A Longitudinal Survey of the Gut Virome of Commercial Broiler Chickens From Prehatch to Slaughter

James Trudgett, David Brennan, Veronica Casement, Paul McAdam, Victoria Smyth
Agri-Food & Biosciences Institute, Belfast, United Kingdom

Introduction
A longitudinal survey was undertaken involving the sampling of seven broiler chicken farms at eleven timepoints across three successive crops. This was done with the aim of elucidating the gut virome (the total collection of viral nucleic acid present) of clinically normal birds and to investigate any correlation between this virome and overall flock performance. These crops were selected to be within the normal range of performance as shown by their feed conversion ratio (FCR) at time of slaughter.

Methods
Fifty samples were taken at each timepoint from prehatch to slaughter. These were pooled into one flock sample per timepoint per crop and per farm. Nucleic acids extracted from the samples were amplified and Illumina next generation sequencing (NGS) libraries were prepared and sequenced using the MiSeq instrument. Sequenced libraries were processed through a bioinformatics pipeline including Viromescan software and the output of this was compared to flock performance as measured by FCR.

Workflow
- Samples collected.
- Samples Pooled (50).
- Disruption / homogenisation.
- Low speed centrifugation.
- 0.22µm filtration.
- 113kG ultracentrifugation.
- Nucleic acid extraction.
- Whole transcriptome amplification.
- NGS library preparation (222 in total).
- Sequencing.
- Quality assessment and trimming.
- Removal of host, human and bacterial reads.
- Viromescan alignment to database.
- Virome comparison to FCR.

Results
Sixty five viral families (including unassigned) were detected in total throughout this study. A total of 9921832 NGS reads were classified as of viral origin. Correlation was observed in 2/3 crops between high viral diversity (measured by the number of viral families detected) and poor performance (higher FCR (Fig 1). The crop where no correlation was observed had a much narrower range of both FCR and viral load.

Five viral families were detected by Viromescan in more than 80% of all data points. These were Astroviridae at 87.8%; Baculoviridae at 80.2%; Coronaviridae at 98.2%; Parvoviridae at 91.4% and Picornaviridae at 99.1%.

It was observed that increasing endemic viral load was found to correlate with worsening FCRs over the 3 crops of the survey, as the Fig 2 graph shows, with the lowest (and best) FCR in crop 1 having least viruses while the worst FCR in crop 3 has the greatest load of viruses.

Conclusions
This survey has allowed us to ascertain the “normal” progression of a number of endemic viruses which a broiler flock would be likely to experience during its lifespan. This knowledge will be useful in the future investigation of a number of disorders and syndromes such as runting stunting syndrome (RSS), malabsorption and uneven flock growth. These conditions are capable of causing significant economic losses to poultry farmers and have previously been linked to a, possibly multifactorial, viral aetiology.