used in experiments to assess recombinant antigens against ticks employ confined cattle.

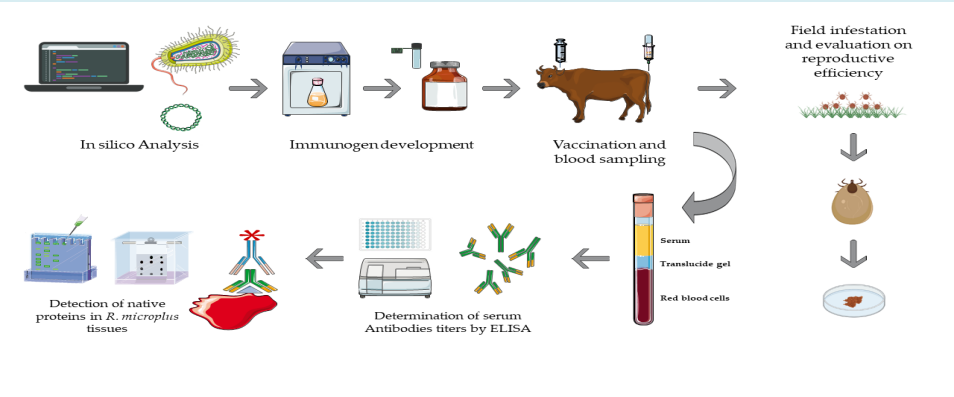

Figure 1: Schematic representation of developing and evaluating vaccines for controlling tick infestations in cattle model.

Current research should focus on optimizing or improving vaccine formulations of existing vaccine or vaccine candidates that are effective against *R. microplus*, and multi-species tick infestations to contribute to the reduction in the use of acaricides in regions where ticks are endemic, as well as the prevention of tick-borne diseases [56]. One of the strategies to improve efficacy in tick vaccines is multi-antigen vaccines, which consist of a combination of two or more antigens [57]. Initial evidence supporting this approach came from a synergistic effect between more than one tick antigen in different immunization trials in cattle [45,47]. These have been administered in the form of chimeric proteins or co-administered simultaneously. However, the results of these trials have been controversial, with some trials reporting disappointing results and others showing attractive results for further evaluation (Table 2).

In conclusion, anti-tick vaccine research has greatly advanced since the development and commercialization of the recombinant Bm86; both single-antigen and multi-antigen vaccines required more research studies to understand specific immunological responses to anti-tick vaccines and improve their efficacy, which depends on a wide array of factors such as host species and/or breed, host age, immunocompetence or prior exposure to ticks, the nature antigen, the type of adjuvant used, the formulation, the route of administration employed, and the immunization scheme [11,58]. For vaccine development and implementation of effective control strategies against ticks, the recent advances in omics technologies and bioinformatics analyses have allowed us to discover new antigens and test them as candidate vaccines much faster and less expensive than in the past [1]. However, further innovation in technologies and strategies will be necessary to turn new antigens into novel vaccines and provide the cattle industry with this safe and effective method for tick population control.

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