Metagenomic analysis of Irish cattle faecal samples using Oxford Nanopore MinION Next Generation Sequencing

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Abstract : The Irish agri-food sector is of major importance to Ireland's manufacturing sector and to the Irish economy through employment and the exporting of animal products worldwide. Infectious diseases and parasites have an impact on farm animal health causing profitability and productivity to be affected. For the sustainability of Irish dairy farming, there must be the highest standard of animal health. There can be a lack of information in accounting for > 1% of complete microbial diversity in an environment. There is the tendency of culture-based methods of microbial identification to overestimate the prevalence of species which grow easily on an agar surface. There is a need for new technologies to address these issues to assist with animal health. Metagenomic approaches provide information on both the whole genome and transcriptome present through DNA sequencing of total DNA from environmental samples producing high determination of functional and taxonomic information. Nanopore Next Generation Technologies have the ability to be powerful sequencing technologies. They provide high throughput, low material requirements and produce ultra-long reads, simplifying the experimental process. The aim of this study is to use a metagenomics approach to analyze dairy cattle faecal samples using the Oxford Nanopore MinION Next Generation Sequencer and to establish an in-house pipeline for metagenomic characterization of complex samples. Faecal samples will be obtained from Irish dairy farms, DNA extracted and the MinION will be used for sequencing, followed by bioinformatics analysis. Of particular interest, will be the parasite Buxtonella sulcata, which there has been little research on and which there is no research on its presence on Irish dairy farms. Preliminary results have shown the ability of the MinION to produce hundreds of reads in a relatively short time frame of eight hours. The faecal samples were obtained from 90 dairy cows on a Galway farm. The results from Oxford Nanopore 'What's in my pot' (WIMP) using the Epi2me workflow, show that from a total of 926 classified reads, 87% were from the Kingdom Bacteria, 10% were from the Kingdom Eukaryota, 3% were from the Kingdom Archaea and < 1% were from the Kingdom Viruses. The most prevalent bacteria were those from the Genus Acholeplasma (71 reads), Bacteroides (35 reads), Clostridium (33 reads), Acinetobacter (20 reads). The most prevalent species present were those from the Genus Acholeplasma and included Acholeplasma laidlawii (39 reads) and Acholeplasma brassicae (26 reads). The preliminary results show the ability of the MinION for the identification of microorganisms to species level coming from a complex sample. With ongoing optimization of the pipe-line, the number of classified reads are likely to increase. Metagenomics has the potential in animal health for diagnostics of microorganisms present on farms. This would support wprevention rather than a cure approach as is outlined in the DAFMs National Farmed Animal Health Strategy 2017-2022.

Keywords : animal health, buxtonella sulcata, infectious disease, irish dairy cattle, metagenomics, minION, next generation sequencing