**Title:** Stimulation of bovine ileal organoids by bovine RANK-L and the uptake of *Mycobacterium avium* subspecies *paratuberculosis* into intestinal epithelial cells

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**Overview:** This study aims to investigate the invasion of *Mycobacterium avium* subspecies *paratuberculosis* (*MAP*), the causative agent of Johne's disease in ruminants, using bovine ileal organoids as an *in vitro* model. We observed that stimulation of organoids with RANK-L leads to increased uptake of *MAP* into intestinal epithelial cells.

**Introduction:** Johne's is a contagious disease that affects the intestine of ruminants. If untreated it results in diarrhoea, weight loss and death. Little is known about the mechanism of *MAP* infection due to a complex host-pathogen dynamic. *MAP* infects the GI tract mainly via M-cells in Peyers patches of the ileum. RANK-L induces differentiation of M-cells thereby increasing uptake of *MAP*. This study aims to develop bovine ileal organoids to study the mechanisms of *MAP* infection and identify factors involved.

**Methods:** Bovine ileal organoids were cultured as previously described by Hamilton *et al.* (2018). Briefly, after 7 days of differentiation and RANK-L stimulation, 3D organoids were challenged with Zymosan bio-particles for 1 h. Similarly, after 9 days of differentiation and RANK-L stimulation, 2D organoid monolayers were challenged with *MAP* for 24 h. After their respective incubations, organoids were fixed in 4 % paraformaldehyde and stained for immunofluorescent imaging.

**Results:** After 7 days of RANK-L stimulation, 3D organoids displayed an increased affinity for bio-particle uptake when compared with no RANK-L. In control and RANK-L stimulated, bio-particle uptake averaged 19 and 32 particles per organoid, respectively. It was also observed that after 9 days of RANK-L stimulation, 2D monolayers showed a trend that *MAP* uptake was increased compared to controls. These results suggest that RANK-L stimulation influences gut cell differentiation with increased transcytosis.

**Conclusions:** We successfully developed 3D and 2D organoid systems and demonstrated their ability to uptake bio-particles and *MAP* in the presence and absence of RANK-L. These models provide the chance to understand the mechanisms of entry of *MAP* into the intestine. Future studies will identify: changes to cellular composition of the gut; cell types involved in *MAP* invasion; changes in the transcriptome after *MAP* infection and factors involved in epithelial attachment and invasion.