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Short communication

Identification and genome characterization of a novel feline picornavirus proposed in the *Hunnivirus* genus

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ABSTRACT

The genus *Hunnivirus*, which has been identified in sheep, cattle, and rats, was first proposed in the family *Picornaviridae* by the International Committee on Taxonomy of Viruses in 2013. In this study, a hunnivirus was detected in fecal samples collected from a diarrheic cat in Southern China in 2017. Genome sequencing and analysis indicated that the novel hunnivirus has the same genome organization as reported for other hunniviruses, 5′UTR-L-P1(VP4-VP2-VP3-VP1)-P2(2A-2B-2C)-P3(3A-3B-3Cpro-3Dpol)-3′UTR, but is genetically divergent. This hunnivirus is proposed as a novel genotype of the species *Hunnivirus A* and provisionally designated *feline hunnivirus*. Our study expands the host range of hunnivirus and enriches knowledge on picornaviruses.

1. Introduction

According to the International Committee on Taxonomy of Viruses (ICTV), picornaviruses in the family *Picornaviridae* are currently divided into 40 genera ([www.picornaviridae.com] February 2018). The genus *Hunnivirus*, which contains a single species, *Hunnivirus A*, was included within the family *Picornaviridae* of the order *Picornavirales* in 2013 (Adams et al., 2014). To date, three genotypes of hunniviruses have been officially proposed by the ICTV, including one hunnivirus isolated from sheep cell cultures in Northern Ireland in 1965 ([https://talk.ictvonline.org/ictv/proposals/2013.008a-dV.A.v2.Hunnivirus.pdf](https://talk.ictvonline.org/ictv/proposals/2013.008a-dV.A.v2.Hunnivirus.pdf)) and bovine and ovine hunniviruses identified in Hungary during 2008 and 2009 (Reuter et al., 2012). From 2014 to 2016, hunniviruses have also been detected in rats in America and China, revealing new hunnivirus genotypes (Du et al., 2016; Firth et al., 2014).

This study is the first to report a novel hunnivirus identified in fecal samples collected from a cat with diarrhea in China. The genome of feline hunnivirus (FeHuV) is genetically divergent from that of other hunniviruses and is proposed as a new genotype of the species *Hunnivirus A*.

2. Materials and methods

2.1. Sample collection

To understand whether hunniviruses are present in cats, we screened 47 and 44 archived fecal samples from diarrheic and healthy cats, respectively. These samples were collected from Guangzhou and Shenzhen, Southern China, from 2016 to 2018. The method of sample collection was conducted under the guidance of the South China Agricultural University Experimental Animal Welfare Ethics Committee. Samples were collected for testing the prevalence of viruses in cats.

2.2. Virus detection and genome sequencing

Viral nucleic acid was extracted from samples using MiniBEST Viral RNA/DNA Extraction Kit (Takara, Japan). The RNA obtained was then reverse-transcribed into cDNA using GoScript Reverse Transcription System with a random primer (Promega, USA). Viral cDNA/DNA was assessed for the presence of known common feline enteric viral pathogens by PCR with PrimeSTAR HS (Takara, Japan). Using a primer...
walking strategy and long-range PCR method, four sets of overlapping primer pairs targeting the near-complete genome of FeHuV were designed using Oligo 7.0 (Fig. 1), and DNA fragments were amplified using Q5 high-fidelity DNA polymerase (NEB, UK). After sequencing, the near-complete genome of one feline hunnivirus strain was assembled from the raw data using SeqMan 7.1.0.

2.3. Phylogenetic analysis

Before constructing the phylogenetic tree, amino acid substitution models were estimated using the “find best protein model” in MEGA 5.05. Three phylogenetic trees based on the putative amino acid sequences of the P1 (778 aa), 2C (323 aa), and 3C (208 aa) coding regions were inferred using the rREV + G + F amino acid substitution model, as based on bootstrap values of 1000 replicates (Fig. 2).

2.4. Virus isolation

Fecal samples testing positive for FeHuV RNA were processed for virus isolation. Culture was performed using a previously described method for bovine and ovine hunniviruses, with slight modification (Reuter et al., 2012). Six cell lines (CRFK, MDCK, A549, PK-15, DF-1, Vero) were used; cultures were incubated for 4 days and observed daily for the cytopathic effect (CPE). After five passages, total RNA was extracted from culture lysates and supernatant for detection of FeHuV by RT-PCR with UNIV-kobu-F/UNIV-kobu-R primers.

![Fig. 1. Sequencing strategy of the feline hunnivirus genome amplified by PCR.](image)

(A) PCR primer-targeting regions are numbered according to the field feline hunnivirus strain FeHuV-1/GZ/2017. The nucleotide (nt) position of each gene is shown above.
(B) Four PCR primer pairs were designed to sequence the feline hunnivirus genome. The primer name and target region are indicated.
(C) Information for the primer sequences. Y: C/T; W: A/T; R: A/G.

![Fig. 2. Phylogram of the feline hunnivirus with other picornaviruses based on the predicted amino acid sequences of P1, 2C, and 3C coding regions.](image)

The field feline hunnivirus strain FeHuV-1/GZ/2017 is labeled with a black circle.

![Diagram](image)
3. Results

3.1. Identification of a novel hunnivirus in diarrheic cat

The primers UNIV-kobu-F/UNIV-kobu-R were utilized in this screening study to assess whether hunniviruses are present in cats. These primers amplify a 216-bp fragment of the 3D RNA-dependent RNA-polymerase (RdRp) gene of both kobuviruses and hunniviruses, and previous studies have reported frequent detection of kobuvirus in stools from diarrheic cats using this primer pair (Chung et al., 2013; Di Martino et al., 2015a).

Among the archived diarrheic fecal samples, PCR products of the expected size were generated using UNIV-kobu-F/UNIV-kobu-R with six samples from stray cats and four samples from pet cats (Supplementary Table 1). After sequencing and blast searches, the species Hunnivirus A was found to be a match for three PCR-positive samples, showing 86.9%–95.3% nucleotide homology with previously identified hunniviruses. This novel hunnivirus in the present study was provisionally designated feline hunnivirus (FeHuV). The three FeHuV RNA-positive samples included two from stray cats and one from a pet cat in Guangzhou; all other samples producing the expected fragment size by RT-PCR using UNIV-kobu-F/UNIV-kobu-R were positive for feline kobuvirus. In addition, feline parvovirus was detected in one FeHuV RNA-positive sample and feline enteric coronavirus in another FeHuV RNA-positive sample. Based on sequencing results, the feline kobuvirus, feline parvovirus, and feline enteric coronavirus in this study have high nucleotide homology, >98%, with previously reported strains. No FeHuV or feline kobuvirus was detected in samples from healthy cats.

3.2. Genome characteristic of the novel feline hunnivirus

After PCR and sequencing, the near-complete genome of one field strain, FeHuV-1/GZ/2017, was assembled, including a partial 5′UTR of 337 nucleotides, a single polyprotein gene of 6825 nucleotides, and a protease-cleavage sites of the hunnivirus.

The genome of FeHuV-1/GZ/2017 has the same genome organization as determined for other hunniviruses: 5′UTR-L-P1(VP4-VP2-VP3-VP1)-P2(2A-2B-2C)-P3(3A-3B-3C-3D)-3′UTR. The putative viral proteins are of 83, 81, 240, 223, 234, 21, 243, 324, 112, 27, 208, and 456 amino acids in length. As reported for bovine and ovine hunniviruses, the putative translation initiation site of FeHuV-1/GZ/2017 contains a nearly optimal Kozak consensus sequence (A/GNNAUGG) (Reuter et al., 2013; Chung et al., 2013; Chung et al., 2014; Melegari et al., 2018; Oem et al., 2014; Reuter et al., 2010). However, these primers are more generic for picornavirus screening kobuviruses (Reuter et al., 2009). Using this primer pair, new picornavirus genera have the closest relationship with those of the Teschovirus genus.

3.4. Virus isolation

CPE was not observed after five passages. In addition, FeHuV RNA was not detected in the culture lysate or supernatant.

4. Discussion

Based on the 3D conserved viral RdRp regions of the Aichi virus, bovine kobuvirus, and porcine kobuvirus, the primer pair UNIV-kobu-F/UNIV-kobu-R was initially designed by Gábor Reuter et al. for screening kobuviruses (Reuter et al., 2009). Using this primer pair, new numbers classified in the genus Kobuvirus have been determined in various animals, including sheep, goat, dog, deer, wolf, fox, and cat (Chung et al., 2013; Di Martino et al., 2013; Di Martino et al., 2015b; Di Martino et al., 2014; Melegari et al., 2018; Oem et al., 2014; Reuter et al., 2010). However, these primers are more generic for picornavirus genera.
than for kobuvirus (Reuter et al., 2012). One novel quail picornavirus and two novel hunniviruses, bovine hunnivirus and ovine hunnivirus, were identified in 2012 using UNIV-kobu-F/UNIV-kobu-R (Pankovics et al., 2012; Reuter et al., 2012). Our study further confirmed the universal applicability of UNIV-kobu-F/UNIV-kobu-R in determining novel picornaviruses. The family Picornaviridae contains clinically important human and animal pathogens and is associated with a series of diseases in the central nervous system, respiratory tract, heart, liver, pancreas, skin and eye (Zell, 2017). However, the transmission routes and pathogenicity of hunniviruses have not been investigated. Both bovine and ovine hunniviruses have been detected in apparently healthy animals. In contrast, FeHuV in this study was detected in diarrheic animals. Further analyses are needed to isolate FeHuV and to clarify its transmission route and relationship with diarrhea in an animal model as well as to assess its zoonotic potential.

In summary, a novel hunnivirus was identified in cats, expanding the host range of hunniviruses. Genomic sequencing and analysis indicated that this hunnivirus is a new genotype. Continued and more large-scale surveillance of this hunnivirus in cat populations is necessary to assess the pathogenicity and risk of zoonotic transmission of this novel pathogen.

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Author contributions

GL and SJL conceived and designed the experiments; GL, MH, XJC, YKS, JH and RJH performed the experiments; GL, MH, XJC, YKS, JH and RJH analyzed the data; JH and RJH contributed reagents/materials/analysis tools; GL drafted the manuscript; SJL revised the manuscript; SJL supervised and approved the message for publication.

Conflict of interest

All authors declare that they have no competing interests.

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