Cryptosporidium parvum dysbiosis of the faecal microbiome of bovine livestock: A computational metagenomic approach

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Introduction

Cryptosporidium is a protozoan parasite and is the causative agent of cryptosporidiosis in humans and animals. Symptoms of the infection may include abdominal pain, vomiting and diarrhoea or may also be asymptomatic. Agricultural losses globally due to *Cryptosporidium* infection in cattle amount to several billion dollars. The severity of infection depends on many factors, including host immunity. Here, the impact of the gut microbiome on infection was studied. The gut microbiome is the community of bacteria resident in the digestive system. Ruminants including cattle have a stomach with four compartments specialised for pre-gastric digestion. It has previously been demonstrated that specific alterations in the bovine microbiome can facilitate growth of cryptosporidial parasites. Therefore, cryptosporidiosis can lead to long-term dysbiosis of the gut. It has been reported that infections with C. parvum are associated with changes in the microbiota as well as a shift in metabolites in the host gut.

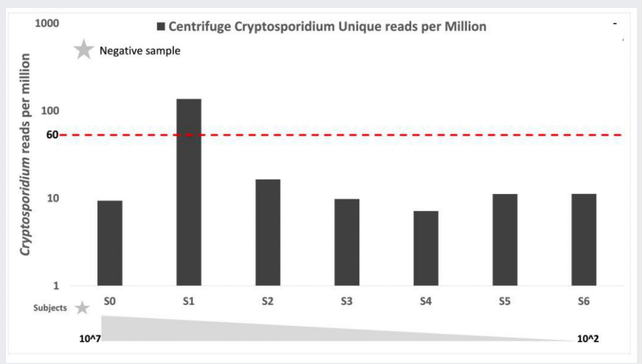
Methods

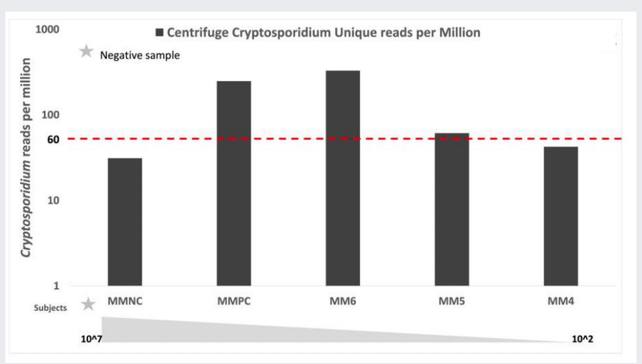
In this study, we developed a bioinformatic pipeline based on shotgun metagenomic sequencing to detect *Cryptosporidium* infection in faecal samples. This was combined with computational analysis of the sequence obtained from faecal DNA to investigate the changes to the gut microbiome between infected and non-infected hosts. Crucial changes to the microbiome in cattle and humans resulting from *Cryptosporidium* infection were uncovered.

Benchmarking of detection of Cryptosporidium directly from faecal samples

Artificially spiked human faecal samples and rabbit samples were used to benchmark detection of *Cryptosporidium* directly from metagenomic shotgun sequence . *Cryptosporidium* reads per million were calculated by CENTRIFUGE (Kim et al, 2016)

Sample ID	Total number of paired-end reads	Positive/ Negative PCR for *Cryptosporidium* after spike-in	*Cryptosporidium* Oocysts per 250 mg aliquot of faecal material	CENTRIFUGE *Cryptosporidium* Unique reads per Million
S0	3,510,890	Pseudo-control	0	9.40
S1	3,911,740	Positive	10^7	136.77
S2	3,587,910	Positive	10^6	16.44
S3	3,869,916	Positive	10^5	9.82
S4	3,499,240	Positive	10^4	7.14
S5	3,573,980	Positive	10^3	11.19
S6	3,130,760	Positive	10^2	11.25
MMNC	3,495,590	Negative	0	31.18
MMPC	3,939,682	Positive	10^7	248.24
MM6	562,221	Positive	10^6	329.05
MM5	3,035,760	Positive	10^5	61.27
MM4	211,433	Positive	10^4	42.57



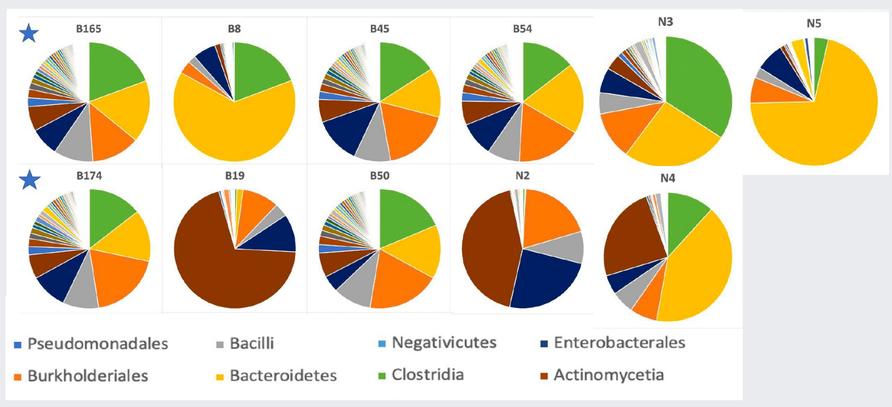


The dotted Red line is to set a detection cut-off at 60 reads per million for *Cryptosporidium* reads given by CENTRIFUGE, where four samples exceed the cut-off,

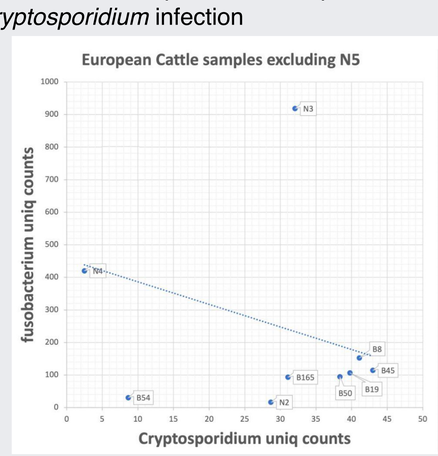
Changes in microbiota associated with infection

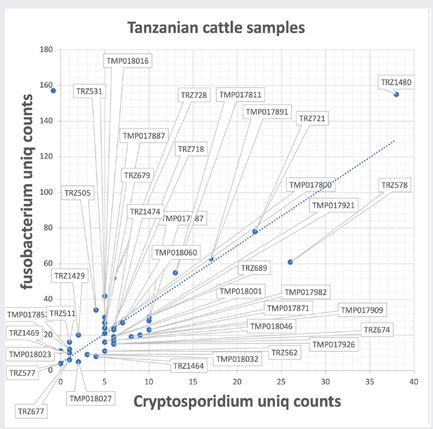
Demonstration on detecting *Cryptosporidium* infection in faecal samples from real infected European cattle, where 2 samples are negative and the rest are positive for *Cryptosporidium* by PCR test

Sample ID	Positive/ Negative PCR for *Cryptosporidium* after spike-in	Total number of paired-end reads in sample from all lanes	CENTRIFUGE *Cryptosporidium* Unique reads per Million
B165	Negative	3,350,120	42.98
B174	Negative	3,799,090	31.06
B19	Positive	3,126,340	8.64
B45	Positive	4,577,790	39.76
B50	Positive	3,967,128	41.09
B54	Positive	3,388,520	38.36
B8	Positive	4,106,290	6.09
N2	Positive	3,243,295	28.67
N3	Positive	3,495,561	32.04
N4	Positive	4,404,238	2.50
N5	Positive	4,393,070	5,054.78



The bacterial component of European cattle samples where sample (N5) shows a severe *Cryptosporidium* infection





No Correlation was observed between *Cryptosporidium* and Fusobacterium in European cattle samples. On the contrary a strong correlation between *Cryptosporidium* to *Fusobacterium* observed in the Tanzanian cattle (Kibegwa et al, 2020) with $R^2 = 0.83$

References

- Kim, D. et al. (2016). Centrifuge: Rapid and sensitive classification of metagenomic sequences, Genome Research. 26(12), 1721-1729.
- Kibegwa, F. M. et al. (2020) A Comparison of Two DNA Metagenomic Bioinformatic Pipelines While Evaluating the Microbial Diversity in Feces of Tanzanian Small Holder Dairy Cattle, BioMed Research International, 2000, 2348560.

Credits

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