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Current approaches in laboratory diagnostic of paratuberculosis

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Abstract
Paratuberculosis is incurable, chronic granulomatous enteritis, caused by Mycobacterium avium subsp. Paratuberculosis. Animals are infected by ingested food and water contaminated with feces of infected animals. In cubation period is long and the causative agents can be secreted for 15-18 months before the clinical signs appear. In cattle the clinical signs appear mostly from 2-6 years of age. The disease spreads slowly and ends with dehydration, cachexia and exhaustion of the animal.

Diagnostics of paratuberculosis can be done in clinical form of the disease and in subclinical infection and there are two main goals – monitoring of the herd and identification of positive animals. Diagnostic is done by direct detection of the causative agent, applying the selective media, from fecal and tissue samples or by detection of agents genome by PCR method. Isolation from fecal samples is demanding but still represents the most reliable method for the diagnostic of paratuberculosis. The isolation is difficult because of the possible contamination of samples, small number of bacteria secreted, intermittent secretation, and especially slow growth of isolates. Different sequences of genomia are identified for molecular identification MAP. Mostly it is the sequence IS900, which appears in 14 to 20 copies in MAP genome. During the last few years, other specific sequences are detected like f57, locus 255, ISMap02 and others. Direct identification MAP in feces or organ samples by PCR method will significantly shorten the time for approoving the infection. Sensitivity of PCR method is limited by the ineffacy of DNA extraction.

Detection of antibodies by ELISA test is a method of choice for diagnostic of paratuberculosis, because it is rapid and with relatively low costs. Agar gel immunodiffusion test (AGID) and complement fixation test are traditional methods for diagnostic of paratuberculosis, which are loosing the significance. Antibody detection can be done from blood or milk samples. In some countries ELISA test is used in control programs for paratuberculosis, instead the cultivation, considering the rapid gain of results and satisfactory specificity and sensitivity.

First serologic study for the presence of paratuberculosis in cattle were done 20 years ago in the region of Vojvodina. Study was done on blood samples of cows from 12 farms and AGID test was applied. Positive results were found in cows from 4 farms, 1.5%. When complement fixation method was applied, 4.1% of positive animals was found.

In a survey 15 years later, we applied ELISA test and found 2.9% of positive animals, what is a sign of good epizootiologic situation.

Lack of precise laboratory tests, long period of incubation and small number of clinical cases, complicates control of paratuberculosis. Control program is based on reduction of agents transmission to susceptible animals, elimination of infected animals, hygiene measures and vaccination. The efficacy of proposed programs directly depends on the elimination of infected animals.

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