Tropical Biomedicine 33(4): 726–738 (2016)

Potential effects on *Rhipicephalus microplus* tick larvae fed on calves immunized with a Subolesin peptide predicted by epitope analysis

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Received 18 April 2016; received in revised form 23 May 2016; accepted 25 May 2016

Abstract. The cattle tick *Rhipicephalus microplus* economically impacts cattle production in tropical and subtropical regions of the world. The recombinant *R. microplus* Subolesin antigen has been shown to protect cattle against tick infestations. In this study, we searched *in silico* protective epitopes in the *subolesin* gene and a recombinant peptide containing the predicted epitopes was expressed, and evaluated against a tick challenge. Two different epitope types, linear B-cells and conformational discontinuous epitopes, were predicted using bioinformatics strategies, in order to synthesize the recombinant peptide. Two Eight-month-old calves European crossbred were immunized with two subcutaneous doses of the subolesin recombinant peptide, emulsified with Montanide ISA 50 V as an adjuvant, at 30-day intervals. The tick challenge was conducted with 5 000 *R. microplus* larvae/animal. ELISA test was used to evaluate the IgG immune response elicited against the peptide. After tick challenge, reduction in the number of engorged females (79%), and reduction in egg hatching (30%) was observed in tick population fed on immunized calves with regards to an untreated control group. The results showed a potential higher effect on tick reproduction for the recombinant peptide compared to other studies reported with Subolesin protein, demonstrating that the use of bioinformatics strategies to predict protective epitopes may lead to improve the immune response elicited against tick recombinant peptides and therefore to prevent cattle tick infestations.

INTRODUCTION

The cattle tick *Rhipicephalus microplus* is the most important ectoparasite affecting the cattle production in tropical and subtropical regions around the world (Peter *et al.*, 2005; Estrada-Peña *et al.*, 2006). Infestations with the cattle tick *R. microplus* have adverse physiological effects on the host and result in decreased live weight gains (Jonsson, 2006). In addition to direct effects, ticks harm their hosts indirectly by transmitting pathogens such as: *Babesia bovis*, *B. bigemina* and *Anaplasma marginale* (Jongejan & Uilenberg, 2004). Global losses produced by tick infestations and tick-borne diseases, have been estimated in the range of $US14 000 – 18 000 million/year (de Castro, 1997; Shakya *et al.*, 2014).

The common method used to control tick infestations is the use of chemical acaricides. However, this form of control present several drawbacks such as development of acaricide resistance, environmental pollution, residues

in food products, and high production costs for farmers (Graf *et al.*, 2004). All of these issues reinforce the need for alternative approaches to control tick infestations such as vaccination (Willadsen & Mckenna, 1991; Willadsen *et al.*, 1996; Imamura *et al.*, 2005).

The only commercially available tick vaccine, based on the recombinant Bm86 protein, has proven its efficacy to control the cattle tick infestations and to lower the prevalence of tick-borne pathogens in some regions; however, variable levels of its efficacy against *R. microplus* strains have been experienced, and sequence variations in the target protein and physiological factors among different tick strains have been found to be associated with variable efficacy (García-García *et al.*, 1999; de la Fuente *et al.*, 2000; Sossai *et al.*, 2005; Popara *et al.*, 2013).

Subolesin was discovered in *Ixodes scapularis* as a candidate tick protective antigen (Almazán *et al.*, 2003; de la Fuente *et al.*, 2005, 2006a). It is the structural and functional ortholog of insects and vertebrates akirins and functions as a transcription factor in regulation of gene expression, thus affecting multiple cellular processes such as tick innate immune response, digestion, reproduction and development (Goto *et al.*, 2008; Galindo *et al.*, 2009; Naranjo *et al.*, 2013). Previous vaccination experiments using recombinant tick Subolesin demonstrated protective efficacy against tick infestations, reduced fertility and/or survival of several arthropod vector species (Canales *et al.*, 2009b; Harrington *et al.*, 2009; Moreno-Cid *et al.*, 2011; de la Fuente *et al.*, 2013) and vectorial capacity of ticks (Canales *et al.*, 2009b; Prudencio *et al.*, 2010; de la Fuente *et al.*, 2011). Moreover, Almazán *et al.* (2010) recorded reduction of 43% in the number of engorged females, and 19% in egg fertility; Merino *et al.* (2013) recorded reduction of 47% in the number of engorged females, and 18% in oviposition compared to ticks *R. microplus* fed on control non-immunized cattle. Recently, Carreón *et al.* (2012), showed a higher control of ticks *R. microplus* in White-tailed deer immunized with Subolesin; they recorded reduction of 53% in the number of engorged females, 21% in oviposition and 51% in egg fertility. These results suggested that the antigen could be used for the development of a universal vaccine, but higher efficacy is required.

Previous immunization experiments on cattle were carried out using synthetic peptides derived from the *R. microplus* gut protein (Bm86). These experiments resulted in significant reduction of the reproductive indices of ticks, represented by the number and weight of adult females, and their oviposition capacity. The use of synthetic peptides increased the efficacy of the recombinant Bm86 vaccine from 51% to 80% (Patarroyo *et al.*, 2002, 2009). A similar approach was used in this study, based on a recombinant peptide derived from *in silico* analysis of linear and conformational epitopes on the *subolesin* gene sequence, instead of synthetic peptides, in order to enhance the protective immune response against *R. microplus* when the recombinant peptide is inoculated into cattle.

MATERIALS AND METHODS

Tick strain

The susceptible *R. microplus* tick strain (Media Joya; Mexico strain), was obtained from a laboratory colony maintained at CENID-PAVET, INIFAP, Jiutepec, Morelos, Mexico. Originally, these ticks were collected from infested cattle in Tapalpa, Jalisco, Mexico. Tick larvae were fed on cattle, collected after repletion, and kept under controlled laboratory conditions for oviposition and hatching in humidity chambers at 12 hours light: 12 hours dark photoperiod, 25–27°C and 80% relative humidity (RH). Larvae were 30 days of age at the time of infestations.

Experimental design

Cattle were randomly assigned to two experimental groups of two animals each, subolesin recombinant peptide-immunized and adjuvant/saline control. Fifteen days after the second immunization, cattle in both immunized and control groups were infested with 5 000 *R. microplus* larvae applied on each animal. Collections of naturally

detached engorged females were recorded from all animals during five days, throughout the engorging period. Adult female ticks were individually counted and weighted daily. After recording the engorged weight, ticks were kept in petri dishes at 25–27°C and 80% RH. Approximately two weeks after tick collection, when oviposition was completed, the egg mass produced by each female was weighted and the egg hatching was checked five weeks after collection.

B-cell epitopes prediction and modeling

The subolesin peptide was designed by prediction of linear B-cell and conformational discontinuous protective epitopes identified by *in silico* analysis of the *subolesin* gene (GenBank accession No. DQ159965.1) using: BCEpred (http://imtech.res.in/raghava/bcepred/bcepred_submission.html) (Saha & Raghava, 2004), Antigenic (http://emboss.bioinformatics.nl/cgi-bin/emboss/antigenic) (Rice *et al.*, 2000), ABCpred (http://imtech.res.in/raghava/abcpred/ABC_submission.html) (Saha & Raghava, 2004) and IEDB/bcell (http://tools.immuneepitope.org/bcell/) (Larsen *et al.*, 2006) programs to predict linear B-cell epitopes on *subolesin* gene sequence. The predicted three-dimensional structure of subolesin and the recombinant peptide was generated using: I-Tasser (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) (Zhang, 2007, 2008) and IEDB/ellipro (http://tools.immuneepitope.org/ellipro/) (Ponomarenko *et al.*, 2008). These algorithms were used to generate the structural prediction models of the conformational discontinuous epitopes in the recombinant peptide.

cDNA Synthesis and PCR conditions

The gene sequence of the predicted peptide was amplified from cDNA by PCR. Approximately 100–150 unfed tick larvae from *R. microplus* were used for the experiment. Total RNA was extracted from homogenized tick samples using TRIZOL reagent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Synthesis of cDNA was obtained from 5 µg of total RNA using RACE cDNA kit (Invitrogen, Carlsbad CA), which was used as template for PCR amplification reactions. Primers Fw 5'-CACCATGAAGCGACGCCGATGTATG-3' and Rev 5'- CTCCTCCCGTATCTTGCTCTC-3' were designed using Primer 3 program, considering sequences flanking antigenic regions of *subolesin* gene. PCR was performed in 25 µl reaction mixture for 40 cycles and amplification conditions 30 s at 95°C and 1 min at 72°C for annealing and extension steps, respectively. PCR products were electrophoresed on 1% agarose gels to check the size of amplified fragments by comparison to a DNA molecular weight marker (1 kb Plus DNA Ladder, Invitrogen).

Cloning and expression of the recombinant peptide

Amplified fragments were cloned into the expression vector pET101/D-TOPO® (Invitrogen, Carlsbad, CA) following the manufacturer's recommendations. Recombinant constructs were transformed into One Shot® Top 10 *Escherichia coli* cells (Invitrogen, Carlsbad, CA). Positive clones were analyzed by PCR as described above. For expression of subolesin recombinant peptide, plasmids were transformed into BL21 Star (DE3) *E. coli* cells (Invitrogen, Carlsbad, CA) and inoculated into Luria broth containing 50 µg/ml ampicillin. Culture was grown at 37°C to optical density (OD) of 600 nm = 0.5. IPTG was then added to a final concentration of 1 mM and then incubated for 5 hours to induce the production of the recombinant peptide. Cells were collected by centrifugation and recombinant proteins were purified by Ni affinity chromatography using the NI-NTA spin kit (Qiagen, Hilden, Germany) following the manufacturer´s instructions.

Western Blot

Expression and purification of the recombinant peptide was confirmed by SDS-PAGE and immunoblotting. Protein samples were analyzed on polyacrylamide gels (Laemmli, 1970) stained with Coomassie Brilliant Blue, and transferred to nitrocellulose membranes (Towbin *et al.*, 1979). Membranes were blocked with 5% skim milk for 60 min at room temperature and then over night at 4°C. Western blot analysis was

performed using anti-His monoclonal antibody (Invitrogen, Carlsbad, CA) for detection of recombinant fusion protein. After washing with TBS, the membrane was incubated with 1:2000 anti-His alkaline phosphatase conjugated (Invitrogen, Carlsbad, CA). The membrane was washed again, and the color was developed using BCIP/NBT alkaline phosphatase substrate (Millipore, Billerica, MA).

Cattle immunization and tick infestation

Calves were immunized with two doses (week 1 and 5) containing 100 µg of recombinant peptide emulsified with Montanide ISA 50 V as an adjuvant. Negative controls were injected with adjuvant/saline alone (placebo). Cattle were subcutaneously injected with 2 ml/dose using a 5 ml syringe and an 18 G needle. All the animals were checked regularly for any signs of local reaction or clinical abnormalities post immunization and received treatment in accordance with the animal experimentation rules described in the Guide for Care and Use of Laboratory Animals for the CENID-PAVET, INIFAP.

The effect of immunization on tick infestation was evaluated on tick number (TN), tick weight (TW), egg mass (EM) and egg hatching (EH), employing the formulae previously used in tick vaccine experiments (de la Fuente *et al.*, 1999). A Student's t-test with unequal variance ($P<0.05$) was used to compare the results of adult female tick number, tick weight, oviposition and egg fertility between immunized and control group.

ELISA Test

After immunization, blood samples were collected weekly from each calf into sterile tubes and maintained at 4°C until arrival at the laboratory. Serum was then separated after centrifugation and stored at -20°C. Serum antibody titers were determined using an antigen-specific indirect ELISA. Purified subolesin peptide (1 µg/well) was used to coat ELISA plates overnight at 4°C. Sera were diluted to 1:100 in PBST (PBS/0.05% Tween 20, pH 7.2) and 5% skimmed milk. The plates were incubated with the diluted sera for 1 hour at 37°C and then incubated with 1:2000 anti-bovine IgG-AP conjugates (Sigma-Aldrich, St. Luis, MI) for 1 hour at 37°C. The color reaction was developed with BCIP/NBT (Millipore, Billerica, MA) and the OD was read at 405 nm in an ELISA reader. After incubation, the plates were washed with PBST. Antibody titers were considered positive when they yielded an OD_{405} nm value at least twice as high the standard deviation of the control group serum samples. Antibody titers between vaccinated and control group were compared by Student's t-test ($P<0.05$).

RESULTS

Structural analysis of the subolesin peptide, linear B-cell and conformational discontinuous epitopes into tertiary structure were predicted *in silico*. The sequence of the subolesin peptide showed four possible linear B-cell protective epitopes with four programs available on the web. To select the best B-cell epitopes, different algorithms were used (ABCpred, BCEpred, Antigenic and IEDB). The best peptide sequences were selected based on the prediction of at least three out of the four algorithms used. The sequence of the first epitope was found on residue 6 to 16 (11 amino acids), second on residue 56 to 67 (12 amino acids), third on residue 78 to 89 (12 amino acids) and fourth on residue 92 to 102 (11 amino acids) (Figure 1A). The sequence of the subolesin peptide contained alpha helices and beta sheets but the structure showed more helical regions and the formation of disulfide bonds between the different cysteins of the protein, thus likely providing a more stable folding and flexibility for this peptide (Figure 1B). The predicted three-dimensional structure contained surface exposed protective epitopes. A set of 10 three-dimensional models was generated for the subolesin peptide (5 from I-Tasser and 5 from IEDB/ellipro). One of the models generated by the IEDB/ellipro was used thereafter for mapping of conformational discontinuous epitopes. Three possible conformational discontinuous epitopes were predicted on the surface of the subolesin peptide. The first conformational

epitope was constituted for 22 amino acids (residues 9 to 30); the second was constituted for 10 amino acids (residues 49, 51-53, 55-60) and the third for 10 amino acids (residues 88-94, 97-99) (Figure 1C). All those epitopes were located within a peptide of approximately 15.5 kDa expressed in *E. coli*, based on the SDS-PAGE electrophoretic analysis and Western Blot (Figure 2). The recombinant peptide was purified to 95% purity by Ni affinity chromatography using the NI-NTA spin kit (Qiagen, Hilden, Germany) and emulsified with Montanide ISA 50 V in order to cattle immunization.

The effect of immunization was evaluated on tick number (TN), tick weight (TW), egg mass (EM) and egg hatching (EH), for ticks detached in both immunized and control groups (Table 1). The results indicate a significant reduction ($P<0.05$) on TN and EH, in the group immunized with the subolesin recombinant peptide, but it did not have a significant effect on TW and EM parameters. Significant differences were found in the time of tick collection, as shown in Figure 3.

Previous to the first immunization, the sera of all cattle were assayed by ELISA for anti-subolesin recombinant peptide antibodies and were found seronegative. Specific IgG antibodies in sera from immunized animals increased after the second inoculation, while sera from control animals remained negative. A rapid increase of antibody levels was observed after the second immunization and the greatest values were obtained 7 days later in immunized animals. A stable level was reached after week 7 and significant differences were maintained until the end of the experiment (week 11) (Figure 4). This level was 3 OD units higher in the experimental group immunized with the subolesin recombinant

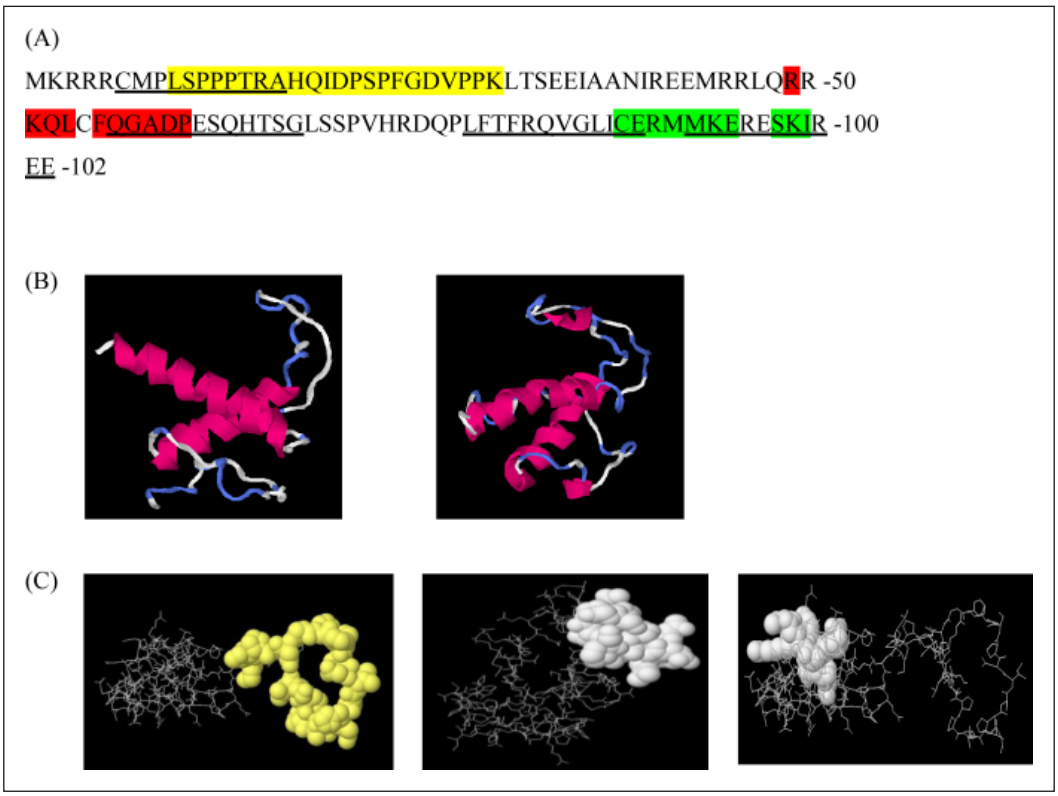

Figure 1. Localization of the predicted linear B-cell and conformational discontinuous epitopes on three-dimensional model of the subolesin peptide. The epitope matching for the subolesin peptide on primary (A) and tertiary structures (C). Linear B-cell epitopes are shown underlined and conformational discontinuous epitopes are shown in yellow, red and green colors. (B) Three dimensional model of the subolesin peptide indicating alpha helices (magenta), beta sheets (blue) and turns (gray line).

peptide compared to the control group. Control animals did not show anti-subolesin peptide antibodies at any time during the experiment. In contrast, the immunized animals developed a strong and specific humoral immune response characterized by significantly high anti-subolesin recombinant peptide IgG levels ($P<0.05$).

DISCUSSION

Tick vaccines are based on the generation of host antigen-specific antibodies that result in injury to the arthropod vector during feeding, reducing the number and weight of engorged female ticks, their egg laying capacity, and their fecundity (Rodríguez *et al.*, 1995; Willadsen *et al.*, 1995; de la Fuente & Kocan, 2003; Canales *et al.*, 2009a,c; Prudencio *et al.*, 2010). Some anti-tick vaccines used to control cattle tick infestations, reduce the incidence of bovine anaplasmosis and babesiosis as demonstrated in different studies (de la Fuente *et al.*, 1998; de la Fuente & Kocan, 2003; Sossai *et al.*, 2005; Canales *et al.*, 2009a,c; Almazán *et al.*, 2010; Popara *et al.*, 2013). The commercial anti-tick vaccines, Gavac and TickGARD, both contain the recombinant antigen Bm86 of *R. microplus*,

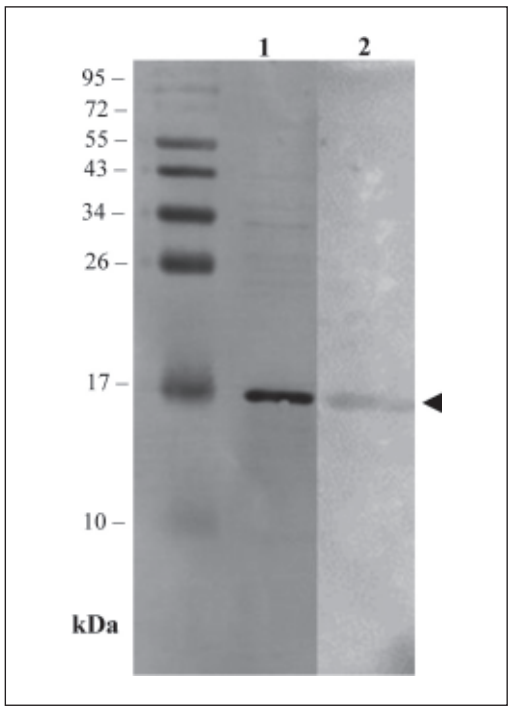

Figure 2. Production of the recombinant peptide. (1) Western blot analysis of the recombinant peptide expressed in *E. coli* and reacted with anti-HIS monoclonal antibody. (2) Five µg of the recombinant peptide purified by Ni affinity chromatography were loaded onto an SDS-PAGE gel and visualized by Coomassie brilliant blue staining. Arrow indicate the size of the recombinant peptide. MW, molecular weight.

Table 1. Reduction (%) of biological parameters of detached *R. microplus* ticks from cattle immunized with the recombinant peptide in relation to ticks detached from animals in control group

Experimental group	Reproductive parameters values evaluated on calves infested with *R. microplus* (Media Joya; Mexico strain) (Average ± standard deviation)			
	Tick Number (TN)	Tick Weight (TW)	Egg Mass (EM)	Egg Hatching (EH)
Recombinant peptide	367 179 (273±133)*	297 307 (302±7)	141 149 (145±6)	0.43 0.45 (0.44±0.01)*
Adyuvant/saline control	1147 1459 (1303±221)*	253 293 (273±28)	118 134 (126±11)	0.59 0.67 (0.63±0.05)*
[a] Overall reduction (%)	79%	(NS)	(NS)	30%

[a] Overall percent reduction was calculated with regards to control group. Data in parenthesis indicate the average ± SD for each reproductive parameter. Abbreviations used: TN (ticks number/animal), TW (individual tick weight in mg), EM (egg mass weight in mg /tick) and EH (Proportion of hatched/non hatched larvae), NS (non-significant). Results from immunized and control groups were compared by Student's t-test (*$P<0.05$).

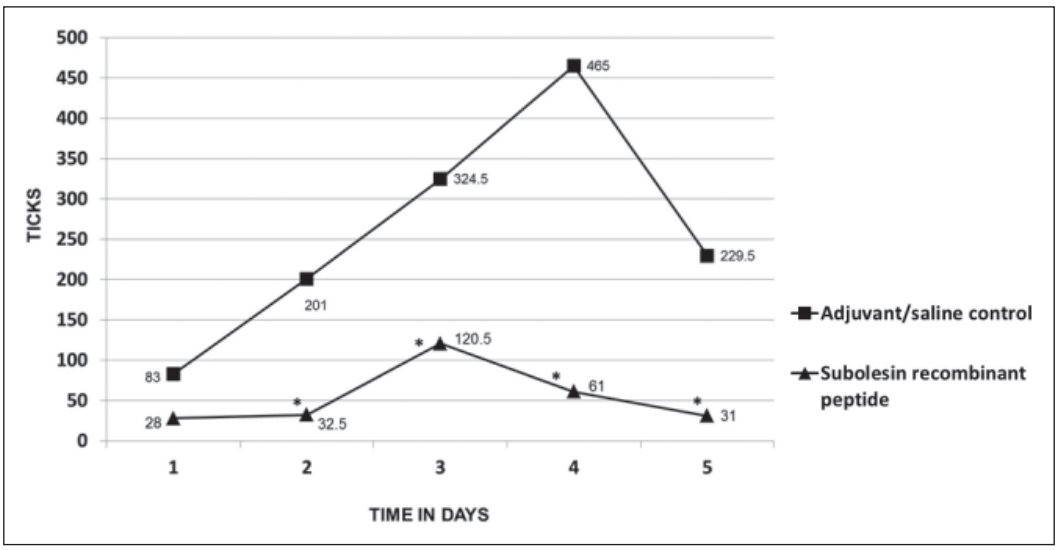

Figure 3. Tick collection per day. Average number of detached engorged female ticks collected per day from immunized and Adyuvant/saline control group, after 5 000 tick larvae infestation. Significant differences were found during tick detachment time (*P<0.05).

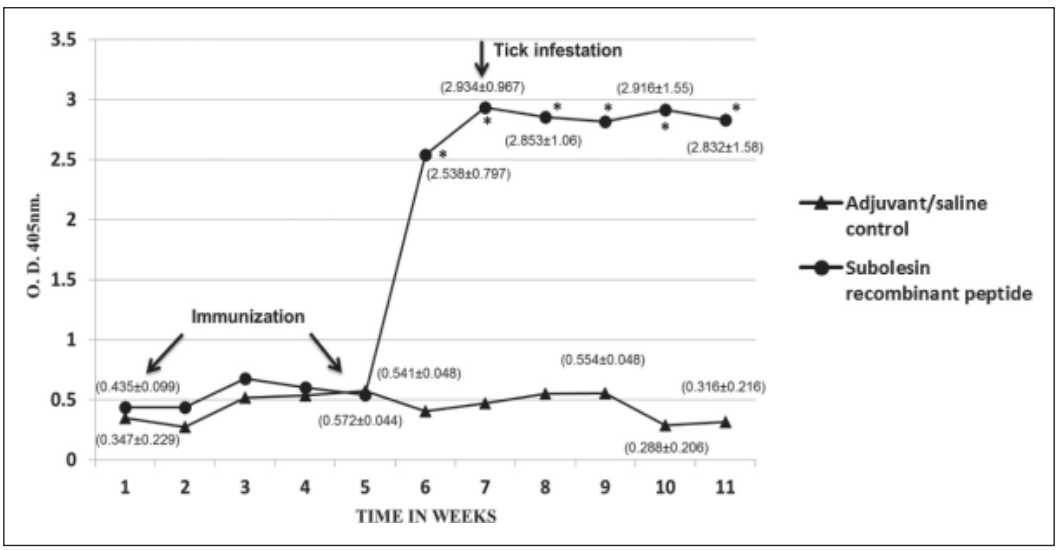

Figure 4. Bovine serum antibody titers to the recombinant peptide were determined by ELISA in cattle immunized and adjuvant/saline control group. Antibody titers in immunized cattle were expressed as the OD_{405} nm value for the serum dilution (1:100). Average ± SD are shown in parenthesis and average comparison between immunized and control cattle was performed by a Student's t-test (*P<0.05). Black arrows indicate immunogen applications and tick infestation.

collected from Cuba and Australia respectively (Rand *et al.*, 1989; Willadsen *et al.*, 1989; Rodríguez, 1994). Both vaccines have significant differences in their efficiency, and sequence divergence have been suggested as a major factor of the variable response in vaccination trials against ticks (de la Fuente *et al.*, 2005; Popara *et al.*, 2013; Shakya *et al.*, 2014), previous reports indicated that variations in the amino acid sequence of the protein expressed higher than 2.8% would be sufficient to not protect

appropriately when recombinant antigens are used (García-García *et al.*, 1999; Shakya *et al.*, 2014). Additionally, it has been shown that tick genetic and physiological differences between tick species and/or geographical strains may also affect tick vaccine efficacy (Popara *et al.*, 2013). Because of these limitations, new antigens have been identified and proposed as new protective antigens for tick vaccine development (de la Fuente *et al.*, 2011; Merino *et al.*, 2013).

Tick subolesin has been shown to control *R. microplus* infestations in vaccinated cattle (Almazán *et al.*, 2010; de la Fuente *et al.*, 2011; Merino *et al.*, 2011, 2013) resulting in a reduction of tick infestations and a reduced infection rate of two different pathogens: *A. marginale* and *B. bigemina* (de la Fuente *et al.*, 2006b; Kocan *et al.*, 2009; Zivkovic *et al.*, 2010). However, the use of the recombinant subolesin has not been totally successful, since only 60% efficiency has been obtained in controlled infestations experiments (Almazán *et al.*, 2010; Merino *et al.*, 2013).

As a proof of concept, Patarroyo *et al.* (2002) reported the use of a synthetic peptide SBm7462 derived from the *R. microplus* gut protein Bm86 as immunogen in an experimental trial in cattle, resulting in significant reduction of the reproductive indices of ticks, represented by the number and weight of adult females, and their oviposition capacity, improving the overall efficacy of the vaccine from 51% (rBm86) up to 81% by using the synthetic peptide SBm7462. A similar approach was proposed in this study focused on the PCR amplification of the immunogenic region of the Subolesin protein to express a recombinant peptide, with an improved immunogenic capacity against tick infestations.

Epitope mapping, was an initial basic tool for understanding the interactions between antigens and antibodies, and is currently being extensively employed to develop new therapeutics (Irving *et al.*, 2001; Prudencio *et al.*, 2010). Analysis of subolesin sequences in *R. microplus* revealed a high degree of sequence conservation among mosquitoes and ticks and suggested the presence of conserved antigenic epitopes (Canales *et al.*, 2009b). As discussed previously, immune cross-reactivity between antigenic epitopes from ticks and mosquitoes Subolesin ortholog proteins with antigenic epitopes might be used to elicit a protective crossed response in immunized hosts (Canales *et al.*, 2009b). In this study, we searched for protective linear B-cell epitopes and conformational discontinuous epitopes on tick Subolesin protein by *in silico* analysis and computational modeling. At present, the crystal structure of Subolesin is not available and therefore the identification of possible conformational discontinuous epitopes, defined by the prediction of protein models, should be considered as preliminary (Prudencio *et al.*, 2010). For tick Subolesin, according to previous results in the sequence of *I. scapularis* (Prudencio *et al.*, 2010; de la Fuente *et al.*, 2011) four linear B-cell epitopes and three possible conformational discontinuous epitopes were found in the sequence of the peptide.

The secondary structure predicted for the subolesin peptide showed helical regions connected by highly disordered loops. Linear B-cell epitopes were located in the stable large helical region of the peptide as well as discussed previously (Prudencio *et al.*, 2010). Therefore, we hypothesized that a subolesin recombinant peptide can be a good candidate to elicit an improved immune response in cattle, based on the previous evidences that Subolesin reduces *R. microplus* infestations as well as the infection levels by the most important pathogens transmitted by cattle ticks, *A. marginale* and *B. bigemina* (de la Fuente *et al.*, 2006b).

Recently, immunization trials with recombinant subolesin have shown protection against ticks and other arthropod vector species (Canales *et al.*, 2009b; Harrington *et al.*, 2009; de la Fuente *et al.*, 2011; de la Fuente *et al.*, 2013; Moreno-Cid *et al.*, 2013). In most of the previous controlled immunization trials with recombinant subolesin, Montanide ISA 50 V was used as adjuvant (Almazán *et al.*, 2010, 2012; Merino

et al, 2011; Moreno-Cid *et al.*, 2013; Shakya *et al.*, 2014). In some of these experiments, anti-subolesin antibody titers increased after the first immunization and then decreased after the second immunization possibly due to instability of the vaccine formulation (Almazán *et al.*, 2010; Shakya *et al.*, 2014). In the results reported herein, antibody titers increased after the second immunization because a specific anti-IgG monoclonal antibody was used. Elicited IgG antibodies positively correlated with the reduction of biological parameters of *R. microplus* after the tick challenge. These results were similar to those obtained earlier using Bm86, Ba86, Bd86 and Haa86 antigens (de la Fuente *et al.*, 1998; Canales *et al.*, 2008, 2009a; Azhahianambi *et al.*, 2009; Jeyabal *et al.*, 2010), suggesting that the effect of the ticks in immunized cattle was the result of the elicited antibody response against subolesin recombinant peptide.

The anti-tick effects evaluated in this study, were higher compared to results reported in previous experiments using vaccination with tick Subolesin (Almazán *et al.*, 2010, 2012; de la Fuente *et al.*, 2011, 2013; Merino *et al.*, 2013; Shakya *et al.*, 2014). As confirmed by the ELISA results, ticks fed on immunized cattle, ingested antibodies anti-subolesin recombinant peptide with the blood meal. The ingestion of blood containing anti-tick antibodies according to previous studies, affects multiple cellular processes such as digestion, development and reproduction (Goto *et al.*, 2008; Galindo *et al.*, 2009; Naranjo *et al.*, 2013).

Interestingly, in this trial the most important effect of the subolesin recombinant peptide immunization was observed on tick number (TN) (79% reduction) and egg hatching (EH) (30% reduction), which has been commonly found in previous experiments with Subolesin vaccines (de la Fuente *et al.*, 2013). Similar to previously results discussed, individual tick weight (TW) and egg mass (EM) were not affected when cattle was immunized with the whole subolesin molecule (Almazán *et al.*, 2010), probably due to variations on the immunogenic region or predicted peptide sequences that are being selected by the immune response induced by the immunization.

This study aims to demonstrate that the IgG elicited response to the recombinant subolesin peptide, affect the reproductive parameters of the cattle tick progeny and therefore enhance its protective capacity as a result of immunization, with a recombinant peptide for the control of cattle tick infestations. A strong immune response was shown by the recombinant peptide used in this study. The presence of linear B-cell and conformational discontinuous epitopes into the sequence of the subolesin peptide was predicted, and elicited protection against *R. microplus* in immunized animals artificially infested was shown.

These results suggest that prediction and mapping of protective epitopes are suitable tools to identify and design recombinant biomolecules that could be used to enhance the efficacy of tick vaccine targets. Although the current results present some limitations regarding to the small number of animals used in the controlled pen trial, those are indicative that a potential efficient control could be obtained under field conditions by using this approach to enhance the efficacy of tick vaccines targets in order to improve in the future the currently used tick integrated control programs.

Acknowledgments. Tick strain used was kindly provided by the Tick Research Unit from CENID–PAVET, INIFAP. We also acknowledge the technical assistance provided by the personnel of the Tick Research Unit from CENID-PAVET, INIFAP, CIATEJ, Parasitology Dpt. at FMVZ-UNAM and BioSA Research Lab at UAGro. Special mention to CONACyT for the scholarship granted to Rodolfo Lagunes, as well as the Infrastructure Project for the creation of BioSA Research Lab, granted to Dr. Delia Domínguez-García and Dr. Rodrigo Rosario-Cruz. We also thank CONACYT for the grant number PDCPN-2013-216321 to Dr. Moisés Martínez Velázquez.

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