

Creating Immune Markers and Complementary Diagnostics for a *Mycobacterium avium* subsp. *paratuberculosis* (MAP) Vaccine Strain

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Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD), a chronic progressive enteritis in ruminants with a high prevalence in Alberta. Despite its economic burden on the dairy industry, present control efforts have not yielded significant results due to poor diagnostic sensitivity related to the insidious nature of the disease and lack of available vaccines. Current vaccines are not made available or tightly regulated due to their inability to differentiate between vaccinated and naturally infected animals. With the high prevalence of MAP infection, the insensitivity of current tests, and difficulty in limiting disease spread, it is clear that the dairy industry needs new ways to protect animals from MAP infection.

Methods: To eliminate the regulatory problems associated with current MAP vaccines, we will modify an attenuated MAP strain such that vaccinated animals can be discriminated from naturally infected animals. This will allow for vaccination while still retaining the ability to serologically detect the presence of natural MAP and *M. bovis* infection. We will insert and eliminate antigens to create positive and negative immune markers, respectively, to be incorporated into the vaccine strain by genetic modification. Immune responses against the positive marker and the lack thereof against the negative marker will clearly demonstrate vaccination. The markers will be assessed in vivo using a calf infection model by measuring immune responses against the markers with a range of diagnostic tests. To ensure maximal marker sensitivity and specificity, we will develop complementary diagnostics, namely ELISA and interferon-gamma release assays, and compare them with commercially available diagnostic tests.

Implications: By creating essential components of a novel marked MAP vaccine strain, this research will bring us one step closer to controlling Johne's disease. This will reduce economic loss and increase the economic competitiveness of Alberta's dairy industry.