

Feline Coronavirus Participation in Diarrhea of Cats

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ABSTRACT. Fecal samples were examined for viruses participated in gastrointestinal disorders of cats, especially focusing on feline coronavirus (FCoV) by a reverse transcriptase-polymerase chain reaction assay. It was found that a primary viral pathogen was feline panleukopenia parvovirus (FPLV; 28.5% of the positive rate) and the secondary was FCoV (10.7%). Commonly reported clinical signs of cats of which feces were FCoV-positive were vomiting, diarrhea and dehydration with an exception of one serious case with concurrent FPLV infection.—KEY WORDS: coronavirus, diarrhea, feline.

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In Japan, viruses detected in feline diarrhea cases include feline panleukopenia parvovirus (FPLV) as a primary pathogen, feline calicivirus, feline rotavirus, and reovirus [20]. Other viruses, on the other hand, such as feline coronavirus (FCoV), feline astrovirus, or feline leukemia virus, are also known to cause clinical enteric diseases in the literature [5]. Furthermore, types 2a and 2b canine parvoviruses (CPV) have been recently recognized as potential pathogens for cats [21, 31].

According to pathobiological properties, FCoV has been classified into two biotypes as feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV), and it has been postulated that FIPV is a highly pathogenic mutant of ubiquitous FECV [28]. The postulation has been evidenced recently by comparative genomic analysis of both biotypes; the order of descent is from FECV to FIPV, suggesting FIPV arises by mutation from endemic FECV in cats [33]. On the other hand, FCoV has been also classified into serotypes I and II defined by neutralization [28]. Type I virus is a genuine coronavirus of cats and type II virus has emerged by the genomic recombination between type I FCoV and canine coronavirus [10, 11, 26, 32]. Type I virus is a predominant in the field [12, 28], and is generally fastidious *in vitro* [28], thus the virus isolation by cell-culturing method for diagnosis appears to be helpless in most cases. Even by using a cell line *felis cutus* whole fetus (fcwf-4) which is highly susceptible to coronaviruses [14, 29], no FCoV was recovered from 335 cat fecal samples examined during the years from 1989 to 1997 (unpublished data). There are only several reports describing spontaneous FCoV enteritis [2, 16, 19], and to the best of our knowledge the FCoV isolation from diarrheal feces of cats has been so far rarely performed in Japan. In the present study, we applied a reverse transcriptase-polymerase chain reaction (RT-PCR) assay to elucidate a significance of FCoV in diarrhea of cats. The RT-PCR assay has been used more frequently for FCoVs than ever, and the epidemiological features have become more evident [1, 3, 4, 6, 8, 9, 15, 17].

Rectal swab samples were submitted from veterinary hospitals in various parts of Japan, and a total of 56 samples, 29 from diarrheal cases and 27 from other conditions, were examined during the time from April, 1998 to January, 1999.

The swab was placed in 1 ml of Eagle's minimal essential medium as described previously [25]. The swab extract was clarified by centrifugation at 8,500 g for 20 min and the resulting supernatant was inoculated into a cell culture dish which contained either Crandell feline kidney or fcwf-4 cell monolayer. The supernatant was additionally examined for FCoV by the RT-PCR assay [8], for parvovirus by the PCR assay [24], and for rotavirus by the reverse passive HA assay [22]. When cytopathic effect was observed in the cell culture, the cells were stained by Giemsa solution and examined cytopathologically. For an identification of the isolate in the cell culture, the infected cells were examined by an indirect immunofluorescence assay (IFA) by using monoclonal antibodies (MAb) against either FPLV [23] or FCoV [13].

Sixteen isolates, 12 from diarrheal and four from the other conditions, of FPLV type parvovirus and one isolate of FCoV were recovered from the samples, but no other viruses were detected. As shown in Table 1, five swab extracts were positive for FCoV by the RT-PCR assay (8.9%) but no FCoV was recovered from them in the fcwf-4 cell culture. On the other hand, the RT-PCR assay was negative for one swab extract from which FCoV was recovered in the cell culture, though the isolate (C427) was reactive by the RT-PCR assay. This may be explained by a probable reason that some PCR-inhibitor existed in the fecal sample. Thus, a total of six samples (10.7%), five from kittens with gastrointestinal and one from a kitten with respiratory disorders, were positive for FCoV in the present survey. Except for the case C427 which was a mixed infection with FPLV and died, the clinical conditions were generally mild and the cats recovered soon. Primary clinical signs reported were vomiting, diarrhea and dehydration.

Although the RT-PCR assay applied in the present study has been already approved to be specific for FCoV [8], we sequenced an RT-PCR product to make sure that the assay was specific. Sequencing was performed by the method described previously [7]. Approximately 220 bp product was expected to be generated by the amplification [8]. The product obtained from the sample C408 was composed of 223 bp and a restriction enzyme *Dra* I site existed inside of it as described previously [8]. The obtained sequence were

Table 1. Clinical conditions of cats of which feces were FCoV-positive by either RT-PCR or cell-culturing assays

Case No.	Age of cat	Fecal appearances reported	Clinical conditions at presentation	Coronavirus isolation <i>in vitro</i>	Other viral agents detected
C407 ^{a)}	2-month	Diarrheal	vomiting, diarrhea, dehydration	–	none
C408 ^{a)}	2-month	Diarrheal	vomiting, diarrhea, dehydration	–	none
C409 ^{a)}	2-month	Diarrheal	vomiting, diarrhea, dehydration	–	none
C420	3-month	Unknown	sneezing, ocular discharge	–	none
C427 ^{b)}	3-month	Diarrheal	vomiting, dehydration, anorexia, depression	+	FPLV ^{c)}
C432	6-month	Unknown	vomiting	–	FPLV ^{d)}

a) The same litter.

b) The cat died on the second day of hospitalization.

c) Feline panleukopenia virus was isolated in the cell culture.

d) FPLV was positive by the PCR assay but not isolated in the cell culture.

compared with the published strains in the database. The data address of the sequence information of this 3' untranslated region of FCoV used here was [X66718] and 98.3% of homology was obtained, indicating positive results by the assay are considered to be specific.

The isolate C427 was serotyped as type II by the IFA with MAbs against FCoV [13]. Biotype of this isolate was unknown because there is no reliable *in vitro* method at present to determine whether it is FIPV or FECV.

FCoV is spread easily by the fecal-oral route and the recent molecular epidemiological studies showed, as ever been suggested seroepidemiologically [18, 27, 30], that a large proportion of cats especially in multiple-cat environment where FCoV was once introduced are chronically infected and shedding virus [3, 9]. The samples examined here were collected from geographically various regions, but the detection rate greater than 10% is considered significant. In conclusion, the results together with the previous observations [21] indicate that FCoV is a secondary important enteric viral pathogen of cats in Japan.

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