THE ONSET OF DETECTABLE IMMUNE RESPONSES AND FECAL SHEDDING AFTER EXPERIMENTAL INFECTION WITH MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

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Introduction
Early detection of Mycobacterium avium subsp. paratuberculosis (Map) infection is hampered by the low sensitivity of the commonly used diagnostics, in part due to the late onset of immune responses and fecal shedding. The immune response in paratuberculosis seems to be driven by strong Th1-type cellular immune responses during the subclinical stages of infection. This Th1 response can be detected by production of IFN-γ by memory T-cells after stimulation with specific antigens. This makes measurement of IFN-γ production after cell stimulation a good candidate for early detection of Map infection. However, reports on the specificity of the IFN-γ assay are conflicting, ranging from 26 to 97.6%

Detection of serum antibodies is associated with the level of Map shedding and the age of the animals. Overall, the sensitivities of serum ELISA when compared to tissue culture are very low and serological detection of subclinical cases is difficult.

Fecal instead of tissue culture is often used as a gold standard for diagnosis of Map for practical reasons. Shedding in the early stages after infection is considered to be low and intermittent, but can potentially lead to infection of pen mates. The aim of this study was to evaluate the onset of cellular and humoral immune responses and fecal shedding with the available diagnostic tests when animals are infected with Map with different doses and at different ages. Tissue culture and histology were used to determine the infection status of the animals.

Materials & Methods
This infection trial was designed based on the guidelines published by Hines et al. (2007). Thirty Holstein-Friesian steer calves were experimentally infected at 5 different ages (2 weeks, 3, 6, 9 & 12 months). In each age group animals were infected with a high (5.10⁹ CFU) or a low (5.10⁷ CFU) dose of Map, on 2 consecutive days. Samples were collected monthly for measuring the onset of fecal shedding and immune responses using serum ELISA, Map-specific IFN-γ induction assay and fecal culture. All calves were euthanized at 17 months of age to confirm infection status by tissue culture and histology. IFN-γ ELISA was performed in 2 stages. In the first stage, blood samples were incubated overnight with a Map-specific antigen (johnin, provided by Canadian Food Inspection Agency, CFIA) and appropriate controls to stimulate the lymphocytes to produce IFN-γ. In the second stage, IFN-γ in the plasma supernatant of each blood aliquot was determined using the BOVIGAM® ELISA (Prionics, USA) according to manufacturer’s instructions.

Serum was tested using the Mycobacterium Paratuberculosis Antibody Test Kit® (IDEXX laboratories, USA).

Fecal and tissue samples were decontaminated and incubated in the TREK ESP® Culture System II (TREK diagnostic systems, USA) for 7 weeks. A confirmatory IS900 PCR was performed on all cultures. The following tissues were selected for tissue culture: ileocecal valve, distal ileum and ileocecal lymph node.

The same tissues and one more (ileal lymph node) were embedded and stained with Hematoxylin-Eosin (HE) and Ziehl-Neelsen (ZN). Interpretation of histology was performed by a pathologist.

Results
A Map-specific IFN-γ response was detected in all age groups starting at 2 months after infection. Calves infected with a high dose of Map responded earlier and stronger than the groups infected with a low dose of Map. After the peak response at 3-4 months post infection, response slightly decreases over time.

Analysis of sera using the serum ELISA showed prolonged increased antibody titers in some animals from all age groups, for the high dose animals as soon as 3 months post-infection.
Fecal shedding was low and intermittently present during the trial in all age groups, except the 9 month infection group. There was no consistent onset of shedding in the different groups. However, high dose calves shed more frequently than low dose calves over the duration of the trial. A combination of tissue culture and histology identified 27 out of the 30 animals as infected.

Discussion

IFN-γ ELISA may offer a powerful tool for early diagnosis of JD. Infected animals respond strongly in the first months after infection. However, a cellular immune response is no proof of established infection, as the cellular immune response is considered most efficient for controlling mycobacterial infections, including paratuberculosis\(^{10,11}\). The observed gradual decrease of the response might potentially lead to negative results when animals are tested long after infection. Serum ELISA also detected animals in earlier stages than previously observed, although not all infected animals produced a detectable antibody titer during the trial. There is no consistent onset of humoral immune responses, but responses can occur in the first 10 months after infection. A combination of tissue culture and histology classified most animals as infected. By tissue culture alone, 16 out of 30 animals tested positive. This may be explained by the fact that only 3 tissues were selected from all the samples collected at necropsy. We have reasons to believe that more animals were actually infected than what tissue culture indicates. For example, some animals were repeatedly shedding while tissue culture had a negative result. We plan to analyse the remaining samples collected at necropsy in order to estimate the optimal-but-practical number of necropsy tissues to collect for culture and histopathology to determine the true disease status of challenged calves.

This study provides information about shedding and cellular and humoral immune responses in the first months after infection, up to 17 months after infection for the group infected at 2 weeks of age. Related to the predefined stages of JD\(^{12}\), this would correspond with the first 2 stages of the disease: the silent infection and carrier stage. This study proves that animals in the silent infection stages can shed the bacteria and can be detected with IFN-γ ELISA and in some cases with serum ELISA. Excretion of Map by young animals may contribute in only a small part to environmental contamination, but it may be a significant risk for transmission of the disease if it occurs when highly susceptible calves are kept in groups\(^{8}\).

Frequent testing and using a combination of available tests would be recommended in order to diagnose Map infection in the early stages of the disease with a high sensitivity and specificity.

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References


