Using spatial transcriptomics to explore the gastric infection of *Ostertagia ostertagi*

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Gastro-intestinal nematodes (GIN) infect millions of cattle globally and represent a major constraint to efficient livestock production. *Ostertagia ostertagi* has a direct life cycle with a pre-patent period of around 18-21 days. After ingestion, infective third stage larvae (iL3) penetrate the gastric glands in the abomasum (gastric stomach) where they develop into fourth stage larvae (L4) before emerging into the lumen at around 10-14 days post-infection to become sexually mature adults. Infection causes significant pathology, particularly when L4 larvae emerge from the gastric gland, causing hyperplasia of the gastric glands, epithelial cytolysis and loss of parietal cells, resulting in elevated abomasal pH and impaired protein metabolism. The mechanisms by which *O. ostertagi* modulates the gastric epithelial function after invasion are poorly understood, due to the inaccessibility of the abomasum for *in vivo* temporal analysis of early host-pathogen interactions. Bulk and single-cell RNA-seq lack the spatial resolution to identify cellular responses in, and surrounding, an infected gland. In this study, day 10 and day 21 post-infection abomasal tissues sections were used to investigate differences in the epithelial transcriptome of infected glands and glands proximal and distal to infected glands. Using a custom bovine RNA probe panel designed for the Nanostring GeoMx® spatial transcriptomic platform, we identified a local loss of parietal cell transcripts and increased mucin gene expression in infected glands, with the effect extending to neighbouring uninfected glands, but absent in distal glands. For the first time we can show the local manipulation of cell populations in direct contact with the parasite and adjacent epithelium at a level of detail and multiplexity which has not been possible up to this point. Follow up studies including additional points during development will improve our understanding of the spatio-temporal interactions in infected glands, clearance and tissue repair and onset of immunity to gastro-intestinal nematodes. In summary, using this approach we were able to identify the localised effect of nematode cellular modulation of the host interface at an unprecedented level of spatial resolution.