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 (PRE) (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 (Suchodolksi, 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 (Suchodolksi, 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...
determine the sequence and prevalence of a unique canine kobuvirus that was identified during initial assessment by viral shotgun 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 0.45μm 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 metagenomic sequencing and the contigs/singlets of prokaryotic and eukaryotic viral families detected by metagenomic sequencing in feces of dogs.

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

2.4. Random amplification, sequence-independent single primer amplification (SISPA) method

Both viral cDNA and genomic DNA were randomly amplified using the SISPA protocol modified from Allander et al (2001) (Allander et al., 2001) and Reyes and Kim, (1991) (Moreno et al., 2017; Reyes and Kim, 1991). Briefly, a second strand synthesis was performed, followed by digestion of the second strand product with a restriction enzyme. Then an adaptor was ligated to the digested DNA followed by PCR amplification of the adaptor-ligated product.

2.5. Viral library preparation and sequencing

The amplified PCR products were cleaned up with WIZARD® SV Gel and PCR clean-up system (Promega®) following manufacturer’s indications. Two libraries with dual indexing for each sample were generated with Illumina Nextera® XT DNA Sample Preparation kit and submitted to the Australian Genome Research Facility (AGRF) for a 250 base paired-end sequencing on the MiSeq® Illumina platform.

2.6. Bioinformatic analyses

Raw sequences were trimmed by quality. The final high-quality reads (HQRs) were used for screening against the dog and bacterial genomes and all dog and bacterial sequences were removed. The host and bacteria free sequences were de novo assembled and the resulting contigs and singletons were used for further analysis (Moreno et al., 2017). All contigs and singletons were analyzed through two different bioinformatic pipelines. Initially, contigs and singletons were compared against the CAMERA Viral Nucleotide Sequence database 10,570.V9, using tBLASTx search with an E-value cut off 10−5. In the second pipeline, contigs and singletons were compared against the NCBI nucleotide database (2012) using BLASTn search with an E-value cut off 1. The tBLASTx and BLASTn files were then analyzed by MEGAN V5.2.1 (Illuson et al., 2011) which identified the lowest common ancestor of known viral sequences.

Finally, all viral contigs/singletons from eukaryotic organisms present in both analyses were assembled and compared against the NCBI reference sequence to evaluate the genome coverage. Sequence analysis software (Sequencher® version 5.0.1, Gene Codes Corporation, Ann Arbor, MI USA) with minimum match percentage 70 and minimum overlap 50 as assembly parameters were used.

2.7. Canine kobuvirus study

The nucleic acid from the fecal sample from the dog with chronic enteropathy (CE9), which had 9 confirmed contigs/singletons identified as canine kobuvirus (according to MEGAN) was used to generate the complete genome sequence (Table S2).

Reverse transcription polymerase chain reaction (RT-PCR) was performed with SuperScript® III One-Step RT-PCR System with Platinum® Taq (Invitrogen™) and PCR conditions used were: 45°C for 60 min and 94°C for 2 min, 40 cycles of 94°C for 45 s, 48°C for 1 min and 72°C for 2 min, and a final elongation step of 72°C for 10 min, followed by final hold at 4°C. PCR products were separated on a 1% agarose TBE gel stained with nucleic acid staining solution RedSafe (iNTRON Biotechnology). All PCR products were gel excised and cleaned up with WIZARD® SV Gel and PCR clean up system (Promega®) following manufacturer’s protocol and sequenced using Sanger sequencing at AGRF. The near complete genome of the canine kobuvirus was assembled using the sequence analysis software (Sequencher® version 5.0.1, Gene Codes Corporation, Ann Arbor, MI USA) with minimum match percentage 80 and minimum overlap 50 as assembly parameters.

Phylogenetic analysis of the polyprotein region of this canine kobuvirus was performed together with its closest viral relatives (best BLAST hits). Twenty three amino acid sequences of the polyprotein region identified in GenBank were aligned using a multiple sequence alignment program CLUSTAL W, from MEGA7 version 7.0 (Kumar et al., 2016) with default settings. A phylogenetic tree with 1000 bootstrap was generated using the Maximum Likelihood method based on the JTT matrix-based model, using MEGA7 version 7.0.

The prevalence of canine kobuvirus was evaluated using a PCR targeting a fragment of the 3D region identified from the confirmed positive sample detected by metagenomic analysis (CE9). DNA and RNA from the additional 35 fecal samples (14 case and 21 control) (Table S1) were then extracted. Briefly, 800 μL of virus dilution buffer (0.01 M Tris solution (pH 7.5), 0.15 M NaCl, 0.01 M CaCl2) was added to 0.2g of fecal material, vortexed until homogenized, centrifuged at 20,000 × g for 3 min and the supernatant harvested. The RNA was extracted from 140 μL of the supernatant using Qiamp® Viral RNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer’s recommendations. In order to amplify a 504 bp fragment from the canine kobuvirus 3D region, two primers were used: CaKV/F and CaKV/R (Di Martino et al., 2013), and RT-PCR was performed using QIAGEN One-Step RT-PCR kit (QIAGEN, Hilden, Germany). The following PCR conditions were used: a RNA denaturation step at 97°C for 3 min in a heat block, prior to the addition of master mix. Subsequently, all samples were placed in the thermo cycler and subjected to 45°C for 60 min and 94°C for 2 min, 40 cycles of 94°C for 45 s, 48°C for 1 min and 72°C for 50 s, and a final elongation step of 72°C for 10 min, followed by final hold at 4°C. All positive samples were visualized on a 1.2% agarose TBE gel.

3. Results

3.1. Overview of the fecal virome of dogs with CE

As a result of bioinformatic analyses, over 52 million MiSeq reads were generated from the eight fecal samples from dogs with chronic enteropathy, of which 45,190,173 sequences were HQRs. From these HQ, all sequences matching to the dog genome and cellular organisms (15,640 and 42,402,966 respectively) were removed. The remaining cleaned up reads, or dog and cellular organisms free sequences, were de novo assembled and 290,225 contigs and singletons were generated (Table S3). Out of the 290,225 contigs and singletons, 11,173 contigs and singletons were identified as viral sequences (Table S3), comprising three viral families that infects eukaryotes, Papillomaviridae, Picornaviridae and Reoviridae, present only in three CE samples. Four families that infect bacteria (bacteriophages) were also identified, Myoviridae, Siphoviridae, and Podoviridae from the dsDNA order Caudovirales and family Microviridae (ssDNA), being present in all CE samples. Overall, bacteriophages comprised 99.8% (11,157 contigs and singletons) of the total number of viral contigs and singletons and were present in all CE samples (Table S3). At the genus level different bacteriophage genera were present in samples from dogs with CE. Bacteriophages that could not be classified to the genus level were also identified (Fig. 1a and b).

Further analysis of eukaryotic viruses identified that one sample (CE1, Table S1 and S3) covered 3.4% of the complete genome of Human papillomavirus type 109 (NC_012485.1) with the largest proportion identical to E1 protein (10.8%) and E2 protein (9%). Reoviridae contigs and singletons were found in two samples (Table S3). Further analysis identified, that these contigs and singletons covered between 13.7% and 15.4% of the VP4 gene segment of the Rotavirus reference sequence for VP4 gene (NC_011510.2). Contigs and singletons matching the Picornaviridae family were used to characterize the complete genome of canine kobuvirus, as explained below.

The fecal virome of eight healthy dogs has been previously described by our group (Moreno et al., 2017). The healthy dog studies identified 3968 viral contigs/singletons. Bacteriophage were found in all samples and 75.9% of viral contigs and singletons were
bacteriophages. The same bacteriophage families were detected in healthy dogs and in dogs with CE, but at the genus level there were some differences between both groups (Fig. 1a and b). Eukaryotic viral families found in the healthy group were Adenoviridae, Papillomaviridae, Parvoviridae, Reoviridae and Coronaviridae (Table S3). Papillomaviridae and Reoviridae families were also present in dogs with CE.

3.2. Canine kobuvirus characterization and prevalence

In one dog with CE we detected, by shotgun sequencing, 9 contigs/singletons which were annotated as Picornaviridae. This sample, a 21-month-old, male, Weimaraner dog with FRD (CE9, Table S1), was chosen for a more detailed follow up. Using Sanger sequencing the near-complete genome of a canine kobuvirus was determined. The total length was 7335 nucleotides, which encode the polyprotein of 2444 aa. The nucleotide composition was 19.8% A, 41% T, 37.1% C. The G/C composition was 58.1%. The genome encoded the complete large open reading frame (ORF) for a polyprotein. The total length was 7335 nucleotides, which encode the polyprotein of 2444 aa. The nucleotide composition was 19.8% A, 20.7% G, 21.9% T, and 37.7% C. The G/C composition was 58.3%.

A phylogenetic tree was constructed by multiple alignment of the full sequence of the polyprotein from the canine kobuvirus characterized in this study (Canine kobuvirus_CE9/AUS/2012, GenBank accession number: MH052678) and 23 other polyprotein kobuvirus sequences reported from different animal species (Table S4). The phylogenetic analysis revealed that the canine kobuvirus identified in our study clustered within the canine kobuvirus clade, with similarities between 96.8% and 99.14%. The most closely related sequences were between 96.8% and 99.14%. The most closely related sequences were from South Korea and China (Oem et al., 2014) (Fig. 2). To evaluate prevalence of canine kobuvirus in a larger population, PCR screening was performed in all dogs used for metagenomics and extra 35 individual dogs with a similar background, 21 healthy client-owned dogs and 14 client-owned dogs with CE. As a result, only one dog was confirmed positive, the same sample already identified as positive (CE9) by shotgun sequencing.

4. Discussion

In this study we described the fecal virome from dogs with chronic enteropathy. We identified both prokaryotic and eukaryotic viruses, whereby prokaryotic viruses (bacteriophage) comprised 93.6% off all contigs/singletons. This high percentage of bacteriophages in dogs with CE is consistent with previous fecal virome studies from humans with IBD or CE (Lepage et al., 2008; Norman et al., 2015). Even though it is known that the SISPA method has some inherent bias with regions of repetitive sequences being generated (Rosseel et al., 2013), we believe that it was the best available technology at the time of the study and the overall findings are consistent and reliable.

Bacteriophages have the ability to modify bacterial populations, both in number and function (De Paepe et al., 2014) and are classified depending on their life cycle as lytic or lysogenic. In the lysogenic cycle, phages are called temperate phages or prophages, and bacteria and bacteriophages live in symbiosis (De Paepe et al., 2014). Nevertheless, bacteriophages can be induced to become lytic or pathogenic, where they then replicate and kill their host (De Paepe et al., 2014). Bacteriophages thus have the ability to transfer their genome into their host bacteria, conveying genetic information and changing the pathogenicity of gut microbial flora or ultimately, modifying bacterial populations through their virulent state (De Paepe et al., 2014).

Recent research analyzing the potential importance of bacteriophages in the immune response of the host and their influence in the dysbiosis present in humans with IBD has been published (Babickova and Gardlik, 2015; Lepage et al., 2008; Norman et al., 2015; Wagner et al., 2013). Various studies analyzing phage populations have found differences between healthy and IBD individuals (Crohn’s disease, CD) (Lepage et al., 2008; Norman et al., 2015) and similarly, differences between phage populations between CD and ulcerative colitis (UC) individuals have been detected (Norman et al., 2015). Despite all these investigations it is not yet clear the exact role that phages may play. It is also possible that bacteriophages may exert a beneficial effect by conferring fitness to a subset of bacteria.

Our current study found different bacteriophage populations between dogs with CE and healthy dogs (i.e.: T7 virus, Bppunalikevirus, Bxzlivirus); hence, it is possible that the difference between bacteriophage populations may be an important factor in preserving or restoring canine intestinal health.

Among eukaryotic viral sequences both, known pathogenic and nonpathogenic viral species were identified in healthy dogs and dogs with CE. Sequences identified as Reoviridae and Papillomaviridae were found in both groups and further analysis identified them as human rotavirus and human papillomavirus. Sequences representing the viral family Picornaviridae were identified in one dog with chronic enteropathy. After further sequence analysis, a canine kobuvirus was characterized. Previous studies using electron microscopy identified picornavirus-like particles in canine fecal samples in 1995 in Australia (Finlaison, 1995). Our study is the first description of a canine...
kobuvirus genome in Australia. A phylogenetic tree, analyzing the complete polyprotein region of this canine kobuvirus and other kobuvirus sequences from different species present in GenBank, showed that it belonged to the canine kobuvirus clade, close to sequences from South Korea (Oem et al., 2014) and China (Li et al., 2016).

The majority of studies evaluating canine kobuvirus have suggested that this enteric virus is more prevalent in animals younger than 2 years (Choi et al., 2014; Di Martino et al., 2013; Li et al., 2011; Oem et al., 2014). Some of these studies have identified canine kobuvirus from both healthy dogs and dogs with diarrhea (Li et al., 2011; Oem et al., 2014). Thus, canine kobuvirus has not been considered a primary causal agent of diarrhea in dogs, mostly because it is regularly found in co-infection with other known enteric viruses (Choi et al., 2014; Di Martino et al., 2013; Li et al., 2016, 2011; Oem et al., 2014). It is currently unknown whether kobuvirus may induce disease in a genetically susceptible animal or interact with bacteria to worsen inflammation. The prevalence study performed in our larger cohort of healthy dogs and dogs with CE did not find any other samples positive for kobuvirus. Further epidemiological studies in a larger cohort may help to better understand the epidemiology of this virus in the canine population and its association with disease.

Although only a small number of dogs with CE were analyzed in this study, there was a difference in the fecal virome when compared to that present in healthy dogs. As a result, it can be hypothesized that the intestinal virome is an important component of the complex interaction between the intestinal immune system, and luminal contents. Further evaluation of the type and function of bacteriophages in particular is warranted alongside evaluation of the intestinal bacteria in future studies of CE in dogs. This is of particular importance when considering the efficacy of potential therapies, including phage, probiotics and fecal microbial transplantation.

Accession number

The GenBank accession number for the Canine kobuvirus sequence is MH052678.

Declarations of interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2018.05.020.

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