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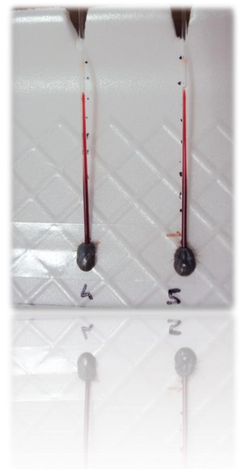
Artificial feeding of *Rhipicephalus microplus* female ticks: effects of anti-TROSPA antibodies on tick and *Babesia bigemina* acquisition



Abstract

Ticks are obligate haematophagous ectoparasites that can transmit a wide variety of pathogens, being considered the principal vectors of disease among animals. The piroplasms *Babesia bigemina* and *B. bovis* are transmitted mainly by *Rhipicephalus microplus* and *R. annulatus* ticks. Anti-tick vaccines despite promising are still scarce mainly due to the lack of effective antigens. Tick proteins involved in tick-pathogen interactions may provide good candidate protective antigens for these new vaccines but their identification and validation are still limiting steps. Appropriate screening procedures are needed to select the best candidates. In this study, we selected TROSPA protein involved in tick-*Babesia* interactions, as showed in a previous study, and used *in vitro* capillary feeding to characterize their potential as antigens for the control of cattle tick infestations and infection with *B. bigemina*.

Purified rabbit polyclonal antibodies were generated against TROSPA and added to uninfected or infected bovine blood to capillary-feed female *R. microplus* ticks. Tick weight, oviposition and pathogen DNA levels were determined in treated and control ticks. Anti rTROSPA antibodies specificity was confirmed by Western blotting and against native proteins in tick tissues using immunofluorescence. Capillary-fed ticks ingested antibodies added to the blood meal and the effect of these antibodies on tick weight and oviposition was shown. However, no effect was observed on pathogen DNA levels. These results highlighted the advantages and some of the disadvantages of *in vitro* tick capillary feeding for the characterization of candidate tick protective antigens. While an effect on tick weight and oviposition was observed, the effect on pathogen levels was not evident probably due to high tick-to-tick variations among other factors. Nevertheless, these results together with previous results of RNA interference functional studies suggest that TROSPA is a good candidate vaccine for the control of *R. microplus* infestations and infection with *B. bigemina*.

Results and Discussion

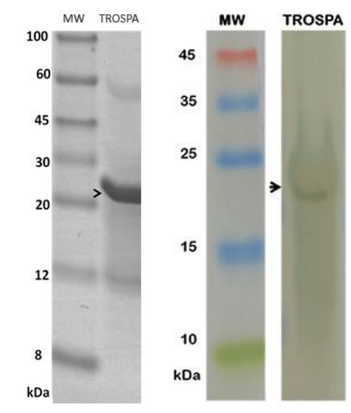


Fig.1 Production of recombinant proteins and antibodies. (A) SDS-PAGE; (B) Western blot analysis of the recombinant proteins using purified IgGs produced in rabbits immunized with these proteins.

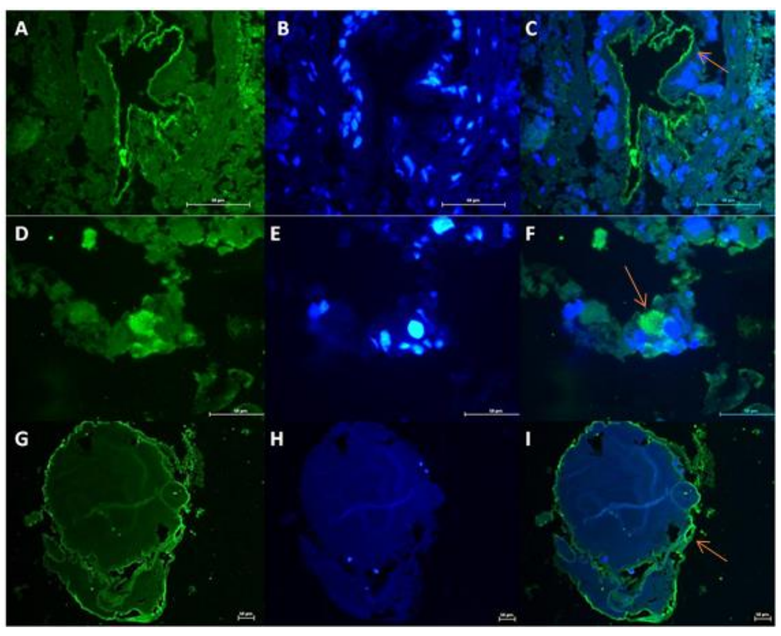


Fig. 2 - Tick tissue sections stained with anti-TROSPA antibodies.

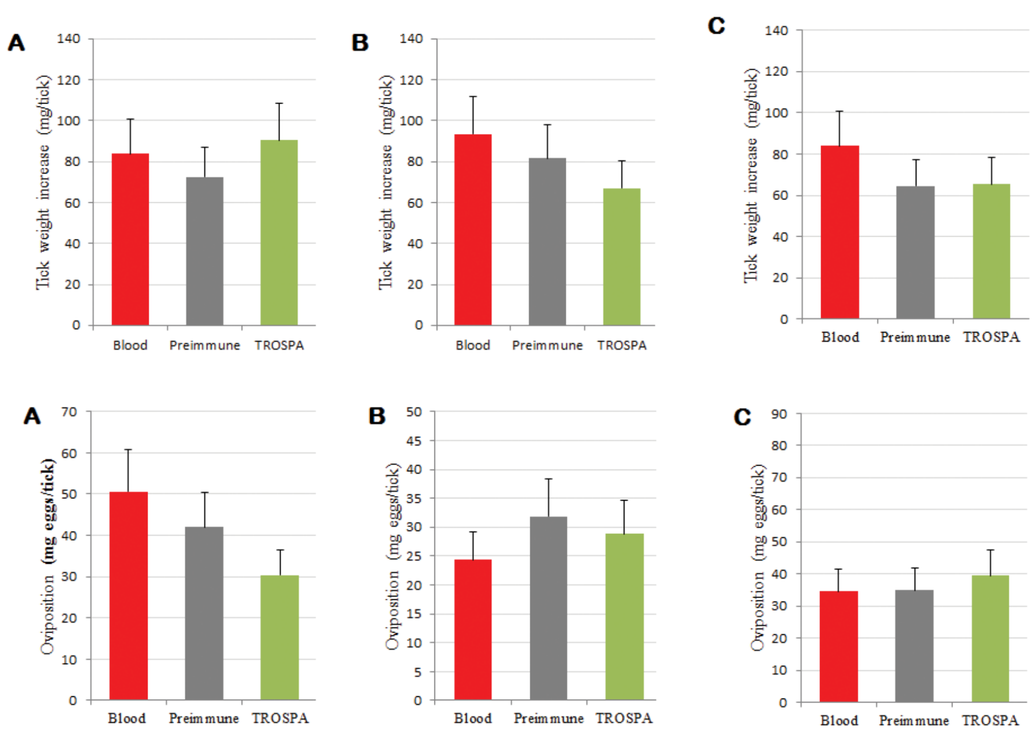


Fig. 3 - Effect of antibodies on 1) tick weight and 2) tick oviposition. (A) Ticks capillary-fed on uninfected blood without (Blood) and with preimmune and antitick protein IgGs (TROSPA). (B) Ticks capillary-fed on *B. bigemina*-infected blood without (Blood) and with preimmune and anti-tick protein IgGs. (C) Ticks capillary-fed on *A. marginale*-infected blood without (Blood) and with preimmune and anti-tick protein IgGs. Ticks (N = 5) were incubated for oviposition after feeding, the egg mass weight was etermined for each tick, expressed as Ave + S.D. (mg eggs/tick) and compared between the group with preimmune antibodies and the other groups by Student's t-test (*P < 0.05).

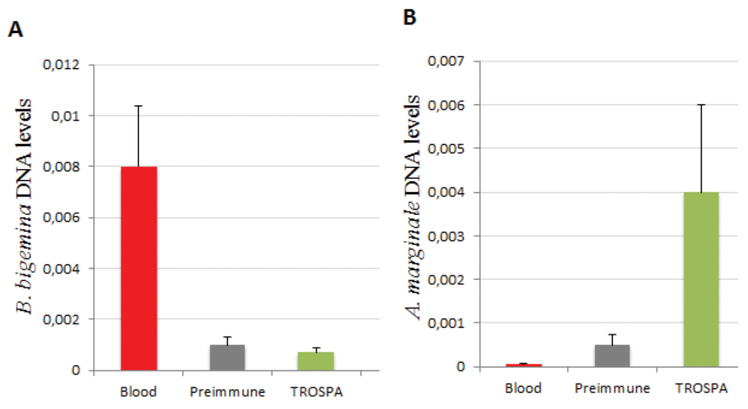


Fig. 4 - Effect of antibodies on pathogen infection and gene expression. (A) *B. bigemina* DNA levels were determined by real-time PCR in ticks capillary-fed on B. bigemina-infected blood without (Blood) and with preimmune and anti-tick protein IgGs (TROSPA). The DNA levels were normalized against tick 16S rDNA, shown as Ave + S.D. normalized Ct values (arbitrary units) and compared between the group with preimmune antibodies and the other groups by Student's t-Test (P > 0.05; N = 10). (B) A. marginale DNA levels were determined by real-time PCR in ticks capillary-fed on A. marginale-infected blood without (Blood) and with preimmune and anti-tick protein IgGs. The DNA levels were normalized against tick 16S rDNA, shown as Ave + S.D. normalized Ct values (arbitrary units) and compared between the group with preimmune antibodies and the other groups by Student's t-Test (P > 0.05; N = 10).

- Capillary feeding showed limitations in the study of pathogen infection in ticks possibly associated with different factors such as tick-to-tick variation in infection levels, parasitemia levels in blood used for capillary feeding, a non-specific effect of the preimmune serum and/or differences in pathogen infection between in vivo tick feeding and in vitro capillary feeding.
- Interactions between antigen-specific antibodies and pathogen in the blood meal could affect the effect on tick weight and oviposition by still unknown mechanisms.
- RNAi alone is not a suitable tool for the identification of tick protective antigens, but a tool to integrate with other methods such as capillary feeding using antibodies against selected proteins for the identification and functional characterization of candidate tick protective antigens.
- TROSPA showed an effect on tick weight and/or oviposition in ticks fed with uninfected or infected blood containing antigen-specific IgGs.
- These results together with previous results of RNAi experiments suggest that TROSPA is a good candidate vaccine antigen for the control of *R. microplus* infestations and infection with *B. bigemina*.
- A recently completed vaccine trial validated this approach for the selection of candidate tick protective antigens and proved the efficacy of TROSPA for the control of *R. microplus* infestations and infection with *B. bigemina*.

References

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This research was supported by project PTDC/CVT/112050/2009 funded by the Fundação para a Ciência e Tecnologia (FCT) and the EU FP7 ANTIGONE project number 278976.