Infectious bursal disease virus (IBDV) replicates in the gut associated lymphoid tissue and alters the gut microbiome of chickens

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1. Background, Aims and Objectives

Aims and Objectives
- Characterise how IBDV affects the microbiome in the gut
- Determine how IBDV affects immune cell populations in the gut
- Determine whether IBDV replicates in the gut

Understanding how IBDV affects the gut environment will help understand how it exacerbates the colonisation of zoonotic gut bacteria of public health importance that will be useful for improving control of these food-borne pathogens.

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- Determine whether IBDV replicates in the gut
- Determine how IBDV affects immune cell populations in the gut
- Characterise how IBDV affects the microbiome in the gut

2. Experimental Design

3. IBDV replicates in the bursa of Fabricius (BF), the Caecal Tonsils (CT) and is shed from the cloaca

4. IBDV infection alters the percentage of B and T cells in the BF and CT

5. IBDV infection alters the microbiome in the caecum and cloaca

6. Conclusions and future work

- IBDV replicates in the gut associated lymphoid tissues of the caecal tonsils (CT) and is shed from the cloaca.
- IBDV infection leads to a reduction in the percentage of B cells in the CT and an increase in the percentage of T cells in the CT.
- IBDV infection leads to a reduction in the percentage of Bacteroïdes and an increase in the percentage of Enterobacteriaceae in the caecum, and a reduction in the percentage of Clostridiales in the cloaca.
- IBDV-mediated changes in gut immunity and/or microbiome may provide a more favourable environment for the colonisation of zoonotic bacteria such as Campylobacter.
- Secretory IgA regulates the composition of the microbiome (Figure 7). We hypothesise that IBDV infects the B cell populations in the gut lamina propria, altering the number and repertoire of IgA-secreting B cells, thereby altering the microbiome.
- Current work is aimed at determining whether live IBDV vaccines lead to these changes.

Figure 1. A bird showing signs of IBDV infection.

Figure 2. A schematic of a bird showing the location of the bursa of Fabricius.

Figure 3. Two experiments were performed using chickens of the Rhode Island Red breed at 2-3 weeks of age, as part of other projects. Samples from day 3 and 10 were used in this project. (A) Chickens were inoculated with either a classical or a very virulent strain of IBDV or were mock-inoculated with PBS alone. Birds were monitored for clinical signs, and at days 1, 2 and 3 post-infection (pi), 6 birds per group were euthanized. Samples obtained from the bursa of Fabricius (BF), the caecal tonsils (CT) and cloacal swabs at day 3 pi were used to quantify viral replication, and samples obtained from the caecum at day 3 pi for use for microbiome analysis. (B) Chickens were either mock-inoculated, or inoculated with a very virulent strain of IBDV. Birds were monitored for clinical signs, and at days 2, 4 and 14 pi, 6 birds per group were euthanized. Samples from the BF and CT obtained at day 14 pi were subject to flow cytometry for analysis of immune cell populations.

Figure 4. IBDV replicates in the bursa of Fabricius, the caecal tonsils (CT) and is shed from the cloaca. Chickens were inoculated with IBDV and the BF and CT harvested at 3 days post infection, and a loop was obtained from the cloaca. RNA was extracted from the organs and reads, and the fold change in the expression of the IBDV VP2 gene determined by RT-qPCR, normalised to a house-keeping gene (RP944), and expressed relative to mock in a delta delta ct analysis.

Figure 5. Upon IBDV infection, the percentage of B cells decreases and the percentage of T cells increases, in the BF and CT, compared to mock-inoculated controls. Chickens were inoculated with IBDV and the BF and CT harvested at 14 days post infection. Mononuclear cells were isolated and stained with anti-CD3-PE (B cells) and anti-CD8-PE (T cells), subject to flow cytometry, and the number of B and T lymphocytes expressed as a percentage of the total number of cells in the sample. A. The percentage of B and T cells in the BF and CT of mock-inoculated birds (white bar) or IBDV-infected birds (black bar). B. Representative dot plots from the CT.

Figure 6. Bacterial communities in the caecum of mock-infected and IBDV-infected chickens. A and C. The percentage of total reads from individual birds from mock, classical and very virulent infected groups. B and D. The average percentage of total reads per group.

Figure 7. Secretory IgA produced from B cells in the lamina propria transcytose into the lumen of the gut and bind commensal bacteria, altering their clearance by mucosa. Therefore the IgA receptor regulates the composition of the microbiome (Roberts et al., 2021).