Disruption of *relA* in *Mycobacterium avium* subsp. *paratuberculosis* impairs survival in the host.

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Paratuberculosis, Johne's disease, is a chronic inflammatory wasting disease in ruminants caused by infection with *Mycobacterium avium subsp. paratuberculosis* (*Map*). The estimated loss in the US dairy industry alone is up to 250 million dollars per year. The disease has been difficult to control due to the lack of an effective vaccine. To address this problem, we generated a deletion mutant (Δ*pknG*) tagged with the green fluorescent protein gene (*gfp*) for a potential live vaccine candidate using the K10 strain of *Map*. RelA is a stringent regulator in several intracellular and extracellular pathogens. It plays an important role in maintaining stationary phase survival of bacteria. Disruption of *relA* in *M. tuberculosis* was shown to decrease survival in a mouse model. Similarly, disruption of *relA* in *Map* impaired survival in vitro and in vivo in this study. In an *in vitro* culture assay with monocyte derived macrophages (MΦ), the survival rate of the Δ*relA* mutant was significantly decreased in MΦ after 6 days post infection compared to wild type K10. In an *in vivo* infection experiment using a cannulated ileum model, 4 - 5 tissue samples out of 9 different tissues from K10 infected calves were culture positive. All tissue samples from Δ*relA* infected calves were culture negative at necropsy 3 months post infection. The findings show the Δ*relA* deletion mutant is a candidate live vaccine. We are currently analyzing the immune response induced by the Δ*relA* mutant.