

TITLE: DEVELOPMENT OF TOOLS FOR THE DIAGNOSIS OF PARATUBERCULOSIS IN BOVINE FECES.

AUTHORS: SOUZA, G.S.; RODRIGUES, A.B.; ROMANO, M.I.; REZENDE A.L.; LASSOUNSKAIA, E.

INSTITUTION: UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO (AV. ALBERTO LAMEGO, 2.000, PARQUE CALIFÓRNIA, CEP 28035-200, CAMPOS DOS GOYTACAZES-RJ, BRAZIL)

ABSTRACT:

Cattle farming business is one of the most important sectors of Brazilian agriculture. Paratuberculosis (PTB), clinically known as Johne's disease, is a chronic intestinal infection of cattle affecting livestock productivity. The disease causes malabsorption syndrome, diarrhea, wasting or loss of body condition, leading ultimately to death. The causative agent, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is excreted by the infected cows in the feces, and to a lesser extent in milk and saliva, contaminating the environment and infecting offspring and other animals. In Brazil, the importance of the disease is underestimated due to the difficulties in detection of infected animals, and lack of reliable, fast and cheap methods of laboratory diagnosis. New immunodiagnostic approaches based on detection and identification of MAP antigens in the feces of infected animals can be useful for diagnosis of PTB. The APA antigen is one of the protein antigens actively secreted by MAP, suggesting that it may be excreted in the bovine feces. For immunodetection of this antigen, we produced murine monoclonal antibodies and chicken IgY against APA protein of MAP (APA-MAP). These antibodies detected the APA-MAP protein in the feces of cows with Johne's disease, but not in those of healthy animals, demonstrating that the APA is a fecal biomarker of the disease. In this work, we produced anti-APA-MAP sandwich ELISA kit indicated for rapid immunodiagnostic of PTB in feces. The produced antibodies may be used for the APA-MAP isolation by immunoprecipitation. Analysis of the APA protein, immunoprecipitated from feces, by Western blot and staining with Schiff's reagent demonstrated that the isolated protein was presented by a glycosylated 60-75 kDa isoform. Additionally, we developed an alternative method for the immunodiagnosis of PTB that was based on detection in feces of antimycobacterial IgA. The obtained results demonstrated the presence of antimycobacterial IgA in the feces of animals with PTB. However, isolated IgA showed cross-reactivity against antigens of *M. avium* and *M. bovis* that did not allow differentiation of PTB and the intestinal form of tuberculosis (TB). It was concluded that immunodetection of the APA-MAP antigen in the feces of cattle is a new and promising approach for the diagnosis of PTB. The tools and protocols for new diagnostic testing have been developed in this work.

Keywords: Paratuberculosis, APA antigen, Immunodiagnosis, Feces

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