Veterinary Parasitology 300 (2021) 109610

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Review article

Ticks and antibodies: May parasite density and tick evasion influence the outcomes following immunization protocols?

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ARTICLE INFO

Keywords:
Rhipicephalus microplus
Tick density
Tick resistance
Antibody response
IGBPs
Anti-tick vaccine

ABSTRACT

Ticks are a major concern to human health and livestock worldwide, being responsible for economic losses that go beyond billions of US dollars per year. This scenario instigates the development of vaccines against these ectoparasites, emphasized by the fact that the main method of controlling ticks still relies on the use of acaricides, what increases costs and may affect the environment as well as human and animal health. The first commercial vaccines against ectoparasites were produced against the tick *Rhipicephalus microplus* and their efficacy were based on antibodies. Many additional attempts have been conducted to produce protective immune responses against ticks by immunization with specific antigens and the antibody response has usually been the main target of evaluation. But some controversy still populates the roles possibly performed by humoral responses in tick-mammalian host relationships. This review focuses on the analysis of specific aspects concerning antibodies and ticks, especially the influence of parasite density and evasion/modulation. The immunization trials already described against *R. microplus* were also compiled and analyzed based on the characteristics of the molecules tested, protocols of immunization and tick challenge. Within these issues, it is discussed if or when antibody levels can be directly correlated with the development of tick resistance, and whether anti-tick protective immune responses generated by infestations may become ineffective under a different tick density. Also, higher titers of antibodies can be correlated with protection or susceptibility to tick infestations, what may be altered following continuous or repeated infestations and differ greatly comparing hosts with distinct genetic backgrounds. Regarding evasion, ticks present a sophisticated mechanism for dealing with antibodies, including Immunoglobulin Binding Proteins (IGBPs), that capture, transport and inject them back into the host, while keeping their properties within the parasite. The comparison of immunization protocols shows a total of 22 molecules already tested in cattle vaccination trials against *R. microplus*, with the predominance of concealed and dual antigens as well as marked differences in tick challenge schemes. The presence of an antibody evasion apparatus and variable levels of tick resistance when facing different densities of parasites are concerns that should be considered when testing vaccine candidates. Ultimately, more refinement may be necessary to effectively design a cocktail vaccine with tick molecules, which may be needed to be altered and combined in non-competing immune contexts to be universally secure and protective.

1. Introduction

Ticks are blood-sucking arthropods responsible for the majority of pathogens transmitted by ectoparasites to mammalian hosts worldwide (Colwell et al., 2011; Dantas-Torres et al., 2012; de la Fuente et al., 2017; Boulanger et al., 2019). They impact even further the livestock by blood spoliation and losses associated with the anti-tick host response (like leather damage and weigh loss caused by excessive itching), resulting in a negative impact that goes beyond billions of US Dollars per year (Grisi et al., 2014; Lew-Tabor and Rodríguez-Valle, 2016; Ndawula and Tabor, 2020). The control of ticks has been dependent on chemical treatments, but it increases costs, is usually not environmentally friendly and may contaminate humans and food-chain products (Abbas et al., 2014). Furthermore, tick acaricide resistance is a common reality (Sagar et al., 2020; Vilela et al., 2020), what emphasizes the rush for the development of additional effective strategies of control (de la Fuente et al., 2016; Kumar et al., 2020). Considering that hosts may develop resistance against ticks, and it involves the immune system,

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https://doi.org/10.1016/j.vetpar.2021.109610
Received 3 June 2021; Received in revised form 7 September 2021; Accepted 19 October 2021
Available online 29 October 2021

immunological control has been raised as an alternative (Jonsson et al., 2014; Rodríguez-Mallon, 2016), and the first commercial vaccines against ectoparasites were afforded against the tick *Rhipicephalus microplus* in the end of the last millennium (Willadsen et al., 1995; Canales et al., 1997). Since then, many efforts have been made to increase the efficacy and feasibility of anti-tick vaccines (Willadsen et al., 1996; Parizi et al., 2011; Maruyama et al., 2017; Blecha et al., 2018; Contreras et al., 2019; Ndawula et al., 2019). In this context, an important role of humoral responses in the development of host resistance against ticks has been recognized since the seminal article of Trager (1939) was published, showing that passive transfer of sera from "hyperimmune" Guinea pigs could avoid *Dermacentor variabilis* to complete its parasitic cycle, as well as implicating antibodies in anti-tick protection. Passive transfer of resistance was also achieved against other ticks, like *R. microplus* in bovines (Roberts and Kerr, 1976) and *Ixodes ricinus* in rabbits (Brossard and Girardin, 1979), although was not effective against *D. andersoni* in Guinea pigs (Wikel and Allen, 1976). Regarding specifically to the anti-tick immunological control perspective, antibody levels against a defined antigen like the midgut protein Bm86 are correlated with protection in commercial vaccines against *R. microplus* (Willadsen et al., 1989; de la Fuente et al., 2020), although this is not universally true to explain protection against ticks following artificial immunizations (Knorr et al., 2018). Also, higher levels of antibodies against tick components following infestations may not be correlated with increased resistance (Kashino et al., 2005; Cruz et al., 2008; Piper et al., 2009; Leal et al., 2013; Évora et al., 2015; Leal et al., 2018) and, surprisingly, artificial immunization with tick antigens may instead enhance tick infestations (Almazán et al., 2020). Therefore, some controversy still populates the evaluation of the roles performed by antibody responses in tick-mammalian host relationships and we intend to briefly review some of these points in the following sections, with a special focus on the *R. microplus*-bovine relationship and the immunization attempts that have been developed.

2. Tick density and antibody responses: how do they interact in the development/maintenance of tick resistance?

Tick resistance can be naturally acquired by hosts after successive infestations; however, it is possible that tick density can hinder the resistance development or alter the sensitivity of ticks to a previously protective immune response, what may include the modulation of tick specific antibody levels produced by the host. Ogden et al. (2002a) reported that sheep naturally exposed to *I. ricinus* during seasons with low tick infestation developed tick resistance, which was not effective when they were exposed to higher numbers of ticks during seasonal peaks of tick activity. Levels of IgG against salivary gland extracts varied among seasons and increased simultaneously with the increased number of adult ticks feeding on sheep. Cruz et al. (2008) evaluated the humoral response of Hereford calves successively infested with *R. microplus* (six heavy infestations with 18,000 larvae followed six light infestations with 800 larvae) and, similarly to Ogden et al. (2002a,2002b), detected higher IgG levels against *R. microplus* gut, salivary gland and larvae protein extracts after heavy infestations. Most of the expression of tick resistance developed after the heavy infestations relied in diminished adult female mean weight. However, after the seventh and eighth infestations (first two light infestations), there was an increase in adult female mean weight to levels similar to host naïve condition. Along the following light infestations tick resistance was recovered but expressed mainly as diminished proportion of ticks that completed the parasitic cycle. Additionally, not only the IgG levels but also the profile of molecules recognized changed during the infestations. Therefore, analogously to what could be seen in the *I. ricinus*-sheep relationship, it seems that the resistance acquired following heavy infestations of *R. microplus* in bovines was not as effective against ticks of the same lineage under light infestations, and a different protective immune response was developed by hosts when dealing with fewer ticks. In this sense, Kashino et al. (2005) reported saliva-specific antibodies from tick-susceptible cattle (Aberdeen) naturally infested with *R. microplus* larvae under low, intermediate, and heavy infestations. Higher IgG1 levels were detected in the animals with heavy infestations, whereas IgG2 levels were similar comparing animals facing different tick loads. Interestingly, IgE levels from moderately infested animals were higher comparing to those found in heavily infested animals. Antibody response of tick-resistant (Nelore) and tick-susceptible (Holstein) breeds to variable infestation loads in the field was also investigated. In this experiment, cattle sera were collected at the end of season of low infestations and the beginning of the season of heavy infestations (November), at the end of heavy infestations (May) and during a new season of light infestations (August). IgE levels were significantly different between the breeds only among May to August, presenting higher levels in susceptible cattle. Concerning the IgG response, similar IgG1 and IgG2 levels were verified in both breeds after low infestations, whereas higher levels were identified in Nelores than Holsteins after heavy infestations, mostly because antibody levels decreased in susceptible animals compared to the previous low infestation season. Conversely, Piper et al. (2009) reported higher IgG1 levels in susceptible breed (Holstein-Friesian) comparing to resistant breed (Brahman) animals after experimental infestations. Nevertheless, differences between the two studies concerning the cattle breeds used, period of sera collection, cattle pre-sensitization and profile of exposure to ticks must be considered. Kashino et al. (2005) reported saliva-specific antibodies from sera of cattle under natural tick infestations (collected during seasons of variable load of ticks) in previously infested animals, whereas Piper et al. (2009) detected antibodies against tick extracts from tick-free bovines subjected to artificial infestations (infested weekly for 7 weeks) simultaneously exposed to ticks in infested pastures.

In an experimental infestation, Santa Gertrudis breed animals (5/8 *Bos taurus* Shorthorn x 3/8 *B. indicus* Brahman) were infested with 10,000 *R. australis* larvae per week during 13 weeks in field condition (exposed to natural infestations). After the first infestation, similar levels of tick specific IgGs and tick loads were detected in susceptible and resistant individuals. After the third infestation, there was a reduction in the tick load and antibody levels in resistant animals, whereas in susceptible animals the antibody levels remained stable, as well as the number of recovered ticks (Piper et al., 2017). Corroborating the data obtained with Holstein-Friesian and Brahman breeds (Piper et al., 2009), susceptible animals developed higher levels of tick specific IgG1 than resistant animals after the third infestation (Piper et al., 2017). Garcia et al. (2017) presented some different results based in an experiment of three successive artificial infestations with 10,000 *R. microplus* larvae each, reporting higher levels of total IgG1 and IgG2 antibodies in susceptible hosts (Holsteins) than resistant hosts (Nelores) before and after the three successive tick infestations. However, levels of IgG1 specific for saliva and salivary glands were higher in Nelore than Holsteins in naïve individuals and after the first infestation, but, after the third infestation, susceptible hosts presented higher specific IgG1 levels than resistant hosts. Levels of IgG2 specific for saliva and salivary glands were similar between breeds before and in the first and second infestations but were also significantly higher in Holsteins in the third infestation (Garcia et al., 2017). Therefore, successive infestations seem to generate different antibody responses when comparing taurine and indicine breeds.

The presence of variable outcomes of tick resistance when facing different densities of parasites is a fact that should be considered in the attempts to test vaccine candidates. It becomes especially important when we compare the protocols evaluating the protective potential of purified molecules against *R. microplus* in cattle, as we can see that the tick density challenge can be highly variable, ranging from 500 to 30,000 larvae in point infestations, but including also description of daily infestations over weeks (see Table 1). The tick density influence, alongside with cattle breed immune response heterogeneity, may deserve a specific analysis when mensurating what an immunization

Table 1
Efficacy and antibody response parameters in cattle immunization trials against *Rhipicephalus microplus* and *R. australis*.

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
Rhipicephalus microplus						
Bm86 (Gavac™)	Two commercial cattle farms were used in this experiment. A total of 98 bovines from Farm 116 (4 ox, 71 calves and 23 heifers) were vaccinated with 3 doses of Bm86 (Gavac™) at weeks 0, 4 and 7. Cattle from Farm 117 (110 calves and 6 heifers) received salina in adjuvant (control). Cattle were challenged with *R. microplus* larvae (Camcord strain) 3 weeks after the last vaccine dose. Freund's complete adjuvant was added to the first dose, while Freund's incomplete adjuvant was used in the remaining doses.	Crossbred Holstein	Antibody titers against Bm86 were higher than control after the 1st immunization and remained until the end of experiment. Anti-Bm86 levels increased with each immunization and the highest levels were detected 2 weeks after the last dose. After reaching the peak, antibody titers decreased until the last measurement.	During 33 weeks of experiment, vaccinated cattle presented less than 5 ticks per animal (except at week 28). In the control group, the rate of infestation remained below 20 ticks per animal (pick at week 31 of 91 ticks/animal). Tick weight and egg laying capacity were lower in the vaccinated group compared to control, however this difference reduced over time. Color change of ticks from vaccinated group was verified.	Not determined	Rodríguez et al., 1995a
Bm86 (Gavac™)	Cattle from a farm were divided in 4 groups. i) Dairy cattle vaccinated (24 animals); ii) dairy cattle unvaccinated (12 animals); iii) Beef cattle vaccinated (21 animals) and iv) Beef cattle unvaccinated (21 animals). Cattle were immunized with rBm86 in Freund's complete adjuvant at week 0 and in Freund's incomplete adjuvant at weeks 4, 7 and 25. Dairy cattle were treated with acaricide at weeks 0 and 4, while Beef cattle were not treated. Cattle were infested under natural conditions for 36 weeks.	Dairy cattle: *B. taurus*, Cross-bred (*B. taurus* x *B. indicus*); Beef cattle: Nelore, Cross-bred (Nelore x Aberdeen Angus).	All immunized cattle produced anti-Bm86 antibodies. The average of antibody titers was 16,000 at week 7 (3rd immunization) and 6000 at week 25 (4th immunization).	Dairy cattle: Infestation below 60 ticks/animal in the vaccinated group (above 60 ticks/animal in the control group). Beef cattle: Crossbred cattle - Tick number was lower in vaccinated than control group after 1st (week 8) and 2nd (week 24) infestation peaks. Total of 0.92 ± 0.5 ticks/animal in vaccinated group at week 24. Similar results were verified in Nelore cattle.	Not determined	Rodríguez et al., 1995b
Bm86 (Gavac™)	Cattle were divided in 2 groups: i) 800 animals were vaccinated with Gavac™ at weeks 0, 4 and 7 (May to July) and revaccinated 4 months after the 1st immunization (week 16, September); ii) 200 animals were not immunized (control). Freund's complete adjuvant was added to the first dose, while Freund's incomplete adjuvant was used in the remaining doses. Animals from vaccinated and control groups were maintained in tick-infested pastures under acaricide treatment.	Crossbred cattle (*B. indicusB. indicus* x Simmental, *B. indicus* x Beefmaster and x Charolaise)	Antibody titers increased after the 3rd immunization and picked after revaccination (4 months post 1st immunization).	Reduction of acaricide treatments from once every 14 days to once every 64 days (maximum interval of 129 days in December). Reduction to almost zero ticks after the 2nd immunization (July 2 to Jan 22) in vaccinated group (lower than the control between July 29th and October 14th). Reduction in egg laying capacity of 35 % (day 56 PPI*) - 100 % (days 83, 125 and 260 PPI*).	Evaluated from 53 % (day 34 PPI*) to 100 % (from day 83 PPI*). Total efficacy of vaccination alone was not determined.	Redondo et al., 1999; Rodríguez et al., 1994, 1995a, 1995b
Bm86 (Gavac™)	Three experiments: i) controlled pen trials -	Diverse: Holstein,	Field condition: Anti-Bm86 antibody titers	Pen trials: Reduction in tick numbers (9–74	Controlled pen trials: 51–91 %; Field trials	de la Fuente et al., 1999

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	Vaccinated and control cattle were experimentally challenged with *R. microplus* larvae; ii) controlled field trials and iii) field trials under production conditions. Gavac™ immunizations were conducted at weeks 0, 4 and 7 (in the field trial cattle received boosters every 6 months). Experiments were carried out in several regions of the world with different *R. microplus* strains and bovine breeds.	crossbred (dairy), crossbred (beef), crossbred (not specified) and *B. indicus*.	varied from 3093 ± 183 to 8968 ± 962 (mean ± SD) in the peak of humoral response (2 weeks after the 3rd immunization).	%), oviposition (8−61 %) and fertility (8.5−13 %).	under production conditions: Increase in time between acaricide treatments by 32 ± 21 days.	
Integrated control using Gavac™ and acaricide	Total of 588,573 cattle from farms of 14 provinces of Cuba were integrated into the program. Animals were immunized with 3 doses (Gavac™ plus Freund's complete adjuvant at week 1 and Gavac™ plus Freund's incomplete adjuvant at weeks 4 and 7), receiving additional boosters every 6 months.	*B. taurus* Holstein Friesian	Anti-Bm86 titers of immunized cattle from 8 farms were evaluated. Antibody titers increased after each booster. The higher titers were obtained in week 9 (after 3rd dose) and declined until week 24.	Increase in the interval between acaricide applications, with reduction of 87 % in the number of treatments, and reduction of morbidity by *Babesia bovis* after integrated control.	Not determined	Rodríguez-Valle et al., 2004; Rodríguez et al., 1994, 1995a, 1995b
Ba86 (*Boophilus annulatus* Bm86)	The trial included 4 groups of 5 animals each - Calves were immunized with 3 doses (weeks 1, 3 and 7) of: i) Ba86 (Israeli strain) with adjuvant (Montanide ISA 50 V); ii) Bm86 (Mozambique strain) with the same adjuvant; iii) Gavac vaccine (Cuba Camcord strain). iv) Control group was inoculated with adjuvant/saline. Cattle were challenged with 10,000 *R. microplus* larvae/animal 20 days after the last dose.	Crossbred calves	Anti-Bm86 levels increased after each immunization in Ba86-vaccinated animals. The higher antibody titers were verified 2 weeks after the 3rd dose, which reduced slightly one month later. The anti-Bm86 titers verified during the experiment were similar to titers of Bm86 (Mozambique strain)-vaccinated cattle. Gavac-immunized animals produced lower antibody levels against Bm86 (Mozambique strain) than animals immunized with Ba86 or Bm86 (Mozambique strain).	Reduction in tick infestation (40 %), tick weight (15 %) and egg fertility (50 %).	71.5 (< Gavac efficacy = 85.2 %; > Bm86 Moçambique strain efficacy = 70.4 %)	Canales et al., 2009b
Bm86 (Gavac™ and TickGard^{PLUS})	Calves were divided in 3 groups (16 animal each): i) vaccinated with Gavac™; ii) vaccinated with TickGard^{PLUS} and iii) unvaccinated. Animals were immunized at weeks 0 and 4. Cattle were infested 3 times on alternated days with 5000 larvae of *R. microplus* (Campo Grande strain).	Crossbred cattle	Immunized calves presented specific IgG antibodies 2 weeks after the 1st vaccination, with higher antibody levels at week 6 (2 weeks after the 2nd vaccination).	Reduction in tick numbers, and tick and egg weight in both vaccinated groups compared to the control group. Tick damage and low reproductive capacity were detected in immunized calves.	49.4 % (Gavac™) and 46.4 % (TickGard^{PLUS})	Andreotti, 2006
Bm86-CG	The trial included 2 groups of 6 animals each: i) immunized	Holstein cattle	Specific antibody levels were higher in immunized than	Reduction in tick numbers (28 %) and fertility (8 %).	31 %	Cunha et al., 2012

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	group was inoculated with recombinant Bm86-CG emulsified with Montanide (ISA 61 VG) and ii) control group was injected with adjuvant only (3-times at 0, 2 and 4 weeks). Animals were challenged with 15,000 *R. microplus*-CG larvae (3 infestations for 1 week).		control cattle 15 days after the 1st immunization. Antibody titers increased after each booster. The peak of anti-rBm86-CG levels was detected 8 days after the tick challenge.			
Bm86 (Gavac^{plus})	Animals from 10 farms were divided in 4 groups and 3 of them were vaccinated with Gavac^{plus}. Groups: i) Cattle from farm 1 (86 animals), farm 4 (102 animals) and farm 7 (192 animals) were vaccinated with Gavac^{TM} at weeks 0, 4 and 7; ii) Cattle from farm 2 (106 animals), farm 6 (161 animals) and farm 9 (150 animals) were vaccinated at weeks 0 and 4; iii) Cattle from farm 3 (116 animals), farm 5 (151 animals) and farm 8 (168 animals) were vaccinated at weeks 0 and 7; iv) Cattle from farm 10 were not vaccinated (control). The vaccines were adjuvated in Montanide 888. Six months after the 1st immunization, calves received an additional booster (week 24). Cattle were infested under natural conditions.	Crossbred cattle (*B. taurus* x *B. indicus*)	All vaccinated cattle groups presented high IgG antibody levels. Anti-Bm86 titers gradually decreased after the vaccination period (weeks 20 and 24) but increased again (week 27) after the additional boost at week 24. The humoral response was not different between groups that received 2 or 3 doses of the vaccine.	Reduction in tick and egg weight in all immunized groups (there was no difference between them). Reduction of hatchability in ticks from cattle of group i and ii. Damage to ticks detached from immunized animals was also perceived.	Not determined	Vargas et al., 2010
synthetic SBm4912	Twenty cattle were divided in 5 groups and immunized with synthetic peptides and adjuvant saponin (groups A, B and C) and control (D and E): A) Immunized with SBm4912; B) immunized with SBm7462; C) immunized with SBm19733; D) control group inoculated with adjuvant and distilled water and E) control inoculated with distilled water. The animals received 3 doses on days 1, 30 and 60. Twenty-one days after the last dose, cattle were challenged with ±1500 larvae (BmUFV1 strain) per day during 3 consecutive days.	*B. taurus* Jersey	Sera from immunized cattle recognized the synthetic protein by Western-blot. Anti-SBm4912 levels increased with each immunization. The peaks of antibody titers were observed 2 weeks after the 2nd and 3rd doses (not different between them). After the last peak, there was a slight reduction in antibodies levels. Control cattle remained negative during the experiment.	Reduction in tick numbers, tick weight, egg laying and fertility.	72.40 % (compared to "distilled water" control); 64.42 % (compared to adjuvant control group).	Patarroyo et al., 2002
synthetic SBm19733		*B. taurus* Jersey				

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	Twenty cattle were divided in 5 groups and immunized with synthetic peptides and adjuvant saponin (groups A, B and C) and control (D and E). A) Immunized with SBm4912; B) immunized with SBm7462; C) immunized with SBm19733; D) control group inoculated with adjuvant with distilled water and E) control inoculated with distilled water. The animals received 3 doses on days 1, 30 and 60. Twenty-one days after the last dose, cattle were challenged with ±1500 larvae (BmUFV1 strain) per day during 3 consecutive days.		Sera from immunized cattle recognized the synthetic protein by Western-blot. Anti-SBm19733 levels increased with each immunization. The higher antibody levels were observed around 2 weeks after the 3rd vaccine dose. After the peak, specific antibodies declined slightly until the end of the trial. Control cattle remained negative during the experiment.	Reduction in tick number, tick weight, egg laying and fertility.	35.87 % (compared to "distilled water" control); 22.57 % (compared to adjuvant control).	Patarroyo et al., 2002
synthetic SBm7462®	Twenty cattle were divided in 5 groups and immunized with synthetic peptides and adjuvant saponin (groups A, B and C) and control (D and E). A) Immunized with SBm4912; B) immunized with SBm7462; C) immunized with SBm19733; D) control group inoculated with adjuvant with distilled water and E) control inoculated with distilled water. The animals received 3 doses on days 1, 30 and 60. Twenty-one days after the last dose, cattle were challenged with ±1500 larvae (BmUFV1 strain) per day during 3 consecutive days.	*B. taurus* Jersey	Sera from immunized cattle recognized the synthetic protein by Western-blot. Anti-SBm7462 levels increased after the 1st immunization. The peak of antibody titers was detected 2 weeks after the 2nd dose. The 3rd immunization increased slightly the antibody levels, but apparently were lower or not statistically different from the antibody titers observed 2 weeks after the 2nd dose. Two weeks after the 3rd dose, antibody levels declined slightly until the end of the trial. Control cattle remained negative during the experiment.	Reduction in tick numbers, tick weight, egg laying and fertility; Dark-red color described in ticks detached from vaccinated cattle.	81.05 % (compared to "distilled water" control); 75.58 % (compared to adjuvant control).	Patarroyo et al., 2002
synthetic SBm7462®	The trial included 3 groups of 4 animals each: i) cattle were immunized with rSBm7462® and saponin as adjuvant dissolved in water; ii) cattle were immunized with supernatant of non-transfected yeast culture of *K.* (*P.*) *pastoris* adding saponin dissolved in water; iii) cattle were immunized with water. Calves were vaccinated on days 1, 30 and 60 and infested with ± 1500 larvae/day during 3 consecutive days (21 days after last immunization).	*B. taurus* Holstein Friesian	Specific-IgG levels increased with each immunization. Cattle presented higher IgG levels 7 days after the 3rd dose and a slight decrease after larvae challenge. The diameter of the germinal centers from bovine lymph nodes were correlated with levels of high-affinity IgG antibodies during the experiment.	Reduction in egg weight (8.59 %) and fertility (17,26 %) from ticks detached from immunized calves.	72.4 %	Patarroyo et al., 2020

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
synthetic and recombinant SBm7462 peptides	Calves were divided in 4 groups of 4 animals each: i) cattle were immunized with the synthetic peptide in adjuvant (saponin) resuspended in water; ii) synthetic control group - cattle were immunized with water; iii) cattle were immunized with recombinant peptide in adjuvant resuspended in water; iv) recombinant control group - cattle were immunized with water. Animals were inoculated on days 1, 30 and 60. Twenty-one days after the 3rd immunization, calves were infested with ± 1500 larvae during 3 consecutive days.	*B. taurus* Holstein Friesian	Kinetics of IgGs of sera from both immunized groups were similar. Specific antibody titers were higher in immunized cattle than control 15 days after the 2nd vaccine dose and increased again after the 3rd immunization, reaching the peak around days 70−84 PPI *. Antibody levels showed a slight drop after the tick challenge.	Reduction in tick numbers (87.7 % and 93.5 %) and oviposition (20 % and 8.6 %) in calves immunized with synthetic and recombinant vaccines, respectively. Decrease in tick weight and egg production index/tick in calves immunized with synthetic peptide. Nutrient index/tick was reduced in bovines vaccinated with recombinant peptide. Changes in tick gut cells, tick size and egg color were reported.	Not determined	Tafur-Gómez et al., 2020
Bm91	Eighteen indigenous *B. indicus* cattle breed (White Lamphun) were divided in 3 groups of 6 animals each: i) cattle immunized with rBm91 (from *R. microplus* strain indigenous of Thailand) adjuvanted with Montanide ISA 50 V in mineral oil; ii) control immunized with PBS and iii) control immunized with adjuvant. Cattle were vaccinated with 3 doses in weeks 0, 3, 6, with an additional booster at week 26. Cattle were infested under natural conditions.	*B. indicus*	Anti-Bm91 titers increased quickly after the 1st immunization and rose slightly after the 2nd dose. Antibody levels remained stable after the 3rd dose until week 12. There was a slight decline in antibody levels until week 26 but increased again after the 4th immunization. Anti-Bm91 titers remained stable until the end of experiment (week 30). Sera from control and adjuvant groups remained seronegative during all trial. Bm91 was recognized by sera of Bm91-immunized cattle from 2nd week until the week 30.	Reduction of the oviposition (5 %) in group immunized with Bm91 compared to control (adjuvant) group; Reduction of the reproductive efficiency index (6 %) and egg viability (8 %) in immunized animals compared to control (PBS) group.	Not determined	Lambertz et al., 2012
Bm95	Controlled pen trial- Cattle were divided in 3 groups (3 animals each): i) immunized with rBm95 expressed in *K.* (*P.*) *pastoris* and Montanide 888 (adjuvant), ii) immunized with Bm86 (Gavac™) and iii) injected with adjuvant only (placebo). Animals were infested with 2000 *R. microplus* larvae of Camcord (Bm86-sensitive) and A (Argentinean Bm86-resistant) tick strains. Field conditions trial- i) Farm 1: 193 unvaccinated bovines (control); ii) Farm 2: 192 animals immunized with Gavac™ and iii) Farm 3, 5, 7−10: 849 animals	Holstein (controlled pen trial); crossbred (1/2 Holstein: 1/2 Zebu) in field trial	Controlled trial- Cross-reactivity between Bm86 and Bm95; similar antibody levels between both proteins. Field trial- Anti-Bm86 and anti-Bm95 levels increased rapidly after the 2nd immunization. The higher antibody levels were observed 2 weeks after the 2nd vaccination, which decreased over the next 6 months.	Controlled pen trial - Vaccination reduced tick number in bovines challenged with Camcord strain tick and reduced tick and egg weight in cattle immunized with both tick strains. Field conditions trial - Bm95-vaccinated cattle had 5 ticks/animal 4 weeks after the last immunization and over 15 ticks/animal after the 8th week (Control group: >15 tick/animal at 4th week). Decrease in the frequency of treatment with acaricides (for every 60 days) compared to Bm86-vaccined cattle (every 47 days) and control (every 27 days).	Controlled trial- 58 % (against A strain) and 89 % (against Camcord strain). Efficacy higher than those obtained after Bm86-vaccination (0 % and 84 %, respectively); Field trial- Not determined.	García-García et al., 2000

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	vaccinated with Bm95. Calves were immunized with 2 doses at weeks 0 and 4.			Reduction in tick weight, eggs laid and reproductive capacity.		
Bm95	Two groups (6 animals each): i) calves immunized with Bm95 recombinant (4 doses at 1-month interval) using Argel (Indican Immunological Ltd., Hyderabad); ii) non-immunized calves. Animals were infested with 1000 *R. microplus* larvae on day 21 after the last dose.	Crossbred calves	Anti-Bm95 titers from immunized calves sera increased after the 1st immunization, with picks after the 3rd and 4th immunizations (days 80 and 110 PPI*). Antibody titers from Group A ranged from 196.1 ± 13.7 (day 0) to 7,979.9 ± 312.5 (day 110). Anti-Bm95 levels drop from day 120 (day 10 after larvae infestation) until the end of experiment (day 150).	Reduction in tick numbers (70.76 %), egg weight (25.35 %), tick weight (8.99 %) and fertility (14 %).	81.27 %	Kumar et al., 2009
BM95-MSP1a: Bacterial membranes containing surface-exposed BM95 fusioned to MSP1a.	Calves were divided in 5 groups (4 animals each) and were inoculated with recombinant proteins adjuvated in Montanide ISA 50 V2: i) BM95-MSP1a, ii) SUB-MSP1a, iii) EF1a-MSP1a, iv) UBQ-MSP1a or v) adjuvant/saline (negative control). Cattle received the chimeras or adjuvant/saline on days 0, 30 and 60. Cattle were infested with 5000 *R. microplus* larvae 2 weeks after the last inoculation.	Beefmaster x Charolais	Anti-MSP1a levels were higher in BM95-MSP1a immunized cattle than control right after the 1st vaccination (day 30). Antibody levels decreased after the 2nd dose (day 60) and increased after the 3rd (day 75), with a slight drop after tick infestation (day 103). Anti-BM95 were observed after the 1st immunization and higher antibody levels were detected after the 2nd dose, with slight decline after 3rd immunization.	Reduction in tick numbers (54 %), tick weight (25 %) and egg fertility (22 %).	64 %	Almazán et al., 2012
Subolesin (SUB)	Cattle were divided in 4 groups (4 calves each) and were immunized with the recombinant proteins adjuvated in Montanide ISA 50 V: i) SUB, ii) UBQ, iii) Bm86 (positive control) or received iv) adjuvant/saline (negative control). Calves received 3 doses (days 1, 4 and 6) of recombinant proteins or adjuvant/saline. Two weeks after the last inoculation calves were infested with 10,000 *R. microplus* larvae.	European crossbred calves	Antibody levels against SUB increased after the 1st immunization but decrease after the 2nd and remain low until the end of experiment.	Reduction of 43 % in tick numbers.	51 %	Almazán et al., 2010
SUB + IV: Subolesin (SUB) and heat inactived *Mycobacterium bovis* (IV)	Cattle were separated in 2 groups: i) 3 calves orally vaccinated with SUB + IV and ii) 2 calves vaccinated only with IV. Cattle were immunized on days 0 and 22 and infested with 500 *R. microplus* larvae on day 43.	Crossbred *B. taurus* calves	Anti-SUB IgG antibodies were detected in the SUB + IV-vaccinated group but varied between the animals. A correlation between increased levels of anti-SUB IgG and reduced number of ticks was observed.	Reduction in tick numbers (51 %) and egg fertility (30 %) in the SUB + IV-vaccinated calves.	65 %	Contreras et al., 2019
SUB vaccination and SUB gene knockdown	Two groups (6 calves each). A) Calves were immunized with subolesin and adjuvant (Montanide ISA 50 V) or B) received	European crossbred calves	Antibody titers increased after the 1st and the 2nd immunizations (peak at day 49) but reduced after the 3rd	i) SUB-vaccinated cattle and infested with control larvae: Reduction in tick numbers; ii) SUB-vaccinated and	i) SUB-vaccinated cattle and infested with control larvae: 44 %; ii) SUB-vaccinated and infested with RNAi larvae: 75 %; iii) SUB-	Merino et al., 2011

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	adjuvant/saline (negative control) on days 0, 28 and 49. Cattle were infested with *R. microplus* larvae 30 days after the last immunization (day 79). The cells used for the infestation contained i) 500 larvae from replete females injected with unrelated GIII dsRNA (control) or ii) 500 larvae from replete females injected with subolesin dsRNA or iii) a combination of 500 control larvae and 500 SUB-RNAi larvae.		immunization (day 79), with lower titers in the end of experiment (day 95). Antibody levels from vaccinated cattle sera were higher than control during all experiment.	infested with RNAi larvae: reduction in tick numbers, tick weight and oviposition. Similar results from experiments of ticks fed in vaccinated and control cattle. iii) SUB-vaccinated and infested with combined larvae: reduction in tick numbers and oviposition.	vaccinated and infested with combined larvae: 22 %	
Ha-SUB: *Hyalomma anatolicum* Subolesin	Cattle were divided in 2 groups (4 animals each): i) immunized with recombinant protein and Montanide ISA 50 V2 (adjuvant) or ii) inoculated with PBS only (control) on days 0, 28 and 49. Calves were infested with *R. microplus* larvae (from 200 mg of eggs) 14 days after the 2nd booster.	Crossbred calves (*B. taurus* x *B. indicus*)	Antibody levels were 11.4- and 13.95-times higher in immunized animals' sera than pre-immune sera on days 17 and 40 PPI*. Antibody titers remained stable until 119 days PPI* with a later decrease trend (9.5 times higher than pre-immune).	Reduction in tick numbers (38.97 %), tick weight (19.49 %) and egg weight (24.53 %).	54 %	Kumar et al., 2017
SUB-MSP1a: Bacterial membranes containing surface-exposed SUB fusioned to MSP1a.	Calves were divided in 5 groups (4 animals each) and were inoculated with recombinant proteins adjuvated in Montanide ISA 50 V2: i) BM95-MSP1a, ii) SUB-MSP1a, iii) EF1a-MSP1a, iv) UBQ-MSP1a or v) adjuvant/saline (negative control). Cattle were immunized 3-times with the chimeras or adjuvant/saline on days 0, 30 and 60. Cattle were infested with 5000 *R. microplus* larvae 2 weeks after the last inoculation.	Beefmaster x Charolais	The group immunized with SUB-MSP1a presented anti-MSP1a levels similar to control group. Higher antibody levels against SUB were detected after 2nd immunization (day 60), with decline after 3rd vaccine dose. Anti-SUB levels remained higher than control group until the end of the experiment (day 103).	Reduction in tick numbers (34 %); tick weight (37 %) and egg fertility (67 %).	81 %	Almazán et al., 2012
SUB	Calves were divided in 3 groups (3 animals each): i) immunized with recombinant protein and adjuvant (Montanide ISA 50 V) on days 0, 28 and 49; ii) injected with adjuvant/saline (placebo) and iii) untreated. All calves were infested with *R. microplus* larvae on days 72, 75 and 77. Cattle groups (except untreated) were also infected with *Anaplasma marginale* and *Babesia bigemina*on days 69 and 92, respectively.	Crossbred calves	Levels of antigen-specific antibodies increased right after the 1st immunization, as well as increased with each immunization (until day 69) and remained stable until day 104 (end of the experiment).	Reduction in tick numbers (47 %), tick weight (9 %) and oviposition (18 %). DNA levels for *A. marginale* and *B. bigemina* were lower in the ticks from vaccinated cattle compared to controls.	60 %	Merino et al., 2013
Q38 (SUB peptides)	Calves were divided in 3 groups (3 animals each): i) immunized with recombinant	Crossbred calves	Levels of antigen-specific antibodies increased right after the 1st immunization, as	Reduction in tick numbers (69 %) and oviposition (20 %). DNA levels for *B.*	75 %	Merino et al., 2013

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	protein and adjuvant (Montanide ISA 50 V) on days 0, 28 and 49; ii) injected with adjuvant/ saline (placebo) and iii) untreated. All calves were infested with *R. microplus* larvae on days 72, 75 and 77. Cattle groups (except untreated) were also infected with *Anaplasma marginale* and *Babesia bigemina* on days 69 and 92, respectively.		well as increased with each immunization (until day 69) and remained stable until day 104 (end of the experiment).	*bigemina* were lower in ticks from immunized cattle than controls.		
rBmSu (SUB)	Ten calves were divided in 2 groups: i) 6 calves were immunized with rBmSu and Montanide 888 in mineral oil (adjuvant) on days 0, 30 and 60; ii) 4 calves received PBS in Montanide 888 in mineral oil (control). Calves were challenged with *R. microplus* larvae (from 100 mg of eggs) 15 days after the 3rd dose (day 75) and again on the 120th day.	Crossbred male calves (*B. taurus* male x *B. indicus* female)	IgG levels increased after the 1st immunization and peaked around day 75 PPI*, with a subsequent decline. IgG1 levels increased after the 1st immunization, while IgG2 levels increased only after the 1st booster. The peak of IgG1 and IgG2 titers was 75−90 days PPI*. Antibody levels decreased after the 90th day.	1st immunization: reduction in fertility (26.1 %); 2nd immunization: Reduction in oviposition (8.6 %) and fertility (24.2 %).	44 % (after 1st immunization) and 37.2 % (after 2nd immunization)	Shakya et al., 2014
Pp0-KLH: synthetic 20 amino acid peptide of the acidic ribosomal protein p0 conjugated to KLH (Keyhole Limpet Hemocyanin)	Two groups (4 animals each) were immunized on days 0, 21, 36 and 60 with: i) P0-KLH adjuvanted in VG Montanide 888 or ii) KLH in adjuvant only (control). Each bovine was infested with ± 3000 *R. microplus* larvae (total) on days 75, 76 and 77.	*B. taurus* Holstein	Anti-Pp0 antibody titers were detected only after the 3rd immunization (day 60). Antibody levels remained high until the end of experiment (day 90). Anti-KLH levels were similar to detected in the control group (immunized with KLH).	Reduction in tick numbers (39 %), tick weight (49 %), oviposition (75 %) and hatched eggs (41 %).	96 %	Rodríguez-Mallon et al., 2015
Pp0-KLH	Cattle were divided in 3 groups (5 animals each) and immunized with: i) pP0–KLH and adjuvant Montanide ISA 50 (SEPPIC, France); ii) pP0–Bm86 and adjuvant and iii) PBS and adjuvant. Bovines received the formulations on days 0, 21 and 36. Fifteen days after the last dose, cattle were infested with ± 1000 larvae per day for 3 days.	Cuban Siboney breed (5/8 Holstein and 3/8 Cebu)	Anti-Pp0 antibody titers increased after each immunization, with effective increased after the 2nd dose. The peak was detected around the day 51 PPI* (Anti-Pp0 titers ± 7000). Anti-KLH antibody levels also effectively increased only after the 3rd immunization (peak on day 51 with titers ± 4000). Both anti-Pp0 and anti-KLH decreased from day 51.	Reduction in tick numbers (84 %) and hatchery (28 %).	89 %.	Rodríguez-Mallon et al., 2020
Pp0-Bm86	Cattle were divided in 3 groups (5 animals each) and immunized with: i) pP0–KLH and adjuvant Montanide ISA 50 (SEPPIC, France); ii) pP0–Bm86 and adjuvant and iii) PBS and adjuvant. Bovines received the formulations on days 0, 21 and 36. Fifteen days after the last dose, cattle were infested	Cuban Siboney breed (5/8 Holstein and 3/8 Cebu)	Anti-Pp0 antibody titers increased after each immunization. The peak was detected around the day 51 PPI* (Anti-Pp0 titers ± 10,000). Anti-Bm86 levels increased with greater intensity after the 3rd vaccine dose (peak on day 51 after the 1st immunization with antibody titers ± 8000). Both anti-Pp0	Reduction in tick numbers (72 %), egg weight (22 %) and hatchery (34 %).	84 %	Rodríguez-Mallon et al., 2020

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	with ± 1000 larvae per day for 3 days.		and anti-Bm86 decreased from day 51.			
BmTI: *R. microplus* trypsin inhibitor	Calves were divided in 2 groups (8 animals each): i) immunized with BmTI in Freund's complete adjuvant or ii) inoculated with adjuvant only (control). Animals were immunized with 3 doses at 21-day intervals. Calves were infested with 20,000 larvae 2 weeks after the last inoculation.	*B. indicus* Nelore	Anti-BmTI antibodies were detected 2 weeks PPI* and increased post 2nd immunization. The anti-BmTI peak was between 21 days after the 2nd dose (day 42 PPI*) and 15 days after the 3rd dose (day 56 PPI*). Antibody levels decreased after tick infestation and remained similar to the titers measured on day 21 PPI* until the end of experiment (day 84 PPI *).	Reduction in tick numbers (69.7 %), egg weight (71.3 %) and engorged weight (69.5 %).	72 %	Andreotti et al., 2002
RmLTI: *R. microplus* larvae trypsin inhibitor	Calves were divided in 2 groups (6 animals each): i) immunized with rRmLTI and Montanide ISA 61 VG (adjuvant) or ii) injected with adjuvant/ saline (negative control). Calves were immunized with 3 doses (days 0, 15 and 30) and infested with 20,000 *R. microplus* larvae on day 51.	*B. taurus* Holstein	IgG levels increased after the 1st immunization, presenting a pick 31 days after the 2nd booster. There was a slight decline in the humoral response after tick infestation.	Reduction in tick numbers (30.15 %), tick weight (24.66 %) and larval hatchability (8.97 %).	32 %	Andreotti et al., 2012
Synthetic BmTI N-terminal fragment	Experiment was conducted under field conditions. Cattle were divided in 2 groups (8 animals each): i) immunized with synthetic BmTI N-terminal fragment in saponin solution or ii) received adjuvant alone (control) every 3 weeks (total of 3 doses). Cattle was challenged 3-times on alternate days with 5000 BmCG strain larvae each time, starting 21 days after the 3rd dose.	Crossbred heifers	Anti-BmTI N-terminal titers increased 2 weeks after the 1st vaccine dose in immunized cattle. The higher antibody levels were detected in the week 9 (15 days after the last immunization). After this period, antibody titers gradually decreased until the end of the experiment (week 15). Antibodies recognized the whole native protein.	Reduction in tick numbers (83.8 %).	18.4 %	Andreotti, 2007
RmLTI-BmCG-LTB: BmCG (Bm86 Campo Grande) and RmLTI (Kunitz protease inhibitors) fused to LTB (heat-labile enterotoxin B subunit from *Escherichia coli*)	Two groups (4 bovines each): i) Cattle were immunized with the chimera and Montanide ISA 61 VG (adjuvant) or ii) treated with adjuvant only (control). The animals were vaccinated 3-times and challenged with 15,000 larvae (21 days after the last immunization).	Angus heifers	Fast antibody production. High antibody levels 15 days after immunization, remaining high on days 30 and 60 post-inoculation. Recognition of RmLTI-BmCG-LTB by sera from vaccinated cattle.	No parameter alone was statistically significant.	55.6 %	Csordas et al., 2018
BrRm-MP4: a metalloprotease of *R. microplus*	Two groups: i) 4 calves were immunized with rBrRm-MP4 and Montanide 888 /Marcol 52 (adjuvant) in PBS (treated group) and ii) 3 calves was injected with PBS emulsified with adjuvant (control group). The 7 calves received 4 doses of vaccine at 15-day intervals. Ten days after	*B. taurus* Hereford	Increase of anti-rBrRm-MP4 antibody levels was verified only after the 3rd booster. High antibody levels after the 4th immunization were detected in all treated animals, remaining high until the end of experiment. Antibody titers were correlated with	Reduction in tick numbers (42.9 %), tick weight (41,46 %), egg laying capacity (148 %) and egg fertility (17.5 %).	60 %	Ali et al., 2015

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Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	the immunizations, the animals were infested with ± 20,000 larvae.		reduction in tick numbers.			
BmVDAC: mitochondrial voltage-dependent anion-selective channel of *R. microplus*	Three groups (5 animals each). Calves were immunized with rBmVDAC and Montanide ISA 71 V G (adjuvant) on days 0, 21 and 42 (groups 2 and 3). Negative control calves were injected with adjuvant in PBS (group 1). Two days after the last immunization, all calves were infested with 10,000 *R. microplus* larvae. Animals from groups 1 and 2 were infected with *B. bigemina*.	*B. taurus* Holstein	Antibody levels raised after 1st vaccine dose in rBmVDAC-immunized groups. Increase of antibody titers in group 3 and stability in group 2 on day 42 (3rd immunization/ infestation onset), indicating higher titers in the group 3. On day 56, antibody levels grow back in group 2 and enhance in group 3 (similar antibody level at day 56 between both groups).	Group 3 (uninfected): Reduction in tick numbers (38 %), tick weight (5 %), oviposition (54 %) and egg fertility (55.8 %); Group 2 (B. bigemina infected): Reduction in egg fertility (32.12 %), whereas other parameters were raised compared to group 1.	Group 2: 34 %; Group 3: 82 %	Ortega-Sánchez et al., 2020
VTDCE	Two groups (4 animals each). Calves were treated with: i) rVTDCE and adjuvant (Marcol 52 and Montanide 888) or ii) PBS and adjuvant. All animals received 5 doses of the preparations at 10-day intervals. Cattle were infested with 20,000 tick larvae 10 days after the last dose. After the challenge, the rVTDCE-immunized group received 5 new boosters.	*B. taurus* Hereford	Increase of the anti-rVTDCE antibody concentration after vaccination, followed by a drop 50 days after the challenge. A new rise was verified after additional five boosters ("development of immunological memory"). Anti-rVTDCE titers reached 16,000 in the immunized calves.	No parameter alone was statistically significant.	21 %	Seixas et al., 2008
GST-HI: *Haemaphysalis longicornis* glutathione S-tranferase	Two groups: i) 4 calves were immunized with rGST-HI in PBS (1st to 4th dose) with addition of Marcol 52/ Montanide 888 (adjuvant) in the 5th and 6th doses; ii) 3 calves received PBS and adjuvant only (control group). The injections were administered at 15-day intervals. Cattle were infested with 20,000 R. microplus larvae 15 days after the last inoculation.	*B. taurus* Hereford	Humoral response was observed after cattle immunizations and after larvae infestation. Sera from immunized cattle recognized native *R. microplus* GST.	Reduction in tick number during heavy infestation period.	57 %	Parizi et al., 2011
native Boophilus Yolk pro-Cathepsin (BYC)	Experiment 1: Two groups (4 animals each). Calves received: i) 4 inoculations of BYC plus Quil A® (adjuvant) in PBS every 2 weeks; or ii) Quil A® (control). Animals were challenged with 30,000 larvae (10-day-old) 2 weeks after the last inoculation. Experiment 2: The same above protocol was applied in this trial, however, calves were challenged with 17-day-old larvae and maintained in the field for 8 months.	*B. taurus* Hereford	The IgG titers peaked after the 4th immunization followed by a reduction after the vaccination period, reaching similar titers to pre-immune sera (11 months later). After a new booster of BYC plus adjuvant, antibody levels increased in five of the six immunized calves.	Experiment 1: Reduction in egg laying capacity (9.2 %) and egg fertility (7.7 %); Experiment 2: Reduction in egg fertility (13.9 %).	Experiment 1: 14 %; Experiment 2: 36%	da Silva Vaz et al., 1998

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
rBYC	Two groups (8 calves each). i) Control group received PBS and adjuvant (Montanide 888/Marcol 52) and ii) treated group received rBYC in PBS emulsified in the same adjuvant at 10-day intervals. Calves were infested with 20,000 *R. microplus* larvae 10 days after the last inoculation.	*B. taurus* Hereford	Antibody titers from vaccinated bovines increased during the vaccination, with peak around the 50th day PPI* (challenge day). Three of the 4 immunized cattle presented antibody response with IgG titers between 1000 and 4000, indicating individual variation in the antibody levels.	Reduction in tick numbers (18.02 %), tick weight (20.21 %), egg laying capacity (1.8 %) and egg fertility (5.96 %).	25.24 %	Leal et al., 2006
RmFER2: *R. microplus* Ferritin 2	Two groups (4 animals each). i) Calves were immunized with 3 doses of recombinant RmFER2 and Montanide ISA 50 V (adjuvant) or ii) received adjuvant and saliva alone (control). Calves were infested with 10,000 larvae 2 weeks after the last inoculation.	European crossbred calves	IgG titers increased with each immunization, presenting higher levels of antibodies after the 3rd immunization (tick challenge day).	Reduction in tick infestation (30 %) and tick weight (12 %).	64 %	Hajdusek et al., 2010
RmAQP1: *R. microplus* Aquaporin 1	Two groups (6 animals each). i) Calves were immunized with recombinant RmAQP1 plus Montanide ISA 61 VG (adjuvant) or ii) injected with adjuvant and PBS alone (negative control). Cattle were immunized 3-times at 2-week intervals and infested with 15,000 larvae 3 weeks after the last inoculation. Two trials were performed: trial 1 (September to December/2010) and trial 2 (March to July/2011).	*B. taurus* Holstein	Anti-rRmAQP1 levels increased after the 1st immunization and continued to increase with the boosters. A peak was verified after larvae infestation (day 62; ± 30 days after 3rd immunization) and a subsequent slight decline was observed.	Reduction in tick number (71 %).	Trial 1: 75 %; Trial 2: 68 %.	Guerrero et al., 2014
Ha-CRT: *Hyalomma anatolicum* Calreticulin	Two groups (4 animals each). i) Cattle were immunized with recombinant Ha-CRT and Montanide ISA 50 V2 (adjuvant) or ii) inoculated with PBS (control) on days 0, 28 and 49. Calves were infested with *R. microplus* larvae (from 200 mg of eggs) 14 days after the 2nd booster.	Crossbred calves (*B. taurus* x *B. indicus*)	Antibody levels increased 10.8-times PPI*, 13.23- and 14.8-times after 2nd and 3rd immunization, respectively, compared to pre-immunized values. Antibody levels remained stable until day 119 PPI* (13.7-times) and decreased on day 139 PPI* (11.14-times higher than pre-immune).	Reduction in tick numbers (23.33 %), tick weight (14.15 %) and egg weight (18.57 %).	37.56 %	Kumar et al., 2017
Ha-CathL: *Hyalomma anatolicum* Cathepsin L-like	Two groups (4 animals each). i) Cattle were immunized with recombinant Ha-CathL and Montanide ISA 50 V2 (adjuvant) or ii) inoculated with PBS (control) on days 0, 28 and 49. Calves were infested with *R. microplus* larvae (from 200 mg of eggs) 14 days after the 2nd booster.	Crossbred calves (*B. taurus* x *B. indicus*)	Antibody levels were 6.9-times higher than pre-immune sera value PPI*, antibody levels raised to 12.45-times from pre-immune sera after 2nd immunization and were maintained until day 62 PPI*. Decrease trend was registered at the end of the trial.	Reduction in tick numbers (4.1 %), tick weight (13.1 %) and egg weight (18.87 %).	22.21 %	Kumar et al., 2017
rBYC, GST-Hl, VTDCE	Two groups (38 calves): i) Treated (18 animals)	Aberdeen Angus and Devon	Antibody titers were 6-, 10- and 2-times higher	Reduction in tick numbers (35.3–61.6	Not determined	Parizi et al., 2012

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	- Calves were vaccinated with a mixture of rBYC, rGST-HI and rVTDCE plus adjuvant (Marcol 52 and Montanide 888) and ii) Control (20 animals) - Calves were injected with PBS plus adjuvant. Animals received 3 boosters at 21-day intervals (days 22, 43 and 64).		against rGST-Hl, rVTDCE and rBYC, respectively, on day 78 than on day 1 (pre-immune sera). IgG levels against GST-Hl remained the same until the end of trial (day 127), whereas IgG titers against VTDCE decreased by half compared to day 78. Anti-BYC reached similar antibody levels from pre-immune sera on day 127.	%) on days 36–127. Greater body weight gain in the treated (39 %) than control group (25 %).		
EF1a-MSP1a: Bacterial membranes containing surface-exposed Elongation Factor 1a (EF1a) fusioned with MSP1a.	Five groups (4 animals each). Calves were inoculated with recombinant proteins adjuvated in Montanide ISA 50 V2: i) BM95-MSP1a, ii) SUB-MSP1a, iii) EF1a-MSP1a, iv) UBQ-MSP1a or v) adjuvant/saline (negative control). Cattle received the chimeras or adjuvant/saline 3-times (days 0, 30 and 60) and were infested with 5000 *R. microplus* larvae 2 weeks after the last inoculation.	Beefmaster x Charolais	Calves immunized with EF1a-MSP1 presented higher antibody levels against MSP1a peptide compared to control in all measurements. Anti-MSP1a antibody titers increased after the 1st, 2nd and 3rd immunizations (days 30, 60 and 75, respectively), with peak on day 75, but decreased after the challenge (day 103).	Reduction in tick numbers (38 %).	38 %	Almazán et al., 2012
Rm39 (glycine-rich protein), **Rm239** (metalloprotease), **Rm76** (IGBP) **and Rm180** (serine protease inhibitor).	Two groups (4 animals each). i) Bovines were vaccinated with the 4 recombinant proteins plus an aluminium hydroxide adjuvant in separate injections 3-times at 3-week intervals (days 0, 21 and 42); ii) Cattle from control group were injected with saline and adjuvant. Two weeks after the last immunization, animals were challenged with 10,000 *R. microplus* larvae.	*B. taurus* Hereford	Rm239 and Rm76 elicited high levels of IgG1 and IgG2 antibodies, respectively. Anti-Rm239 IgG1 levels presented a pick 1 week after immunizations (day 49) and a subsequent decline. Antibody levels increased 2 days after larvae challenge (day 57) and remained stable until the end of infestation. IgG2 levels against Rm76 reached higher titers on day 55 (larvae challenge) and declined 2 days later. Anti-Rm76 IgG2 levels increased again after infestation with adult ticks (day 72).	Reduction in tick numbers (525 %) and tick weight (552 %).	73.2 %	Maruyama et al., 2017
SILK	Calves were divided in 3 groups (3 animals each): i) immunized with recombinant SILK and Montanide ISA 50 V (adjuvant) on days 0, 28 and 49; ii) injected with adjuvant/saline (placebo) and iii) untreated. All calves were infested with *R. microplus* larvae (three cells with 500 larvae each) on days 72, 75 and 77. Calves (except	Crossbred calves	Levels of antigen-specific antibodies increased right after the 1st immunization, as well as increased with each immunization (until day 69) and remained stable from day 69 until day 104 (end of the experiment).	Reduction in tick numbers (58 %) and oviposition (9 %). DNA levels for *A. marginale* were lower in ticks fed in vaccinated cattle compared to controls.	62 %	Merino et al., 2013

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	untreated) were also infected with *Anaplasma marginale* and *Babesia bigemina* on days 69 and 92, respectively.					
R. australis						
Bm86 (TickGard^{PLUS})	The experiment of immunization with TickGard^{PLUS} was performed from August 1997 to February 1998. Cattle were divided in 4 groups (10 animals each): i) Vaccinated cattle that returned immediately to pasture after morning milking; ii) vaccinated cattle confined for 2 h after morning milking; iii) unvaccinated cattle that returned immediately to pasture after morning milking (placebo); iv) unvaccinated cattle confined for 2 h after morning milking (placebo). Cattle were immunized on 16 September, 14 October, and 22 December 1997 and challenged with 2500 larvae on 12, 19, 26 August, 2 September, and 28 October 1997.	Holstein	Anti-Bm86 antibodies increased 2 weeks after the 2nd immunization. Anti-Bm86 titers in the 8th week (after the 3rd immunization) was lower than 2 weeks PPI *.	TickGard^{PLUS}-immunized cattle gained more weight (52.5 kg) than placebo groups (33.9 kg). Reduction in hatchability and tick numbers in the vaccinated groups.	Not determined	Jonsson et al., 2000
Bm86 (TickGard^{TM})	Calves were divided in 2 groups (2 animals each): i) vaccinated and ii) control. Cattle were immunized with TickGard^{TM} and, 4 weeks later, received a booster (6 weeks before tick infestation). Calves were infested with 1500 larvae/animal.		High anti-Bm86 titers were detected in all vaccinated animals.	Reduction in tick numbers, tick weight and weight of eggs/gram of ticks, leading to a reduction of 74 % in egg laying.	Not determined	de Vos et al., 2001
Bm86	Sixteen Charolais cattle were infested with *R. australis* prior to the experiment for the cattle would reach the same level of naturally acquired immunity. Animals were divided in 2 groups: i) immunized with rBm86 produced in *E. coli* emulsified in Montanide^{TM} ISA 61 (adjuvant) or ii) injected with adjuvant only. Cattle received the injections on days 0 and 28 and were infested with 1000 larvae on days 39, 41, 43, 53, 55 and 57.	Charolais cattle	A strong anti-Bm86 antibody response was detected 14 days after the 1st immunization. Antibody levels increased after the 2nd immunization (day 42) and remained high. A slight apparent decline was observed from 70th day.	Reduction in tick numbers (51.2 %), tick weight (35 %), egg laying per tick (51.2 %) and fertility (18.8 %).	74 %	Hüe et al., 2017
Bm91	Two fractions (GF4 and GF5,6) from crude membrane material of adult ticks, containing a 86 kDa protein (Bm91) were obtained by		Sera from GF4 or GF5,6 or Bm91-vaccinated cattle recognized recombinant Bm91 in all trials.	Reduction in tick numbers, tick weight in all trials. Decrease of egg weight/tick weight in trials 1 and 3	Not determined	Riding et al., 1994

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Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	different protocols. Three trials were carried out. Trial 1- 9 animals (3 animals/ group) were immunized with GF4 and GF5,6 fractions plus Quil A (adjuvant) or adjuvant only; Trial 2- 4 animals (2 per group) were immunized with GF5,6 plus Quil A or adjuvant only; Trial 3 - 6 animals (3 per group) received Bm91 plus CFA (1st dose) and Bm91 plus IFA (2nd dose) or were not vaccinated (control). Two weeks after the last immunization, cattle were challenged with 1000 larvae/day for 21 days.			(not determined in trial 2).		
Bm86 e Bm91	All vaccine formulations were adjuvated in Montanide 888 (Seppic) and Marcol 52. First and 2nd immunizations were injected on different sides of the neck. Trial 1 - 100 ug of each antigen (residues 229−621 of Bm91 and Bm86). Trial 2 - 100 ug of each antigen (amino acids 59−621 of Bm91 and Bm86). Trial 3 - Bm86 and Bm91 (residues 30−640) were inoculated separately into each bovine. In all trials 6 animals were vaccinated with Bm86 only, 5−7 animals with Bm86 and Bm91 and 3 were not vaccinated. Cattle were infested with 1000 larvae (Yeerongpilly strain tick).	*B. taurus* (Hereford)	Cattle vaccinated with both antigens presented anti-Bm86 and Bm91 antibodies (mean log titers of 3.46 and 3.82, respectively). They presented anti-Bm86 antibody levels in similar proportion to vaccinated with Bm86 alone.	Anti-Bm86 were the major antibodies responsible for vaccine efficacy in cattle vaccinated with both antigens, but anti-Bm91 antibodies have shown to contribute to reducing the weight of eggs laid.	Not determined	Willadsen et al., 1996
BMA7	Three trials: Trial 1 - Cattle were divided in 2 groups: vaccinated and control (3 animals each). Cattle were vaccinated with native BMA7 plus CFA as adjuvant (1st immunization) and BMA7 plus IFA as adjuvant (2nd immunization). Trial 2 -Two groups: vaccinated and control (4 animals each). Cattle were immunized with native BMA7 plus Montanide ISA 70 (adjuvant).		Trials 1 and 2 - Anti-BMA7 antibody levels were higher in vaccinated animals with reduced oviposition per day, indicating correlation between egg yield and anti-BMA7 titers.	Trials 1 and 2 - Three of the 7 bovines showed reduction in egg weight/day (there was no statistical difference between the 2 trials).	Not determined	McKenna et al., 1998
BMA7 and Bm86	Twelve cattle were divided in 2 groups (6 animals each) and were		Animals vaccinated with Bm86 and BMA7 produced anti-BMA7	Reduction in egg weight/day compared	Efficacy of BMA7+Bm86 was ±	McKenna et al., 1998

(*continued on next page*)

Table 1 (*continued*)

Antigen	Immunization protocol	Host	Humoral response	Biological parameters	Overall eficacy	Reference
	immunized with Montanide ISA 70 (adjuvant) plus i) Bm86 alone or ii) Bm86 and BMA7. Control group was the same of the trial 2 above (4 animals). Animals were immunized with 2 doses at 4 weeks-interval and infested 2 weeks after the last immunization with 1000 larvae/day for 3 weeks.		antibody levels against native and recombinant BMA7. The mean of anti-Bm86 antibody titers was higher in animals immunized with Bm86 alone than immunized with both antigens, but it was not statistically significant.	to cattle immunized with Bm86 only.	twice the efficacy of Bm86 alone.	
5′ nucleotidase; 5′ nucleotidase and Bm86	Cattle were divided in 4 groups (4 animals each), which were immunized as follows: i) 100 μg of recombinant Bm86; ii) 100 μg of recombinant 5′ nucleotidase; ii) recombinant Bm86 and recombinant 5′ nucleotidase (100 μg of each); iv) adjuvant alone (control). The vaccines were formulated using ISA50 or QuilA as adjuvant. The animals were vaccinated 3-times at 1-month interval and challenged with 1000 *R. microplus* larvae/day for 21 days (1 week after the 3rd immunization). Six sheep were also immunized with r5'nucleotidase (with ISA50 or ISA773 as adjuvant) and 2 control groups received adjuvant alone.	*B. taurus* (Hereford x Angus)	Vaccination with 5′nucleotidase alone induced a protective immune response in sheep but failed to do the same in cattle. Differences in antibody levels were not likely to explain this dissemblance. Vaccination with Bm86 induced protection levels like those already reported previously. Combination of both antigens did not produce any increase in the protection parameters compared to immunization with Bm86 alone.	5′ nucleotidase: No effect. 5' nucleotidase + Bm86: Reduction in tick numbers, tick weight, fecundity, and reproductive potential.	5′ nucleotidase: 0%; 5' nucleotidase + Bm86: Efficacy was ± half than obtained with Bm86 (81 %)	Hope et al., 2010

* PPI: post-primary immunization.

protocol may actually bring under variable field conditions, as it has been shown to range from a few to thousands of adult females per host and many times changing markedly over seasons (Seifert, 1971; Lima et al., 2000; Martins et al., 2002; de Clercq et al., 2013; Ferraz da Costa et al., 2014; Reck et al., 2014; Nicaretta et al., 2020).

3. Ticks and antibodies: how do they duel within the parasite?

Antibodies ingested from blood meals can cross the midgut of *R. microplus* and keep binding activity within the adult female (da Silva Vaz et al., 1996). On the other hand, it is reported the presence of an active process of binding and carrying antibodies within ticks performed by Immunoglobulin Binding Proteins (IGBPs), which are able to sequester IgG from the midgut, transport them into the hemolymph to the salivary glands and reintroduce them into the host via saliva (Wang and Nuttall, 1994, 1995a, 1999). IGBPs seem to be widespread in ticks and present different affinities concerning IgGs from different host species (Wang and Nuttall, 1995b; Gong et al., 2014) as well as being part of the "mating care" performed by males in the behavior of feeding/salivating close to adult females (Wang et al., 1998). Indeed, higher presence of IGBPs seems to be related to the preparation for the adult female rapid engorgement phase (Gong et al., 2014). Poorly evaluated in other ticks, IgE binding was demonstrated by an IGBP from *R. appendiculatus* and claimed for use in therapies for hypersensitivity I (Wang and Nuttall, 2013). In relation specifically to *R. microplus*, the analysis of transcriptomes of different developmental stages indicated the presence of 26 putative IGBPs (Garcia et al., 2020). They were shown to be highly expressed in males and differences in bovine IgG allotypes influencing the binding affinities with IGBPs are suggested to play a role in the variable levels of tick resistance developed by bovines following infestations (Carvalho et al., 2011; Garcia et al., 2020). Furthermore, males allowed to feed on *B. indicus* hosts presented higher levels of IGBPs transcription in salivary glands than those feeding on *B. taurus* (Garcia et al., 2020). It has been also shown that successfully infesting larvae present higher expression of five IGBPs when feeding on *B. indicus* comparing to unfed and frustrated larvae (Rodríguez-Valle et al., 2010). With the aim to overpass the evasion of the antibody response, a putative IGBP was included in a cocktail immunization protocol which resulted in an overall protection of 73,2 % when challenged with 10,000 larvae (Maruyama et al., 2017). Additionally, *Anaplasma marginale* infection in males down regulated IGBP expression (Zivkovic et al., 2010), pointing to a possible interaction of these proteins with the arthropod-pathogen/bacteriome relationship. Another *R. microplus* protein capable of binding IgG is paramyosin (Ferreira et al., 2002) and, although it has been described primarily as a muscle protein, it is widely present in adult tissues, being highly expressed in fat body and gut,

therefore presenting characteristics consistent with roles such as IgG clearance (Leal et al., 2013). This continuous delivery of IgG back to the host may increase their local concentration and influence immune responses by antibody feedback (Zhang et al., 2013; Xu et al., 2018), what would help to explain the presence of this transport system apparatus. In this context, Fcγ receptors may be one of the targets of the IgG enriched saliva, highlighting a possible modulation of FcRn functions. FcRn is present in multiple cell types and is responsible for IgG recycling, as well as participates in the stimulation of IgG-immune complex mediated immune responses (Schneider et al., 2015; Challa et al., 2019; Toh et al., 2019). Interestingly, FcRn also participates in albumin recycling (Leblanc et al., 2019; Toh et al., 2019), and ticks seem to also deliver albumin back into the host via saliva (Tirloni et al., 2014, 2015; Kim et al., 2016). Therefore, it can be inferred that host proteins may themselves be used by ticks in order to circumvent protective immune responses, or at least the humoral IgG-mediated response.

4. Artificial immunization and protection against ticks: what is the scenario and how much are antibodies involved?

The first vaccines against ectoparasites were alleged based on the development of specific antibodies against the *R. microplus* midgut glycoprotein BM86, which were shown to inhibit endocytosis, damage midgut cells, with the possible involvement of the complement system (Kemp et al., 1989; Willadsen et al., 1989; Tellam et al., 1992), and interfere *in vivo* with feeding and progeny viability (Rodríguez et al., 1995a; Willadsen et al., 1995; Canales et al., 1997; Rodríguez-Valle et al., 2004). The vaccines TickGARD and GAVAC were able to significatively control *R. microplus* populations but showed variability in their effectiveness according to the locality and tick strains. The presence of variations when comparing the Bm86 sequences of tick species and strains seems to explain these differences, which prevent the already commercialized vaccines from becoming universal (García-García et al., 1999; Blecha et al., 2018). Additionally, crosses between tick populations, phylogenetic and taxonomic analyzes indicated that the Australia's Yeerongpilly strain (previously considered as *R. microplus*), which was used to produce the TickGARD vaccine, could belong to a different species, later renamed as *R. australis* (Labruna et al., 2009; Estrada-Peña et al., 2012). In this regard, additional analyses comparing different tick isolates and based on mitochondrial genetic markers recognize five closely related genetic clades, being three clades corresponding to *R. microplus* sequences, one clade to *R. annulatus* and one clade to *R. australis* (Burger et al., 2014; Low et al., 2015). Potential improvements departing from the Bm86-based vaccines have been evaluated specially against *R. microplus*, including associations with other known and/or new potential antigens. A compilation of studies using known molecules able to elicit specific antibodies following immunization and that resulted in protection of cattle at some degree against *R. microplus* is shown in Table 1. It can be easily seen the great variation of anti-tick expression of resistance comparing the different analyses, although it must be emphasized that most vaccination/tick challenge trials followed different protocols, what turns into a risk direct comparisons. The twenty-two tick antigens present in the publications compiled in Table 1 were also categorized in Table 2 based on their characteristics, in order to evaluate and compare the profiles of the molecules that successfully induced protective anti-*R. microplus* immune responses in cattle.

Antigens can be divided into "concealed" (which do not elicit a host immune response during a natural infestation or do not come into contact with the host's immune system) and "exposed" (which elicit a response from the host's immune system). The term "concealed" was coined originally to indicate that the lack of the immune response was related to the antigen location within the parasite. Concealed antigens, such as Bm86, are not secreted during feeding and therefore the host's immune system does not produce antibodies against these proteins during a tick infestation. However, after the immunization with a concealed antigen, specific antibodies can be produced against the target protein, enabling the ingest of protective host antibodies during tick feeding (Willadsen and Kemp, 1988; Tellam et al., 1992). The possible reduction in the levels of specific antibodies after the immunizations with concealed antigens can be a disadvantage, as it may demand additional vaccine boosters. This limitation theoretically is not faced when using exposed antigens, as tick infestations themselves could serve as boosters, increasing the levels of antibodies with each infestation (Nuttall et al., 2006; Maruyama et al., 2017). Additionally, there are molecules that may be classified as 'dual', since they share characteristics of both exposed and concealed antigens, what may provide a higher probability of harming more efficiently the tick as well as naturally boost the immune system with salivation (Nuttall et al., 2006). Table 2 shows that 17 of the 22 antigens evaluated against *R. microplus* could be putatively classified as dual or concealed, as only five could be clearly classified as exposed. The presence of functional anti-tick cattle antibodies in the hemolymph of ticks fed on immunized cattle (da Silva Vaz et al., 1996) indicated the capability of these antibodies to reach antigens present in internal organs and tissues (da Silva Vaz et al., 1998; Hajdusek et al., 2010; Seixas et al., 2012), reinforcing the search for protective concealed or dual antigens. Furthermore, the anti-tick efficacy following subolesin immunization, an intracellular protein, was suggested to be explained by internalization of antibodies within arthropod cells, enabling intracellular neutralization and lowering subolesin mRNA levels (de la Fuente et al., 2011). In this sense, some proteins considered as intracellular and without containing a signal peptide such as ribosomal proteins, GAPDH, heat shock proteins, ubiquitin, enolase, cytoskeletal proteins (for instance, actin and paramyosin), among others, have also been found in tick saliva (Díaz-Martín et al., 2013; Tirloni et al., 2014, 2015, 2017; Kim et al., 2020). So, these molecules seem to be secreted into the extracellular environment by a non-classical pathway (Zhan et al., 2009; Aguilera et al., 2012; Nawaz

Table 2
Overview of the general characteristics of 22 molecules used in cattle vaccination trials against *Rhipicephalus microplus*.

Categories	Number of molecules	References
Putatively concealed	7	Willadsen et al., 1996;
Putatively exposed	5	McKenna et al., 1998;
Putatively "dual"	10	Andreotti et al., 2002;
Proteins used in associated form (cocktail)	13	Patarroyo et al., 2002;
Proteins mostly associated with adult gut	9	Hajdusek et al., 2010;
Secreted proteins	16	Almazán et al., 2012;
Proteins with housekeeping functions	5	Parizi et al., 2012;
Proteins involved in the modulation of the host immune system and/or homeostasis	8	Seixas et al., 2012; Díaz-Martín et al., 2013;
Proteins mostly associated with oogenesis, egg and larvae	7	Merino et al., 2013;
Proteins associated with detoxofication	1	Ali et al., 2014; Guerrero et al., 2014; Radulović et al., 2014; Tirloni et al., 2014; Richards et al., 2015; Rodríguez-Mallon et al., 2015;
Proteins putatively involved in tick-adhesion	2	Tirloni et al., 2015; Kumar et al., 2017; Maruyama et al., 2017; Tirloni et al., 2017; Csordas et al., 2018; Kim et al., 2020; Ortega-Sánchez et al., 2020

Twenty-two different *R. microplus* molecules associated with humoral responses in cattle vaccination trials were distributed into 11 categories related to their tissue distribution and characteristics, according to the available literature. The categorization in "secreted proteins" and "putatively concealed, exposed or dual" was also performed based on the analysis of published tick saliva proteomes. Each protein may have been included in more than one category.

et al., 2020a) and present a role in the parasite-host interface unrelated to their already known intracellular roles (Kim et al., 2020). But the presence of such moonlighting proteins (proteins that perform different roles within an organism) is not unexpected. Moonlighting proteins are ubiquitous among prokaryotes and eukaryotes, are a common finding relating to pathogens virulence, and most of them are defined canonically as housekeeping proteins (Jeffery, 1999; Franco-Serrano et al., 2018; Liu and Jeffery, 2020; Singh and Bhalla, 2020). Concerning ticks, on the other hand, it was raised the possibility that the high content of these proteins in tick saliva could be influenced by artificial salivation methods (Mans et al., 2016). Although moonlighting proteins are mostly identified originally as intracellular and do not present secretion motifs or signal peptides, they can be found outside the cells (Franco-Serrano et al., 2018; Liu and Jeffery, 2020), and many different unconventional protein secretion mechanisms have been described that could possibly explain their secretion without direct involvement with the endoplasmic reticulum and Golgi apparatus (Dimou and Nickel, 2018; Cohen et al., 2020). The presence of these proteins in tick saliva may be explained by apocrine secretion (Shaw and Young, 1995). Additionally, extracellular vesicles were demonstrated to be present in tick saliva, being able to participate in host immune modulation and interfere with pathogen transmission (Zhou et al., 2018; Nawaz et al., 2020a; Oliva Chávez et al., 2021). Furthermore, they were shown to present a diverse array of proteins, including housekeeping and host proteins (Nawaz et al., 2020a), as well as microRNAs (Nawaz et al., 2020b), what seems to occur in several other parasite-host relationships (Sultana and Neelakanta, 2020; Khosravi et al., 2020). Housekeeping proteins are highly conserved and their use as vaccine antigens has been under debate, as they can lead to the production of autoantibodies in immunized animals (Canales et al., 2009a; Rodríguez-Mallon et al., 2012; Franco-Serrano et al., 2018; Kim et al., 2020). However, the strategy employed in the case of the ribosomal protein P0, which is highly conserved in vertebrate hosts, involved the selection of an immunogenic and specific portion of 20 amino acids with low similarity with vertebrates, resulting an overall protection of 96 % against *R. microplus* in vaccinated cattle (Rodríguez-Mallon et al., 2015). Other housekeeping antigens have also shown some degree of efficacy against *R. microplus* without an autoimmune response in the immunized hosts (Almazán et al., 2010; Kumar et al., 2017). Therefore, even conserved proteins represent an open window of opportunities for the development of safe vaccines.

Concerning exposed antigens, Narasimhan et al. (2020) showed that Guinea pigs immunized with *I. scapularis* saliva presented high levels of anti-saliva antibodies, erythema at the bite site and reduced female recovery when compared to the control group and suggested that saliva immunization was sufficient to elicit the resistance phenotype in Guinea pigs challenged with *I. scapularis*. In *R. microplus*, sera from repeatedly infested bovines were able to neutralize anti-thrombotic and host endothelium activation activities of tick saliva (Reck et al., 2009). A cocktail vaccine containing four salivary antigens identified from a sialotranscriptome of upregulated proteins from ticks fed on Holsteins (susceptible cattle) elicited specific IgG antibodies after the immunizations. The antigens correspond putatively for a metalloprotease, a proteinase inhibitor, an immunoglobulin-binding protein, and a glycine-rich protein, and were named, respectively, as Rm239, Rm180, Rm76, and Rm39. The antibody levels against two of them (anti-Rm76 and anti-Rm239) increased after the infestation with adult *R. microplus*, suggesting that the secretion of these proteins during the tick challenge acted as a booster (Maruyama et al., 2017; Tabor, 2018). Perner et al. (2020) showed that secreted metalloproteases of *I. ricinus* are the main target of antibody responses in rabbits, which when treated with specific inhibitors impaired the tick development while feeding on susceptible hosts but failing to do so when evaluated in an artificial feeding apparatus with either inhibitors or antibodies. These results emphasize the caution that must be taken in extrapolating results from artificial conditions to the actual parasite life cycle in its full complexity. The cattle vaccination with a metalloprotease (rBrRm-MP4) of *R. microplus* was also able to elicit antibodies and to maintain the antibody titers until the end of infestation, showing an overall protective efficacy of 60 % (Ali et al., 2015). Indeed, the gene silencing of two putative secreted metalloproteases from *R. microplus* also showed to diminish egg weight and oviposition (Barnard et al., 2012). On the other hand, it must be emphasized that salivary antigens can be constantly secreted during parasitism and essential molecules may have undergone selective pressures to remain concealed from the host's immune system (Opdebeeck, 1994; Mulenga et al., 1999). Strategies such as exposing proteins similar to host molecules or exposing highly immunogenic protein regions while hiding the most important regions for protein function were described (Bishop et al., 2002; Havlíková et al., 2009). Additionally, ticks are suggested to present higher rates of gene duplication for secreted proteins, what helps to explain the origin for the functionally redundant paralogous proteins displayed in saliva (Mans et al., 2017). The presence of this myriad of salivary proteins enables ticks to alternate the expression of certain genes throughout the feeding period (Maruyama et al., 2010; Bullard et al., 2016; Tirloni et al., 2020), providing an antigenic shift to be recognized by the host immune system (Valenzuela et al., 2002; Chmelař et al., 2016; Ribeiro and Mans, 2020). This sialome switch has been described at diverse tick-host relationships (Karim and Ribeiro, 2015; Kim et al., 2016; Tirloni et al., 2017; Narasimhan et al., 2019; Tirloni et al., 2020; Kim et al., 2020) and its context has been further expanded as saliva content may also differ when ticks face different hosts (Tirloni et al., 2017; Narasimhan et al., 2019) or different host's immune status (Perner et al., 2018). Therefore, ticks may present different means to keep essential physiological processes out of the reach of the host's immune system.

Due to the difficulty in identifying a target that possess all the desired characteristics for a protective anti-tick vaccine, such as the production of long-lasting antibodies, broad spectrum, viable cost and application mode, it is suggested the development of vaccines containing a cocktail of antigens (Ndawula and Tabor, 2020). The blending of molecules has been carried out in cattle vaccination trials against *R. microplus* and most of them were based on antigens that already presented a protective potential when tested individually (see Table 1). The combinations of Bm86 and Bm91 (Willadsen et al., 1996), Bm86 and BMA7 (McKenna et al., 1998) and, later, the association of VTDCE, BYC and Hl-GST (Parizi et al., 2012), which indicated greater body weight gain in vaccinated cattle compared to the control group, suggest advantages with the use of a cocktail vaccine. On the other hand, Parizi et al. (2012) showed that sera from immunized cattle presented higher levels of antibodies against Hl-GST compared to BYC and VTDCE. Similarly, the combination of *R. appendiculatus* antigens subolesin and 64TRP with the *Theileria parva* sporozoite antigen p67C resulted in high levels of antibodies against subolesin, but very low levels against p67C (Olds et al., 2016), indicating that antigenic competition can reduce the effectiveness of antigen combinations. The already mentioned cocktail vaccine containing four salivary antigens of *R. microplus* induced a protection of 73.2 %, but only two of them elicited most of the IgG response (Maruyama et al., 2017). In another case, sera from rabbits immunized with *R. appendiculatus*, *R. decoloratus*, *R. microplus*, *Amblyomma variegatum* and *Haemaphysalis longicornis* GSTs cocktail showed lower antibody titers against GSTs of different tick species when compared to sera from rabbits immunized only with *R. decolatus* or *A. variegatum* GSTs (Ndawula et al., 2019). Indeed, in Table 2 we can see that 13 out of 22 molecules tested against *R. microplus* in cattle were already evaluated in mixed formulations, but with variable results, not always additive (see Table 1). Although these results indicate the feasibility of using mixed heterologous antigens as components of multispecies anti-tick vaccines, strategies must be designed to surpass eventual antigen competition negative effects.

Antigens with epitopes conserved among different species or strains of ticks have also been studied to develop a broad-spectrum vaccine, such as glutathione S-transferases (GST) (Parizi et al., 2011; Ndawula et al., 2019), cystatins (Parizi et al., 2020), subolesin (SUB) (Hassan

et al., 2020; Kasaija et al., 2020), Bm86 (Fragoso et al., 1998; Vos et al., 2001; Kopp et al., 2009; Rodríguez-Valle et al., 2012) and a cocktail containing subolesin (SUB), calreticulin (CRT) and cathepsin L-like cysteine (CathL) (Kumar et al., 2017). Indeed, cross-protection against *R. microplus* infestation was demonstrated in cattle vaccination with Ba86 (*R. annulatus* Bm86) (Canales et al., 2009b), *H. longicornis* GST (Parizi et al., 2011) as well as with *Hyalomma anatolicum* SUB-CRT-CathL cocktail (Kumar et al., 2017).

The search for antigens that elicit high levels of specific antibodies against ticks usually evaluates possible correlations of antibody titers with tick biological parameters, such as number of ticks that complete the parasitic phase, engorged weight, oviposition, and hatching. Positive correlations may indicate that the overall antibody titers should be responsible for positive outcomes found in vaccination trials (Parizi et al., 2012; Contreras and de la Fuente, 2016; Kasaija et al., 2020). But, as pointed before, it may not be always the case (Knorr et al., 2018; Almazán et al., 2020). These apparent contradictions may be at least partly explained by the presence of immunodominant regions within antigens. For instance, the protein 64 P from *R. appendiculatus* presented protection against tick infestations, but when truncated forms of the protein were tested, the most immunogenic recombinant forms failed to induce protection (Trimnell et al., 2002). Analogously, a 5′-nucleotidase of *R. microplus*, when tested in sheep and cattle, showed to induce protection when inoculated in sheep, what was correlated with the antibody titers, but failed to do so in cattle. A competitive ELISA analysis suggests that most of the antibodies generated by sheep and cattle recognize different parts of the protein (Hope et al., 2010). Therefore, different regions of an antigen may bring different outcomes when targeted by antibodies, and not necessarily the overall antibody titer against the full protein may reflect it, an issue that should be evaluated when testing antigens in immunization protocols, especially those particularly aiming the neutralization of specific functions in the tick physiology.

5. Conclusion

The antibody response still brings some surprises in the tick-host relationship. On one hand it is recognized as responsible for the effectivity of the first commercial vaccines against ectoparasites. On the other hand, it may sometimes be associated with an opposite effect. This apparent contradiction can be explained by the complexity of the race of arms disputed and under constant mutual challenge by these organisms. The protocols of immunization described in the literature show great heterogeneity and provides several alternatives to project how to reduce infestations, but also with the limitation that no one has ever been able to completely demonstrate the killing of a tick generation or fully abrogate its progeny development. Therefore, different strategies must be taken into action by ticks in order to circumvent even protective immune responses, which includes host molecules themselves as can be easily exemplified by IGBPs. Additionally, parasite density clearly affects some tick induced immune responses that may be as contrasting as departing from a protective response turning into a naïve-like condition. Molecular and physiological adjustments may be taking place in the determination of parasite survival, or not, and the development of immune strategies to effectively imper the tick parasitic cycle in all conditions faced in real life must deal with all these aspects. A special concern must be taken for the humoral response to be produced, as antibodies represent the most common vaccination-induced immune weapon evaluated. Moreover, the standardization of some parameters used in tick vaccine development, as proposed previously (Schetters et al., 2016), would facilitate data comparisons and increase clarity of antigens immunization efficacy. Ultimately, more refinement may be necessary to effectively design a cocktail vaccine with tick-derived molecules, which may be needed to be modified to optimize protective responses, and combined in non-competing immune contexts, in order to give a step ahead in herds protection.

Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – [Finance Code 001], INCT-Entomologia Molecular, CNPq and FAPERGS.

CRediT authorship contribution statement

Bruna Ferreira Leal: Data curation, Writing – original draft, Writing – review & editing, Visualization. **Carlos Alexandre Sanchez Ferreira:** Conceptualization, Data curation, Writing – original draft, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgements

BFL received a scholarship from CAPES, Brasil.

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