Ciência Rural, Santa Maria, v.54:03, e20230161, 2024 http://doi.org/10.1590/0103-8478cr20230161

Ciência Rural

ISSNe 1678-4596
PARASITOLOGY

Vaccination against *Rhipicephalus microplus*: an alternative to chemical control?

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ABSTRACT: *Rhipicephalus (Boophilus) microplus* is a hard tick endemic in livestock-growing regions and causes economic losses in the largest beef-producing countries, including Brazil, Mexico, Argentina, Australia and Uruguay. The use of chemical acaricides is still the main strategy to control *R. microplus* infestations. Nevertheless, immunological control of *R. microplus* with an anti-tick vaccine is a suitable alternative and has manifold advantages because it can avoid drug-resistance and the presence of acaricide residues in milk, beef and in the environment. Indeed, vaccines based on the Bm86 antigen have had relative commercial and technical success to control *R. microplus* in some regions. Although, the efficacy of such vaccines varies among tick populations and is insufficient to provide an acceptable level of protection. Therefore, the need to search for better antigens is impelling. This review focused on the restrictions imposed on the use of acaricides in Brazil and in the European Union, as well as on the impacts of Bm86-based vaccines on *R. microplus* control. The efficacy of experimental anti-tick vaccines (based on subolesin, glutathione S-transferase, ferritin 2; voltage-dependent anion channel; aquaporin, 60 S acidic ribosomal protein, metalloprotease and trypsin) that can elicit an immune response against the physiological functions of various ticks is discussed.
Key words: *Rhipicephalus microplus*, vaccine, acaricides, Brazil, food contamination.

Potenciais vacinas contra *Rhipicephalus microplus*: uma alternativa ao controle químico?

RESUMO: O *Rhipicephalus (Boophilus) microplus* é um carrapato duro que é endêmico de regiões de pecuária e causa perdas econômicas nos maiores países produtores de carne bovina, incluindo Brasil, México, Argentina, Austrália e Uruguai. O uso de acaricidas ainda é a principal estratégia para controlar infestações por *R. microplus*. No entanto, o controle imunológico do *R. microplus* com uma vacina contra carrapatos é uma alternativa adequada e possui diversas vantagens, por evitar a seleção de populações de carrapato resistentes a drogas, evitar a presença de resíduos de acaricidas no leite, na carne e no ambiente. As vacinas baseadas no antígeno Bm86 tiveram relativo sucesso comercial e técnico no controle do *R. microplus* em diversas regiões. No entanto, a eficácia dessas vacinas varia entre as populações de carrapatos e é insuficiente para fornecer um nível aceitável de proteção. Portanto, há uma necessidade de procurar novos antígenos. Esta revisão foca nas restrições impostas ao uso de acaricidas no Brasil e na União Europeia, bem como nos impactos das vacinas baseadas em Bm86 no controle do *R. microplus*. Também é discutida a eficácia de vacinas anti-carrapatos experimentais (baseadas em subolesina, glutationa S-transferase, ferritina 2; canal aniônico dependente de voltagem; aquaporina, proteína ribossômica ácida 60S, metaloprotease, tripsina) que podem elicitar uma resposta imune contra as funções fisiológicas de vários carrapatos.
Palavras-chave: *Rhipicephalus microplus*, vacina, acaricida, Brasil, contaminação de alimentos.

INTRODUCTION

Ticks are responsible for considerable morbidity and mortality (unless controlled), and economic losses, both directly through blood sucking and indirectly as vector of pathogens (JONGEJAN & UILENBERG, 2004). Ticks constitute a threat to public and animal health, with major effects on livestock (DE LA FUENTE et al., 2016). It is estimated that approximately 80% of the world's cattle population is exposed to tick infestation (SNELSON, 1975, cited by MCCOSKER, 1979). The cattle tick *Rhipicephalus (Boophilus) microplus* is responsible for economic losses in the livestock industry, due to decreased production of milk and meat, as well as impairing leather quality. These effects are not only caused by the tick infestation itself but also by the pathogens transmitted to bovines, mainly protozoa (e.g., *Babesia bovis* and *Babesia bigemina*) and bacteria (*Anaplasma marginale*). These parasites are

Received 03.16.23 Approved 06.14.23 Returned by the author 07.14.23
CR-2023-0161.R2
Editor: Rudi Weiblen

responsible for high bovine mortality (DU PLESSIS et al., 1994; MOLOSSI et al., 2021). In addition, treatment with chemical acaricides is costly and increasingly less effective. In Brazil, it is estimated that the annual costs associated with *R. microplus* infestation are around US$ 3.2 billion (GRISI et al., 2014). In Brangus cattle breed, the cost of *R. microplus* infestation in Brazil has been estimated to be US$ 34.61 per animal in the backgrounding phase (from weaning to feedlot placement) and US$ 7.97 per animal in the finishing phase (when cattle are fed until they reach market weight). Even in Nellore cattle, a pure *Bos indicus* breed that is relatively resistant to *R. microplus*, infestation costs in Brazil have been estimated at US$ 4.66 and US$ 1.18 per animal in the backgrounding and finishing phases, respectively (CALVANO et al., 2019). Significant annual losses due to *R. microplus* infestation have been reported for other countries, such as US$ 573.61 million in Mexico (RODRÍGUEZ-VIVAS et al., 2017) and US$ 128 - 146 million in Australia (MEAT & LIVESTOCK AUSTRALIA, 2005).

Various strategies to control this tick have been used, such as acaricides (the main control method), pasture management, vaccine, nutritional management, and selection of resistant hosts (RODRÍGUEZ-VIVAS et al., 2018). The major classes of acaricides currently in use are amidines, organophosphates, organochlorines, synthetic pyrethroids, insect growth regulators, phenylpyrazoles, and macrocyclic lactones (RODRÍGUEZ-VIVAS et al., 2018). However, all these acaricides have major drawbacks due to the increasing level of acaricide resistance among tick populations (CUTULLE et al., 2013; KLAFKE et al., 2017; LOVIS et al., 2013; RECK et al., 2014).

Immunization to control tick populations is an interesting alternative because it avoids or reduces the use of acaricides. This may lead to decreased food and environmental contamination with pesticide residues, and to a reduced selection-pressure for acaricide-resistance. (GUERRERO et al., 2012a). Among the advantages of using immunological control are the absence of a resting period after the use of chemical acaricides, safety in the application of vaccines, and avoiding even the possibility of the presence of acaricide residues in animal products intended for human consumption (CANALES et al., 2010; DE LA FUENTE, 2016; DE LA FUENTE & CONTERAS, 2015).

Acaricide control of R. microplus and food contamination concerns

According to the United Nations Food and Agriculture Organization (FAO, 2012), insecticide resistance is defined as “a heritable change in the sensitivity of a pest population that is reflected in the repeated failure (more than one instance) of a product to achieve the expected level of control when used according to the label recommendation for that pest species”. The difficulties in cattle tick control due to drug resistance come from the increasing number of tick populations that are unaffected by acaricides in subsequent generations (FAO, 2012). Since 1936, it has been known that there are acaricide-resistant *R. microplus* populations of (GEORGE et al., 2008). Nowadays, there are cattle tick acaricide-resistant populations in Africa, Asia, Central America, South America, and Oceania (reviewed by DZEMO et al., 2022). In addition, the need to discard milk and not to slaughter the animal during the resting period after the application of acaricides increases the costs of tick control (DALLEGRAVE et al., 2016; DALLEGRAVE et al., 2018; DE MENEGHI et al., 2016). These practices are essential, given acaricides and/or its metabolites accumulate in animal fat and could be hazardous to human health.

Due to their highly lipophilic nature, residues of ivermectin (a macrocyclic lactone) persist in milk and dairy products, the use of ivermectin in lactating animals must; therefore, be avoided (ESCRIBANO et al., 2012). Although, ivermectin residues are less persistent during cheese production processes, it has been reported that 65% of the drug remains in the raw milk used to produce cheese (CERKVENIK et al., 2004). In Brazil, a study conducted under the auspices of the Official Program for Analysis of Residues of Veterinary Drugs in Foods of Animal Origin detected ivermectin residues in samples of dairy products - in 42% of ultra-high temperature milk samples, in 11% of pasteurized milk samples, and in 59% of powdered milk samples; - although, the maximum residue limit (MRL) was not exceeded in any of the samples it is motive of concern (NOVAES et al., 2017). Despite the fact that the use of ivermectin during lactation is not recommended, it is reported to be common in some regions of Brazil (NOVAES et al., 2017).

Cypermethrin, a synthetic pyrethroid, is another acaricide that is widely used for control of *R. microplus* in livestock in Brazil (KLAFKE et al., 2017; PICININ et al., 2017). Pyrethroids are also fat-soluble pesticides and contamination of meat and milk by these chemicals has also been reported (DALLEGRAVE et al., 2016). The resting period must be at least 14 days in lactating cows (BASTOS et al., 2011; HERNANDES et al., 2009). However, that guideline is not always followed in Brazil, where 15%

of milk producers are reportedly unaware of a resting period for any acaricide (NASCIMENTO et al., 2021). Residue levels of pyrethroid and other acaricides have been detected in up to 15.1% of milk samples, with some samples (until 6.8%) exceeding the legal limit (PICININ et al., 2016; PICININ et al., 2017; CISCATO et al., 2002; OLIVEIRA et al., 2023).

Fluazuron is a benzoylphenyl urea derivative that impairs chitin synthesis in ticks, affecting their ecdysis and oviposition (JUNQUERA et al., 2019). Recently, it was reported that nursing calves had higher plasma levels than did their lactating dams that had been treated with fluazuron, indicating that the compound is passed through milk and accumulates in calves due to the continuous intake (EMA, 2018; SUAREZ et al., 2021).

The organophosphate diazinon is used to control cattle ticks in various regions. Organophosphate residues have been detected in raw milk (FAGNANI et al., 2011; JARDIM et al., 2018; NERO et al., 2007; SILVA et al., 2014). Organophosphate residues detected in raw milk are similar to those detected in animal feed (FAGNANI et al., 2011; SILVA et al., 2014). It has been suggested that the levels of organophosphate contamination of milk can be explained by the animal feed, in which organophosphates are present, since they are widely used in crops used to produce animal feed (FAGNANI et al., 2011).

In October 2017, European Union regulations prohibited the use of fipronil in farm animals to control tick infestations (EU, 2017). However, in Brazil, the use of fipronil is allowed for crop protection against some pests (ANVISA, 2020) and for tick control (KLAFKE et al., 2017; NASCIMENTO et al., 2021; RECK et al. 2014). This is an issue of major concern because European Union (a very important market for Brazilian beef) could ban beef and milk from countries when the use of this drug is allowed. Pesticide residues are transferred from feed to cow milk (FAOUDER et al., 2007), and it is possible that milk can be contaminated indirectly from feed or directly from the acaricide used in the herd. In fact, contamination of raw milk with fipronil has been reported in Brazil (OLIVEIRA, 2016).

Legislation regulating the use of acaricides in Brazil and in the European Union

In a society increasingly concerned with human and animal health issues, the contamination of animal products with acaricides presents a serious obstacle for cattle farming. Recently, the Brazilian Health Regulatory Agency (ANVISA) issued Resolution No. 328 and Normative Instruction No. 51 (ANVISA, 2019a; ANVISA, 2019b). Those documents define the maximum residue limits (MRLs) in foods of livestock origin and the acceptable daily intakes (ADIs) of acaricides (Table 1). Legislation in Brazil and the European Union is similar regarding the MRLs and ADIs for amitraz, fluazuron, flumethrin, and ivermectin (Table 1). However, the legislation is more restrictive in the European Union than in Brazil. The MRLs and ADIs for cypermethrin and ivermectin are lower in the European Union than in Brazil. Besides fipronil, the European Union has also banned the use of fluazuron and ivermectin in dairy cattle (Table 1), which effectively blocks the exportation of dairy products from Brazil to the European Union.

Immunological control of R. microplus with Bm86- and Bm95-based vaccines

In a breakthrough research, ALLEN & HUMPHREYS (1979) showed that immunization of hosts using tick proteins induces an immune response which confers high levels of protection against tick infestation (ALMAZÁN, 2022). This historic achievement is the basis of all subsequent landscape in anti-tick vaccine development. Indeed, first commercial vaccine against any ectoparasite was an anti-tick vaccine based on Bm86 protein (Bm86), an *R. microplus* gut glycoprotein. This vaccine was pivotal because it established the concept of a concealed antigen. A concealed antigen is defined as an antigen that is not encountered by the host immune system under natural infestation and; consequently, the host cannot mount an immune response but when a parasite-derived molecule is injected, the host produces antibodies against it. Actually, functional antibodies present in the blood meal reach the midgut and also other tissues of the parasite (VAZ et al, 1996). Although, this type of vaccine does not avoid host infestation, because the effect comes after the blood meal, it does reduce the size of the next tick generation and the parasite propagation is inhibited along the time (WILLADSEN & KEMP, 1988).

Indeed, further *in silico* analysis suggested that Bm86 has characteristics of both exposed and concealed antigens, since its localization and presence of a signal peptide do not fit perfectly as a truly concealed antigen (TRIMNELL et al., 2002; NUTTALL et al., 2006; TABOR, 2018). In the case of rBm86-based vaccines, bovine antibodies are ingested by ticks in the blood meal, encounter Bm86 on the apical surface of *R. microplus* gut cells, and disturbs gut function, thus impairing the parasite fitness (RAND et al., 1989; WILLADSEN & KEMP, 1988).

Table 1 - Acaricides approved for use in Brazil and in the European Union, together with their acceptable daily intakes, marker residues in tissues, and maximum residue limits.

	---------------ADI-------------				-----------MRL-----------	
Acaricide	Brazil (µg/kg BW)	EU (µg/kg BW)	Marker residue	Tissue	Brazil[a,b] (µg/kg BW)	EU (µg/kg BW)
Amitraz (amidine)	0–0[a]	0–3[c]	Sum of amitraz and all of its metabolites containing the 2,4-DMA fraction	Muscle	NN	NN[c]
				Liver	200	200[c]
				Kidney	200	200[c]
				Fat	200	200[c]
				Milk	10	10[c]
Cypermethrin (synthetic pyrethroid)	0–20[a]	0–15[d]	Total cypermethrin residues	Muscle	50	20[d]
				Liver	50	20[d]
				Kidney	50	20[d]
				Fat	1000	200[d]
				Milk	100	20[d]
Flumethrin (synthetic pyrethroid)	0–1.8[a]	0–1.8[e]	Flumethrin (sum of trans-Z isomers)	Muscle	20	10[e]
				Liver	20	20[e]
				Kidney	10	10[e]
				Fat	150	150[e]
				Milk	30	30[e]
Diazinon (organophosphate)	0–2[a]	0–0.2[f]	Diazinon	Muscle	20	20[f]
				Liver	20	30[f]
				Kidney	20	30[f]
				Fat	700	70[f]
				Milk	20	20[f]
Fluazuron (insect growth regulator)	0–40[a]	0–43[g]	Fluazuron	Muscle	200	200[g]
				Liver	500	500[g]
				Kidney	500	500[g]
				Fat	7000	7000[g]
				Milk	200	N/A[g,*]
Ivermectin (macrocyclic lactone)	0–10[a]	0–10[h]	22,23-Dihydro-avermectin B1a	Muscle	30	30[h]
				Liver	800	100[h]
				Kidney	100	30[h]
				Fat	400	100[h]
				Milk	10	N/A[h,*]
Fipronil (phenylpyrazole)	0.2[j]	N/A[i,†]	Fipronil (sum of fipronil and sulfone metabolites)	Muscle	ND	5[i,‡]
				Liver	ND	
				Kidney	ND	
				Fat	ND	
				Milk	ND	

ADI = acceptable daily intake; MRL = maximum residue limit; EU = European Union; BW = body weight; NN = not necessary; DMA = dimethylamine; N/A = not applicable; ND = not determined.
Sources: [a]Anvisa (2019[a]); [b]Anvisa (2019b); [c]EFSA (2016); [f]EMA (1995); [e]EMA (1998); [b]EMA (2004); [h]EMA (2014); [g]EMA (2018), [i]EU (2017); [j]Anvisa (2002).
[*]Not approved for use in animals from which milk is produced for human consumption.
[†]Not approved for use in food-producing animals.
[‡]Limit of analytical determination.

The antithesis of the concept of concealed antigens is that of exposed antigens. Exposed antigens are secreted in tick saliva during attachment and feeding. Vaccines based on exposed antigens induce an immune response which can be enhanced by subsequent natural infestations (NUTTALL et al., 2006). The host immune response against tick-derived exposed antigens is subject to host immune-evasion strategies develop along the co-evolution of the parasite and the host (reviewed by ALI et al., 2022; WILLADSEN & KEMP, 1988). So, antibodies induced by salivary proteins could be less effective in impairing tick physiology. In addition, variations in the composition and expression of proteins during tick feeding have been observed and are thought to be a strategy to evade host immunity (KIM et al., 2020; TIRLONI et al., 2014; TIRLONI et al., 2015). However, various anti-tick vaccination experiments have shown that exposed and concealed antigens can induce some degree of protective immune response against ticks (PEREIRA et al., 2022; SEIXAS et al., 2012; TRENTELMAN et al., 2019). The first rBm86-based vaccine became available in 1994 and was marketed in Australia as TickGARD (WILLADSEN et al., 1995). That vaccine was taken off the market for several reasons, including its low efficacy in some *R. microplus* populations and the fact that it did not exert the knockdown effects exhibited by chemical acaricides (TRENTELMAN et al., 2019). Additionally, 3–4 vaccinations per year are necessary and this is impractical and even incompatible within extensive beef cattle production farms (TABOR, 2021). Another rBm86-based vaccine, marketed under the name Gavac, has been shown to have a positive economic impact on the cattle industry in several countries (CANALES et al., 1997; DE LA FUENTE et al., 1998). More recently, a rBm86-based vaccine Bovimune Ixovac was launched in Mexico (LAPISA, 2018). The effectiveness of rBm86-based vaccines is highly variable among tick populations, it ranges from 51% to 91% (DE LA FUENTE et al., 2000; DE LA FUENTE et al., 1999; HUE et al., 2017; PATARROYO et al., 2002; RODRIGUEZ et al., 1995; WILLADSEN & KEMP, 1988).

In some *R. microplus* populations, the low efficacy of rBm86-based vaccines was overcome by using the Bm95 protein as the vaccinal antigen. In a study conducted in Argentina, Bm95 was identified in a population of *R. microplus* and was shown to have 91.4% amino acid similarity with Bm86 (GARCIA-GARCIA et al., 1999). Recombinant Bm95 was found to protect cattle from tick infestation in Argentina and Cuba, demonstrating its efficacy against some tick populations refractory to immunization with rBm86 (GARCIA-GARCIA et al., 1999). An inverse correlation was observed between vaccine efficacy and variation in the Bm86/Bm95 locus, suggesting that an amino acid sequence variation greater than 2.8% is enough to diminish the efficacy (GARCIA-GARCIA et al., 1999).

Integrated control of R. microplus with Gavac and acaricides

Integrated pest control management is defined as using a combination of common-sense practices to take advantage of environmental factors and the population dynamics of a pest species in order to control that species. Information about the life cycles of ticks and their interaction with the environment, as well as climatologic data and vector control methods (including the use of pesticides), are critical to designing effective strategies to reduce tick infestations (RODRÍGUEZ-VIVAS et al., 2018).

Effectively, vaccines are an additional tool in the tick-control arsenal. In a study conducted in Cuba (RODRIGUEZ-VALLE et al., 2004), this approach was taken with the Gavac vaccine, and the use of such vaccine resulted in an increase in the interval between acaricide treatments in *Bos taurus* and an 87% decrease in the total number of acaricide treatments required. Similar results were obtained in *B. indicus*, in which there was also an increase in the interval between acaricide treatments, together with a 68% decrease in the total number of acaricide treatments required (RODRIGUEZ-VALLE et al., 2004). In another study, conducted in Mexico, Gavac vaccine was used in combination with an amidine for the control of *R. microplus*, resulting in a lower number of acaricide treatments in cattle that had received the anti-tick vaccine (REDONDO et al., 1999). Over a 9-year period, cattle on a ranch in Mexico were immunized with Gavac, and the annual number of acaricide treatments decreased from 24 in 1997 to 7–8 in 2006, the number of ticks per animal decreased from 100 to < 20 over the same period (DE LA FUENTE et al., 2007).

In Venezuela, 1.9 million cattle on nearly 40,000 ranches were vaccinated with Gavac via the national integrated program for bovine tick control, as reported by SUAREZ et al. (2016). The authors found that, by the end of the second year, the use of chemical acaricides had been reduced by 83.7%, corresponding to a reduction of more than 260 tons, and that there had been an 81.5% reduction in the economic costs (i.e., savings in acaricide purchases). These data indicate that the success of an anti-tick vaccine relies on its integration into a tick control management strategy that includes acaricide treatments and other measures. Undoubtedly, vaccines constitute a useful tool to prolong the useful lifespan of a given acaricide, given that they delay the selection of acaricide-resistant tick populations. The use of vaccines can decrease the amount of chemicals applied, thus reducing the risk of food and environmental contamination.

Although, Bm86-based vaccines were developed some time ago, they are still in use in less extensively grazed herds in some regions such as Cuba (WILLADSEN, 2006; VARGAS-HERNÁNDEZ et al., 2018). In addition, because the global market for acaricides and tick repellents is huge and more profitable than anti-tick vaccines, there has been limited investment and interest to developed novel ectoparasite vaccines, even those that have proven effective (DE LA FUENTE & ESTRADA-PENA, 2019). Antiparasitic treatment is the usual method to control ticks and other parasites in cattle, so alternative control strategies, including vaccination, remain largely unknown and many livestock farmers do not have a good understanding of the efficacy of vaccines to control parasites. Furthermore, vaccines do not control ticks as effectively as acaricides, and it is essential to educate livestock producers on the fundamentals of correct use of antiparasitic vaccines. Despite the technical and marketing problems related to the Bm86-based anti-tick vaccine against *R. microplus*, vaccines still remain as a promising alternative because the widespread use of acaricides leads to the selection of acaricide-resistant tick populations and because consumer concerns impel legislation to be more and more restrictive in relation to the presence chemical residues in food. In fact, anti-tick vaccines could make possible not just to reduce hazardous residues in meat and dairy products but also to produce those products by processes that eliminate even the risk of contamination by acaricides and their residues. Therefore, it is imperative to identify and validate better antigens. In addition to looking for antigens with greater efficacy against different populations of *R. microplus*, it is also useful to identify those able to cross react and be able to induce protection against more than one tick species (ALMAZAN et al., 2018; GUERRERO et al., 2012b).

Potential antigens for the control of R. microplus

More than two dozen tick proteins have been tested as antigens for anti-tick vaccines. However, only a few have shown potential as promising viable candidates (Table 2). Several comprehensive reviews focusing on different aspects of tick vaccine development have recently been published (RODRIGUEZ-VIVAS et al., 2018, LEAL et al., 2021; PEREIRA et al., 2022; ABBAS et al., 2023). Here, the intent is to present a brief overview of the processes of antigen discovery and characterization and development of anti-tick vaccines considering the discovery and characterization of new antigens which can elicit an immune response that impairs tick physiological functions.

In general, such antigens should encounter immunoglobulins entering the hemolymph (or gut) and are associated with some crucial function for the tick survival or fitness (NUTTALL et al., 2006). Their efficacy must be evaluated in anti-tick vaccine trials. In such trials, it is necessary to calculate the overall efficacy, that is, considering the overall effect on the size of the next tick generation. It is important to standardize the results and compare the efficacy showed by different research groups with different antigens. In general, the efficacy is calculated as a percentage considering the difference between an immunized group and an unvaccinated control group in terms of the number of fully engorged ticks, their egg laying capacity, and egg fertility, in other words, an indication of the overall impact in the next tick generation. (CUNHA et al., 2013).

Subolesin (SUB) is an intracellular regulatory protein involved in signal transduction that affects multiple cellular processes in ticks, such as the innate immune response, feeding, reproduction, and development (NARANJO et al., 2013). Knockdown of *SUB* by RNA interference (RNAi) has been shown to lead to a more than 90% reduction in oviposition and progeny in five tick species (DE LA FUENTE et al., 2006), evidence of physiological importance of this protein and its usefulness for the development of an anti-tick vaccine. Also, cattle immunized with the recombinant protein and challenged with *R. microplus* showed a 47% decrease in the number of engorged females, and the overall efficacy of the vaccine was 60% (ALMAZAN et al., 2010; MERINO et al., 2013). In another study, quantitative PCR was used in order to measure the presence of *B. bigemina* and *A. marginale* DNA in ticks feeding on SUB-vaccinated and control cattle (MERINO et al., 2013). The authors found that SUB was capable of controlling tick infestation and tick-borne pathogens in cattle. In crossbred *B. taurus–B. indicus* cattle, the efficacy of the SUB vaccine was reported to be 44 % and 37% after the first and second challenges, respectively (SHAKYA et al., 2014).

Ferritin 2 (FER2) has been confirmed as the primary transporter of nonheme iron between the tick gut and the peripheral tissues in *Ixodes ricinus*, a vector of tick-borne encephalitis and Lyme borreliosis. In RNAi experiments, HAJDUSEK et al. (2009) demonstrated the relevance of FER2 in iron metabolism, showing that it is involved tick development and reproduction. In that study, the authors immunized cattle with the recombinant *R. microplus* FER2 homologue (RmFER2), expressed in *E. coli*. Results indicated that RmFER2 is a protective antigen with an efficacy of 64% (Table 2). Artificial feeding with cattle blood containing antibodies against recombinant *I. persulcatus* FER2 has been

Table 2 - Efficacy of antigens used in immunizing cattle against *Rhipicephalus microplus*.

Antigen	Efficacy (%)	Formula to calculate vaccine efficacy(%)	Reference
Subolesin	60	=100 [1 - (CRT * CR0 * CRF)]	(ALMAZAN et al., 2010)
	44.0 and 37.2*	=100 [1 - (CRT * CR0 * CRF)]	(SHAKYA et al., 2014)
	54	= 100 [1 - (CRT * CR0)]	(KUMAR et al., 2017)
Glutathione S-transferase	57	=100 [1 - (NFE * WE * WL)]	(PARIZI et al., 2011)
Voltage-dependent anion channel	82	=100 [1 - (CRT * CR0 * CRF)]	(ORTEGA-SANCHEZ et al., 2020)
Metalloprotease	60	=100 [1 - (NFE * WE * WL)]	(ALI et al., 2015a)
Trypsin inhibitors	72.8	=100 [1 - (NET * EW * EF)]	(ANDREOTTI et al., 2002)
Low trypsin inhibitor	32	=100 [1 - (CRT * CR0 * CRF)]	(ANDREOTTI et al., 2012)
Aquaporin 1	68 and 75†	=100 [1- (NET * EWPF * H)].	(GUERRERO et al., 2014)
60 S acidic ribosomal protein (P0)	96	=100 [1- (RA * PA *VA * OA * FE)]	(RODRIGUEZ-MALLON et al., 2015)
Ferritin 2	64	=100[1 – (CRT * CR0 * CRF)]	(HAJDUSEK et al., 2010)
Flagelliform silk protein	62	= 100 [1 - (CRT * CR0)]	(MERINO et al., 2013)

CRT, NFE, NET = reduction in the number of adult female ticks as compared to the control group; CRO, WE, EW or EWPF = reduction in oviposition as compared to the control group; CRF, WL, EF or H = reduction egg fertility as as compared to the control group. RA = reduction in the number of adult female ticks as compared to the control group; PA = reduction in the number of adult female ticks as compared to the control group; VA= reduction in the number of female viability (able to lay eggs) as compared to the control group; OA = reduction in oviposition as compared to the control group; FE = reduction egg fertility as as compared to the control group.
*In the first and second challenges, respectively.
†In two independent cattle pen trials.

shown to decrease the weight and engorgement of *R. microplus* females (XAVIER et al., 2021).

Glutathione S-transferase (GST) is widely distributed among organisms and plays a role in the detoxification of endogenous substances and xenobiotics (PAVLIDI et al., 2018). In arthropods, GST plays a pivotal role in one of the mechanisms of pesticide detoxification. It has been demonstrated that GST metabolizes insecticides by facilitating a reductive dehydrochlorination or by conjugating them with reduced glutathione, as well as contributing to the removal of toxic free radical oxygen species produced through the action of pesticides (ENAYATI et al., 2005). Reports on GST over expression in pesticide-resistant strains have shown that elevated pesticide metabolism is not the only effect of such over expression. Increased GST expression can attenuate oxidative stress or sequester the pesticide rather than metabolizing it (FEYEREISEN et al., 2015).

Several acaricides are substrates for *R. microplus* GST, suggesting that this enzyme plays a role in pesticide detoxification (DA SILVA VAZ et al., 2004a). In addition, serum from rabbits immunized with recombinant GST from *Haemaphysalis longicornis* (rGST-Hl) has been shown to recognize recombinant *R. microplus* GST, confirming cross immunization (DA SILVA VAZ et al., 2004b). In fact, rGST-Hl has been shown to induce a protective response against *R. microplus* in cattle, with an efficacy of 57% (PARIZI et al., 2011). In one of the few field trials for tick vaccines, rGST-Hl was used in combination with other antigens and that the number of semi-engorged female ticks was significantly lower among the vaccinated cattle. Surprisingly, cattle weight gain in the vaccinated group was 56% bigger in comparison with the unvaccinated cattle (body weight gain was 39% and 25% in 127 days, respectively)). Among the various antigens tested in that trial, rGST-Hl elicited the most persistent humoral response (PARIZI et al., 2012a; PARIZI et al., 2012b). Immunization with rGST-Hl has also been shown to provide cross protection against other hard tick species (NDAWULA et al., 2019; SABADIN et al., 2017). A cocktail of recombinant GST from *Rhipicephalus decoloratus* and *A. variegatum* has been shown to induce a protective response in rabbits and to reduce the number of *Rhipicephalus sanguineus* adult females by 35.3% (NDAWULA et al., 2019). Since *R. decoloratus* and *A. variegatum* are also cattle parasites a broad-spectrum universal anti-tick vaccine could be prepared based on GST as the major antigen.

Previous studies on *Drosophila melanogaster* (FROLOV & BIRCHLER, 1998) and *Aedes albopictus* (JAYACHANDRAN & FALLON, 2003) have demonstrated the crucial role of a 60S ribosomal proteins (P0) in regulating gene expression in arthropods, as evidenced by gene disruption experiments. In a subsequent study, an immunogenic region in *Rhipicephalus* sp. P0 was identified and a 20 amino acid synthetic peptide corresponding to host-non homologous region was inoculated in rabbits (RODRIGUEZ-MALLON et al., 2012). After a challenge with *R. sanguineus*, the protective efficacy of this peptide was found to be 90%. A subsequent vaccine trial demonstrated that the same peptide is protective against *R. microplus* in cattle, with an efficacy of 96% (RODRIGUEZ-MALLON et al., 2015). Another successful approach with P0 was obtained by chemically conjugating the peptide to Bm86. The use of such a pP0–Bm86 construct in cattle resulted in 84% reduction of *R. microplus* (RODRÍGUEZ MALLÓN et al., 2020).

Immunization with a *R. microplus* metalloprotease has been shown to be 60% efficacious against that same tick (ALI et al., 2015a). Metalloproteases have been shown to be essential for diverse biological functions in organisms, including modulation of host innate immune responses, as well as inhibiting host angiogenesis and blood coagulation (ALI et al., 2015b; SIMO et al., 2017).

Peptidase inhibitors play a pivotal role in tick parasitism because they interfere with several systems and also in defense-related host peptidases, thus facilitating blood feeding (PARIZI et al., 2018). Some peptidase inhibitors, such as several serpins and cystatins, have been identified in cattle tick saliva (FENG et al., 2019; TIRLONI et al., 2014). The immunogenic properties of these inhibitors and their potential as multispecies anti-tick vaccines have been described (PARIZI et al., 2020; TIRLONI et al., 2016). In one study, trypsin inhibitors were used as vaccines against *R. microplus*. Effectively, they confer partial protection in the immunized cattle (ANDREOTTI et al., 2002). In a subsequent study (ANDREOTTI et al., 2012), a tick-derived recombinant trypsin inhibitor was found to have an efficacy of 32% against *R. microplus* infestation in cattle (Table 2).

A flagelliform silk protein has been identified in *R. microplus*, *Dermacentor andersoni* (ALARCON-CHAIDEZ et al., 2007; SANTOS et al., 2004), and *R. appendiculatus* (MULENGA et al., 2007). Also, the recombinant silk protein induced a partial protection against *R. microplus* (MERINO et al., 2013) and a reduction in *A. marginale* DNA levels, suggesting that immunization with the silk protein confers some protection against this bacterium.

Aquaporins are channel pore-forming membrane proteins that are able to transport water across the cell membrane. Hereafter, in one transcriptomics study (GUERRERO et al., 2014), a sequence encoding an aquaporin from *R. microplus* was identified and cloned in an expression vector. In that study, the recombinant protein was expressed in *P. pastoris* and it was found to have an efficacy of 75% and 68% in two independent cattle pen trials (GUERRERO et al., 2014), as shown in table 2. Recently, synthetic peptides corresponding to predicted extracellular domains from another aquaporin of *R. microplus* led to an overall reduction of tick-numbers on cattle by 25% (SCOLES et al., 2022). Another channel protein identified in *R. microplus* is a mitochondrial voltage-dependent anion channel, designated BmVDAC (RODRIGUEZ-HERNANDEZ, 2012). Among cattle immunized with recombinant BmVDAC, the efficacy against *R. microplus* was found to be 82%. Interestingly, when ticks were infected with *B. bigemina*, the reported efficacy of recombinant BmVDAC decreased to 34% (ORTEGA-SANCHEZ et al., 2020).

CONCLUSION AND PERSPECTIVES

As an anti-tick vaccine, Bm86 is far from being a complete technical and economic success. However, it has proven that an anti-tick vaccine is feasible and can be a valuable tool for the control of *R. microplus*, which constitutes a huge problem for livestock production. A number of antigens conferring a considerable degree of protection against *R. microplus* have been identified. Also, some of these antigens confer cross protection against other tick species besides the species from which they were obtained. Thus, the identification of new proteins with potential use in vaccines, a deeper characterization of proteins already identified, as well as the study of the multi-antigen vaccines, are necessary to increase the protection already reached. This approach also requests to discover and characterize analogous proteins in different tick species in order to develop a vaccine efficient against various tick species simultaneously.

Studies to develop a commercially acceptable vaccine against ticks is still in progress. Traditional vaccine design processes, which involve identifying putative antigens through costly and time-consuming *in vivo* tests, have limitations. In contrast, the development of reverse vaccinology methodologies that utilize bioinformatics approaches has led to the discovery of new vaccine candidates and can short the time to achieve a vaccine that fulfil all the requirements to have a spread use. Indeed, several recent studies have successfully identified new tick antigens using this strategy (DE LA FUENTE & MERINO, 2013; MARUYAMA et al., 2017; PÉREZ-SÁNCHEZ et al., 2019; NDAWULA et al., 2020; TIRLONI et al., 2020; TRENTELMAN et al., 2020; ALI et al., 2021; COUTO et al., 2021; PÉREZ-SÁNCHEZ et al., 2022). In addition, a multi-antigen tick vaccine will be useful to control multiple tick species, particularly in areas where animals are parasitized by more than one tick species.

ACKNOWLEDGEMENTS

This research was supported by grants from the Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, National Council for Scientific and Technological Development; grant no. 465678/2014-9), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Coordination for the Improvement of Higher Education Personnel; grant no. 88881.068421/2014-01 and Finance code 001), the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, Carlos Chagas Filho Foundation for the Support of Research in the State of Rio de Janeiro; grant no. E-26/210.012/2018), and the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, Foundation for the Support of Research in the State of Rio Grande do Sul; grant no. 21/2551-0002221-3).

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The research was conducted in accordance with the Norms for Animal Experimentation Ethics Committee of Universidade Federal do Rio Grande do Sul.

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