IVD_23 - Mycobacterial antigens as a new target for detection of *M. avium paratuberculosis* in milk from cows with paratuberculosis

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Introduction: Bovine paratuberculosis (PTB), or Johne's disease, is a chronic intestinal infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) that causes great damage to the dairy industry. MAP is eliminated in the feces and milk of infected animals, both during the clinical and subclinical phases of infection. The presence of MAP has been demonstrated in raw and pasteurized milk, and in white cheese. Humans are exposed to mycobacteria through the consumption of dairy products, and there are reports of a possible relationship between MAP infection and Crohn's disease. Laboratory diagnosis of PTB is based on the isolation of bacteria or detection of DNA, using high-cost imported kits and reagents. Therefore, new diagnostic approaches based on the detection of bacterial antigens may be useful for the bacteriological quality of milk and PTB control. Recently, we produced monoclonal antibodies against one of the antigens secreted by MAP (APA protein), which were used in the ELISA kit indicated for fecal diagnosis (PTB-Detect kit).

Objectives: The aim of this work was to verify the presence of APA protein in raw milk from cows with PTB using immunochemical methods.

Methodology: Milk samples were collected from herds in the farms of northwest region of Rio de Janeiro. Firstly, these samples were subjected to the ELISA test, using the commercial kit PARAS-4P ID-Vet Screen® Paratuberculosis Indirect Screening test (France) to detect anti-MAP antibodies in the milk of animals with PTB. Positive samples were subjected to immunodetection of the APA antigen, using an immunoprecipitation technique with subsequent analysis by Western blot.

Results: The APA -MAP antigen was found in approximately 5% of these samples. Our results demonstrate that APA is being secreted in the milk of cows with PTB in its glycosylated 70 kDa isoform, and the protein can be isolated from milk through immunoprecipitation.

Conclusion: Finally, the isolation of APA antigen in milk can be a useful tool in the immunodiagnosis of PTB and the sensitivity and specificity of the test is being compared with a commercial kit (VetMAX™ MAP Real- Time PCR, USA) indicated for the detection of PTB DNA MAP.

Keywords: Immunodiagnosis; Milk; Bovine paratuberculosis