

Tick Transmitted diseases



Proteases from Babesia sp.

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Babesiosis is a human malaria-like disease that has recently been considered as an emerging zoonosis. Babesiosis is a tick borne disease (TBD) caused by parasites of the genus *Babesia*, with considerable worldwide economic, medical, and veterinary impact.

Proteases are involved in a multitude of physiological reactions from simple digestion of food proteins to highly-regulated cascades. Tick proteolytic enzymes in the midgut may play critical roles in host blood meal digestion and pathogen transmission.

Our recent work on this field was based on using *Babesia* protease genes to built a new PCR method for the detection of *Babesia bigemina* and *Babesia bovis* on samples collected in Mozambique.

Our studies on the detection of *Babesia bigemina* and *Babesia bovis* on those samples was done using two distinct PCR methods. The DNA samples were analyzed using a previously described nested PCR and a novel hot-start PCR method. Primers were selected for the hot-start PCR based on the putative gene of an undescribed aspartic protease named babesipsin, present in both *B. bovis* and *B. bigemina*. The combination of

hot-start polymerase and long primers (29–31 bp) were in this study determinant for the successful amplification and detection in only one PCR. With a seminested approach the sensitivity was further increased. The babesipsin seminested hot-start PCR was in this study more sensitive than the nested PCR. A total of 117 field samples were tested by

seminested hot-start PCR, and 104 were positive for *B. bigemina* (90%), 97 were positive for *B. bovis* (82%), 86 were mixed infections (52%) and only 2 were negative for both *Babesia* species (1.7%). The

results confirm that this area of Mozambique is endemic for babesiosis, and that this TBD should be regarded as a threat for imported cattle.

Selected Publications:

1. Tiago M. Martins, Olívia C. Pedro, Rubina A. Caldeira, Virgílio E. do Rosário, Luís Neves, Ana Domingos (2008) Detection of bovine babesiosis in Mozambique by a novel seminested hot-start PCR method. *Veterinary Parasitology* 153: 225–230.

Comunicações orais:

T. Martins, C. Novo, A. Domingos (2006). Identification of bovine babesiosis proteases as diagnostic and drug targets. Seminário em “New targets for parasitic diseases therapy” organizado por UTPAM/Departamento de Biotecnologia do INETI em 18 de Junho de 2006.

T. Martins, C. Novo, A. Domingos (2006). Identification of bovine babesiosis proteases as diagnostic and drug targets. Seminário em “New targets for parasitic diseases therapy” organizado por UTPAM/Departamento de Biotecnologia do INETI em 18 de Junho de 2006.

T.M. Martins, V.E. do Rosário, L. Neves e A. Domingos (2007) Development of a new Hot-start PCR method for the detection of bovine Babesiosis in Mozambique, 23-28 de Setembro ICTTD3 Coordinated Action meeting, Zanzibar, Tanzânia.

Posters:

Tiago M. Martins, Carlos Novo, Virgílio E. do Rosário and Ana Domingos (2007) New cysteine proteases from the cattle parasite *Babesia bigemina*. Drug Development for Parasite Diseases - COST B22 Annual Congress, 10 - 13 June 2007, University of Dundee, Scotland, UK.

Tiago M. Martins, Carlos Novo, Virgílio E. do Rosário, Luis Neves and Ana Domingos (2007) Bovine Babesiosis in Mozambique detected by a novel Nested Duplex PCR method. Apicomplexan Biology in the post-genomic era - COST Action 857, 4st Annual Workshop 4-7 Maio, Ajaccio, France.

2. Proteases as potential drugs for malaria

Parasite cysteine proteases have been shown to be immunogenic and are being exploited as serodiagnostic markers and vaccine targets among parasitic diseases. Due to the fact that there are a few new drugs available and in spite of recent promising developments have spurred new hopes on the development of malaria, none have been approved, so far, for general public use, new approaches are needed.

Cysteine proteases (falcipains) of *P. falciparum* are potential targets for antimalarial chemotherapy, since they have been shown to be involved in important cellular functions such as hemoglobin degradation and invasion/rupture of red blood cells during parasite life cycle.

Our main interest on this subject was to identify new cysteine proteases from a malaria animal model that could be used to identify new targets for treatment. In these studies, genes encoding *P.*

chabaudi cysteine proteases chabaupain-1 and chabaupain-2 were cloned and further expressed in *E. coli*.

Both proteases identified encode fairly typical papain-family cysteine proteases, as expected, sharing a number of unusual features with the falcipains. They contain unusually large prosequences showing predicted membrane-spanning domains near the N-terminal. Near active-site residues, unusual sequences are also conserved as observed among other proteases from *Plasmodia*.

To obtain soluble active protein after expression, it was designed an optimization protocol. Our results showed that the expression attains its maximum at 4 hours post-induction and does not depend on the concentration of the inductor; by lowering the temperature during the induction period, the protein expressed in soluble form increased.

In parallel, two different culture media were further assayed. Strains and tag effects on expression were also evaluated and different culture media and two different expressing cell lines were tested. Biologically active falcipain-1 was obtained; a number of falcipain-1 constructs were designed and different refolding conditions were as well tested.

Our purpose was also to study the presence of chabaupain-1 in *Anopheles* biological material after infection with *Plasmodium*. Assays were performed in midguts, hemolymph and fat body after infection and compared with samples from uninfected mosquitos. The results, showing the presence of CP-1 from *P. chabaudi* in *Anopheles* midguts, also support that this protease is probably implicated on parasite egress from oocysts.

The antibody used in these analysis appears to be specific to CP-1 since it doesn't recognize CP-2, therefore it can be useful for further studies on localization and/or function studies of both proteases.

Future work will be developed to explore the immunogenicity of anti-CP-1

Selected Publications:

1.R.L. Caldeira, T.M. Martins, H. Silveira, V. do Rosário, C. Novo, A. Domingos (2006) Cystein Proteases from *Plasmodium chabaudi* an Important Target on Malaria Therapy, *International Journal of Infectious Diseases*, vol 10 - supl. 1: S298.

2.Rubina L Caldeira; Lídia M Gonçalves, Tiago M Martins; Carlos M Novo, Virgílio E do Rosário, Ana G Domingos. *Plasmodium chabaudi*: expression of active recombinant chabaupain-1 and localization studies in *Anopheles* sp.- submitted in August 2008 to the *Experimental Parasitology* journal.

Comunicações orais:

R. Caldeira, T. Martins, H. Silveira, V. do Rosário, C. Novo, A. Domingos (2006) Chabaupain-1 a cystein protease can be detected in protein midgut extracts of *Plasmodium* infected *Anopheles* mosquitoes. 3rd Annual Joint WGs Meeting Cost Action B22-Drug Discovery & Development For Parasitic Diseases, 1 a 4 Outubro, Atenas, Grécia.

R. Caldeira, T. Martins, C. Novo, A. Domingos (2006) Proteases from *Plasmodium* can be a good therapeutic target for malaria therapy. Seminário em “New targets for parasitic diseases therapy” organizado UTPAM do Departamento de Biotecnologia do INETI em 18 de Junho de 2006.

Posters:

R.L. Caldeira, T.M. Martins, H. Silveira, V. do Rosário, C. Novo, A. Domingos (2006) Cystein Proteases from *Plasmodium chabaudi* an Important Target on Malaria Therapy, poster apresentado no 12th International Congress on Infectious Diseases, 15 a 18 de Junho, Lisboa, Portugal.

R. Caldeira, T. Martins, , H. Silveira, V. do Rosário, C. Novo, A. Domingos (2006) Cystein proteases from *Plasmodium* as therapeutic targets, poster apresentado no 3rd Annual Workshop COST- European Cooperation in the field of Scientific and Technical Research- Action 857, 17 a 20 de Maio, Dresden, Alemanha



Carlos Novo



Ana Armada



Tiago Martins

Tick Transmitted Diseases

Diagnosis

Bovine Babesiosis in Mozambique detected by a novel Nested Duplex PCR method

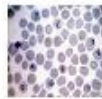


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ABSTRACT

Babesiosis is an emerging, tick-transmitted, zoonotic disease caused by parasites of the genus *Babesia*. In this work it was our purpose to study the incidence of *B. bigemina* and *B. bovis* in Mozambique. Blood samples were collected in two different farms located near Maputo city.



After DNA extraction, the samples were analyzed using RLB and a novel Nested Duplex PCR method. Primers were selected from the *B. bigemina* Genome database (Sanger Institute) and the *B. bovis* Genome Database (Washington State University). Primers were selected for the putative gene of undescribed aspartic proteases (babepsin). Some of the positive PCR amplified fragments from *B. bovis* were cloned and sequenced. Results show the high prevalence of both *Babesia* species in the samples (more than 70%), and as expected, the Nested PCR is more sensitive than the RLB.

MAIN OBJECTIVES



- Blood collection from cattle naturally infected by *B. bovis* and *B. bigemina* in Mozambique
- Identification of new coding genes for proteases
- Polymorphism studies
- Development of diagnostic methods based on ELISA
- Technology transfer to Veterinary Faculty, Maputo

SAMPLES COLLECTION



- 79 from Matatane district
- 117 from Boane district

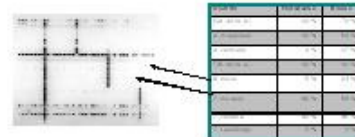
Babesia

Bovine babesiosis is a febrile, tick-transmitted zoonotic disease of cattle, caused by one or more protozoan parasites of the genus *Babesia* and generally characterized by extensive erythrocytic lysis leading to anemia, icterus, hemoglobinuria and death.

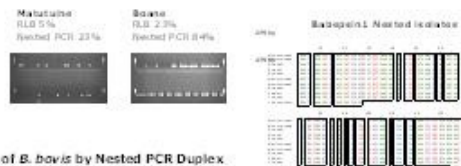
Parasites of the genus *Babesia* are some of the most ubiquitous and widespread blood parasites in the world that infect livestock, and consequently have considerable worldwide economic, medical, and veterinary impact. There are probably at least six *Babesia* species responsible for bovine babesiosis, but one of most concern is *B. bovis*, which is transmitted primarily by *Boophilus* ticks.



RLB - Reverse Line Blot analysis



DETECTION OF *B. BOVIS* BY NESTED PCR



BABESIA SPP. PROTEASES:

Babesia proteases are thought to have similar functions as those from malaria parasites, thus the inhibition of APs from *Babesia* parasites is a very promising approach to *Babesia* therapy. In *B. bovis*, virulence depends on protease content. *B. equi* propagation *in vitro* was significantly reduced in the presence of the cysteine protease inhibitor E64d.

Proteases represent 2% of all expressed genes

B. bigemina -> 3%

Gene	Accession	Length (bp)	GC (%)	Start	Stop	ORF (aa)
BabP1	U00001	1080	48.5	1	1079	359
BabP2	U00002	1080	48.5	1	1079	359
BabP3	U00003	1080	48.5	1	1079	359
BabP4	U00004	1080	48.5	1	1079	359
BabP5	U00005	1080	48.5	1	1079	359
BabP6	U00006	1080	48.5	1	1079	359
BabP7	U00007	1080	48.5	1	1079	359
BabP8	U00008	1080	48.5	1	1079	359
BabP9	U00009	1080	48.5	1	1079	359
BabP10	U00010	1080	48.5	1	1079	359
BabP11	U00011	1080	48.5	1	1079	359
BabP12	U00012	1080	48.5	1	1079	359
BabP13	U00013	1080	48.5	1	1079	359
BabP14	U00014	1080	48.5	1	1079	359
BabP15	U00015	1080	48.5	1	1079	359
BabP16	U00016	1080	48.5	1	1079	359
BabP17	U00017	1080	48.5	1	1079	359
BabP18	U00018	1080	48.5	1	1079	359
BabP19	U00019	1080	48.5	1	1079	359
BabP20	U00020	1080	48.5	1	1079	359
BabP21	U00021	1080	48.5	1	1079	359
BabP22	U00022	1080	48.5	1	1079	359
BabP23	U00023	1080	48.5	1	1079	359
BabP24	U00024	1080	48.5	1	1079	359
BabP25	U00025	1080	48.5	1	1079	359
BabP26	U00026	1080	48.5	1	1079	359
BabP27	U00027	1080	48.5	1	1079	359
BabP28	U00028	1080	48.5	1	1079	359
BabP29	U00029	1080	48.5	1	1079	359
BabP30	U00030	1080	48.5	1	1079	359
BabP31	U00031	1080	48.5	1	1079	359
BabP32	U00032	1080	48.5	1	1079	359
BabP33	U00033	1080	48.5	1	1079	359
BabP34	U00034	1080	48.5	1	1079	359
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BabP36	U00036	1080	48.5	1	1079	359
BabP37	U00037	1080	48.5	1	1079	359
BabP38	U00038	1080	48.5	1	1079	359
BabP39	U00039	1080	48.5	1	1079	359
BabP40	U00040	1080	48.5	1	1079	359
BabP41	U00041	1080	48.5	1	1079	359
BabP42	U00042	1080	48.5	1	1079	359
BabP43	U00043	1080	48.5	1	1079	359
BabP44	U00044	1080	48.5	1	1079	359
BabP45	U00045	1080	48.5	1	1079	359
BabP46	U00046	1080	48.5	1	1079	359
BabP47	U00047	1080	48.5	1	1079	359
BabP48	U00048	1080	48.5	1	1079	359
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BabP51	U00051	1080	48.5	1	1079	359
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BabP54	U00054	1080	48.5	1	1079	359
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BabP58	U00058	1080	48.5	1	1079	359
BabP59	U00059	1080	48.5	1	1079	359
BabP60	U00060	1080	48.5	1	1079	359
BabP61	U00061	1080	48.5	1	1079	359
BabP62	U00062	1080	48.5	1	1079	359
BabP63	U00063	1080	48.5	1	1079	359
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BabP65	U00065	1080	48.5	1	1079	359
BabP66	U00066	1080	48.5	1	1079	359
BabP67	U00067	1080	48.5	1	1079	359
BabP68	U00068	1080	48.5	1	1079	359
BabP69	U00069	1080	48.5	1	1079	359
BabP70	U00070	1080	48.5	1	1079	359
BabP71	U00071	1080	48.5	1	1079	359
BabP72	U00072	1080	48.5	1	1079	359
BabP73	U00073	1080	48.5	1	1079	359
BabP74	U00074	1080	48.5	1	1079	359
BabP75	U00075	1080	48.5	1	1079	359
BabP76	U00076	1080	48.5	1	1079	359
BabP77	U00077	1080	48.5	1	1079	359
BabP78	U00078	1080	48.5	1	1079	359
BabP79	U00079	1080	48.5	1	1079	359
BabP80	U00080	1080	48.5	1	1079	359
BabP81	U00081	1080	48.5	1	1079	359
BabP82	U00082	1080	48.5	1	1079	359
BabP83	U00083	1080	48.5	1	1079	359
BabP84	U00084	1080	48.5	1	1079	359
BabP85	U00085	1080	48.5	1	1079	359
BabP86	U00086	1080	48.5	1	1079	359
BabP87	U00087	1080	48.5	1	1079	359
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BabP89	U00089	1080	48.5	1	1079	359
BabP90	U00090	1080	48.5	1	1079	359
BabP91	U00091	1080	48.5	1	1079	359
BabP92	U00092	1080	48.5	1	1079	359
BabP93	U00093	1080	48.5	1	1079	359
BabP94	U00094	1080	48.5	1	1079	359
BabP95	U00095	1080	48.5	1	1079	359
BabP96	U00096	1080	48.5	1	1079	359
BabP97	U00097	1				