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Research paper

A novel feline norovirus in diarrheic cats

Barbara Di Martino [a,*], Federica Di Profio [a], Irene Melegari [a], Vittorio Sarchese [a], Maria Assunta Cafiero [b], Serena Robetto [c], Giovanni Aste [a], Gianvito Lanave [d], Fulvio Marsilio [a], Vito Martella [d]

[a] Faculty of Veterinary Medicine, Università degli Studi di Teramo, Italy
[b] Experimental Zooprophylactic Institute of Puglia and Basilicata, Foggia, Italy
[c] Experimental Zooprophylactic Institute of Piemonte, Liguria e Valle d'Aosta, Centro di Referenza Nazionale per le Malattie degli Animali Selvatici (CeRMAS), Italy
[d] Faculty of Veterinary Medicine, Università Aldo Moro di Bari, Valenzano, Italy

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ABSTRACT

By screening a collection of fecal samples from young cats housed in three different shelters in South Italy, noroviruses (NoVs) were found in 3/48 (6.2%) specimens of animals with enteritis signs while they were not detected in samples collected from healthy cats (0/57). Upon sequence analysis of the short RNA-dependent RNA polymerase (RdRp) region, the three strains displayed the highest nucleotide (nt) and amino acid (aa) identities to the prototype GIV.2 strain lion/Pistoia/387/06/ITA (91.0–93.0% nt and 97.0–98.0% aa). The sequence of ~3.4-kb portion at the 3′ end of the genome of a NoV strain, TE/77-13/ITA, was determined. In the full-length ORF2, encoding the VP1 capsid protein, the virus was genetically closest to the canine GVI.2 NoV strains C33/Viseu/2007/PRT and FD53/2007/ITA (81.0–84.0% nt and 93.0–94.0% aa identities), suggesting a recombination nature, with the cross-over site being mapped to the ORF1-ORF2 junction. Based on the full-length VP1 amino acid sequence, we classified the novel feline NoV, together with the canine strains Viseu and FD53, as a genotype 2, within the genogroup GVI. These findings indicate that, as observed for GIV NoV, GVI strains may infect both the canine and feline host. Unrestricted circulation of NoV strains in small carnivores may provide the basis for quick genetic diversification of these viruses by recombination. Interspecies circulation of NoVs in pets must also be considered when facing outbreaks of enteric diseases in these animals.

1. Introduction

Noroviruses (NoVs), Caliciviridae family, have been identified as the most common cause of viral gastroenteritis in humans. NoV infections affect persons of all age groups and are predominantly transmitted through the fecal–oral route, either indirectly through contaminated food, water or surfaces or directly from person to person (Patel et al., 2008).

Drop virions are nonenveloped and approximately 30 to 35 nm in diameter. The RNA genome is organized into three open reading frames (ORFs) (Green, 2007). ORF1 encodes a polyprotein that is cleaved by the virus-encoded protease to produce several nonstructural proteins, including the RNA dependent RNA polymerase (RdRp), ORF2 encodes a major capsid protein (VP1) and ORF3 encodes a small basic protein (VP2) that has been associated with the capsid stability (Bertolotti-Ciarlet et al., 2003).

Based on the full-length VP1 amino acid sequence, NoVs have been divided into six genogroups (GI to GVI) and several genotypes (Zheng et al., 2006; Martella et al., 2009; Green, 2013). Only GI, GII, and GIV NoVs infect humans, with GII strains being the most prevalent worldwide (Green, 2007). NoVs genetically similar to human NoVs have been recently found in dogs and cats (Martella et al., 2007, 2008; Summa et al., 2012; Pinto et al., 2012; Soma et al., 2014), raising public health concerns of potential cross-species transmission due to the strict social interaction between humans and pets.

Feline NoVs were first detected in the stools of 8–12-week-old kittens from a feline shelter with an outbreak of diarrhea in New York State (Pinto et al., 2012). In the VP1 encoding gene, the feline NoVs displayed the highest amino acid (aa) identity (97.9%) to the prototype NoV strain GIV.2/Pistoia/387/06/ITA, detected in a captive lion cub with severe hemorrhagic enteritis (Martella et al., 2007) and to the canine strain GIV.2/Bari-170/07/IT (90.4% aa), detected in a young dog with diarrhea (Martella et al., 2008). Using baculovirus-expressed VP1 of the lion NoV strain GIV.2/Pistoia/387/06/ITA, antibodies specific for GIV NoVs have been identified in 16.1% of cats in Italy (Di Martino et al., 2010), providing indirect evidence for the circulation of these NoVs in felines.

In addition, the RNA of GIV.2 NoVs has been detected in 1.2% of fecal samples of cats with enteritis in Japan (Soma et al., 2014). Upon genome sequencing, the feline NoV strain cat/GVI.1/JPN/2012/M49 (Takano et al., 2015) was found to be more similar (87.0% aa identity) in the full-length ORF2 to the canine NoV GVI.1/Bari/91/07/IT (Martella et al., 2009).

* Corresponding author.
E-mail address: bdimartino@unite.it (B. Di Martino).

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Altogether these findings indicate that diverse NoV strains may infect cats, as observed in dogs, and that the feline and canine host may be infected by the same NoV strains, thus constituting an enlarged host reservoir for these animal NoVs. In order to draw a more complete picture of NoVs molecular epidemiology in cats, in this study a collection of fecal specimens from diarrheic and healthy animals was screened using either broadly-reactive primers for caliciviruses and primers specific for NoVs.

2. Materials and methods

2.1. Sampling

A total of 105 stool samples from domestic cats aged 2–12 months were collected from April to July 2013 in three different shelters located in South Italy. The fecal panel consisted of 48 samples from cats with signs of mild to severe gastroenteritis and 57 samples from asymptomatic animals. All the samples were stored at −80 °C until use.

2.2. RNA and DNA extraction

Fecal specimens (10%) were re-suspended in phosphate-buffered saline pH 7.2, and the debris were removed by centrifugation at 8000 ×*g* for 5 min. DNA and RNA extracts were prepared using the DNAeasy® and QIAamp® viral RNA kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions and stored at −80 °C until use.

2.3. Screening by RT-PCR and PCR

To assess the presence of NoV RNA, the samples were screened using a broadly reactive primer pair, p289–p290, targeted to highly conserved motifs DYSKWDST and YGDD of the RNA-dependent RNA polymerase (RdRp) region of the polymerase complex (Jiang et al., 1999). In the samples yielding amplicons of the expected sizes, the presence of NoV was confirmed using norovirus-specific primer pair JV12Y–JV13I (Vennema et al., 2002). All the fecal samples were also tested by PCR or RT-PCR for feline parvovirus (FPV) (Buonavoglia et al., 2001), feline enteric coronavirus (FECV) (Gunn-Moore et al., 1998) and feline kobuviruses (FeKoV) (Di Martino et al., 2015).

2.4. Sequence and phylogenetic analysis of the NoV strains

The amplicons were excised from the gel and purified using a QIAquick gel extraction kit (Qiagen GmbH, Hilden, Germany). The fragment was then subjected to direct sequencing using BigDye Terminator Cycle chemistry and 3730 DNA Analyzer (Applied Biosystems, Foster, CA). Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov) and FASTA (http://www.ebi.ac.uk/fasta33) with default values were used to find homologous hits. The sequence of ~3.4-kb fragment at the 3′ end of the genome of one such strain, TE/77-13/ITA, including the partial RdRp gene and the complete ORF2 and ORF3 genes, was determined by 3′ RACE protocol, as previously described (Scotto-Lavino et al., 2006). cDNA was synthesized by SuperScript III First-Strand cDNA synthesis kit (Invitrogen Ltd., Milan, Italy) with primer QT. PCR was then performed with TaKaRa La Taq polymerase (Takara Bio Europe S.A.S. Saint-Germain-en-Laye, France) with forward primer p290 and reverse primers QO and QI. Finally, the amplicons were purified and cloned by using TOPO® XL Cloning Kit (Invitrogen Ltd., Milan, Italy). Additional primers were designed to determine the complete 3.4 kb sequences by an overlapping strategy (Table 1). Sequence editing and multiple alignments were performed with the BioEdit software package, version 2.1 (Hall, 1999). Phylogenetic trees were generated using Bayesian analysis with MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The appropriate substitution model settings were derived using jModelTest (Posada, 2009). The sequence obtained was analyzed with Simplot (Lole et al., 1999) using a window size of 200 and step size of 20, with gap strip off and Hamming correction on. Additionally, recombination analysis was carried out with different algorithms implemented in the Recombination Detection Program v.4.43 (RDP4) (Martin et al., 2010), with default settings.

3. Results

Out of 105 samples, 3 (2.8%) contained NoVs RNA, either alone (0.9%, 1/105) or in mixed infections with FeKoV or FECV (1.9%, 2/105). Sixteen samples (15.2%) were found to contain FPV DNA alone. All the NoV positive samples were identified from diarrheic cats with a prevalence rate of 6.2% (3/48), while they were not detected from asymptomatic animals (0/57). By sequence comparison in the short RdRp fragment, the viruses TE/68-13, ME/78-13 and TE/77-13/ITA shared 89.9–93.2% nt and 90.8–95.3% aa identities to each other and displayed the highest

Table 1
Oligonucleotides used for cDNA synthesis and amplification in this study. Nucleotide position refers to the sequence of the feline NoV cat/TE/77-13/ITA (GenBank accession no. KT245136).

Oligonucleotide	Position	Sequence (5′ to 3′)	Sense	Reference
p290	2–24	GATTACTCCAAGTGGGACTCCAC	+	Jiang et al. (1999)
p289	299–317	TGACAATGTAATCATCACCATA	−	Jiang et al. (1999)
JV12Y	40–60	ATACCACCTATGATGCAGAYTA	+	Vennema et al. (2002)
JV13I	292–312	TCATCATCACCATAGAAGAG	−	Vennema et al. (2002)
FeNoV-201	201–221	TCAACAGCATCGCCCACTGGA	+	This study
FeNoV-550	550–572	GCATCATCGTCTCGTTGGGGTCC	−	This study
FeNoV-852	852–870	CCAGAGAGTCAACAAGAGG	+	This study
FeNoV-1026	1026–1043	AGGGCCAAGCTCGAGATC	−	This study
FeNoV-1084	1084–1114	TGGAGGGATGGAAGTGCAGAT	+	This study
FeNoV-1581	1581–1601	ATCCAGGGTGCACCTGCCATT	−	This study
FeNoV-1581	1581–1601	AATGGCAGGTGCACCCTGGAT	+	This study
FeNoV-2094	2094–2113	TACAACGGGGCCATAGGGGA	+	This study
FeNoV-2094	2094–2113	TCCCCTATGGCCCCGTTGTA	−	This study
FeNoV-2452	2452–2472	CAGTTCCCACAGGGCTGAGTG	−	This study
FeNoV-2729	2729–2749	GAAGCCGCCCTTGCGCAACGC	−	This study
FeNoV-2729	2729–2749	GCGTTGCGCAAGGGCGGCTTC	+	This study
FeNoV-2851	2851–2872	GGTGGCCATGCCAGAGTACCCT	−	This study
FeNoV-3198	3281–3297	TTGGACTCACCTCTGCG	−	This study
Q_T	3′/5′ end	*CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTTTTTTTTTTTTTTT*	+/−	Scotto-Lavino et al. (2006)
Qo	3′/5′ end	CCAGTGAGCAGAGTGACG	+/−	Scotto-Lavino et al. (2006)
QI	3′ end	GAGGACTCGAGCTCAAGC	−	Scotto-Lavino et al. (2006)