

PZ-39 ISOLATION OF A *Babesia*-FREE STRAIN OF *Boophilus microplus*, AND THE BIOLOGICAL PURIFICATION OF THE PROTOZOAN PARASITES

Farias, N.A.R., Chies, J.M., Santos, T.R., Ozaki, L.S.*, Sant'Anna, D.M., Silva, C.H., Dabdab, K. & Gonzales, J.C.

Faculdade de Veterinária, Divisão de Entomozooses, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9060, 91500, Porto Alegre, *Departamento de Biotecnologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil.

The main vector of the bovine parasites *Babesia bovis* and *B. bigemina* is the tick *Boophilus microplus*. In the field the tick is normally infected with both parasites species. In order to study the pathogenicity of these protozoa, *Babesia*-free ticks, as well as isolates of both parasites, were obtained by experimental manipulations and from natural sources. A strain of *Babesia*-free tick was obtained by treating an infested animal with benzimidazole based babesicide. A naturally free strain was obtained in Santa Vitória do Palmar, RS, as ascertained by microscopic examination of haemolymph. Both strains of ticks were confirmed to be free of parasites by passages on splenectomized animals for twelve generations. Isolates of *B. bovis* and *B. bigemina* were obtained by standard biological manipulations based on instar specific infection of each parasite. We now have available: a) strains of ticks free of *Babesia*; b) isolates of both *B. bovis* and *B. bigemina*; and, after manipulating these biological materials; c) strains of ticks infected with either *B. bovis* or *B. bigemina*.

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PZ-40 INSERTION OF CYTOTOXIC EPITOPES FROM PLASMODIUM BERGHEI IN SALMONELLA FLAGELLIN

Cury, M.F.S.; Zavala, F.* & Newton, S.M.C.

Departamento de Microbiologia, Instituto de Ciências Biomédicas II, Universidade de São Paulo, CEP 05508 - São Paulo, SP, Brasil

* New York University, Department of Molecular Parasitology

Sporozoites, the infective stage of Plasmodium sp, are attractive targets for immunization against malaria. Indeed, it is well established that antibodies directed to sporozoites are able to confer protection. The main target antigen for antibody production is the CSP (circumsporozoite protein), which covers the whole parasite. In this protein, most antibodies, if not all, are directed to a repeated region. However, there is evidence that other mechanisms of protection are also involved: it is possible to protect mice that have been depleted of B-cells, and by adoptive transfer of T-cells. Recently, a cytotoxic epitope from Plasmodium berghei CSP was described (Romero et al, Nature 341:323, 1989). CD8⁺ cytotoxic T-cells specific for this epitope, when transferred to naive mice, were able to protect them against challenge with live sporozoites.

We are constructing attenuated, *aroA*⁻ strains of Salmonella expressing chimeric flagellar filaments, harboring the cytotoxic epitope on their surface. The construction of hybrid flagellin genes involves insertion of oligonucleotides in the hypervariable region of the gene, which codes for flagellar antigenic determinants. Oligonucleotides were designed to allow sequential insertions in the hypervariable region. Our results indicate that it is possible to insert at least two copies of the cytotoxic epitope in Salmonella flagellin without affecting bacterial motility.

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