A pilot study on feline astrovirus and feline panleukopenia virus in shelter cats in Erzurum, Turkey

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SUMMARY

The aim of the study was to investigate the presence and molecular character of feline astrovirus and feline panleukopenia virus in cats in Erzurum, Turkey. Fifty feces from cats of which 13 with clinical signs and 37 with clinically healthy were analysed using reverse transcription polymerase chain reaction for ORF2 gene of astrovirus and VP2 gene of panleukopenia virus. Upon reverse transcription polymerase chain reaction, sequence analyses, bioinformatics analyses and phylogeny was performed. Of the cats 8 % (4/50) were positive for FAstV and 10 % (5/50) were positive for FPLV. Stool samples from the astrovirus and panleukopenia virus positive cats were negative for rotavirus and coronavirus. When positive astrovirus amplicons were sequenced, the feline astrovirus strains appeared to be genetically more related to human astroviruses than to porcine, canine, bat, and avian astroviruses. Result of the sequence analysis of the feline panleukopenia virus positive PCR products indicate that it was closely related to Asian strains in the cluster of G1. This study also has shown that the genotypes of the feline panleukopenia virus found in this study were different from previously reported a single study from Turkey and were closely related to European strains. This is the first report on the presence and molecular characterization of FAstV in cats from Turkey. While role of parvovirus in gastroenteritis cases was well known, role of astrovirus is remain unclear in cats.

Keywords: Diarrhoea, feline astrovirus, feline panleukopenia virus, molecular characterization, Turkey

RÉSUMÉ

Une étude pilote sur l’astrovirus félin et le virus de la panleucopénie féline chez les chats dans des refuges à Erzurum, Turquie


Mots-clés: Diarrhée, astrovirus félin, virus de la panleucopénie féline, caractérisation moléculaire, Turquie

Introduction

Cats are one of the most common pets, with a worldwide population estimated at ~200 million. Interaction with human being presumably accelerated following the domestication of cats ~9500 years ago [5, 6, 10].

The feline astrovirus (FAstV) and feline panleukopenia virus (FPLV) are important viral enteric pathogens in cats. These viruses are the leading cause of diarrhoea and gastrointestinal upset in cats and also induce the death of immature and young adult cats [13, 15]. The astrovirus is a member of Astroviridae, and its name means “star-like particle.” Astroviruses are small, non-enveloped viruses that have a single-stranded RNA genome composed of three open reading frames. In terms of the host, there are two genera: Avastrovirus and Mamastrovirus. Although the Avastrovirus infects avian species, Mamastrovirus infect mammals, including humans, ruminants, and carnivores [2]. FAstV was first identified by electron microscopy in the stool obtained from a domestic young cat with diarrhoea in 1981, and it was subsequently reported in Australia and Europe, including in England, Germany, and Italy [2, 9]. Although the role of the primary pathogen or synergistic remains unclear, FAstV has been accepted as an important agent of feline enteritis [16].

Parvovirus are a member of the family of Parvoviridae, which are small and single-stranded DNA viruses. Parvoviruses infect a variety of animal species. Canine and feline parvoviruses cause haemorrhagic gastroenteritis and leukopenia and also have a high mortality rate,
especially in pups and kittens [3, 20]. Although the genome organization of the parvovirus is deoxyribonucleic acid (DNA) conformation, it is highly susceptible to mutation, as in ribonucleic acid (RNA) viruses [3]. Canine parvoviruses (CPVs) are composed of the canine parvovirus (CPV), feline panleukopenia virus (FPLV), and mink enteritis virus (MEV), which are closely related viruses. The viruses located in this group cause serious diseases, especially in young cats [17]. Antigenic variants of parvovirus (CPV-2a, CPV-2b, and CPV 2c) were detected in different countries [4, 15]. This variants are separated from each other by five or seven amino acid changes in the VP2 protein gene [3]. Currently, there are limited studies on FPLV in Turkey. According to the database resources of The National Center for Biotechnology Information (NCBI), there is limited information on FAstV and FPLV sequence data. To date, the genotypes of FAstV have not been analysed in Turkey. The aim of this pilot study was to investigate the relationships of FPLV and FAstV to gastroenteritis in cats and to determine the FAstV and FPLV strains by phylogeny in Erzurum, Turkey.

**Materials and methods**

**SPECIMEN COLLECTION**

Animal shelters generally contain old cats and sometimes kittens that were either idle cats or had been freed. All animals living in an animal shelter are in close contact and share the same environmental area, food, and water. A total of 50 cats from a municipal shelter from September 2015 to April 2016 were included in the study. Sample size was determined by shelter limitations and was too difficult to bring out precise estimates of prevalence. Clinical symptoms in the cats included diarrhea, anorexia and vomiting. Information about sampled animals, such as sample type, breed, sex and clinical sign is shown in Table I. Information of vaccination status was not known. Feces of symptomatic (n=13) and clinically healthy (n=37) cats were picked up in separate sterile tubes and were sent to the virology laboratory of the veterinary faculty at Ataturk University. All samples were frozen at -20 °C until use.

**SAMPLE PREPARATION, REVERSE TRANSCRIPTION AND PCR**

The feces samples were suspended in 1 mL of DNAse-RNase–free water and then stirred. The mixture solution was centrifuged, and the upper portion of the liquid was transferred into a new sterile tube. The total RNA was extracted from a 300-μL fecal suspension using a GF-1 nucleic acid extraction kit (Vivantis, Malaysia). Three microliters of extracted RNA were reverse transcribed using a cDNA synthesis kit (Thermo Scientific, Germany) according to the manufacturer’s instructions.

To determine the astrovirus and CPV-2 types, we used specific primers. The presence of the astrovirus RNA was confirmed by RT-PCR using specific primers for astrovirus: Mon 269 and Mon 270, which target the capsid region (ORF2) of the astrovirus. PCR was performed according to the method reported by Moschidou et al., [16]. For the parvovirus, we performed PCR using a Hfor-Hrev primer set to amplify a part of the VP2 gene as reported by Buonavoglia et al., [1] (Table II).

<table>
<thead>
<tr>
<th>ID</th>
<th>Sample</th>
<th>Sex</th>
<th>Breed</th>
<th>Clinical sign</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAstV-TR-Erz4</td>
<td>Rectal swab</td>
<td>F</td>
<td>Van</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>FAstV-TR-Erz5</td>
<td>Fresh stool</td>
<td>F</td>
<td>Mixed</td>
<td>-</td>
</tr>
<tr>
<td>FAstV-TR-Erz6</td>
<td>Fresh stool</td>
<td>M</td>
<td>Mixed</td>
<td>-</td>
</tr>
<tr>
<td>FAstV-TR-Erz9</td>
<td>Fresh stool</td>
<td>F</td>
<td>Mixed</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>FPLV/TR/Erz/1</td>
<td>Fresh stool</td>
<td>M</td>
<td>Mixed</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>FPLV/TR/Erz/7</td>
<td>Fresh stool</td>
<td>F</td>
<td>Van</td>
<td>Anorexia, vomiting, diarrhea</td>
</tr>
<tr>
<td>FPLV/TR/Erz/11</td>
<td>Rectal swab</td>
<td>F</td>
<td>Mixed</td>
<td>Anorexia, diarrhea</td>
</tr>
<tr>
<td>FPLV/TR/Erz/14</td>
<td>Rectal swab</td>
<td>F</td>
<td>Mixed</td>
<td>-</td>
</tr>
<tr>
<td>FPLV/TR/Erz/20</td>
<td>Fresh stool</td>
<td>M</td>
<td>Mixed</td>
<td>Diarrhea</td>
</tr>
</tbody>
</table>

**Table I**: ID, Sample type, sex, breed and clinical remark of cats infected with FPLV and Astrovirus

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5′-3′</th>
<th>Polarity</th>
<th>Specificity</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hfor</td>
<td>CAGGTGATGAATTGCTACA</td>
<td>+</td>
<td>FPLV</td>
<td>630</td>
</tr>
<tr>
<td>Hrev</td>
<td>CATTTGGATAAACCTGGTGT</td>
<td>-</td>
<td>FPLV</td>
<td></td>
</tr>
<tr>
<td>Mon 269</td>
<td>CAACCTAGGAACAGGGTGT</td>
<td>+</td>
<td>FAstV</td>
<td>450</td>
</tr>
<tr>
<td>Mon 270</td>
<td>TCAGATGCATTGTCATTGTT</td>
<td>-</td>
<td>FAstV</td>
<td></td>
</tr>
</tbody>
</table>

**Table II**: Oligonucleotide details used in the study

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The PCR products were separated by electrophoresis and illuminated with ultraviolet (UV) light, and then, the PCR products were purified for sequencing using a commercial cleanup kit (QIAquick PCR Purification Kit, QIAGEN, Germany).

SEQUENCING AND PHYLOGENETIC ANALYSIS

Nucleotide sequencing was performed using a Big Dye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, USA) according to the manufacturer’s protocol. Multiple sequence alignments were assembled with ClustalW using BioEdit software package version 7.0.5.3 and compared to related sequences using BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) [7]. Phylogenetic analyses were conducted using MEGA, version 6.0, with the neighbor-joining method, and bootstrap values were calculated with 1,000 replicates [19].

Results

In the present study, gastrointestinal infections by FAstV and FPLV were investigated in cats. A total of 50 cats were enrolled in the study. Of the cats, 8% (4/50) were positive for FAstV, and 10% (5/50) were positive for FPLV (Table I). Regarding the presence of co-infection, all samples were evaluated for the rotavirus and coronavirus, but co-infection was not seen.

When positive samples were characterized by sequence analysis, the results demonstrated that both FAstV and FPLV strains are circulating among domestic cats in Erzurum, Turkey. Upon the sequence analysis of the FPLV, the sequences separate within three groups as G1, G2, and G3. Parvovirus sequence analysis demonstrated the highest nucleotide identity in the VP2 gene for feline panleukopenia virus subgroup G1 and our strains were located with Japan, Korea, and China strains in the cluster of G1. All four positive strains were found to be closely related to FPLV (Figure 1).

Figure 1: Phylogenetic tree of the nucleotide sequences of the FPLV positive samples based on partial VP2 region.
The sequences of FAstV occur in several different clusters, including feline, human porcine, bat, canine or avian. All FAstV strains appeared within same groups as FAstV. Astrovirus sequence analysis demonstrated the highest nucleotide identity in the capsid protein gene for the other FAstV in the NCBI (Figure 2). This is the first identification and molecular characterization study in Turkey for the FAstV.

Nucleotide sequence accession numbers; the partial genome sequences of feline astrovirus and feline panleukopenia virus have been submitted to GenBank under the accession no. KY563080 (FAstV-TR-Erz4), KY563081 (FAstV-TR-Erz5), KY563082 (FAstV-TR-Erz6), KY563083 (FAstV-TR-Erz9), KY563084 (FPLV/TR/Erz/11), KY563085 (FPLV/TR/Erz/14), KY563086 (FPLV/TR/Erz/20), KY563087 (FPLV/TR/Erz/1), KY563088 (FPLV/TR/Erz/7).

Discussion

Viral gastroenteritis is one of the common causes of morbidity in young domestic animals in the world. In this study, we investigated the FPLV and FAstV in feces on a molecular scale. FPLV is an important pathogen of cats [1, 18]. The new antigenic variants or the relocation of the dominant type of the parvovirus are emerging in different countries. Although they seem similar, CPV and FPLV are associated with different evolutionary models [3, 11]. On the other hand, the canine parvovirus (CPV) have different antigenic variants: CPV-2a, CPV-2b, and CPV-2c, as it is known. FPLV has a certain genetic stability [4, 15]. This three variants of CPV2 and FPLV are separated from each other by five or seven amino acid changes in the VP2 protein gene. These amino acid differences in the VP2 coding region enables detect currently known variants of CPV-2 [3]. In the current study, phylogeny showed that all strains were located in cluster G1 (Figure 1). These results are not in line with a single previously reported study that found a cluster of G3
in Turkey [17]. Vaccine-associated infections could occur after immunization for FPLV, but our strain was located in a different cluster with vaccine strains (FeloCell, Purevax). Therefore, vaccination research for FPLV is needed. The FPLV strains of Italy, Russia, the United Kingdom (UK), and Austria have been located in the same cluster with vaccine strains. According to the phylogenetic analyses, the FPLV strains in our study were more closely related to Asian strains than to European strains. Previously, a single study was reported for FPLV in Turkey, which was located in cluster G3 [17]. In this study, FPLV strains were closely related to European strains. The detection of two different genotypes in different regions highlights the diversity of the circulating FPLV genotypes in cats in Turkey.

Natural infection by an astrovirus has been reported in household cats with anorexia, vomiting, diarrhea, partial dehydration, and mild fever [8, 9]. Due to the possibility of the interspecies transmission of the astroviruses, calculations of genetic distance are performed [14]. Sequence information has been shown in some earlier studies to analyse the nucleotide similarity among FAstV [2, 13, 16]. Considering the large territory and location of Turkey, no research has been conducted in Turkey to analyse FAstV. In our study, four astrovirus strains from shelter cats were sequenced, all being characterized as true FAstV. All of our FAstV strains were located in the same group (Figure 2). Transmission of the astrovirus between human begins and feline has been hypothesized and feline like astrovirus has recently been reported in a child [16]. Although feline astroviruses are closely related to human astroviruses, our strains partially took part in a separate clade with human astroviruses [12]. The FAstV sequences detected in our study displayed similarities based on the phylogeny with Korea, the USA, China, and Italy (Figure 2). This study shows preliminary results and highlights the presence of FAstV in Turkey. To our knowledge, it is the first detection and results and highlights the presence of FAstV in Turkey. To our knowledge, it is the first detection and molecular characterization of FAstV and FPLV (G1 cluster) in Turkey. Based on the limited studies available and on the data obtained in this study, further studies are required to fully assess the aetiology and epidemiology of FAstV in Turkey.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research.

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References