Interim report - The role of the microbiome in the pathology of cutaneous and renal glomerular vasculopathy (CRGV) – a shotgun metagenomic sequencing approach

September 2024

Produced by – Jack Whitehouse

Hypothesis: Does the gut microbiota play a role in the disease process of CRGV?

Importance: Establishing differences in the bacterial species in the gut microbiota of dogs suffering from CRGV compared to dogs suffering from Acute Kidney Injury, and Medically healthy dogs would improve our chances of identifying bacterial species that may be responsible for CRGV, either as a direct cause or as a contributing factor.

Background

Cutaneous and Renal Glomerular Vasculopathy (CRGV) is an emerging disease affecting dogs in the UK. CRGV was first reported in Alabama, United States in the mid-1980s affecting racing greyhounds. The location of some of the cases resulted in the colloquial term 'Alabama Rot'. Of unknown aetiology, the disease has contributed to ~ 324[1] dogs dying of clinical abnormalities associated with CRGV in the UK alone. There is no known cause or proven link to an infectious agent and treatment is limited in most cases, thus leading to poor prognosis. Dogs develop cutaneous lesions on their extremities before variably developing acute kidney injury (AKI), typically four days after lesion presentation[2]. During the development of AKI, subsequent clinical abnormalities may also occur (e.g., anaemia, thrombocytopenia) before the onset of clinical signs associated with death or euthanasia[2]. The lack of understanding regarding a definitive diagnosis relies on the identification of pathology, such as thrombotic microangiopathy (TMA) in renal tissue during a *post-mortem* examination. TMA is a pathology characterised by inflammation and damage, including endothelial damage and thrombosis within the microvasculature[2]. Little is known about the pathology and aetiology of CRGV and what is known does little to advance the treatment, diagnosis, and survival rates of affected dogs.

The faecal microbiota encompasses a diverse range of organisms including bacteria that perform a variety of functions influencing the overall health of the host, such as nutrient metabolism, immune regulation, and host defense[3]. Changes in the abundance of bacterial species have previously been associated with several diseases[3]. For example, inflammatory bowel disease in dogs was associated with reduced faecal bacterial diversity with increases in the taxa Gammaproteobacteria and Bacilli[3]. *Aeromonas hydrophilia* has been reported as a potential aetiological agent for CRGV[4] because of the geographical distribution of cases across wet environments (e.g., wet flood meadows) and the ability of *A. hydrophilia* to cause skin lesions in a range of species including humans, and *Leptospira*-like kidney lesions in dogs[4&6]. Shiga toxin-producing *Escherichia coli* which is associated with Haemolytic Uraemic Syndrome (HUS) in humans and shares key disease hallmarks with CRGV[2 & 5] may also be a potential aetiological agent. However, to date, investigations into these two possible aetiological agents have been limited and their role in CRGV remains unclear.

Characterising the structure of the faecal microbiota during health and disease has previously paved the way for determining the function and role of species in the development of disease. In the proposed study a shotgun metagenomic sequencing approach will be employed. Shotgun metagenomic sequencing is a high-throughput microbial genomic sequencing method, providing an unbiased and sensitive approach to comprehensively reviewing all bacterial species present in a faecal sample[7]. This high-throughput method performs deep sub-sampling and detects very low abundance constituents of the microbial community that may be unique to CRGV-affected dogs.

Progress against the original deliverables

1. Recruit veterinary clinics for the collection of AKI faeces
2. Creation of a whole genome library
3. Assembly and processing of data
4. Draft a manuscript

Current Progress

1. Assembly and processing of data

The data has now been processed using the SqueezeMeta bioinformatic piepline[8] and is currently undergoing analysis using R Studio to determine the bacterial species associated with health, and the species level profile of the CRGV-affected gut microbiota. The expected data outputs include, but are not limited to taxonomic assignment, functional annotation (e.g., what are the microorganisms doing?), and microbial community structures of the faecal microbiota of CRGV-affected dogs compared to AKI and medically healthy dogs. The main data analysis should be completed by mid-November 2024.

2. Draft manuscript

Once the data have been assembled and processed, a concise and robust bioinformatic report will be produced for ARRF and a manuscript drafted for publication will be drafted and submitted to a high impact-peer reviewed journal.

Supporting references

1. Alabama Rot – Anderson Moores Veterinary Specialists (2022). [Online]. Available from: https://www.alabama-rot.co.uk/.
2. Walker, J. J. A., *et al*. (2021) 'Clinicopathological features of cutaneous and renal glomerular vasculopathy in 178 dogs', *Vet Rec*, 189 (4). doi: 10.1002/vetr.72.
3. Pilla, R., *et al*. (2019) 'The Role of the Canine Gut Microbiome and Metabolome in Health and Gastrointestinal Disease', *Front Vet Sci*, 14 (6), pp. 498. doi:10.3389/fvets.2019.00498.
4. Macdonald, F. A. (2015) 'Role of *Aeromonas hydrophila* in CRGV in Dogs', *Vet Rec*, 177 (23), pp. 602. doi:10.1136/vr.h6667.
5. Mayer, C. L., *et al*. (2012) 'Shiga Toxins and the pathophysiology of haemolytic uremic syndrome in humans and animals', *Toxins*, 8 (11). doi: 10.3390/toxins4111261.
6. Stevens, K. B., *et al*. (2018) 'Spatiotemporal patterns and agroecological risk factors for cutaneous and renal glomerular vasculopathy (Alabama Rot) in dogs in the UK', *Vet Rec*, 183 (16), pp. 502. doi: 10.1136/vr.104892.
7. Quince, C., *et al.* (2017) 'Shotgun metagenomics, from sampling to analysis', *Nat Biotechnol*, 35 (9), pp. 833 – 44. doi: 10.1038/nbt.3935.
8. Tamames, J., and Puente-Sanchez, F. (2018) 'SqueezeMeta, A Highly Portable, Fully Automatic Metagenomic Analyse Pipeline', *Frontiers in Microbiology*, 9. doi: 10.3389/fmicb.2018.03349.