Production of purified protein derivative (PPD) from *Mycobacterium aviumhominisuis* to improve the comparative intradermal tuberculin skin test on swine - Pandolfi J.R.[1]*, Silva V.S.[1], Kramer B.[1], Morés N.[1], Loyola W.[1], Grings V.H.[1], Coldebella A.[1], Sluszz T.[1], Bordin L.C.[1], Lazarotti M.[1], Lopes L.S.[1], Ibelli A.M.G.[1]

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Swine are susceptible to mycobacterial infections such as *Mycobacterium avium* complex (MAC) and *M. tuberculosis* Complex (MTBC) that includes *M. bovis*. MAC bacteria are more prevalent and cause economic losses on swine production as they induce granulomatous gross lesions identical of those observed on swine tuberculosis. Regardless of the National Program to Control and Eradicate Brucellosis and Tuberculosis, some diagnostic tools are needed to differentiate MAC and TB infections in swine. Mycobacteria share antigens and this is aconstraint to distinguish animals infected by MAC from those infected by MTBC agents. The comparative intradermal tuberculin skin test is employed to evaluate the presence of tuberculosis on herds. However this test providesonly herd information, and this is a huge problem when sanitary measures must to be taken to clean up a positive herd. The aim of this project is to improve the tuberculin skin test to swine, by the production of a *M. aviumhominisuis* PPD (PPDH). Thus, the PPDH will be produced, purified, standardized such as the commercial ones and compared to them. The comparison will be done on 180 pre-immunized (with *M. aviumavium, M. aviumhominisuis or M. bovis*, *M. aviumavium and M. bovis, M. aviumhominisuis and M. bovis and a* negative control) adult swine. The test will be performed after 63 days of immunization and comparisons between PPDH and avian PPD and PPDH and bovine PPD will be performed. The health profile of tested animals will be evaluated by pathological, microbiological and molecular techniques.

Key-words: *Mycobacterium aviumhominissuis*, tuberculosis, intradermal tuberculin skin test, PPD

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Production of purified protein derivative (PPD) from *Mycobacterium avium hominisuis* to improve the comparative intradermal tuberculin skin test on swine.

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Introduction

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Materials & Methods

Antigens production

M. Avium hominisuis, M. avium avium D4 strain and M. bovis AN5 strain were produced by growing in Middelbrook 7H9 (with ADC or OADC) for 3 months. After that mycobacteria were inactivated. Cell mass were recovered by centrifugation. PPD precipitation was performed by TCA protocol (as described below). Then protein quantification was done by Bradford. The antigen was standardized on a 5mg/mL concentration on a buffer solution (PBS+glicerol+phenol) and a new autoclavation step was done. After that the sterility of antigens was checked by growing on Middelbrook 7H9, blood agar and BHI culture.

Figure 1. Mycobacterial Antigen production **A.** *M. avium hominisuis* culture for antigen production. **B.** *M. bovis* culture for antigen production.

Materials & Methods

PPD production

the PPDH was produced, purified, standardized such as the commercial ones.

Briefly a M. avium hominisuis strain was cultured into a Middlebrook 7H9 broth for 3 months. Then, cells were centrifuged and supernatant was recovered and used to PPD precipitation with TCA (4%). PPD was centrifuged (3.000 xg for 10 min.). Pellet was washed once with TCA (40% stock solution) and twice with NaCl solution (10%). Then pellet was ressupended on a buffer solution (PBS+5%glicerol+0,5%phenol) and total protein was quantified and the PPD solution was standardized to a 0,5mg/mL as the commercial PPDs.

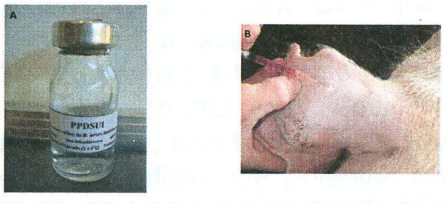

Figure 2. PPDSUI and tuberculin injection. **A.** *M. avium hominisuis* PPD - PPDSUI. **B** Tuberculin intradermic injection. Picture B is a courtesy of Nelson Morés.

The comparison will be done on 180 pre-immunized (with *M. avium avium , M. avium hominisuis or M. bovis, M. avium avium and M. bovis, M. avium hominisuis and M. bovis and a* negative control) adult swine. The test will be performed after 63 days of immunization and comparisons between PPDH and avian PPD or PPDH and bovine PPD will be done. The health profile of tested animals will be evaluated by pathological, microbiological and molecular techniques.

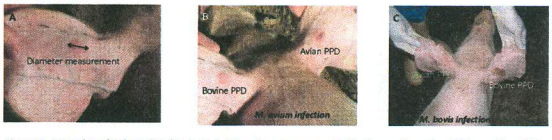

Figure 3. Examples of tuberculin skin test. **A.** Diameter measurement of skin reaction after 48 hours. **B** and **C.** Diferences between lesions induced by bovine or avian PPD under different infection conditions. Pictures are a courtesy of Nelson Morés.