Detection of Ascitic Feline Coronavirus RNA from Cats with Clinically Suspected Feline Infectious Peritonitis

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ABSTRACT. Ascitic feline coronavirus (FCoV) RNA was examined in 854 cats with suspected feline infectious peritonitis (FIP) by RT-PCR. The positivity was significantly higher in purebreds (62.2%) than in crossbreds (34.8%) (*P*<0.0001). Among purebreds, the positivities in the Norwegian forest cat (92.3%) and Scottish fold (77.6%) were significantly higher than the average of purebreds (*P*=0.0274 and 0.0251, respectively). The positivity was significantly higher in males (51.5%) than in females (35.7%) (*P*<0.0001), whereas no gender difference has generally been noted in FCoV antibody prevalence, indicating that FIP more frequently develops in males among FCoV-infected cats. Genotyping was performed for 377 gene-positive specimens. Type I (83.3%) was far more predominantly detected than type II (10.6%) (*P*<0.0001), similar to previous serological and genetic surveys.
KEY WORDS: domestic cat, effusive FIP, feline coronavirus, Japan, RNA.

Feline infectious peritonitis (FIP) is an immune-mediated progressive infectious disease of domestic cats and wild felids caused by infection with feline coronavirus (FCoV), a single-stranded RNA virus classified as *Alphacoronavirus* [1, 28]. It is divided into effusive FIP, in which exudate is observed in the body cavity, and non-effusive FIP, in which multiple pyogranuloma lesions are formed, but differences in lesions are influenced by the immunity of individuals [3]. This disease is considered to be induced by a mutant of feline enteric coronavirus (FECV), FIP virus (FIPV) [8, 26]. The incidence is generally about 1% in FCoV-infected cats, but it varies depending on the age, breed, environment and superinfection with other viruses [7, 8, 17, 23]. There are 2 genotypes of FCoV, and FCoV type II is considered to arise by recombination between FCoV type I and canine coronavirus (CCoV) [9, 27]. Based on serological and genetic investigations, FCoV type I is overwhelmingly dominant over FCoV type II, and mixed infection with both types is not rare [6, 11, 14, 15]. Since FIPV and FECV cannot be distinguished serologically or genetically, it is generally difficult to diagnose FIP [1], but it has been stated that the demonstration of FCoV RNA in ascites is one of most reliable diagnostic indicators of FIP [3, 10]. Several results concerning the state of FIP incidence have been reported overseas [6, 15, 16, 18, 29]. In Japan, there have been several reports on the prevalence of FCoV infection [11, 13, 20, 24], but, to our knowledge, no studies on FIP prevalence have been reported. Thus, in this study, to investigate the state of FIP incidence in Japan, we examined FCoV RNA in ascites of domestic cats suspected of having FIP using RT-PCR and investigated the genotype of detected FCoV RNA.

Ascites was obtained from a total of 854 privately owned domestic cats that were clinically suspected of having FIP from veterinary clinics throughout Japan in the period of June 2008 to October 2012. Viral RNA was extracted using a QIAamp Viral RNA Mini Kit (Qiagen, Tokyo, Japan). Detection of FCoV RNA was performed using a QIAGEN OneStep RT-PCR Kit (Qiagen) with the P205-P211 primer pair providing a 223 bp amplicon of the 3'-untranslated region (UTR) gene of FCoV [10], according to the manufacturer's instructions. FIPV strain 79-1146 (American Type Culture Collection, Rockville, MD, U.S.A.) was used as a positive reference virus. Furthermore, for samples in which the 3'-UTR gene was detected, genotyping was performed with the nested RT-PCR reported by Addie *et al.* [4], providing 360 and 218 bp amplicons of FCoV types I and II, respectively. FIPV strain UCD1 (kindly provided by Azabu University) and FIPV strain 79-1146 (same as above) were used as positive reference viruses for the types I and II, respectively. Gene positivity was analyzed by chi-square test (StatView, Adept Scientific, Herts, U.K.), and a *P* value of <0.05 was considered statistically significant.

The 3'-UTR gene was detected in 377 of 854 cats (44.1%). Grouped by breed, the gene positivity was significantly higher in purebreds (62.2%; 181 of 291) than in crossbreds

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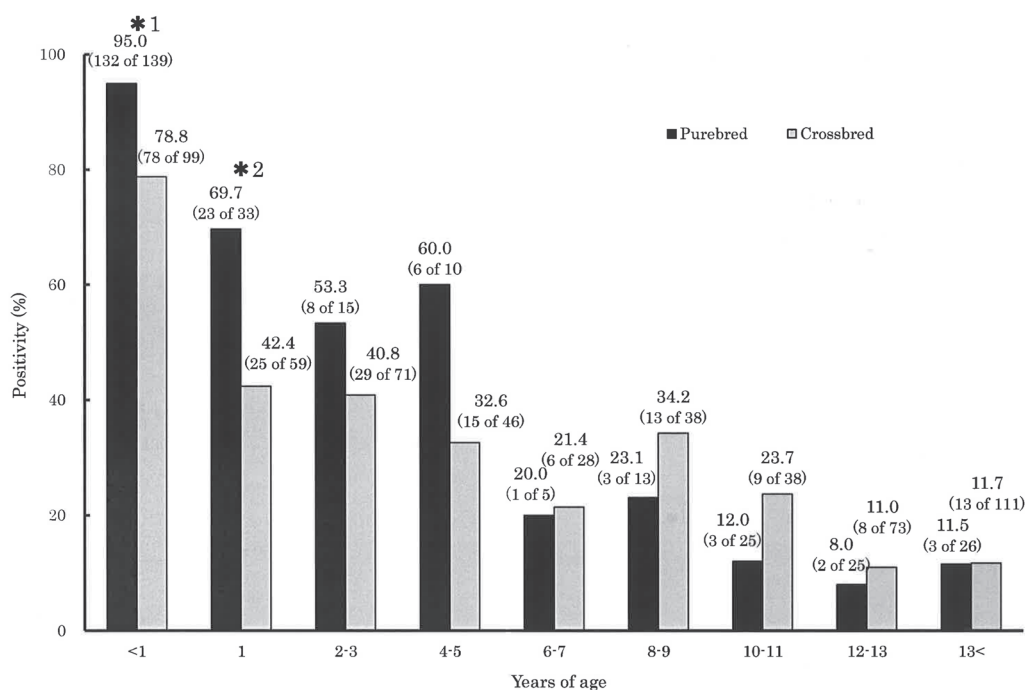
Fig. 1.   Age frequency of the ascitic feline coronavirus (FCoV) 3'-untranslated region (UTR) gene from cats with clinically suspected feline infectious peritonitis (FIP). Black and grey bars indicate the positivity of purebreds and crossbreds, respectively. *Significant differences were observed between the purebreds and crossbreds (chi-square test). *1 Less than a year of age; P=0.0001, χ^2=14.6. *2 A year of age; P=0.0119, χ^2=6.3.

Table 1.   Detection of the ascitic feline coronavirus (FCoV) 3'-untranslated region (UTR) gene from cats with clinically suspected feline infectious peritonitis (FIP) grouped by breed

	Breed	Positivity	P value vs average of purebreds
	Abyssinian	47.6% (10 of 21)	>0.05
	American shorthair	30.0% (24 of 80)	<0.0001 (χ^2=26.3)
	Maine coon	60.0% (9 of 15)	>0.05
	Norwegian forest cat	92.3% (12 of 13)	0.0274 (χ^2=4.9)
Purebred	Persian	76.7% (23 of 30)	>0.05
	Russian blue	69.2% (9 of 13)	>0.05
	Scottish fold	77.6% (45 of 58)	0.0251 (χ^2=5.0)
	Others	80.3% (36 of 45)	
	Subtotal (avarege)	62.2% (181 of 291)	
	Crossbred	34.8% (196 of 563)	<0.0001 (χ^2=58.4)
	Total	44.1% (377 of 854)	

The breeds that consisted of fewer than 10 cats were classified together as others. A significance test (χ^2 test) was performed between each breed and the average of purebreds (62.2%).

(34.8%; 196 of 563) (P<0.0001, χ^2=58.4). Thus, age-related changes in positivity were investigated in purebreds and crossbreds. As shown in Fig. 1, the highest positivity was noted in cats younger than 1 year of age in both breeds and decreased with aging after 1 year of age. The positivity was higher in the purebreds than in the crossbreds until 5 years of age, and a significant difference was noted between the purebreds and crossbreds at 1 year of age and younger (P=0.0001, χ^2=14.6 and P=0.0119, χ^2=6.3, respectively).

Then, the positivity was investigated in various purebreds. As shown in Table 1, the positivities in the Norwegian forest cat (92.3%) and Scottish fold (77.6%) were significantly higher than the average for purebreds (62.2%) (P=0.0274, χ^2=4.9 and P=0.0251, χ^2=5.0, respectively), whereas that in the American shorthair (30.0%) was significantly lower than the average for purebreds (P<0.0001, χ^2=26.3); the positivity in the American shorthair was also lower than that in crossbreds, although the difference was not significant. The

Table 2. Genotyping of feline coronavirus (FCoV) gene from cats with clinically suspected feline infectious peritonitis (FIP) positive for the 3'-untranslated region (UTR) gene

Years of age	Breed	Genotype			
		Type I	Type II	Types I and II	Not detected
Younger than 1	Purebred n=132	99 (75.0%) [b]	31 (23.5%) [c]	8 (6.1%)	10
	Crossbred n=78	74 (94.9%) [b]	3 (3.8%) [c]	1 (1.3%)	2
1 to 4	Purebred n=32	27 (84.4%)	1 (3.2%)	0	4
	Crossbred n=58	52 (89.7%)	2 (3.5%)	1 (1.8%)	5
5 or older	Purebred n=17	15 (88.2%)	1 (5.8%)	1 (5.8%)	2
	Crossbred n=60	47 (78.3%)	2 (3.3%)	2 (3.3%)	13
Total n=377		314 (83.3%) [a]	40 (10.6%) [a]	13 (3.4%)	36

Significant differences were shown (χ^2test). a) $P<0.0001$, χ^2=399.8. b) P=0.0003, χ^2=13.3. c) P=0.0002, χ^2=13.9.

positivity was low in spring-summer and high in fall-winter, although no significant differences were observed (38.8, 40.9, 47.3 and 49.5% in spring, summer, fall and winter, respectively). Grouped by gender, the positivity was significantly higher in males (51.5%; 235 of 456) than in females (35.7%; 142 of 398) ($P<0.0001$, χ^2=21.7). The positivity was investigated in 10 regions of Japan (Hokkaido, Tohoku, Shinetsu, Kanto, Hokuriku, Tokai, Kinki, Chugoku, Shikoku and Kyushu), but no marked regional difference was noted (data not shown).

Furthermore, the genotype was investigated in 377 specimens positive for the 3'-UTR gene. As shown in Table 2, FCoV type I (83.3%) was more predominantly detected than FCoV type II (10.6%) ($P<0.0001$, χ^2=399.8), and both types were detected in 3.4% of specimens. In 36 specimens, no amplicons were observed; therefore, their genotypes could not be identified. There were no significant differences in the positivity of either type between the purebreds and crossbreds older than 1 year of age. But, at younger than 1 year of age, the positivity of FCoV type I was significantly higher in the crossbreds (94.9%) than in the purebreds (75.0%) (P=0.0003, χ^2=13.3), whereas that of FCoV type II was significantly higher in the purebreds (23.5%) than in the crossbreds (3.8%) (P=0.0002, χ^2=13.9). Of 40 cats in which FCoV type II was detected, 31 (77.5%) were purebreds younger than 1 year of age.

Since demonstration of FCoV RNA in ascites of FIP-suspected cats using RT-PCR was a useful indicator to diagnose FIP [3, 10], the results of this study likely represent the epidemiology of effusive FIP in domestic cats in Japan. However, whether or not cats negative for the 3'-UTR gene were infected with FCoV was unknown. This status needs to be investigated further, such as by antibody test and quantitative RT-PCR.

In previous surveys, the incidence of FIP was higher in purebreds than in crossbreds, and it was particularly high in specific breeds [16–19, 29]. Similarly, in this study, the positivity of the 3'-UTR gene was significantly higher in purebreds than in crossbreds, particularly in the Norwegian forest cat and Scottish fold, whereas the positivity was lower in the American shorthair. However, in previous reports from the U.S.A. and Australia, the incidence of FIP was high in the Abyssinian, Bengal, Birman, Himalayan, Ragdoll and

Rex and the Australian mist, British shorthair, Burmese and Rex, respectively, and low in the Burmese, Exotic shorthair, Manx, Persian, Russian blue and Siamese and the Persian, respectively [16, 18]. These breeds are inconsistent with our findings. The breeds that had high and low positivity in this study are consistent with FCoV antibody prevalence in Japan [24]. These findings indicate that countries and regions vary in the incidence of FIP by breed, and the prevalence of FCoV in breeding colonies seems to be closely related to the incidence, rather than bloodlines.

The positivity was highest in cats younger than 1 year of age and markedly decreased with aging. These findings are consistent with the incidence of FIP in previous reports [2, 6, 16, 17, 19, 29]. At younger than 1 year of age, the positivity was significantly higher in purebreds than in crossbreds, indicating high prevalence of FCoV in multi-cat households, such as pet shops and breeding catteries [8]. The positivity was also significantly higher in males than in females, as previously reported [6, 16, 17, 29]. However, no gender difference has generally been noted in the antibody prevalence in previous studies [5, 12, 20, 24], indicating that FIP more frequently develops in males among FCoV-infected cats, although no serological data were shown in this study. This may be due to the influences of the stress of fighting and infectious diseases, such as feline leukemia virus and feline immunodeficiency virus infections, since these viruses more frequently infect males than females [22]. On investigation by season, the positivity tended to increase in fall-winter. A similar tendency has been reported in the U.S.A. [19], suggesting the seasonality of FIP development. This may be related to weather and the estrus cycle.

FCoV type I was far more predominant than FCoV type II, and this is consistent with previous serological and genetic surveys [6, 11, 14, 15]. However, most of the FCoV type II cases (77.5%) were detected in purebreds younger than 1 year of age. Most purebred cats were likely to have been closely maintained with dogs in pet shops. Considering the fact that the CCoV infection rate is very high in dogs maintained in multi-dog households [21, 25], this finding epidemiologically may indicate the possibility that FCoV type II is a recombinant of FCoV type I and CCoV [9, 27], although further detailed analyses are necessary for clarifying this relationship.

The above findings may help to clarify the state of FIP incidence in pet cats in Japan. But, the results were from cats with effusive FIP and are not necessarily applicable to the non-effusive FIP. Moreover, genotype-associated differences in the pathogenicity could not be investigated, because the severity and course of symptoms of the cats from which the test specimens were collected were unclear. It may be necessary to perform epidemiological surveys based on further detailed information and to investigate specimens from cats with non-effusive FIP.

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