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Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

USE OF NOVEL RECOMBINANT ANTIGENS IN THE INTERFERON GAMMA ASSAY FOR DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INFECTION IN CATTLE

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Early stage *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection can be detected by measuring antigen specific cell mediated immune responses by the interferon gamma (IFN-γ) assay. Available IFN-γ assay use purified protein derivate of Johnin (PPDj) leading to low specificity. The objectives of the study were to evaluate immunogenicity and specificity of 14 novel recombinant antigens for use in the IFN-γ assay and to assess the consistency of IFN-γ responses. The antigens used were 4 ESAT-6 family members, 4 latency proteins, 4 secreted proteins including Ag85B, 3 other antigens and PPDj. The study included blood samples from 26 heifers of a MAP infected herd, collected three times with 4 and 5 week interval and blood samples from 60 heifers of a MAP non-infected herd collected once. The IFN-γ responses of the non-infected heifers were used to establish cut-off values for each antigen. Animals of the MAP infected herd were grouped as cases or non-cases by a case definition that was: an animal with ≥2 positive tests for ≥4 antigens, which resulted in 13 cases and 13 non-cases. Based on the case-definition, immunogenicity and specificity of each antigen were calculated. IFN-γ levels of the infected and non-infected herds were significantly ($P<0.05$) different and IFN-γ levels of cases were significantly higher than non-cases ($P<0.05$). The results of the IFN-γ assay using PPDj did not correlate with the results using the novel antigens since 5 of the 17 animals that were positive to PPDj were non-cases and one case were negative to PPDj but positive to all other tested antigens. Furthermore, PPDj produced elevated IFN-γ responses of both the infected and non-infected herds and showed low consistency. Immunogenicity was highest for the group of latency proteins (0.65-0.85) that also had high specificity (0.92-1.00). Three latency proteins showed positive IFN-γ tests that correlated highly with the case definition and one of these antigens (LATP-2) had no homologue sequence in the *M. avium* subsp. *avium* or *M. bovis* genome and could be a promising diagnostic antigen. The combination of antigens for use as a cocktail should be further investigated. However, to detect the animals defined as cases, 8 of the novel antigens and Ag85B would have to be included.