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## Introduction:

The population of microbes within the gastrointestinal tract and their collective genomes is known as the gastrointestinal microbiome (McDermott and Huffnagle, 2014).

Enteric pathogens have the potential to cause changes in the gut microbiota (Carding *et al.*, 2015). Johne's disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in domestic and wild ruminants (cattle, sheep, deer). It is characterized by chronic granulomatous enteritis seen mainly in the ileum (Singh *et al.*, 2013). Infection with MAP usually occurs most commonly in the neonatal stages via oral route due to ingestion of contaminated materials (soil, faeces, MAP infected milk or colostrum).

Sheep are infected with intestinal worms causing diseases and economic losses (Roeber *et al.*, 2013). Helminths such as *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* are known to cause economic losses in the sheep industry. Nematode larvae can become contaminated with MAP serving as vectors for the transmission of Johne's disease (Whittington, Lloyd, & Reddacliff, 2001).

## Results and Discussion:

PCR was carried out from 80 samples of DNA extracted from faeces. Figure 2 shows a representative image of the PCR products. 95% (76) of samples successfully processed for sequencing.

PCR product →

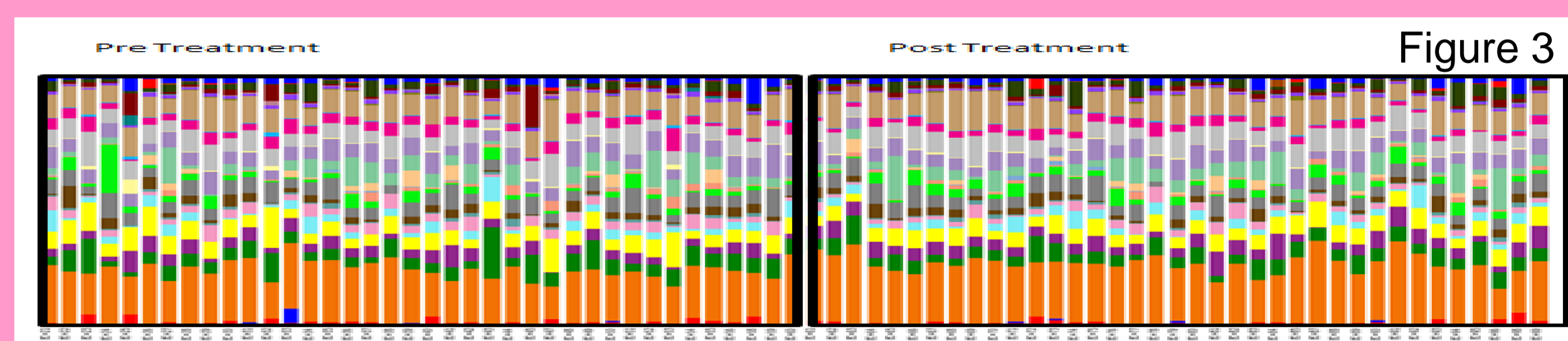


Figure 2: 1% agarose gel in 1xTAE using Gel-Red DNA stain. Visualised under UV. Lane 1: 100 bp ladder, Lanes 2 (negative kit control), 3 -15 are the PCR bands from unknown samples (400bp). Note: Where PCR failed (lane 6), reaction were repeated at optimized DNA concentrations.

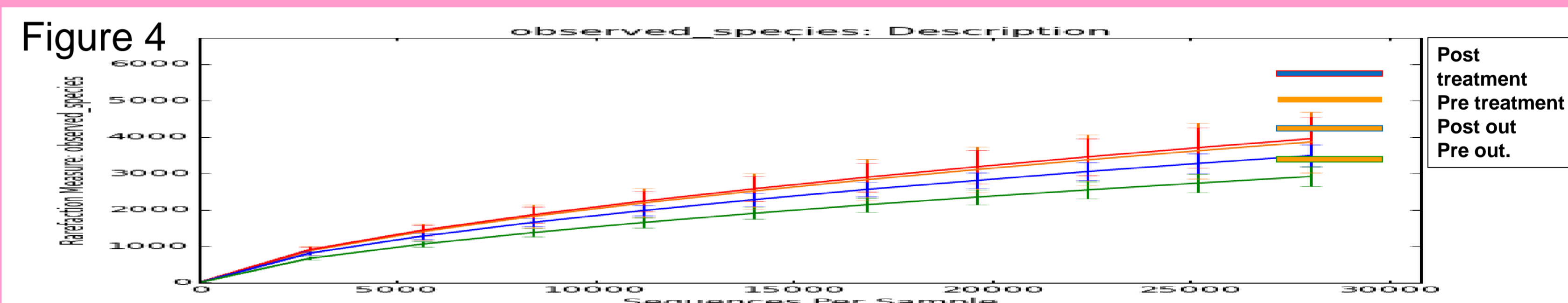
## Data processing and a Analysis:

QIIME: Used to analyse and compare microbial communities in the sequenced data between the microbiome of sheep before and after treatment with anthelmintics.

- Taxonomic plot for bacterial and archae communities of nematode infected group (Pre and Post Treatment) at genus level (figure 3).



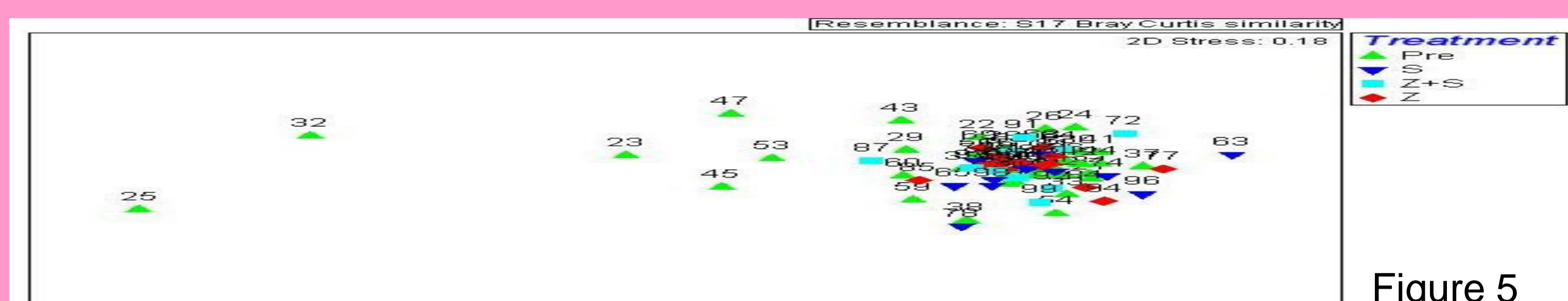
- A plot of the number of species as a function of the number of samples (known as the Rarefaction curves) was performed. The gradient of the curve represents the diversity of the operational taxonomic units within the biological group (figure 4).



Post treatment (red) has the steepest gradient closely followed by pre treatment (orange) samples. The post treated and pre treated are not significantly different but have a higher diversity that is significantly different ( $P < 0.05$ ) than the post treated outliers (blue) and the pre treated outliers (green). The pre treated outliers (green) have the least diversity than all the other sample groups.

## Primer and Permanova used to analyse QIIME output data.

Bray-Curtis similarity matrices show similarity between the Pre-treated and post-treated animals.



Bray-Curtis similarity curve shows clustering of the pre and post treatment. The pre outliers and post outliers do not cluster due to differences in diversity.

## Conclusions:

This data suggest that anthelmintic treatment has little effect on the gastrointestinal microbiome of sheep. Even those sheep with a different microbiome diversity pre treatment showed a shift of diversity towards the sample mean after treatment.

Future work will compare Johne's disease sample between different sampling times and between sheep with worms only and dully infected (Johne's disease and worms).

## Aim of Project:

To carry out an analytical study to understand the relationship between Johne's disease pathogenesis, the gastrointestinal microbiome and gastrointestinal parasites with the aim of developing improved preventative control strategies.

The project was divided into 2 areas:

- 1) to study parasitic infection in sheep with no history of Johne's before and after anthelmintic treatment;
- 2) to find out the relationship of worm burden and Johne's disease in sheep with dual infections (gastrointestinal nematodes and MAP)

To determine whether the presence of gastrointestinal parasite alter the gut microbiota to facilitate infection of MAP especially in the multibacillary form of the disease in farm conditions.



Figure 1

## Materials and method:

Rectal faecal samples of sheep were collected as surrogate for the small intestine content of sheep from a number of commercial farms with and without a history of Johne's disease (figure 1).

Experiment 1: Sheep naturally infected with intestinal worm with no history of Johne's disease

- 40 faecal samples collected before worm treatment.
- 40 faecal samples collected after worm treatment.

Experiment 2: Sheep naturally infected with both MAP and worms.

- 57 faecal samples collected.

Faecal Sample Processing:

- DNA was isolated from faeces using the MOBIO Powerfecal® DNA Isolation kit.
- DNA was quantified using nanodrop
- PCR (Polymerase chain reaction) amplification of DNA, using Illumina® bar-coded primers was carried out (Caporazo *et al.*, 2012).
- amplified DNA was purified (using Wizard SV Gel and PCR clean up kit), quantified (QuantiFluor® ONE dsDNA System) and pooled.
- Pooled library of 76 amplicons submitted to Edinburgh genomics for Illumina MiSeq processing.