ACTIVATION OF HOST IMMUNE RESPONSES IN NEONATAL CALVES AND INTERFERENCE WITH TB DIAGNOSTICS AFTER IMMUNIZATION WITH A COMMERCIAL HEAT-KILLED VACCINE

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Abstract:
A major drawback of current whole-cell vaccines for *Mycobacterium avium* subsp. *paratuberculosis* is the potential interference with diagnostic tests for bovine tuberculosis and paratuberculosis. The current study was designed to explore cross-reactivity of the current USDA commercial vaccine for MAP with diagnostic tools for bovine TB and to assess host responses to vaccination. Neonatal dairy calves were assigned to treatment groups consisting of: 1) Control – no vaccine (n = 5); and 2) Vaccinate – Mycopar vaccine (n = 5).

Blood and fecal samples were collected prior to the initiation of the study for pre-vaccination measurements. Calves were vaccinated subcutaneously with a 0.5 ml dose in the dewlap-brisket area as per standard procedure with the wild-type commercial vaccine that consists of a heat-killed whole cell suspension of MAP in oil (Mycopar). Calves were sampled throughout the study on days 7, 14, 28, and at 3, 6, 9, and 12 months. Comparative cervical skin testing was performed at 6 months both as a diagnostic tool and to determine in vivo cell-mediated response to vaccination. For determination of *M. bovis*-specific antibody, the TB Stat-Pak assay and the dual-path platform (DPP) VetTB assay (Chembio Diagnostic Systems) were performed. Peripheral blood mononuclear cells were isolated before and after vaccination and stimulated in vitro for measurement of interferon-(IFN)-γ, interleukin (IL)-4, IL-10, and IL-12, and to assess differences in lymphocyte populations by flow cytometry. Results from this study demonstrated a rapid initiation of MAP-specific IFN-γ in Vaccinate calves by 7 days, with robust responses continuing throughout the study.

Vaccinate calves also had IFN-γ responses to BoPPD, with moderate reactivity to ESAT-6/CFP-10, an *M. bovis* recombinant fusion protein. Interestingly, IL-4 and IL-10 were markedly decreased in Vaccinate calves only on days 7 and 14 of the study and thereafter were similar to Controls. Vaccinate calves began to seroconvert at 4 months with all calves having detectable MAP antibody by 6 months. Only one Vaccinate calf had a positive (suspect) skin test response to *M. bovis* PPD and none of these calves reacted in *M. bovis* serologic tests. These results suggest that vaccination with Mycopar will interfere with diagnostic tools for the detection of paratuberculosis but have low interference with *M. bovis* diagnostics.

Introduction
New serologic tests for the detection of *M. bovis* infection have recently been developed and are demonstrating high levels of sensitivity and specificity in the detection of bovine tuberculosis (Lyashchenko et al., 2008). However, there are no available data to determine if these new serologic test platforms will reduce cross-reactivity with MAP antigens associated with the paratuberculosis vaccine. Further, a more thorough assessment of host immune responses to vaccination will provide us with information about protective correlates associated with reduced clinical disease. The proposed research will explore cross-reactivity of the current USDA commercial vaccine for MAP with new serologic diagnostic tools for bovine TB.

Materials and Methods
Neonatal calves were randomly assigned to treatment groups consisting of: 1) Control – no vaccine; n = 5; and 2) Vaccine group – Mycopar vaccine; n = 5. After pre-vaccination sampling on days -2 and 0, calves were vaccinated intramuscularly (IM) in the brisket area as per standard procedure with the wild-type commercial vaccine (heat-killed whole cell suspension of MAP in oil; Mycopar, Fort Dodge Animal Health, Ft. Dodge, IA). Skin testing was performed at 6 months both as a diagnostic tool and to determine in vivo cell-mediated response to vaccination. To assess the effects of vaccination on standard diagnostic tools for MAP and *M. bovis*, samples were monitored for the presence of antibody and IFN-γ responses. Plasma was harvested after incubation of whole blood with medium only, ConA,
MAP sonicate, JPPD, BoPPD, and Esat-6/CFP-10 fusion protein and assayed for IFN-g by ELISA (Bovigam, Prionics). Serum was harvested from whole blood and assayed for the presence of MAP antibodies by commercial ELISA (Herdchek) and Western blot. For determination of *M. bovis*-specific antibody, the TB Stat-Pak assay (Chembio Diagnostic Systems, Inc., Medford, NY) and the dual-path platform (DPP) VetTB assay (Chembio Diagnostic Systems). Peripheral blood mononuclear cells were stimulated in vitro with medium control (NS), ConA (10 μg/ml), MPS (10 μg/ml), JPPD (10 μg/ml), BoPPD (10 μg/ml), and rEC (1 μg/ml) to evaluate immunologic parameters such as cytokine secretion (IFN-g, IL-4, IL-10, IL-12), and changes in cell populations.

**Results**

MAP-specific IFN-g was highly upregulated in calves within 30 days of vaccination. There were cross-reactive IFN-g responses to BoPPD stimulation in vitro in Vaccinate calves that paralleled responses to JPPD and MPS (Figure 1). However, responses to rEC were not different between Control and Vaccinate calves.

CCT (skin test) responses were highly specific to AvPPD and did not yield false positive results with BoPPD for vaccinated calves as demonstrated on the scattergram depicting zones of positive and negative results (Figure 2). MAP-specific antibody was demonstrated by 4 months post-vaccination using Western blot analysis (data not shown). Sera from vaccinated calves did not demonstrate any cross-reactivity with the TB Stat-Pak or DPP VetTB assays for *M. bovis* (data not shown).

Vaccination resulted in the increased expression of CD25 and CD26 activation markers on CD4 cells by 6 months.

![Image](image1.png)

**Figure 1.** Interferon-gamma (IFN-g) responses for total PBMCs at 1 month (A) and 12 months (B) after vaccination of calves

![Image](image2.png)

**Figure 2.** Delayed-type-hypersensitivity responses to *M. avium* PPD and *M. bovis* PPD were determined by the comparative cervical (CCT) test (means ± standard errors) in Control (n = 5) and Vaccinate calves (n = 5). Responses represent the change in skin thickness relative to pre-skin test (A) with results depicted on scattergram for interpretation of results (B).
Discussion and Conclusions

Vaccination of calves with a commercial heat-killed vaccine invokes an early cell-mediated immune response that is maintained through 12 months and is concurrent with the appearance of MAP antibody. There are several TB test options including the rEC-mediated IFN-g test, the CCT test, and newly developed *M. bovis* antibody tests that do not result in false positive results in MAP-vaccinated animals and could be used effectively in TB control programs.