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Short Communication

Complete genome sequence of canine astrovirus with molecular and epidemiological characterisation of UK strains

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ABSTRACT

Astroviruses are a common cause of gastroenteritis in children worldwide. These viruses can also cause infection in a range of domestic and wild animal species. Canine astrovirus (CaAstV) was first identified in the USA, and has since been reported in dogs from Europe, the Far East and South America. We sought to determine whether CaAstV is circulating in the UK dog population, and to characterise any identified strains. Stool samples were collected from pet dogs in the UK with and without gastroenteritis, and samples were screened for CaAstV by qPCR. Four CaAstV positive samples were identified from dogs with gastroenteritis (4/67, 6.0%), whereas no samples from healthy dogs were positive ($p < 0.001$). Sequencing of the capsid sequences from the four CaAstV strains found significant genetic heterogeneity, with only 80% amino acid identity between strains. The full genome sequence of two UK CaAstV strains was then determined, confirming that CaAstV conforms to the classic genome organisation of other astroviruses with ORF1a and ORF1b separated by a frameshift and ORF2 encoding the capsid protein. This is the first report describing the circulation of CaAstV in UK dogs with clinical signs of gastroenteritis, and the first description of the full-length genomes of two CaAstV strains.

1. Introduction

Astroviruses are small non-enveloped, positive sense RNA viruses with a wide host range. Astroviruses are classified into two genera; the *Mamastrovirus* genera includes astroviruses isolated from humans, pigs, cattle, cats and dogs, whereas astrovirus isolates from birds are categorised into the *Avastrovirus* genera (King et al., 2011). Astroviruses were first identified in 1975 in the stools of children with diarrhoea (Appleton and Higgins, 1975; Madeley and Cosgrove, 1975), and are now estimated to cause up to 10% of gastroenteritis cases in children worldwide (Moser and Schultz-Cherry, 2005).

Canine astrovirus (CaAstV) was first described in the USA, following the identification of star-shaped particles in diarrhoeic stools from a litter of beagles (Williams, 1980). Later studies have identified CaAstV in Italy, France, China, Korea and Brazil (Castro et al., 2013; Choi et al., 2014; Grellet et al., 2012; Martella et al., 2011; Zhu et al., 2011).

The genome of astroviruses is typically 6.4–7.3 kb and divided into three open reading frames (ORFs), ORF1a, ORF1b and ORF2 with a 5′ untranslated region and a 3′ poly-A tail (King et al., 2011). ORF1 codes for the non-structural proteins and ORF2 encodes the capsid precursor protein. The complete coding sequence of ORF2, and a partial sequence of ORF1b has previously been determined for a number of CaAstV isolates (Toffan et al., 2009; Zhu

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et al., 2011), but to date, no full-length genome sequence of CaAstV has been reported.

The purpose of this study was to determine the prevalence of CaAstV in the UK dog population and to obtain where possible the complete genetic sequence of circulating strains. Four CaAstV strains were identified in dogs showing clinical signs of gastroenteritis and sequencing of ORF2 found significant genetic diversity between strains. The first full-length sequences of two CaAstV strains was subsequently determined.

2. Methods

2.1. Sample collection

Stool samples were collected from dogs admitted to five participating veterinary clinics and a single animal shelter in counties in the south and east of England; Cambridgeshire, Kent, Lincolnshire, Middlesex and Suffolk. Ethical approval was not required for this study as all samples collected were animal waste products. With owner consent, dogs were recruited to the study if they passed stools whilst hospitalised. Stool samples were collected from dogs from the animal shelter if they passed diarrhoea. All stool samples were stored at −20 °C until and during transportation to the laboratory, where they were stored at −80 °C prior to nucleic acid extraction. As controls, stool samples were also collected from healthy dogs owned by veterinary staff at each clinic, as well as from dogs at participating boarding kennels. Basic case data was recorded for each dog from which a stool sample was collected, including age, breed, sex, reason for admission, and any recent history of enteric disease.

2.2. Detection of CaAstV in clinical samples

Stools were diluted to a final concentration of 10% w/v in phosphate-buffered saline, pH 7.2, and solids were removed by centrifugation at 8000 × *g* for 5 min. Viral nucleic acid was extracted from 140 μl of each clarified stool suspension using the GenElute™ Mammalian Total RNA Miniprep Kit (Sigma–Aldrich) according to the manufacturers' instructions. An internal extraction control (equine arteritis virus RNA) was added during the nucleic acid extraction process as previously described (Caddy et al., 2013).cDNA was generated by reverse transcription using MMLV reverse transcriptase enzyme (Life Technologies) and random hexamers (Life Technologies) with the reaction performed at 42 °C for 1 h, followed by an inactivation step at 70 °C for 10 min. qPCR was performed using primers targeting a conserved region of the viral RNA-dependent RNA polymerase as previously described (Martella et al., 2011). The sequences of primers used for PCR in this study are presented in Table 1. qPCR reactions were prepared using the MESA Blue qPCR MasterMix Plus for SYBR Assay (Eurogentech). Briefly, 2 μl cDNA was mixed with 2× MasterMix and 0.5 μM primers, then incubated at 95 °C for 10 min. The thermal cycle protocol used with a ViiA7 qPCR machine (AB Applied Biosystems) was as follows: 40 cycles of 94 °C, 15 s; 56 °C, 30 s; 72 °C, 30 s, followed by generation of a melt curve. Viral genome copy number was calculated by interpolation from a standard curve generated using serial dilutions of a standard DNA amplicon cloned from a positive control. The limit of detection was determined by the lowest dilution of control standard CNA reproducibly detected in the assay. All samples were additionally screened for the presence of canine parvovirus (CPV), canine enteric coronavirus (CECoV) and canine norovirus (CNV) using a 1-step qRT-PCR protocol (Caddy et al., 2013).

2.3. Capsid sequencing

All samples positive to CaAstV by qPCR were subjected to conventional PCR to confirm the presence of CaAstV and to enable genome sequencing. The capsid of all positive samples was amplified from cDNA synthesised using SuperScript II Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol with 0.5 μM AV12 primer (Table 1). The PCR reaction was performed using KOD hot start polymerase (EMD Millipore), with reverse primer s2m-rev, and the forward primer 625F-1 from the original qPCR assay. The amplification programme consisted of an initial 5 min step at 95 °C, followed by 35 cycles with 95 °C for 20 s, 58 °C for 30 s and 72 °C for 90 s. A final elongation step at 72 °C for 5 min was performed, followed by chilling to 4 °C.

PCR products were subsequently cloned into pCR-Blunt™ using the Zero Blunt PCR Cloning Kit (Life Technologies) according to the manufacturers protocol. Sequencing of the 5′ and 3′ regions of the plasmid insert was performed using pCR-Blunt™ specific primers by the University of Cambridge Biochemistry DNA Sequencing Facility. Sequencing primers for the central region of insert were then designed based on the primary sequence data to give a 200 nt overlap with each predecessor, and a second round of sequencing reactions was performed (sequencing primer details available on request). The complete capsid

Table 1
CaAstV specific primers used in this study.

Primer target		Sequence	Reference
RdRp	625F-1	GTACTATACCRTCTGATTTAATT AGACCAARGTGTCATAGTTCAG	Martella et al. (2011)
ORF1b–ORF2	AV12 s2m-rev	TTT TTT TTT TTT TTT TTT GC CCC TCG ATC CTA CTC GG	Toffan et al. (2009)
ORF1a	683	TGAAGGACTGCTCAGAGTG	This paper
5′ RACE	682	ATGCAGCGACAAACACAACA	This paper
3′ RACE	894	CACAGCCCATTGAAGATG	This paper