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Vaccination with proteins involved in tick–pathogen interactions reduces vector infestations and pathogen infection

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ABSTRACT

Tick-borne pathogens cause diseases that greatly impact animal health and production worldwide. The ultimate goal of tick vaccines is to protect against tick-borne diseases through the control of vector infestations and reducing pathogen infection and transmission. Tick genetic traits are involved in vector–pathogen interactions and some of these molecules such as Subolesin (SUB) have been shown to protect against vector infestations and pathogen infection. Based on these premises, herein we characterized the efficacy of cattle vaccination with tick proteins involved in vector–pathogen interactions, TROSPA, SILK, and Q38 for the control of cattle tick, *Rhipicephalus (Boophilus) microplus* infestations and infection with *Anaplasma marginale* and *Babesia bigemina*. SUB and adjuvant/saline placebo were used as positive and negative controls, respectively. The results showed that vaccination with Q38, SILK and SUB reduced tick infestations and oviposition with vaccine efficacies of 75% (Q38), 62% (SILK) and 60% (SUB) with respect to ticks fed on placebo control cattle. Vaccination with TROSPA did not have a significant effect on any of the tick parameters analyzed. The results also showed that vaccination with Q38, TROSPA and SUB reduced *B. bigemina* DNA levels in ticks while vaccination with SILK and SUB resulted in lower *A. marginal*e DNA levels when compared to ticks fed on placebo control cattle. The positive correlation between antigen-specific antibody titers and reduction of tick infestations and pathogen infection strongly suggested that the effect of the vaccine was the result of the antibody response in vaccinated cattle. Vaccination and co-infection with *A. marginale* and *B. bigemina* also affected the expression of genes encoding for vaccine antigens in ticks fed on cattle. These results showed that vaccines using tick proteins involved in vector–pathogen interactions could be used for the dual control of tick infestations and pathogen infection.

1. Introduction

Ticks greatly impact human and animal health and are considered the most important vectors of pathogens that cause disease in cattle [1,2]. The cattle ticks, *Rhipicephalus (Boophilus) microplus*, are distributed in tropical and subtropical regions of the world, where they economically impact cattle industry by reducing weight gain and milk production, and by transmitting pathogens that cause babesiosis (protozoal piroplasms *Babesia bovis* and *Babesia bigemina*) and anaplasmosis (bacterium *Anaplasma marginale*) [2–7].

The development of tick vaccines with the dual effect of reducing tick infestations and the incidence of tick-borne diseases such as bovine anaplasmosis and babesiosis while minimizing acaricide applications is essential toward improving cattle health and production in tropical and subtropical regions of the world [8–10]. Tick vaccines based on *R. microplus* BM86/BM95 antigens have proven their efficacy for control of cattle tick infestations and the prevalence of tick-borne pathogens in some regions [11,12]. However, these vaccines do not affect tick vector capacity and new vaccines are required to improve the control of tick infestation and the infection and transmission of tick-borne pathogens [13]. Previously,

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Labuda et al. [14] showed that a tick vaccine containing the *Rhipicephalus appendiculatus* tick cement protein 64P protected mice against tick infestations and tick-borne encephalitis virus (TBEV) transmitted by infected *Ixodes ricinus* ticks. Recently, cattle vaccinated with Subolesin (SUB), a protein involved in vector–pathogen interactions [10,15,16], resulted in reduced *R. microplus* infestations and pathogen levels for two different cattle tick-borne pathogens, *A. marginale* (Rickettsiales: Anaplasmataceae) and *B. bigemina* (Piroplasmida: Babesiidae) [17,18]. These results suggested that it is possible to reduce tick infestations while affecting tick vector capacity and prompted us to select tick proteins involved in tick–*Anaplasma* (SILK [15,19]) and tick–*Babesia* (TROSPA [15,20]) interactions to characterize their efficacy as vaccines for the control of cattle tick infestations in cattle and pathogen levels for *A. marginale* and *B. bigemina* in ticks.

2. Materials and methods

2.1. Experimental design

The experimental design for this study is described in Fig. 1. This study was carried out in strict accordance with the Guide for Care and Use of Laboratory Animals for the University of Queretaro and the protocol was approved by the Committee on the Ethics of Animal Experiments (Permit no.: 23FCN2012).

2.2. Ticks

The *R. microplus* ticks (Acaricide-susceptible and *Anaplasma* and *Babesia* free Media Joya strain, CENAPA, Mexico [21–23]) were obtained from a laboratory colony maintained at the University of Queretaro, Mexico. Originally, these ticks were collected from infested cattle in Tapalpa, Jalisco, Mexico. Tick larvae were fed on cattle and collected after repletion to allow for oviposition and hatching in humidity chambers at 12 h light:12 h dark photoperiod, 22–25 °C and 95% relative humidity. Larvae were used for infestations at 15 days after hatching from eggs.

2.3. Cattle vaccination

Recombinant *R. microplus* TROSPA, SILK, SUB and Q38 chimera (Genbank accession numbers JK489429, GO496219, GQ456170 and JX193856, respectively) were expressed in *Escherichia coli* from synthetic genes optimized for codon usage in *E. coli* and purified by Ni affinity chromatography to 80–90% purity. Protein adjuvation was done by mixing a solution of anhydromannitoletheroctodecenoate (Montanide ISA 50 V; Seppic, Paris, France) with the recombinant protein solution in batch-by-batch processes using a high-speed mixer Heidolph DIAX 900 (Heidolph Elektro, Kelheim, Germany) at 8000 rpm and the vaccine was filled manually under sterile conditions in glass bottles of 20 ml (Wheaton, Millville, NJ, USA) at a concentration of 100 μg/2 ml dose. Quality controls were made by testing mechanical and thermal stability of vaccine emulsions as described previously [24]. Calves were immunized with 3 doses (days 0, 28 and 49) containing 100 μg/dose of purified recombinant proteins formulated as described above. Negative controls were injected with adjuvant/saline alone (placebo) or left untreated. Cattle were injected intramuscularly with 2 ml/dose using a 5 ml syringe and an 18G needle.

2.4. Tick infestation, data collection and analysis

On days 23, 26 and 28 after the last immunization (days 72, 75 and 77), cattle in vaccinated, placebo and untreated control groups were infested with *R. microplus* larvae. Three tick infestations treatments were evaluated on each animal in individual cells glued on the back of the calf. The cells were infested with 500 larvae each. Adult engorged female ticks dropping from cattle were daily collected, counted and weighted. All the collected adult female ticks were assessed for oviposition [21]. The personnel collecting the ticks were 'blinded' as to which group animals belonged. The effect of each treatment on cattle tick infestations was evaluated employing the formulae used before in tick vaccine experiments [21]. The average ± S.D. for adult female tick number, weight (mg), and oviposition (egg weight (mg)/tick) were calculated and compared between vaccinated and placebo control cattle by

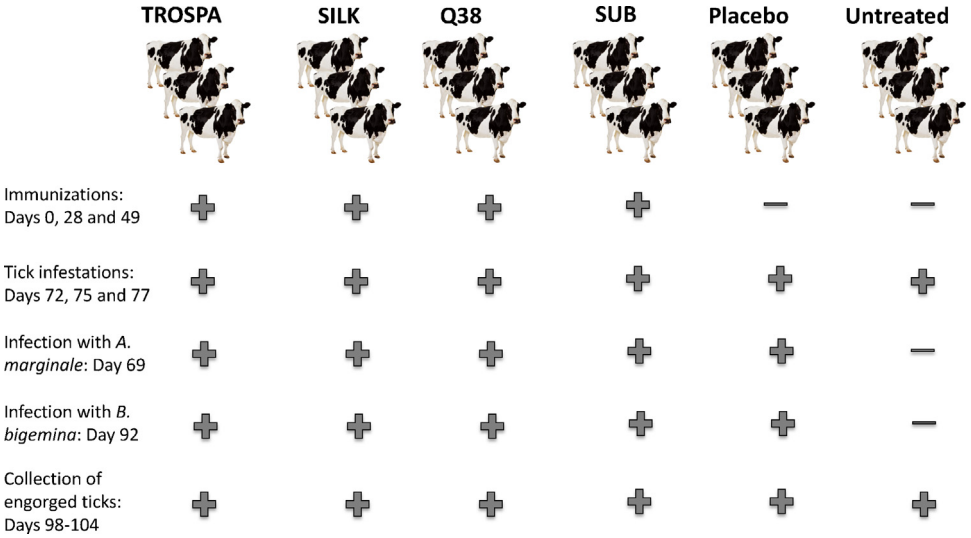

Fig. 1. Experimental design. Seven-month-old crossbred *Anaplasma* and *Babesia* free calves were purchased from a tick-free area and randomly assigned to 6 experimental groups of 3 animals each: vaccinated with TROSPA, vaccinated with SILK, vaccinated with Q38, vaccinated with SUB (positive control), injected with adjuvant/saline alone (placebo) and untreated (uninfected) controls. Calves were immunized with 3 doses on days 0, 28 and 49. Animals in vaccinated and control groups (placebo and untreated) were infested with *R. microplus* larvae in three separate cells for each animal on days 72, 75 and 77. Animals in vaccinated and placebo groups were then infected with *A. marginale* and *B. bigemina* on days 69 and 92, respectively. This experimental design allowed ticks to feed on vaccinated or placebo control cattle co-infected with both pathogens as well as on untreated and uninfected animals. Calves were evaluated for antibody response to vaccination and pathogen infection. Engorged female ticks dropped from the host on days 98–104 and were collected, counted and evaluated for tick weight, oviposition and pathogen infection levels. The mRNA levels of genes encoding for protective antigens were also characterized in engorged ticks.