Prospection and production of recombinant antigens for the diagnosis of animal tuberculosis - Pandolfi J.R.[1]*, Silva V.S.[1], Kramer B.[1], Lobo F.P.[2], Zanelli C.F.[3], Araújo F.R.[4], Calich V.L.G.[5], Peixoto J.O.[1], Cantão M.E.[1], Morés N.[1], Loyola W.[1], Grings V.H.[1], Coldebella A.[1], Sluszz T.[1], Bordin L.C.[1], Lazzarotti M.[1], Lopes L.S.[1], Ibelli A.M.G.[1], Amaral A.L.[1], Tessmann A.L.[1]

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Tuberculosis is a major problem of public health worldwide. The disease, characterized by granulomatous lesions, which the major etiological agent in animals is the *Mycobacterium bovis*, has serious economical and zoonotic implications. Screening of mycobacteria infections in animals is done using intradermal skin test. However the tuberculin skin test is unable to distinguish between bacteria from *M. avium* Complex (MAC) and *M. tuberculosis* Complex (MTBC), which includes *M. bovis*. In addition, in swine the skin test does not provide individual interpretation. Therefore, the aim of this project is to prospect and produce specific *M. bovis* recombinant antigens and evaluate their employment in two distinct in vitro tuberculosis diagnostic tests. To this end, a network of multi institutional and multidisciplinary research was established to carry out this proposal. The exploration of these antigens will be made through bioinformatics tools, proteomics analysis and literature review of candidate genes. The selected antigens will be cloned, expressed and purified to be then evaluated by two humoral and cellular immunity based assays, screening antibodies or gamma interferon production. Besides the application of these antigens in diagnostic assays to cattle and swine production, these antigens may also be used in other domestic animal species and could also have application in monitoring wildlife populations.

Key-words: animal tuberculosis, *Mycobacterium bovis*, recombinant antigens

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Prospection and production of recombinant antigens for the diagnosis of animal tuberculosis.

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Introduction

Tuberculosis is a major problem of public health worldwide. The disease, characterized by granulomatous lesions, which the major etiological agent in animals is the *Mycobacterium bovis*, has serious economical and zoonotic implications. Screening of mycobacteria infections in animals is done using intradermal skin test. However the tuberculin skin test is unable to distinguish between bacteria from *M. avium* Complex (MAC) and *M. tuberculosis* Complex (MTBC), which includes *M. bovis*. In addition, in swine the skin test does not provide individual interpretation. Therefore, the aim of this proposal is to prospect and produce specific *M. bovis* recombinant antigens and evaluate their employment in two distinct in vitro tuberculosis diagnostic tests. To this end, a network of multi institutional and multidisciplinary research was established to carry out this proposal. The exploration of these antigens will be made through bioinformatics tools, proteomics analysis and literature review of candidate genes. The selected antigens will be cloned, expressed and purified to be then evaluated by two humoral and cellular immunity based assays, screening antibodies or gamma interferon production.

Materials & Methods

1. Candidates prospection

- Bioinformatics study - This step aims to achieve in silico exploration of potential antigens for the diagnosis of animal tuberculosis. Thus, specific proteins of *M. bovis*, that are potentially immunogenic (ie proteins possibly extracellular and / or transmembrane with epitopes predicted B lymphocyte or T), will be searched for the prioritization of possible targets for use in diagnostic tests.

- Proteomic study - This action plan aims the exploration and identification of specific proteins of *M. bovis* by performing two-dimensional electrophoresis of protein extracts from *M. bovis* and *M. avium*. After electrophoresis, a software will then make the comparison and determination of the spots to be excised and sent to the identification of molecules through mass spectrometry (MALDI-TOF).

- Literature candidates – A strategy to eventual failure of bioinformatics and proteomic steps is the employment of candidate antigen genes mined from literature.

2. Antigen production

Mycobacterial growth and DNA extraction will be carried out at Embrapa Swine and Poultry Biosecurity lab. Then, DNA samples will be employed to gene cloning, protein expression and purification at Embrapa Beef Cattle ands Embrapa Swine and poultry laboratories. All expressed antigens will be evaluated by humoral and cellular immunological approachs.

3. Immunological assays

- Humoral approach: A immunoenzymatic assay carried on a nitrocellulosis membrane will be employed to verify antigens quality.

- Cellular approaches – Flow citometry and ELISA will be used to verify γ –IFN production by white blood cells.

Future Directions

Besides the application of these antigens in diagnostic assays to cattle and swine production, these antigens may also be used in other domestic animal species and could also have application in monitoring wildlife populations.