



NOTE

Virology

Prevalence of astrovirus and parvovirus in Japanese domestic cats

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ABSTRACT. Feces obtained from 204 domestic cats with gastrointestinal symptoms were genetically examined for feline astrovirus (FeAstV) and feline parvovirus (FPV), both of which are known feline gastroenteric viruses. FeAstV detection rates were significantly higher in winter (44.4%) than in other seasons, and in cats under a year old (27.8%) than in a year or older ones (12.4%) ($P < 0.05$). In contrast, no significant seasonal and age differences were obtained in FPV detection rates. Upon FeAstV ORF2 sequence analysis, the 23 present isolates were classified into the same clade (*Mamastrovirus 2*) as the 18 reference strains from other countries. Our findings suggest that FeAstV is already circulating in Japan, and it is more prevalent in juvenile cats in winter, unlike FPV.

KEY WORDS: astrovirus, cat, Japan, parvovirus

Astroviruses (AstVs) are small round, non-enveloped viruses with a single-stranded positive-sense RNA genome that were discovered in feces from an infant with acute gastroenteritis in 1975 [12]. AstVs are important gastrointestinal infectious factors, and the risk of gastroenteritis is high in young children, the elderly, and immunocompromised patients [6]. AstVs have been also detected in many animal species besides humans. They are classified into two viral genera, *Mamastrovirus* (MAstV) and *Avastrovirus* (AAstV), detected in mammals and birds, respectively, and 19 (MAstV1-19) and three genotypes (AAstV1-3) have been reported in each species, respectively [3, 7]. AstV particles were detected using electron micrography in feces from a domestic cat in the USA in 1979 [9]. Previous genetic analyses showed that AstV isolated from cats (feline astrovirus; FeAstV) can be classified into MAstV2 and discriminated from AstVs of other mammals [3]. FeAstV has been detected in feces of domestic cats in the USA, UK, New Zealand, Italy, Turkey, Hong Kong, China, South Korea, and Australia [2, 4, 8, 9, 11, 13, 15, 19, 21]. In addition, viruses very closely related to FeAstV have been detected in cheetahs and tigers, suggesting that this virus is also present in related felines [1, 22]. Pathogenicity of FeAstV for cats is still under discussion, but diarrhea has been reproduced in an experimental infection using specific pathogen-free (SPF) cats [8]. The virus was detected at a high rate in cats with diarrhea compared to non-symptomatic cats in several epidemiological surveys [20, 21, 23], showing that FeAstV is an important factor for gastrointestinal infection in cats. In addition, FeAstV frequently co-infects with other viruses causing diarrhea like feline parvovirus (FPV); the clinical importance of these cases is under investigation [2, 15, 23]. No AstV infection of cats has been reported in Japan. To clarify the presence and prevalence of FeAstV in Japan, this study examined FeAstV and FPV to assess their detection rates in the feces of domestic cats submitted to a veterinary laboratory to diagnose FPV infection. Furthermore, the base sequence of a partial ORF2 gene encoding capsid, which is the main AstV component protein, in the FeAstV isolates was compared to that of isolates from other countries.

The fecal samples obtained from 204 household cats, which were submitted to a commercial veterinary laboratory (Marupi Lifetech, Osaka, Japan) to diagnose FPV gastroenteritis from veterinary clinics in Japan between 2014 and 2019, were used to examine FeAstV and FPV genes. The sample was suspended in sterile saline, and nucleic acid was isolated from the supernatant using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Samples were preserved at -45°C before testing. Although this kit is designed for DNA purification, RNA can also be comparatively efficiently extracted and purified according to the attached instructions and a previous study [5]. AstV was examined using RT-PCR with the Mon269-Mon270 primer pair, yielding a 450-bp amplicon of the AstV ORF2 gene [16]. The RNA was examined using a QIAGEN OneStep

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RT-PCR kit (Qiagen). The reaction mixture was prepared following the manufacturer's instructions. The RNA was reverse transcribed at 50°C for 30 min, followed by inactivation of reverse transcriptase and denaturation of cDNA at 95°C for 15 min. The cDNA was amplified in 40 sequential cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, followed by a final extension of 72°C for 7 min. The PCR product was electrophoresed at 100 V for 35 min in a 2% agarose gel. The gel was stained with ethidium bromide and visualized under UV illumination. The PCR product was purified using ExoSAP and subjected to direct sequencing in both directions using the dye-terminator cycle sequencing methodology (Genewiz Japan, Saitama, Japan). Sequence analysis was conducted using the MEGA X software [10]. Nucleotide sequences were aligned using the ClustalW method, and a phylogenetic tree was constructed using the neighbor-joining method with 1,000 bootstrap replicates and pairwise distances. Detection of FPV DNA was performed using PCR with AmpliTaq Gold DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA) and the P2s-P2as primer pair, yielding a 681-bp amplicon of the parvoviral VP2 gene [17]. This primer pair can specifically detect FPV and the original type canine parvovirus (CPV) that cannot infect felines, but not the variant type CPVs that can also infect felines. Gene positivity was analyzed using the chi-square test (StatView, Adept Scientific, Herts, UK), and a *P* value of <0.05 was considered statistically significant.

Of 204 cases, FeAstV and FPV were detected in 43 (21.1%) and 58 cases (28.4%), respectively, whereas both viruses were detected in ten cases (4.9%). The FPV detection rate was 23.3% (10/43) when limited to FeAstV-detected cases. Grouped by season, the FeAstV detection rate was 44.4% in winter (December to February), being the highest. This differed significantly from the detection rates obtained in spring (March to May), summer (June to August), and autumn (September to November), as shown in Table 1. No significant seasonal differences were noted in FPV, however, the detection rate was the lowest in winter (19.4%). Grouped by age, the FeAstV detection rates in cases aged under a year and a year or older were 27.8% (32/115) and 12.4% (11/89), respectively; the differences between the groups were significant ($P=0.0072$, $\chi^2=7.215$). Upon further analyses, FeAstV positive rates in cases aged under 3 and 3–11 months were significantly higher than in cases aged 12–24 months (Table 1). No significant differences were noted in the FPV detection rates between the age groups. Furthermore, the analyses of FeAstV and FPV positive rates were also made by year, season, area, breed, and gender; however, no significant differences were noted with respect to these parameters.

On base sequence analysis, the 23 present isolates were classified into the same clade as the 18 reference AstV strains of the felines obtained from the GenBank database, excluding the D1 strain (Accession No. KM017741). The isolates were relatively dispersed within the group as shown in Fig. 1. The sequence identities among the present isolates were 83.3–100% and those of these isolates with the strains from the felines excluding the D1 strain were 82.8–97.2%. The identities of the present isolates with the D1 strain were 51.0–53.8%, which is extremely low.

Here, AstV was detected at a high rate from Japanese cat feces. Gene sequence analysis of the ORF2 region showed that these isolates have high identity with previously reported AstV strains from the felines, indicating that FeAstV is already circulating among domestic cats in Japan like in other countries. These isolates were dispersed within the MAstV2 clade, with FeAstVs isolated in the USA, Europe, and other Asia countries on the phylogenetic tree. This result suggests that the FeAstVs currently spread in Japan did not uniquely evolved in Japan, and it may move relatively easily between the countries. The identities of the D1 strain isolated from cats of a shelter in the USA [24] with the present isolates were 51.0–53.8%, which is extremely low, and its clade differed compared to MAstV2 on the phylogenetic tree. To clarify the prevalence of AstVs closely related to this strain in Japanese cats, it is advisable to perform a follow-up test.

Since FeAstV has also been detected in cats without gastrointestinal symptoms at a relatively high rate [13, 19], the pathogenicity of this virus in cats certainly remains unclear. However, the symptom was reproduced in an experimental infection of FeAstV [8]. Moreover, a higher FeAstV detection rate has been reported in several epidemiological surveys in cats with gastroenteritis compared to non-symptomatic cases [20, 21, 23]. Unfortunately, non-symptomatic cats could not be examined for FeAstV in this study. So, the investigations grouped by symptoms should be conducted to elucidate the gastrointestinal pathogenicity of FeAstV.

In dogs, coronavirus is known as an aggravating factor

Table 1. Prevalence of feline astrovirus (FeAstV) RNA and feline parvovirus (FPV) DNA in Japanese domestic cats with gastrointestinal symptoms, grouped by year, season, area, age, breed, and gender

		(Number)	FeAstV (%)	FPV (%)
Year	2014	(40)	17.5	27.5
	2015	(57)	26.3	36.8
	2016	(8)	25.0	25.0
	2017	(23)	26.1	30.4
	2018	(21)	19.0	19.0
	2019	(55)	16.4	23.6
Season	Winter	(36)	44.4 ^{c,d,e}	19.4
	Spring	(46)	10.9 ^e	28.3
	Summer	(60)	13.3 ^d	35.0
	Autumun	(62)	22.6 ^e	27.4
Area	Kanto	(83)	20.5	24.1
	Kinki	(86)	20.9	32.6
	Others	(35)	22.9	28.6
Age	<3 months	(63)	28.6 ^f	33.3
	3–11 months	(52)	26.9 ^g	26.9
	12–24 months	(39)	10.3 ^{f,g}	30.8
	>24 months	(50)	14.0	26.0
Breed ^{a)}	Mixed breed	(160)	21.9	28.8
	Purebred	(29)	13.8	20.9
Gender ^{b)}	Male	(95)	21.1	24.2
	Female	(100)	20.0	31.0
Total		(204)	21.1	28.4

a) No data of breed was available in 15 cats. b) No data of gender was available in 9 cats. Significant differences; c) $P=0.0005$, $\chi^2=11.950$, d) $P=0.0006$, $\chi^2=11.618$, e) $P=0.0236$, $\chi^2=5.126$, f) $P=0.0289$, $\chi^2=4.776$, g) $P=0.0482$, $\chi^2=3.901$.

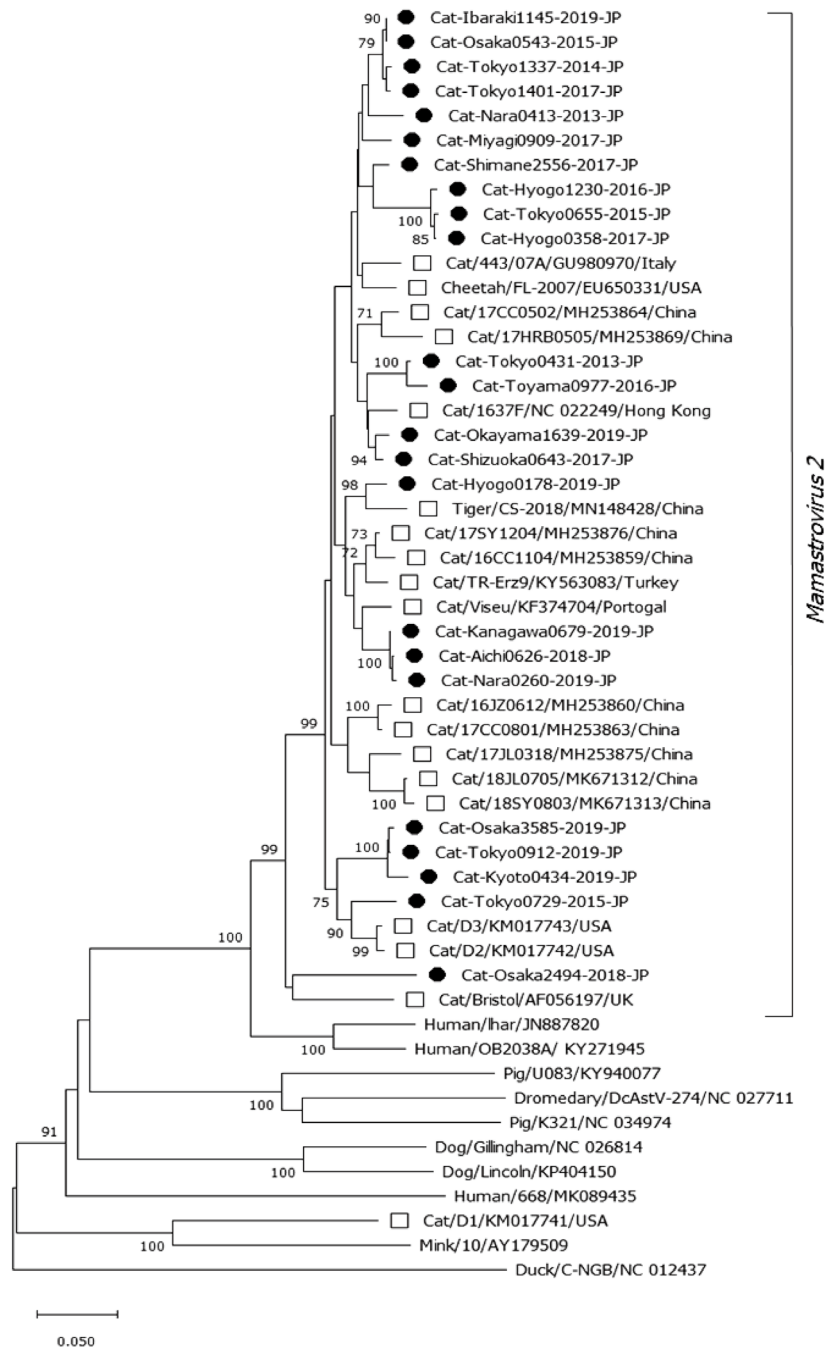


Fig. 1. The phylogenetic tree of the amplicons consisting of a partial astroviral ORF2 gene based on the neighbor-joining method with 1,000 bootstrap replicates and pairwise distances. Bootstrap values >70% are displayed in the tree. Circles and squares stand for 23 present strains and 19 reference strains of the felines obtained from the GenBank database, respectively.

of parvovirus infection [18]. For FeAstV, the importance of mixed infection with FPV has been proposed in several reports [2, 15, 23], and an epidemiological survey performed in China showed that 73.7% (28/38) of FeAstV-infected cats were also infected with FPV [23]. However, the rate was extremely low in this study (23.3%) compared to the previous result. Here, the FeAstV detection rate increased in winter. Similar seasonal variation was also observed in human AstV outbreaks, suggesting that temperature may be involved in virus stability [3]. In contrast, the FPV detection rate in this study was low in winter. This differing seasonality may cause the low rate of mixed infection by both viruses in Japan.

FeAstV infection may lead to the manifestation and aggravation of symptoms when factors like age, maintenance environment, and other gastrointestinal pathogens like feline coronavirus, feline bocavirus, feline kobuvirus, and feline norovirus overlap, but FeAstV infection alone may not cause acute fatal disease. Unfortunately, detailed data like the clinical symptom severity of cats from which fecal samples were collected and their maintenance environment, were unavailable in this study. Further

epidemiological investigations are necessary to clarify the pathogenic roles of FeAstV in cats.

The risk of FeAstV infection is ubiquitous, because it is resistant in the environment for long periods [14]. This virus may seem to be highly prevalent especially in the multi-cat environments since it infects via the fecal-oral route, and transmission via fomites and animal-to-animal spread is likely to be common [3]. The FeAstV vaccine is unavailable for clinical use. Hence, the need for its development should be also debated especially for juvenile cats kept in breeding facilities, pet shops, and animal shelters, since a high FeAstV detection rate was shown in cases aged under a year in this study.

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