Cloning and expression of two proteases from *Babesia bovis* genome.

Lu[1], S., González[1], Y.G., Torquato[1], R.J.S., Tanaka[1], A.S.

[1]Departamento de Bioquímica, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil.
lu.stephen@unifesp.br

Introduction: The *Babesia* genus comprises a group of the second most commonly found parasites in mammal's bloodstream and is responsible for major problems in public and animal health. In livestock the tick *Rhipicephalus microplus* acts as the vector of *Babesia* and *Anaplasma* which leads to almost 7 billion USD in losses for cattle production. Despite the economic and health relevance of *Babesia* genus the knowledge about parasite invasion and survival is still sparse. **Objectives:** Functional characterization of two proteases from *B. bovis* and their relation in host-parasite biology. **Materials and methods:** Using the genome of *Babesia bovis* we perform a search for putative proteases and select two sequences for further studies, one cysteine-like peptidase (XP_001612131) named BbCp and one subtilisin-like (XP_001610126) named BbSp. BbCp sequence was amplified from a cDNA preparation of red blood cells from infected bovines. BbCp pro-enzyme (PRO) and its catalytic domain (CD) were cloned in the pPICZαB vector for yeast expression. The genomic analyses of BbSp sequence reveal the absence of intron sequences and thus gDNA amplification was carried for cloning in the pGEM-T vector and DNA sequencing. **Results:** Although we were able to select *P. pastoris* clones containing $_{PRO}$BbCp or $_{CD}$BbCp, enzymatic activity was not observed in yeast supernatant. SDS-PAGE was also performed and no protein of the expected molecular mass was observed. DNA sequencing of BbSp.pGEM-T construction confirms the absence of introns in the genomic sequence. **Conclusion and discussion:** Since neither protein nor enzymatic activity of BbCp was observed in yeast supernatant we decide to carry protein expression in the bacteria system, thus $_{PRO}$BbCp sequence was subcloned in the pET14B vector and different strains of bacteria will be used in the attempt to acquire the active enzyme. The BbSp.pGEM-T construction will be used to clone $_{PRO}$BbSp and $_{CD}$BbSp for expression in yeast and bacteria.

Supported by: FAPESP, CNPq and INCT-Entomologia Molecular.

Keywords: Babesia, Proteases, and Apicomplexa.