Is it possible to Diagnose Subclinical Infection with *M. paratuberculosis* in Cattle younger than 2 years of Age?

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**Introduction**

Paratuberculosis has emerged to become a serious disease of dairy cattle in industrialised countries. The exact prevalence of paratuberculosis in Danish dairy herds is not known, but recent pilot projects suggested that approximately 50% of the herds are infected (Nielsen et al., 2000a; Nielsen et al., 2000b). In Denmark, diagnosis of infection with *M. paratuberculosis* is made most commonly through detection of the pathogen by cultivation of faecal samples. However, cultivation-positive results cannot be expected until late in the subclinical stage, when infected animals are 2 years of age or older. Similarly, antibody detection methods such as the absorbed indirect enzyme-linked immuno sorbent assay (ELISA) may not reveal infected animals until late in the course of infection. However, ELISA is widely used for diagnosis of paratuberculosis due to easy performance and high accuracy (Collins, 1996). Test for the cellular immune response, that is the first response elicited to infection with mycobacteria and other intracellular pathogens (Chiodini, 1996), may result in an earlier diagnosis than by faecal cultivation or serum ELISA. Detection of secreted interferon-gamma (IFN-γ), following antigen stimulation of peripheral blood lymphocytes, has been evaluated as a diagnostic tool for identification of young cattle infected with *M. paratuberculosis* (McDonald et al., 1999). Problems with non-paratuberculosis specific reactions and uncertain interpretation of assay results limited the diagnostic potential in calves. Results from recent Danish studies indicate that infected animals may be identified by the IFN-γ test from approximately 1 year of age (Jungersen et al., 2002; Huda et al., 2003). This presentation reports results from testing of IFN-γ test and the application of IFN-γ test compared to serum and milk ELISA to cattle in different age groups.

**Materials and methods**

During a 2-year period 371 cattle were sampled in a prospective cohort study in order to evaluate immunological diagnostics for paratuberculosis. Two hundred and fifty-two animals were selected from dairy herds with known history of paratuberculosis and 119 animals from herds presumably free from infection with *M. paratuberculosis*. The animals were from 1 month to 4-5 years of age at the time of the first sampling. Animals were subjected to repeated sampling 4-5 times per year with varying intervals, in total, 10 sampling rounds were performed. At each sampling heparin-stabilized blood for IFN-γ test, non-stabilized blood and individual cow milk sample for ELISAs, and a faecal sample for cultivation of *M. paratuberculosis* was collected.

IFN-γ test was performed as previously described (Jungersen et al., 2002). Briefly, within 8 hours from collection whole blood samples were incubated over night in separate wells with PBS (nil antigen) and 10 µg/ml *M. paratuberculosis* purified protein derivative (PPDj; Danish Veterinary Institute, Copenhagen, Denmark), and enriched plasma supernatant was assayed for secreted IFN-γ by a commercially available sandwich ELISA (BOVIGAM™; CSL Ltd., Parkville, Australia). Results were interpreted using average OD value of PPDj stimulation subtracted by average OD value of nil antigen stimulation. By an estimated 95% cut-off point (Jungersen et al., 2002), an animal was considered positive if the OD difference PPDj-PBS > 0.25.

Test for serum and milk antibody response was performed using an in-house absorbed indirect IgG ELISA using a commercially available antigen (PPA; Allied Monitor, USA), using a protocol with few modifications compared to that described by Klausen et al. (Nielsen et al., 2001; 2003).

Cultivation of faecal samples was performed as briefly described in the following. Faecal samples were decontaminated with sodium hydroxide and oxalic acid (Beerwerth, 1967) supplied by malachite green, neomycin and amphotericin B. Decontaminated samples were...
inoculated onto modified Löwenstein-Jensen medium (Jorgensen, 1982); after 12 weeks of incubation samples were inspected for colonies compatible with *M. paratuberculosis*.

**Results and discussion**

The number of positives by IFN-γ test was compared to the number of cultivation positives after the three first sampling rounds. In total, 97 (47%) animals from infected herds tested positive at least at one sampling by IFN-γ test, of these, 14 (7%) were cultivation positive at least at one sampling. Number of IFN-γ positive animals from non-infected herds was 5 (5%). The youngest IFN-γ positive animal was 4 months of age, average of test-positive animals was 26 months. By faecal cultivation, the youngest positive animal was 10 months of age and cultivation positive animals were in average 37 months of age. The frequency of IFN-γ positives was highly dependent on age and markedly increased from 7-12 months of age. However, a majority of test-positive animals presented variable test results at the 3 repeated samplings, indicating that reactors do not necessarily mount a stable cellular immune response. High proportion of fluctuating test results in youngest animals indicated that IFN-γ test could be applied to cattle from 1 year of age.

Performance of IFN-γ test and serum and milk ELISAs for revealing infection with *M. paratuberculosis* were evaluated in different age groups (1 year, 2 years, and 3 years and older) of infected and non-infected animals. The average result of each test representing the chronologically last three samplings of the animal in a particular age group was applied. IFN-γ test had the best performance in age groups 1 year and 2 years, and in age groups 3 years and older, the tests performed almost equally well. The results support that cellular immune responses are the earliest detectable responses against infection with *M. paratuberculosis*. The present protocol for IFN-γ test implied the application of fresh whole blood samples, as previous testings of day-old samples showed significantly reduced IFN-γ secretion to antigen response (Jungersen et al., 2002). Application of IFN-γ test under field conditions requires investigations of possibilities to avoid the demand of fresh blood samples.

Results indicate that IFN-γ test can be applied for screening of cattle at 1 and 2 years of age for exposure to *M. paratuberculosis* and thus identify animals at risk of developing paratuberculosis as adults.

**References**


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