INTRODUCTION

Rotavirus (RV) is a highly infectious pathogen responsible for acute diarrhoea in infants and animals worldwide. Enteritis is the most common cause of mortality in neonatal calves in Northern Ireland. AFB Veterinary Science Division identified rotavirus as the causative agent of enteritis in 28% of young calves and 48% of piglets faecal samples.

- RV is classified into groups from Group A to I and has a causative agent of enteritis in 28% of young calves and 48% of piglets faecal samples.
- Rotavirus has a dsRNA segmented genome with 11 RNA segments.
- Genotyping is based on the dual viral outer proteins VP7 glycoprotein (G) and VP4 protease sensitive hemagglutinin (P) or whole genome classification.
- New RV strains are classified after submission to the Rotavirus classification working group.

AIM of STUDY

1) Complete a molecular detection rotavirus study in Northern Ireland livestock, exotic animals and wildlife.
2) Phylogenetic diversity study of the genotype rotavirus (Group A).
3) Investigation of the associated enteric virome of clinically diseased animals to detect possible co-pathogens.
4) Identify if re-assortment and interspecies transmission occurs.

MATERIALS and METHODS

1) Sample biobank and screening for RV VP6 gene

<table>
<thead>
<tr>
<th>Sample Bank</th>
<th>Animal species</th>
<th>Sample</th>
<th>RVA</th>
<th>RVA P0.01</th>
<th>RVA P0.02</th>
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2) Next Generation Sequencing (NGS)

2a) Viral Enrichment method:

- 20% Hemagglutination
- Precise Amplification
- Contribution

2b) Library preparation method:

- Quantification
- Library index and PCR
- Library sequencing: indexed pairs
- Requantification and index PCR
- Denaturation Library and run

2c) NGS run method:

- 5 MiSeq (illumina) runs - V3 600 Cycles Viral metagenome denovo pair-end reads.
- Bioinformatics programs for quality trim and to filter reads, assembly, align and taxonomically classify before visualisation.

RESULTS – Snapshot of enteric virome and RV phylogenetics

Pig samples

- Porcine microbiome
- Bovine & Ovine Virome
- Equine, wildlife & exotic microbiome

3) VISUALISATION

- 3a MEGAN - Bovine WTA 18
- 3b Korna chart

CONCLUSIONS and FUTURE WORK

The faecal virome of enterically diseased animals is dominated by dsDNA bacteriophage Cauloviridae order and families. Siphoviridae, Myoviridae and Podoviridae as demonstrated in the porcine WTA 79. Korna virome chart and heat maps. Rotavirus is the most abundant virus of these clinically diseased livestock animals and some wildlife virome alongside the bacteriophages and coexists with other enteric viruses. The preliminary mapping of reads shows other viruses present in the enteric virome which is dependant on animal species. Most commonly found viruses observed with rotavirus were ungulate bocaparvovirus, aichivirus, astrovirus, mastadenovirus A and stoll-associated circular virus. Insect and plant viruses were also shown in viromes of animals indicative of ingested viruses from their diet.

Preliminary phylogenetics of RVA found common genotypes were present in porcine samples such as G3 P[13] and bovine G6 P[11] however novel strains of RVA were also present in bovine, pudu and ovine samples. It remains unclear if rotavirus inter-species transmission or reassortment has occurred at this stage of analysis although the porcine strain of RVA is most similar to a human strain.

Future work will be the continual review of analysis NGS bioinformatics pipelines for suitable semi-quantitative, sensitive and accurate analysis that represents the virome samples. Reference genome mapping of the RVA and a comprehensive phylogenetic mapping from all animal species in this study will be completed to demonstrate the diversity of rotavirus within the Northern Ireland region. Lastly, unclassified reads will be studied to identify possible new viruses.