Artificial feeding of *Rhipicephalus microplus* female ticks: effects of serum enriched with anti-calreticulin antibodies on tick and *Babesia bigemina* acquisition

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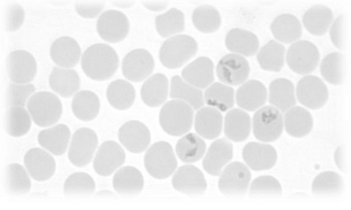
Background

Babesia spp. are tick-borne pathogens that cause a disease called babesiosis in a wide range of animals. Particularity, *B. bovis* and *B. bigemina* are transmitted by cattle ticks, *Rhipicephalus annulatus* and *R. microplus* being considered the most important cattle ectoparasites with major economic impact on cattle production. The use of chemicals to control ticks has drawbacks supporting the need to pursue alternative methods. Anti-tick vaccines are considered a cost-effective and environmentally safe strategy but are still scarce mainly due to the lack of effective antigens. Calreticulin has been identified as being involved in *B. bigemina* infection in *R. annulatus* ticks. Herein, *R. microplus* females were successfully artificially fed using capillary tubes, a low cost alternative to test antigens allowing achieve critical data. Additionally, recombinant calreticulin (rCRT) protein was expressed in an *E. coli* expression system and antibodies were raised against rCRT. Anti-rCRT serum was supplemented to a blood meal, offered to partially engorged *R. microplus* females and their effect in feeding process as well as infection by *B. bigemina* was analyzed. No significant reductions in tick and egg weight were observed when ticks fed with anti-rCRT serum. Also, *B. bigemina* infection levels did not show a statistically significant decrease when ticks fed with anti-rCRT antibodies. In spite of these results artificial capillary feeding can be a useful technique for the characterization of candidate tick protective antigens.



Results and Discussion

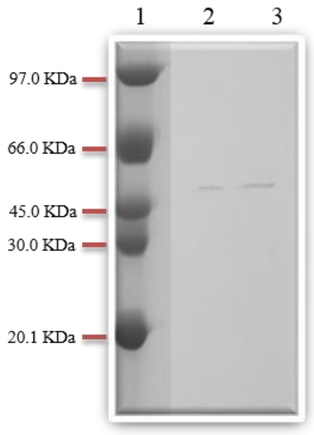


Fig.1 - Purified rCRT. SDS-PAGE gel at 12.5% stained with Coomassie-blue.

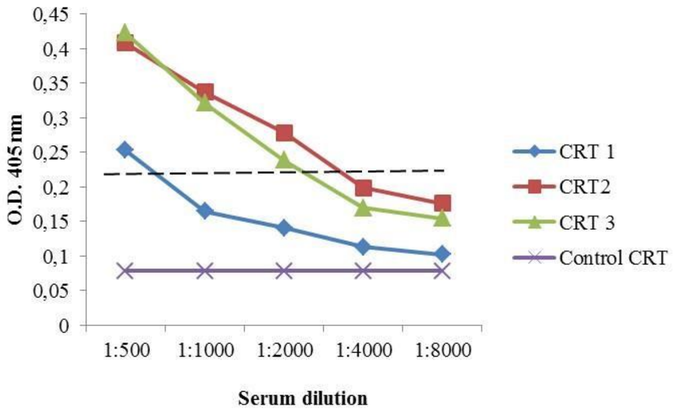


Fig. 2 - Serum titer of each mouse after four rCRT immunizations. The dashed line represents three times the O.D. of the control serum.

Table 2 - Levels of infection acquired.

	Infection levels by *B. bigemina* (AVG ± SD)		
Infected blood *B. bigemina*	7,89E-03	±	2,25E-02
Infected blood + Control serum	4,36E-03	±	9,07E-03
Infected blood + Anti-CRT serum	2,74E-03	±	7,96E-03

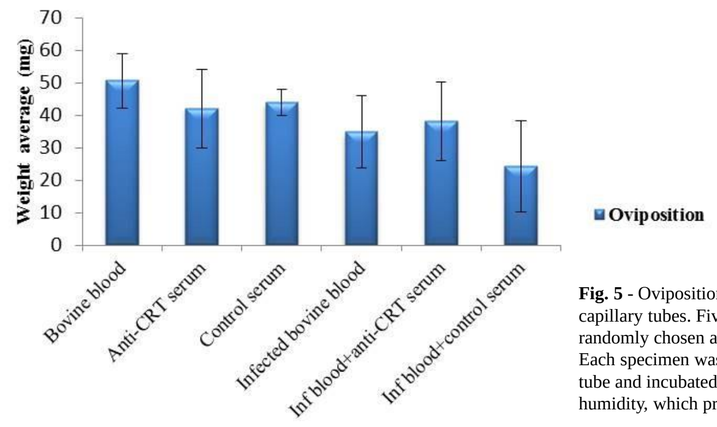


Fig. 5 - Oviposition average in tick groups fed by capillary tubes. Five females of each group were randomly chosen and oviposition was allowed. Each specimen was placed into a 2 mL eppendorf tube and incubated at 28ºC with an 85% relative humidity, which promotes oviposition.

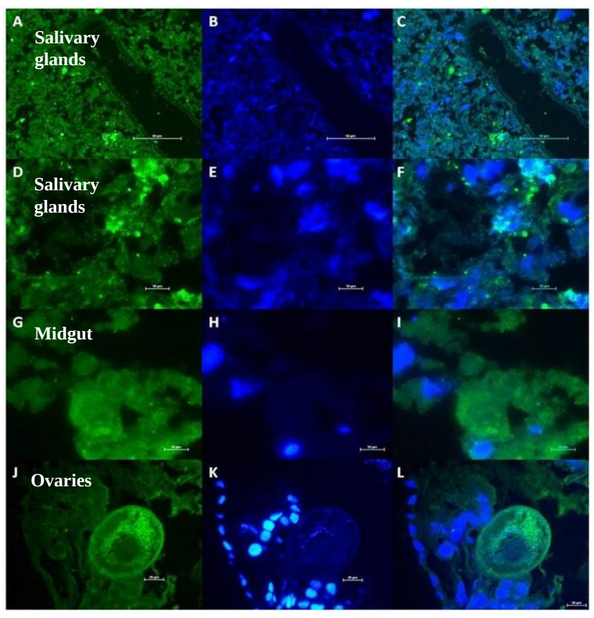


Fig. 3 - Immunofluorescence analysis of cattle tick *R. microplus* tissues probed with anti-CRT antibodies. Nuclear DNA was stained with DAPI. (A, D, G, J) fluorescein filter at 400x amplification; (B, E, H, K) DAPI staining; (C, F, I, L) Merge of both staining. Bars: 50 µm (A, B, C); 10 µm (D-I) and 20 µm (J-L).

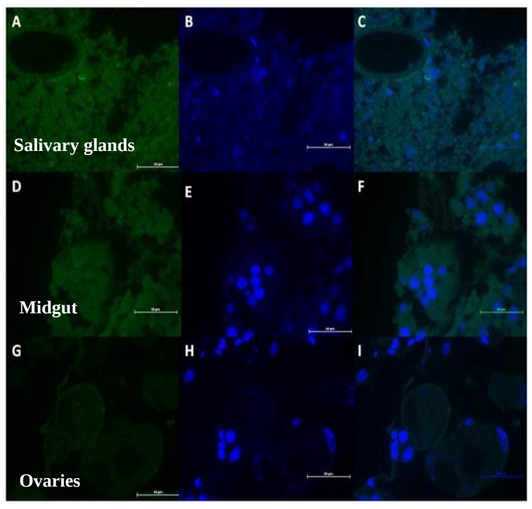


Fig. 4 – Immunofluorescence analysis of cattle tick tissues probed with pre-immune serum (1:100). Nuclear DNA was stained with DAPI. (A, D, G) fluorescein filter; (B, E, H) DAPI staining; (C, F, I) Merge of both stainings. Bars: 50 µm.

Table 1 - Weight gain with capillary tube artificial feeding.

Groups	Weight (mg) AVG ± SD		Weight increment	
	Before AF	After AF	(mg) AVG ± SD	% AVG ± SD
A-Bovine blood	32,7±6,8	116,6±19,9	83,8±20,7	134,1±48,1
B- Infected blood (*B. bigemina*)	32,9±9,4	126,2±41,6	93,3±36	195,2±45,6
C- Blood + Anti-CRT serum	29,7±2,7	110,9±20,8	81,2±21,7	139,1±47,8
D- Blood + Control serum	30,1±4,6	127,3±29,2	97,2±27,1	162,1±41,7
E- Infected blood + Control serum	30,3±8,3	91,4±29,2	61,1±26,1	104,5±43,3
F- Infected blood + Anti CRT serum	36,7±7,6	125,7±37,6	89,1±33,6	122,8±42,6

- **rCRT showed to have a low immunogenic capacity in mice (Fig. 1 & 2);**
- **Produced antibodies against rCRT recognized native CRT in tick tissues (Fig. 3 & 4);**
- ***R. microplus* females were successfully artificially fed with remarkable gain weight in all experimental groups (Table 1);**
- **After artificial feeding with infected and uninfected blood, the *crt* levels were found to be statistically different between groups in accordance to previous studies;**
- **No significant reductions in tick body and egg weight were observed when ticks were fed with anti-rCRT serum (Fig. 5);**
- **Regarding *B. bigemina* infection levels, in groups fed with infected blood containing anti-rCRT antibodies did not show a statistically significant decrease (Table 2);**
- **These results suggests that rCRT is not a good candidate for anti-tick vaccine development.**

Study supported by FCT: PTDC/CVT/112050/2009

References

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