Detection of Mycobacterium avium subsp. Paratuberculosis in Cattle by using Indirect Absorbed ELISA (enzyme-linked immunosorbent assay) system and culture in Alborz Province, Iran

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ABSTRACT

Objective/Background: Mycobacterium avium subsp. paratuberculosis is a slow growing, Gram-positive, and acid-fast bacillus. It is the causative agent of the chronic enteritis disease of ruminants called Johne's disease. Most of the infected animals are young. However, most clinical cases belong to adult cattle aged 3-5 years. Due to a prolonged incubation period, identification of subclinically infected animals is one of the most crucial problems in Johne's disease. The aim of this study was to detect M. avium subsp. paratuberculosis in cattle using indirect absorbed enzyme-linked immunosorbent assay (ELISA) and culture for positive samples in Alborz Province, Iran.

Methods: Briefly, 384 blood samples were taken from cattle of five areas of Alborz Province. Blood samples were tested by indirect absorbed ELISA (Razi paratuberculosis kit, Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran). Soluble antigens of Mycobacterium phlei were used to remove nonspecific antibodies in the bovine serum. Before transferring serum samples of cattle to the plate that coated with (MAP) antigens, serum samples of cattle were diluted and pre-incubated with a dilution buffer containing M. phlei antigens. During this project, we cultured fecal samples of cattle who displayed positive results for ELISA test. Anti-ruminant immunoglobulin G and Horse Radish Peroxidase (HRP) were added to all microwells. After washing, the substrate solution tetra-methyl benzidine (TMB) was added to eliminate the excess conjugate. The microplate was read using a spectrophotometer (ELISA reader, Bio Rad-Model 620) at 450 nm in the Razi Vaccine and Serum Research Institute (Karaj, Iran). All ELISA-positive samples were cultured in media tubes (3 tubes of Herrold's egg with Mycobactin and 1 tube of Herrold's egg without Mycobactin for every sample).

Results: In total, 3.19% of cattle serum samples showed significant antibodies titer to infection with M. avium subsp. paratuberculosis in all the areas of sample source, whereas 96.81% serum samples were negative. Of the 12 ELISA-positive samples, six samples showed growth in the media.

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Conclusion: Because there is no treatment or cure for Johne's disease, detection of infected cattle and subsequent culling is very important for preventing infection in other cattle. Fecal culture is a standard method for the identification of the disease. However, in Johne's disease, due to prolonged incubation and shedding of the disease, the probability of isolating the responsible agent is very low. To identify the infection, the indirect absorbed ELISA method is used for eradication. This technique is considered as one of the most reliable for identification of the disease worldwide due to its ease of use and low cost. However, for confirmation of ELISA-positive results, culture method has been recommended.

Conflicts of interest

The authors have no conflicts of interest to declare.