

Development of a Marked Johne's Disease Vaccine Strain: Using Immune Markers to Differentiate Vaccinated From Naturally Infected Animals

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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 overwhelming results due to poor diagnostic sensitivity and ineffectiveness of current vaccines in preventing infection. There are also no MAP vaccines approved in Canada due to their inability to differentiate between vaccinated and naturally infected animals with JD or *M. bovis*. With the high prevalence of MAP infection, the insensitivity of current tests, and difficulty in limiting disease spread, it is clear that the Canadian dairy industry needs new ways to protect animals from MAP infection.

Methods: We will avoid the regulatory problems associated with current MAP vaccines by modifying an attenuated MAP strain such that vaccinated animals can be easily 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 replace an immunogenic MAP gene with a foreign epitope to create a positive and negative marked MAP strain. 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, in which newborn calves from low MAP seroprevalence farms will either be infected with the marked strain, a wild-type field strain, or remain non-infected controls. Immune responses against the markers will be measured by custom-made ELISA and IFN-gamma release assays; these complementary diagnostics will be compared with commercially available diagnostic tests.

Implications: Adding immune markers to a live attenuated MAP vaccine strain will bring us one step closer to controlling Johne's disease. The markers will allow compliance with regulatory restrictions, leading to the widespread availability of an effective JD vaccine. This will reduce dairy-related economic loss and increase the economic competitiveness of Alberta's dairy industry.