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Characterization of the fecal virome in dogs with chronic enteropathy

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ABSTRACT

The fecal virome has been investigated in humans and various animal species using next generation sequencing. However, limited information is available about the fecal virome of dogs with chronic enteropathy (CE). We aimed to characterize the canine fecal virome of dogs with CE and compare it with the virome of previously analyzed healthy dogs.A total of 16 adult dogs; 8 healthy dogs (data from a parallel study) and 8 dogs with CE had fecal samples assessed by viral shotgun sequencing. Fecal samples were subjected to enrichment of viral nucleic acids prior to sequencing and metagenomic analyses. Characterization of the complete genome of a canine kobuvirus was performed by Sanger sequencing. An additional 21 healthy dogs and 14 dogs with CE were further analyzed for the prevalence of canine kobuvirus.Three fecal samples from dogs with CE contained in total 3 eukaryotic viral families. In contrast, 4/8 fecal samples previously identified from healthy dogs, contained 5 eukaryotic viral families with 2 families exclusive to this group. Bacteriophages were identified in all fecal samples from CE and healthy dogs. Canine kobuvirus was identified in one dog with CE, by shotgun sequencing, and the complete genome was then characterized. This kobuvirus was classified within canine kobuvirus group, being similar to strains from Korea and China. The larger prevalence study did not detect additional samples positive for canine kobuvirus. The fecal virome of dogs with CE differs in number and type of viral families from healthy dogs. The first Australian canine kobuvirus sequence was identified and characterized from a dog with CE.

1. Introduction

The virome comprises all viruses, including those infecting eukaryotic and prokaryotic organisms within a biological or environmental sample. Recent improvements in molecular diagnostic techniques, have allowed better identification and discovery of known and new viruses (Delwart, 2007; Rosario and Breitbart, 2011). Viruses infecting bacteria (bacteriophages) are the predominant viral component in feces of multiple animal species (Breitbart et al., 2003; Moreno et al., 2017; Norman et al., 2015; Reyes et al., 2010). The interplay between intestinal bacteria, viruses and the host immune system has been a major focus of research in host health and disease, especially in conditions such as inflammatory bowel disease (IBD) (Babickova and Gardlik, 2015; De Paepe et al., 2014; Wagner et al., 2013).Chronic enteropathy (CE) is the clinical term for what used to be called IBD in dogs, and is characterized by gastrointestinal clinical signs that persist longer than 3 weeks without any specific pathogenic, mechanical or other extra-intestinal cause of diarrhea (Dandrieux, 2016). Canine CE has recently been classified according to the method by which clinical resolution is achieved: food-responsive (FRE) (Allenspach et al., 2007), antibiotic-responsive (ARE) (Hall, 2011) and immunosuppressant-responsive enteropathies (IRE) (Allenspach et al., 2007; Dandrieux, 2016). Dysbiosis in dogs with CE has been well established (Suchodolski, 2016), but it is unknown if there are differences in the microbiome between the types of CE, whether the changes are biologically relevant and indeed whether the dysbiosis is a cause or consequence of the inflammation (Suchodolski, 2016). Similarly, no information about the intestinal virome in dogs with CE has been published.

The purpose of this study was to characterize the virome in feces collected from dogs with CE and compare it to the fecal virome present in healthy dogs (Moreno et al., 2017). A secondary aim was to

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determine the sequence and prevalence of a unique canine kobuvirus that was identified during initial assessment by viral shot gun sequencing.

2. Material and methods

2.1. Clinical CE cases

Eight client-owned dogs were recruited into the study by U-Vet Hospital, The University of Melbourne, for investigation of chronic gastrointestinal clinical signs, vomiting, diarrhea, weight loss, for more than 3 weeks of duration. Full, written owner consent was obtained and the project was approved by the University of Melbourne Animal Ethics Committee (study ID: 1112072.2), which operates according to the National Health and Medical Research Council (NHMRC) guidelines for use of animals in research. No dog had received dietary, antibiotic or anti-inflammatory treatment in the preceding 4 weeks, and all had active clinical signs of CE at the time of assessment. Each dog had full hematology, biochemistry, including serum cobalamin and canine pancreatic lipase immunoreactivity, urinalysis, fecal analysis and abdominal ultrasound performed. After no specific abnormalities were identified, the gastrointestinal tract was assessed by upper and lower gastrointestinal endoscopy and endoscopic biopsies obtained from the stomach, duodenum, ileum and colon for histopathology. Dogs were then recruited fully into the study if no disease other than CE was observed. Treatment comprised initially of a hydrolyzed/hypoallergenic prescription veterinary diet, followed by antibiotics, and then immunosuppressives based on clinical response. Response to a treatment was defined by a 75% reduction in canine chronic enteropathy clinical activity index (CCECAI) (Allenspach et al., 2007) within 2–3 weeks of the commencement of treatment. The type of chronic enteropathy (CE) was ultimately defined by at least an 8-week reduction (> 75%) in CCECAI to either diet, antibiotic or immunosuppressive treatment.

Fecal samples from these dogs were used for metagenomic sequencing and were collected at the first visit prior to diagnostic or therapeutic interventions, between June 2010 and March 2013. All dogs were aged between 1.5 and 5 years, median 2.8 years; and comprised 3 females and 5 males of different breeds. The final CE classification was 4 FRE, 3 ARE and 1 IRE (Table 1). An additional 14 dogs with CE,7 FRE, 5 ARE and 2 IRE, (Table S1) were recruited under the same inclusion criteria as the initial 8, to specifically evaluate prevalence of canine kobuvirus (Table S1). These 14 dogs were recruited between June 2010 and Sept 2015 and fecal samples were also obtained at the first visit, from 7 males and 7 females of different breeds. The age range varied between 7 months and 11 years old, median 4.8 years.

2.2. Control dogs

Eight fecal samples from healthy dogs kept in a shelter were collected for a parallel project analyzing the fecal virome of healthy dogs and dogs with acute diarrhea (Moreno et al., 2017). These dogs were 6 males, 2 females, different breeds, age range: 6 months to 7 years, median 3.5 years (Table 1).

An additional 21 healthy dogs had fecal samples collected to evaluate the prevalence of canine kobuvirus. These samples were collected from client or staff-owned dogs from the U-Vet Hospital, between September 2014 and February 2015, from 8 males and 13 females of different breeds (Table S1). The age range was between 3 months and 15 years old (median 5 years). All healthy dogs were considered to be healthy based on absence of signs of clinical disease with no antibiotic administration for more than 6 months, and full worming and vaccination prophylaxis completed.

All fecal samples were initially stored at 4 °C before being stored at − 80 °C within 6 h of collection. Information about age, sex, breed, diet, vaccination and deworming status was recorded for each dog.

2.3. Sample preparation and fecal extract preparation

Fecal samples were processed as described previously (Moreno et al., 2017). Briefly, fecal samples were dissolved in saline buffer, vortexed and filtered through a 0.45 µm filter by centrifugation at 3800 × *g* for 5 min. Before nucleic acid extraction all samples were subjected to a viral enrichment protocol using a mixture of DNases and RNase A, as described previously (Moreno et al., 2017).The nucleic acid extraction was performed using QIAamp® Viral RNA mini kit (QIAGEN, Hilden, Germany) according to manufacturer's recommendations. Each sample was then divided into two aliquots. One aliquot was used for genomic DNA analysis and the second aliquot was used for a second DNase step to remove genomic DNA. Following the second DNase step, the leftover viral RNA was transcribed with Sensiscript Reverse Transcriptase kit (Sensiscript RT kit; QIAGEN, Hilden, Germany) to generate complementary DNA (cDNA), according to manufacturer's instructions with the same modifications as specified previously (Moreno et al., 2017).

Table 1
Summary of signalment and the contigs/singletons of prokaryotic and eukaryotic viral families detected by metagenomic sequencing in feces of dogs.

SAMPLES	AGE	SEX	BREED	DIAGNOSTIC	PROKARYOTIC VIRAL CONTIGS/ SINGLETONS	EUKARYOTIC VIRAL FAMILIES (Nº OF CONTIGS/SINGLETONS DETECTED)
HD4	7 y	MN	Maltese X	Healthy	1012	*Reoviridae* (39)
HD5	2 y	MN	Jack Russell	Healthy	209	None
HD6	5 y	F	Maltese/Shih Tzu	Healthy	953	None
HD7	4 y 7 m	FS	Staffy	Healthy	731	None
HD8	7 y	MN	Labrador X	Healthy	82	None
HD9	8 m 1 w	MN	Mastiff/Staffy	Healthy	5	*Parvoviridae* (3)
HD10	8 m	M	Maltese X	Healthy	2	*Coronaviridae* (912)
HD11	2 y 6 m	MN	Maltese/Shih Tzu	Healthy	18	*Adenoviridae* (1) *Papillomaviridae* (1)
CE1	2y 8m	FS	Labrador	FRE	214	*Papillomaviridae* (1) *Reoviridae* (2)
CE4	5y	M	Rottweiler	IRE	449	None
CE5	2y 5m	MN	Bull Terrier	FRE	227	None
CE8	1y 6m	F	German Shepherd	ARE	499	None
CE9	1y 9 m	M	Weimaraner	FRE	8777	*Picornaviridae* (9)
CE10	2y	M	Basset hound	ARE	319	None
CE11	4y 5m	MN	Labradoodle	ARE	468	*Reoviridae* (4)
CE13	5y	FS	Terrier cross	FRE	202	None

HD: healthy dogs, CE: dogs with chronic enteropathy, MN: male neutered, M: male, FS: female spayed, F: female, FRD: food responsive enteropathy, IRE: immunosuppressive responsive enteropathy, ARE: antibiotic responsive enteropathy.