

Novel antigens used to detect cell-mediated immune responses over time in *Mycobacterium avium* subsp. *paratuberculosis* infected cattle

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Paratuberculosis is a chronic, granulomatous enteric infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in ruminants. Early-stage MAP infection can be detected using diagnostics for cell mediated immune responses, e.g. the whole blood interferon gamma (IFN- γ) test. Available IFN- γ tests are using purified protein derivatives of MAP (PPDj) which are crude products consisting of undefined antigens with possible cross reactions toward other environmental bacteria. The IFN- γ test is known to have low specificity, especially in young calves. The objective of the study was to optimize the IFN- γ test using different types of novel antigens for stimulation to determine if some antigens could be excluded or combined.

Fourteen novel antigens were selected for testing, including 4 members of the ESAT-6 family, 4 hypothetical latency proteins, 3 secreted proteins, 2 proteins not present in *Mycobacterium avium* subsp. *avium* (MAA) and 1 selected from an immunological hot spot region. Variation of IFN- γ responses in different antigen groups was determined based on 3 repeated tests performed on the same 30 heifers 15-24 months of age from a herd with known MAP infection, with 4 and 5 week intervals.

Cut-offs used to discriminate test-positive and test-negative for each antigen was based on samples from 60 heifers from a non-infected herd. Based on PPDj stimulations approximately half of the heifers in the infected herd tested positive at each sampling. Fewer animals tested positive using recombinant antigens. Latency proteins and secreted proteins antigens resulted in 40% test positive or fewer. Hypothetical antigens based on sequences not present in MAA tested approximately one third of the animals as positives. The results showed that PPDj resulted in the highest percentage test positive animals. However, this crude antigen mixture is expected to induce non-specific IFN- γ production. Higher specificity might be obtained by combining some of the novel antigens.

However, validation of these antigens is hindered by the lack of a gold standard.