

Short Communication

Title: Genetic diversity of feline morbilliviruses isolated in Japan

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Running head: Genetic diversity of FmPV

Abstract

Feline morbillivirus (FmoPV) is an emerging virus in domestic cats and considered to be associated with tubulointerstitial nephritis. Although FmoPV was first described in China in 2012, there has been no report of the isolation of this virus in other countries. In this study, we describe the isolation and characterization of FmoPV from domestic cats in Japan. By using RT-PCR, we found that 3 of 13 urine samples from cats brought to veterinary hospitals were positive for FmoPV. FmoPV strains SS1 to SS3 were isolated from the RT-PCR-positive urine samples. CRFK cells exposed to FmoPV showed cytopathic effects with syncytia formation, and FmoPV N protein was detected by indirect immunofluorescence assays. In addition, pleomorphic virus particles with apparent glycoprotein envelope spikes were observed by electron microscopy. By sequence analysis of FmoPV H and L genes, we found that FmoPVs showed genetic diversity, however signatures of positive selection were not identified.

Keywords

cats, feline morbillivirus, genetic diversity, phylogenetic analysis

Morbilliviruses belong to the family *Paramyxoviridae* and are negative-sense single-stranded RNA viruses that include pathogens of humans and animals, including measles, rinderpest, and canine distemper (CDV) viruses (Lamb & Parks, 2013). Although CDV infections are sporadically observed among large felids (Deem *et al.*, 2000; Meli *et al.*, 2010; Quigley *et al.*, 2010), the presence of morbilliviruses in domestic cats (*Felis catus*) was unknown until Woo *et al.* (2012) reported the isolation of feline morbillivirus (FmoPV) in domestic cats in Hong Kong. Recently, Furuya *et al.* (2013) demonstrated evidence for the presence of FmoPV in Japanese domestic cats by reverse transcription (RT)-polymerase chain reaction (PCR); however, FmoPV has not been isolated in countries other than China (Hong Kong). Genetic analysis revealed that although FmoPV isolates in Hong Kong were members of the morbillivirus lineage, they were substantially different from any reported previously (Woo *et al.*, 2012). Importantly, a histological study suggested that there might be a correlation between FmoPV infection and tubulointerstitial nephritis which is commonly seen in cats (Woo *et al.*, 2012). In this report, we describe the isolation and genetic diversity of FmoPV for the first time to our knowledge from domestic cats in Japan.

Urine samples were randomly collected from cats brought to veterinary clinics in Kyoto and Ibaraki prefectures, Japan. These cats were admitted to the clinics for a variety of reasons. RNA was extracted from the samples using Viral RNA Kits (Qiagen) following the manufacturer's instructions. From the extracted RNA, a part of L-gene of FmoPV was amplified using SuperScript III® (Invitrogen) with random primers according to the manufacturer's instructions, followed by PCR with a set of specific FmoPV primers (5'-GGAACATGGCCTCCTGTAGA-3'/5'-CTCCATTGGCAATCAGGTTT-3') used previously

(Furuya *et al.*, 2013), yielding the final product of 487 bp in size. For sequencing, amplicons were purified from agarose gels with Gel Extraction Kit (Qiagen) and nucleotide sequences were determined by a DNA sequencing service (FASMAC Co., Ltd., Kanagawa, Japan). We found that 3 out of 13 cats were positive for FmoPV genomic RNA and the cDNA showed 90-99% nucleotide identity to the HK isolates (Woo *et al.*, 2012) (data not shown).

Next, we attempted to isolate FmoPV from urine samples. Five hundred microliters of the urine samples were centrifuged at 3,000 rpm for 5 min to remove debris, and filtered through 450nm disc filters (PALL). Then *L*-1-tosylamide-2-phenylethyl chloromethyl ketone-treated trypsin (Sigma-Aldrich) was added to the samples at the final concentration of 0.1 $\mu\text{g ml}^{-1}$ and they were incubated at 37 °C for 15 min. The mixture was inoculated into Crandell-Rees feline kidney (CRFK) cells grown in 25cm² flasks in serum-free Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich) supplemented with penicillin (100 units ml^{-1}) and streptomycin (100 $\mu\text{g ml}^{-1}$) (Invitrogen). After 16 h, inocula were replaced by DMEM supplemented with 2 % heat-inactivated fetal calf serum (FCS) and antibiotics (see above). Cultures were incubated at 37 °C in a humidified atmosphere with 5 % CO₂ and observed daily for cytopathic effect (CPE) by light microscopy. Subsequently, CRFK cells inoculated with three urine samples (cats #138, 140 and 143) that were positive for FmoPV RNA showed syncytia formation (Fig. 1a). Using RT-PCR, the culture supernatants and cell lysates of these three cultures were found to be positive for FmoPV RNA (data not shown).

To detect viral antigens, we conducted indirect immunofluorescence assay (IFA) using a specific antibody against FmoPV N produced as described previously (Fischer *et al.*, 2003, Krach *et al.*, 2000).

Briefly, rabbits were immunized with a mixture of three synthetic peptides (VTAPDTAAEAENRR, DNINDRGEDQSNSI and SGVTNHYNDTDLK) conjugated with keyhole limpet hemocyanin. CRFK cells showing CPE were fixed in 4% paraformaldehyde at room temperature for 15 min. The fixed cells were incubated with 1:200 diluted anti-FmoPV N rabbit antibody, followed by 1:1,000 diluted anti-rabbit IgG conjugated with Alexa Fluor 488 (Life Technologies). Cells were embedded in VECTASHIELD Mounting Medium (Vector Laboratories Inc.) and subjected to confocal fluorescence microscopy (Digital Eclipse Spectral Imaging Confocal Laser Microscope C1si, Nikon Inc.). Uninfected cells were used as a negative control. We observed specific green granular and diffuse cytoplasmic fluorescence in CRFK cells showing CPE (Fig. 1b). We designated the three FmoPV isolates from cats #138, 140 and 143 as strains SS1, SS2 and SS3, respectively.

The morphology of SS1 was examined by electron microscopy (EM). CRFK cells collected 72 h after viral inoculation were fixed with 1.5 % glutaraldehyde in 0.1M phosphate buffer, postfixed in 1 % osmium tetroxide and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under Hitachi H-7500 transmission electron microscope (Hitachi Corp.). By EM, pleomorphic virus particles that appear to have glycoprotein envelope spikes were observed (Fig. 1c). The virions were highly variable in size, ranging from 100 to 500 nm in diameter. There were also rare aggregates of filamentous virus nucleocapsids in the cytoplasm of the infected cells. These ultrastructural findings are consistent with those reported in other morbilliviruses such as measles virus (Nakai & Imagawa, 1969).

To detect antibodies against FmoPV, plasma samples from the 13 cats we investigated were subjected to immunoblot analysis using FmoPV strain SS1 propagated in CRFK cells as antigens.

Immunoblot analysis was performed as described previously (Nakaya *et al.*, 2013) using 1:1,000 dilutions of cat plasma samples and 1:30,000 dilution of horseradish peroxidase-conjugated goat anti-cat IgG antibody (Bethyl). As a result, one of the three RT-PCR-positive (33 %) and two of the 10 (20 %) RT-PCR-negative samples were positive for anti-FmoPV N IgG antibodies (Fig. 1d). Moreover, patterns of FmoPV proteins recognized by cat plasma were different in the positive cats.

Nucleotide sequences of six genes (N-P/V/C-M-F-H-L) of one strain (SS1) and one (H) gene of two strains (SS2 and SS3) were determined and deposited in the DDBJ and assigned accession numbers AB910309, AB910310, AB910311, respectively. Pairwise comparisons of nucleotide and amino acid sequence identities of FmoPV H and L genes were shown in Table 1. Note that no gaps were observed in the alignments. As a result, nucleotide and amino acid sequence identities of six FmoPV H genes obtained from three Japanese and three Hong Kong strains were 89.8-98.8 % and 93.3-99.0 %, respectively (Table 1a). Interestingly, we found that SS3 (Jpn) and M252A (HK) H genes showed the highest nucleotide and amino acid identities (98.8 % and 99.9 %, respectively) among the six strains (15 pairwise combinations). On the other hand, SS1 H gene is distant from those of the other strains (Table 1a). Similarly, nucleotide and amino acid sequences of SS1 L gene are found to be relatively distant from those of the other three Hong Kong strains (Table 1b). These observations were also supported by phylogenetic analyses of FmoPV H (Fig. 2a) and L genes (data not shown). We also calculated the ratio of non-synonymous to synonymous substitution rates (dN/dS) of each branch of FmoPV H and L maximum likelihood (ML) trees using the codeml program in PAML 4.7 (Yang, 2007). We could not detect any evidence that positive selection may have operated during evolution of FmoPV strains (mean dN/dS = 0.0752 and 0.0392 for H and L gene trees, respectively). In addition, variable

selective pressures along branches are not statistically supported for both FmoPV H and L trees (data not shown).

To infer the phylogeny of FmoPVs to other viruses, we conducted phylogenetic analyses using amino acid sequences of H/G (Fig. 2b) (G proteins of hendraviruses and some of the unclassified paramyxoviruses are equivalent to H proteins of morbilliviruses and FmoPVs) and L genes (data not shown) of FmoPVs, morbilliviruses, henipaviruses, and unclassified paramyxoviruses (Table S1). This procedure was the same as we previously reported (Shimode *et al.*, 2014). In both trees, FmoPV strains formed a distinct clade that is distantly related to the cluster consisting of the other morbilliviruses. In addition, the FmoPV clade is also distant from henipaviruses and other unclassified paramyxoviruses. These results indicate that FmoPVs do not belong to morbilliviruses but are unclassified paramyxoviruses.

In this study, we report the isolation of FmoPV from domestic cats in Japan. Although FmoPV Hong Kong isolates have been shown to be a single species (Woo *et al.*, 2012), our present study revealed that FmoPV is genetically diverse. Phylogenetic analyses using nucleotide sequences of the H and L genes showed that Japanese strain SS1 was relatively distantly related to Hong Kong isolates (Fig. 2a). Low dN/dS values between isolates may indicate that FmoPV had not been subjected to positive selection to escape from the cat immune system, possibly because the infections are limited and result in low levels of virions. These results may reflect the findings that we could not detect any anti-F and -H antibodies in RT-PCR-positive cats (Fig. 1d).

Intriguingly, one of our isolates, strain SS2, had a high similarity to strain M252A from Hong Kong. Similarly, Furuya *et al.* (2013) reported that a partial sequence of FmoPV in a cat living in

Tokyo was quite similar to the strain M252A. These data may suggest that the FmoPV had been transmitted by unidentified vector(s) which traversed between China and Japan. According to phylogenetic analyses, bats and rodents have morbilli-related viruses (Drexler *et al.*, 2012). As several species of bats migrate very long distances (Calisher *et al.*, 2006), it is possible that FmoPV-infected bats flew to Japan from China, or vice versa. Alternatively, there can be additional explanations, *i.e.*, transportation of virus-carrying cats between the two regions and/or unusually high genetic constraints of this virus.

The isolation rate of FmoPV was relatively high in cats with inhabit in Kyoto. Further, antibody positive rates were also high in both RT-PCR positive and negative cats. From our data, we suspect that FmoPV may be prevalent in cats from the Kyoto area, and some cats may be persistently infected with FmoPV irrespective of detectable antibodies; however, the sample size was too small to draw any significant conclusions.

The humoral immune responses in cats against FmoPV were varied in immunoblot analysis (Fig. 1d). In support of this observation, it was reported that persistently CDV-infected dogs also showed different reaction to envelope and core antigens (Krakowka *et al.*, 1975). These data indicate that individual cats elicit antibodies against FmoPV proteins in different ways. However, we cannot exclude the possibility that FmoPV strains have different antigenicity. Furthermore, Appel *et al.* (1974) reported that about 10 % of serum samples originating from cats in the United States had neutralizing antibodies against CDV. We also reported that certain cat populations in Japan and Taiwan had neutralizing anti-CDV antibodies (Ikeda *et al.*, 2001). Because anti-CDV dog sera responded to FmoPV antigens and anti-FmoPV N antibody detected CDV N protein (Fig. 1e),

cross-reactivity between FmoPV and CDV should be considered in interpretation of immunoblot analyses. Further studies are needed to discriminate anti-FmoPV from anti-CDV antibodies in cats.

Previous report by Woo *et al.* (2012) suggested that FmoPV infection is related to tubulointerstitial nephritis. However, in this study, we could not find any clear relationship between FmoPV infection (determined by the presence of FmoPV RNA in urine and/or anti-FmoPV antibodies) and nephritis. Further epidemiological studies with larger number of cats and experimental infection are needed to know the pathogenesis of FmoPV in cats.

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Figure legends

Fig. 1. Characterization of FmoPV in CRFK cells. (a) CPE of CRFK cells with syncytia formation. Cells were stained with Hemacolor (Merck Millipore). Arrowheads indicate the syncytia formation. (b) Indirect immunofluorescent assay of CRFK cells infected with FmoPV strain SS1 using an anti-serum against FmoPV N protein. Specific fluorescence was observed in the cytoplasm (stained green) of the infected CRFK cells. Cell nuclei were stained with DAPI and are shown in blue. (c) EM examination of CRFK cells infected with FmoPV strain SS1. Arrowheads indicate pleomorphic viral particles with envelope spikes. An arrow shows the early stage of viral budding process with spike-like structure on the cell surface. N: nucleus. Bar = 500 nm (d) Immunoblot analysis with cat plasmas samples tested against FmoPV strain SS1 virions. Lane 1: pre-immune rabbit serum (pre); Lane 2: rabbit serum immunized with synthetic peptides of FmoPV N protein (α -N); Lane 3: negative control using distilled water instead of 1st antibody (DW); Lanes 4 to 11; cat plasmas. Results of RT-PCR and virus isolation tests determined by the appearance of CPE in CRFK cells are shown under each lane. (e) Cross-reactivity between FmoPV and canine distemper virus (CDV). Antiserum from rabbit immunized with synthetic peptides of FmoPV N protein (α -N) and anti-CDV dog serum (α -CDV) were reacted with FmoPV strain SS1 propagated in CRFK cells and CDV strain Snyder Hill propagated in Vero cells.

Fig. 2. Maximum likelihood phylogenetic trees. (a) Phylogeny determined from the nucleotide sequence of the H gene of FmoPV isolates. RAxML v7.2.8 (Stamatakis, 2006) was used for the

analysis applying the General Time Reversible (GTR) model of nucleotide substitutions with gamma-distributed rate heterogeneity and an estimated proportion of invariable sites. Percent of 1,000 fast bootstrapping tests are shown at each node. The accession numbers used in the present study are as follows: 761U HK (JQ411014, China), 776U HK (JQ411015, China), M252A HK (JQ411016, China), SS1 Jpn (AB910309, Japan), SS2 Jpn (AB910310, Japan), and SS3 Jpn (AB910311, Japan).

(b) Phylogenetic trees determined from the amino acid sequence of the H or G gene of paramyxoviruses including FmoPV isolates, morbilliviruses, henipaviruses, and unclassified paramyxoviruses. The viral genes were chosen based on the results of tblastx homology search (data not shown) as well as previously related works (Woo *et al.*, 2012 and Marsh *et al.*, 2012). The accession numbers used in the present study were listed in the Table S1. RAxML v7.2.8 (Stamatakis, 2006) was used for the analysis applying FLU amino acid replacement model (Dang *et al.*, 2010) with gamma-distributed rate heterogeneity and an estimated proportion of invariable sites. This amino acid replacement matrix with corrections were selected by the Akaike Information Criterion (AIC) obtained from ProtTest 3 (Darriba *et al.*, 2011). Only values of $\geq 80\%$ of 1,000 fast bootstrapping tests were shown at each node.

Abbreviations: BatPV, bat paramyxovirus; BeiPV, Beilong virus; CDV, canine distemper virus; CedPV, Cedar virus; DmoPV, dolphin morbillivirus; HeV, Hendra virus; JPV, J-virus; MeV, measles virus; NarPV, Nariva virus; NiV, Nipah virus; PPRV, peste-des-petits-ruminants virus; PdiPV, phocine distemper virus; RPV, rinderpest virus; SalPV, Salem virus; TlmPV, Tailam virus; TupPV, Tupaia paramyxovirus. Each gene name used in the analysis was also shown.

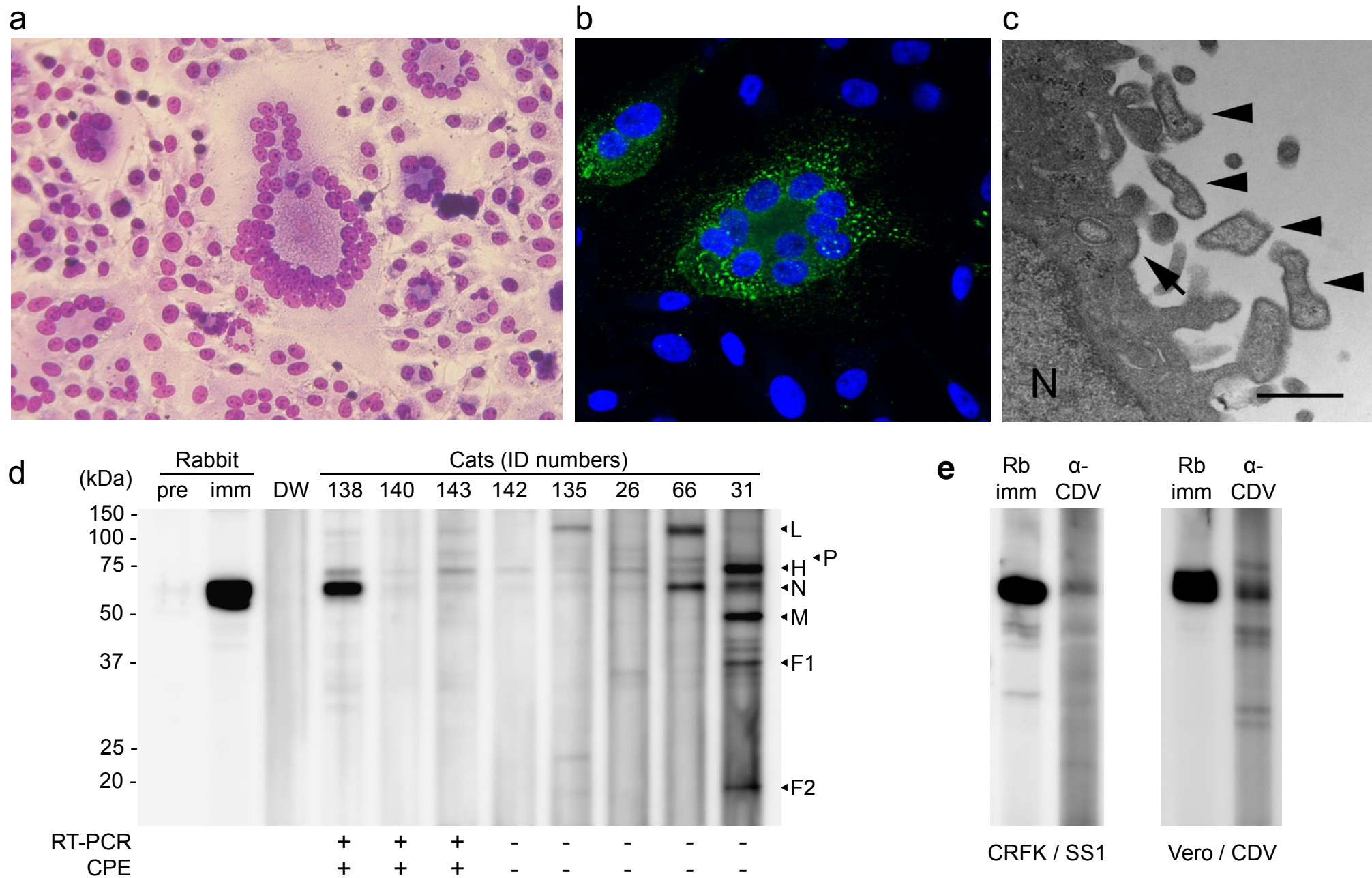


Fig. 1

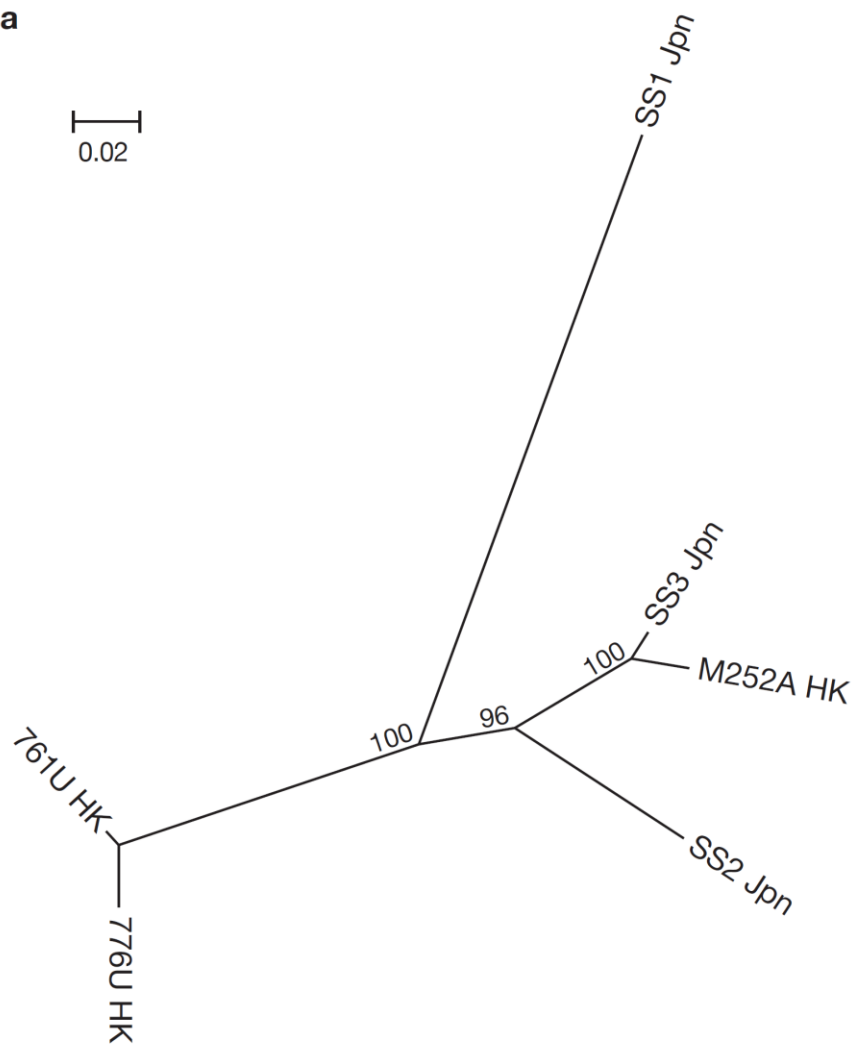
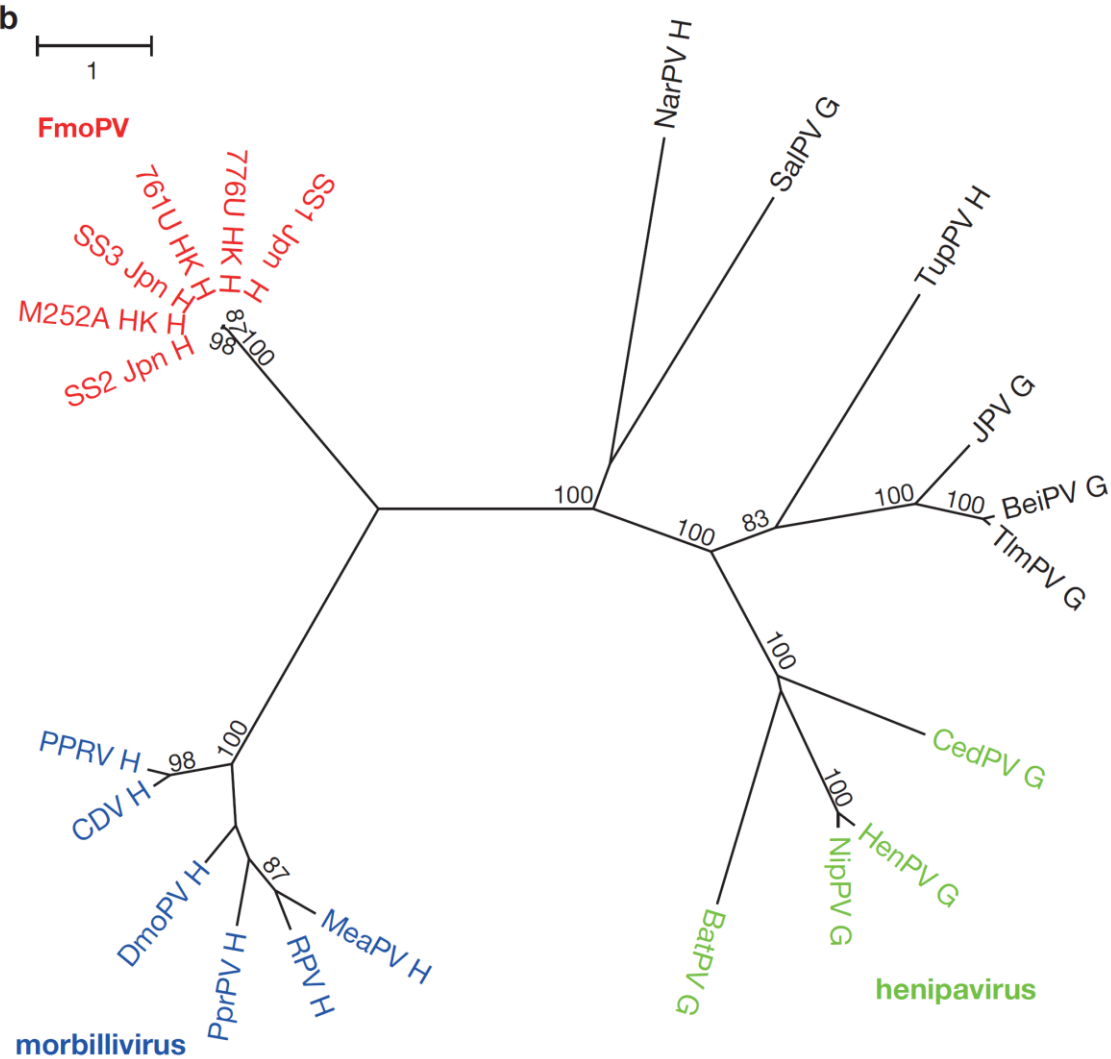
a**b****Fig. 2**

Table.1a Pairwise comparison of nucleotide (bottom left) and amino acid (upper right) identity of FmoPV H genes.

% identity	SS1 Jpn	SS2 Jpn	SS3 Jpn	761U HK	776U HK	M252A HK
SS1 Jpn		93.6	94.1	94.3	93.3	94.1
SS2 Jpn	90.6		96.8	96.0	95.0	96.8
SS3 Jpn	90.7	95.5		96.3	95.3	99.0
761U HK	90.3	92.8	93.3		99.0	96.3
776U HK	89.8	92.6	92.8	98.9		95.3
M252A HK	90.7	95.2	98.8	93.0	92.7	

Table. 1b Pairwise comparison of nucleotide (bottom left) and amino acid (upper right) identity of FmoPV L genes.

% identity	SS1 Jpn	761U HK	776U HK	M252A HK
SS1 Jpn		95.9	96.2	96.4
761U HK	89.7		99.4	97.0
776U HK	89.7	98.5		97.3
M252A HK	90.3	93.3	93.3	

1 Note

2 Virology

3 *In vitro* host range of feline morbillivirus

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15 Running head: HOST RANGE OF FMOPV

16

17 ABSTRACT.

18 Feline morbillivirus (FmoPV) is an emerging virus in cats, which is associated with
19 tubulointerstitial nephritis. To study the *in vitro* host range of FmoPV, we inoculated FmoPV
20 strain SS1 to 32 cell lines originated from 13 species and cultured for two weeks, followed by
21 RNA extraction and reverse-transcription-polymerase chain reaction for FmoPV detection. As a
22 result, only cell lines derived from cats and African green monkeys were susceptible to FmoPV.
23 FmoPV infects diverse feline cell lines: epithelial, fibroblastic, lymphoid and glial cells. These
24 results indicate that the receptor(s) for FmoPV are ubiquitously expressed in cats. No infectivity
25 of FmoPV was observed in human cell lines, which suggests least threatening of cross-species
26 transmission of FmoPV from cats to humans.

27
28 KEY WORDS: cats, cell tropism, cross-species transmission, feline morbillivirus, host range

Feline morbillivirus (FmoPV) is a negative-strand RNA virus in a morbillivirus family. Most morbilliviruses infect dendritic cells through the respiratory route and cause lymphopenia and immune suppression [1, 15, 18]. Subsequently, the virus is disseminated to peripheral tissues, resulting in large-scale infection of epithelial cells. FmoPV was initially reported in 2012 in Hong Kong, and this virus is considered to be associated with renal failure in cats, in particular, tubulointerstitial nephritis [19]. FmoPV RNA was detected in over 10% of stray cats in Hong Kong and mainland China. Furuya *et al.* also reported the existence of FmoPV infection in Japan [5]. By analyzing paraffin-embedded kidney tissues of cats with nephritis, 40% of domestic cats in Japan were positive for FmoPV. This pathological relationship is a big concern for veterinarians and cat owners, because chronic renal disease is commonly seen in the over 7-year-old cats, and tubulointerstitial nephritis is the most common renal failure in cats [4]. Recently, we demonstrated that although FmoPVs display genetic diversity in general, particular isolates from Japan and Hong Kong showed nearly identical nucleotide sequence [12]. This fact may suggest that there are natural vectors and/or reservoirs. Phylogenetic and recombination analyses indicated that the recombination has occurred within the F and H genes between Japan and Hong Kong isolates [11]. Although it was reported that Vero cell originated from African green monkey is susceptible to FmoPV [19], it has never been examined whether FmoPV infects other species. In this investigation, we examined *in vitro* host range of FmoPV to assess the possibility of cross-species infection.

To prepare stock virus of FmoPV, we inoculated FmoPV strain SS1 [12] into Crandell-Rees feline kidney (CRFK) cells (ATCC, CCL-94). Two weeks after inoculation, culture supernatants were harvested, filtered through a 450-nm membrane filter (PALL, Ann Arbor, MI, U.S.A.) and stored at -80°C as stock virus. The virus titer was determined as described before

[7]. To study the *in vitro* host range of FmoPV, infectivity assay was performed using 32 cell lines originated from 13 species, *i.e.*, human embryonic kidney (HEK) 293T cells (ATCC, CRL-11268), TE671 cells (human rhabdomyosarcoma) (ATCC, CCL-8805), HT1080 cells (human fibrosarcoma) (ATCC, CCL-12012), HeLa cells (human epithelioid carcinoma) (ATCC, CCL-2), MT-4 cells (human T cell leukemia) (Public Health England, 08081402), Molt-4 cells (human lymphoblastic leukemia) (ATCC, CRL-1582), Vero cells (African green monkey kidney cells) (ATCC, CCL-81), Cos-7 cells (African green monkey kidney cells) (ATCC, CRL-1651), CRFK cells (feline kidney cells) (ATCC, CCL-94), FEA cells (feline embryonic fibroblast), G355-5 cells (feline astrocyte) (ATCC, CRL-2033), AH927 cells (feline fibroblast), FER cells (feline embryonic fibroblast) (a kind gift from Prof. Jarrett), QN10S cells (feline fibroblast), fcwf-4 cells (feline whole fetus fibroblast) (ATCC, CRL-2787), 3201 cells (feline thymic lymphoma) (ATCC, CRL-10909), MCC cells (feline large granular lymphoma) [2], FL-4 cells (feline T cell) (ATCC, CRL-10772), FL-74 cells (feline lymphoma) (ATCC, CRL-8012), MDCK cells (canine kidney cells) (ATCC, CCL-34), Cf2Th cells (canine thymus fibroblast) (ATCC, CRL-1430), MDTF cells (*Mus dunni* tail fibroblast) (ATCC, CRL-2017), NIH3T3 cells (mouse fibroblast) (ATCC, CRL-1658), HSN cells (rat sarcoma) [3], C6 cells (rat glioma) (ATCC, CCL-107), SIRC cells (rabbit cornea) (ATCC, CCL-60), MPf cells (ferret brain fibroblast) (ATCC, CRL-1656), Mv1Lu cells (mink lung fibroblast) (ATCC, CCL-64), QT6 cells (quail fibrosarcoma) (ATCC, CRL-1708), MDBK cells (bovine kidney cells) (ATCC, CCL-22), FHK-Tcl3.1 cells (horse kidney cells) [8] and PK15 cells (porcine kidney cells) (ATCC, CCL-33) (Table 1). The cells were maintained in T25 flasks, trypsinized at 70 to 90% confluence and seeded T25 flasks with FmoPV strain SS1 at a multiplicity of infection of 1. The cultures were incubated at 37°C in a humidified atmosphere with 5 % CO₂ in air and observed daily for cytopathic effect under a light

microscope. After serial passages for two weeks, we extracted RNA from the cells using QIAamp Viral RNA kit (Qiagen, Valencia, CA, U.S.A.) following the manufacturer's instruction. Extracted RNA was reverse-transcribed using SuperScript III (Invitrogen, Carlsbad, CA, U.S.A.) with random primers, followed by PCR amplification targeting a 487 base pair (bp) fragment of the L gene by using specific primers (5'-GGAACATGGCCTCCTGTAGA-3' and 5'-CTCCATTGGCAATCAGGTTT-3') [5] (Fig. 1A). The PCR mixture (10 μ l) contained cDNA, PCR buffer (10 nmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 3 mmol/l MgCl₂ and 0.01% gelatine), 200 μ M of each dNTP and 1.0 units of ExTaq polymerase (TaKaRa, Otsu, Japan). PCR condition amplifying the DNA was as follows: 35 cycles of 94 °C for 30 sec, 48 °C for 30 sec and 72 °C for 30 sec.

As a result, by RT-PCR amplifying the 487-bp fragment in the L gene of FmoPV, we revealed that only cell lines derived from cat and African green monkey were susceptible to FmoPV (Table 1). The difference of intensity of PCR bands is presumably due to replication efficacy in each cell line. Representative RT-PCR results are shown in Fig. 1B. Intensity of the PCR bands of FmoPV-infected Vero and COS-7 cells became stronger after three weeks post inoculation (Fig. 1 for Vero cells; data not shown for COS-7 cells), however, FmoPV was not detected from FmoPV-inoculated human cell lines and negative controls. CPE of FmoPV-infected Vero cells were also observed (data not shown). To further examine the possibility of viral adaptation in African green monkey cells to become infectious to human cells, we collected supernatants from FmoPV-infected Vero and Cos-7 cells and then inoculated them into human cell lines listed in Table 1. Again, we could not obtain any indication of FmoPV infection in human cell lines by RT-PCR.

Measles virus and canine distemper virus (CDV) use two cellular receptors: CD150 expressed on subsets of immune cells [17] and nectin-4 expressed in epithelial cells [9, 10]. Measles virus infects immune cells expressing CD150, and the infected immune cells travel to the draining lymph nodes. After amplifying the virus, cell-associated viremia spread the infection to other organs including kidney, gastrointestinal tract and liver. Late in pathogenesis, the virus infects airway epithelial cells expressing nectin-4 via the basolateral surface, followed by replication and shed apically from the infected cell into the airway lumen [10]. In this study, we found that FmoPV infected variety of cell types including epithelial, fibroblastic, lymphoid and glial cells. This indicates the FmoPV receptor(s) are expressed in a variety of cat tissues. The association between FmoPV and interstitial nephritis was reported, but relationships with other diseases are still unclear. CDV is known to cause lethal systemic diseases including central nervous system (CNS) disease in large felids [16]. FmoPV may have a potential to cause other diseases, such as CNS disease, since G355-5 cells originated from feline astrocytes are susceptible to FmoPV (Table 1).

The different morbilliviruses most likely evolved from a common ancestral virus that has adapted to their respective mammalian hosts, indicating that morbilliviruses have an intrinsic capacity to adapt to new host species [6]. Having cats might be a big zoonotic concern, because they are one of the most popular companion animals, and human may expose to FmoPV through physical contacts with cats. Our results indicate that FmoPV possibly infects African green monkeys but not humans. There was no adaptation to human cell lines through replication in African green monkey cell lines. Therefore, here, we provisionally conclude that there are least threatening of cross-species transmission of FmoPV from cats to humans. However, CDV is known to cause lethal diseases not only in carnivores but also in other species. CDV infects

human cells only by one amino acid substitution [13, 14]. Thus, we cannot exclude the possibility that FmoPV would become a threat for other animals in the future. Although a current study showed that cell lines except for ones from cats and African green monkeys are insensitive to FmoPV *in vitro*, further investigation and monitoring might be needed.

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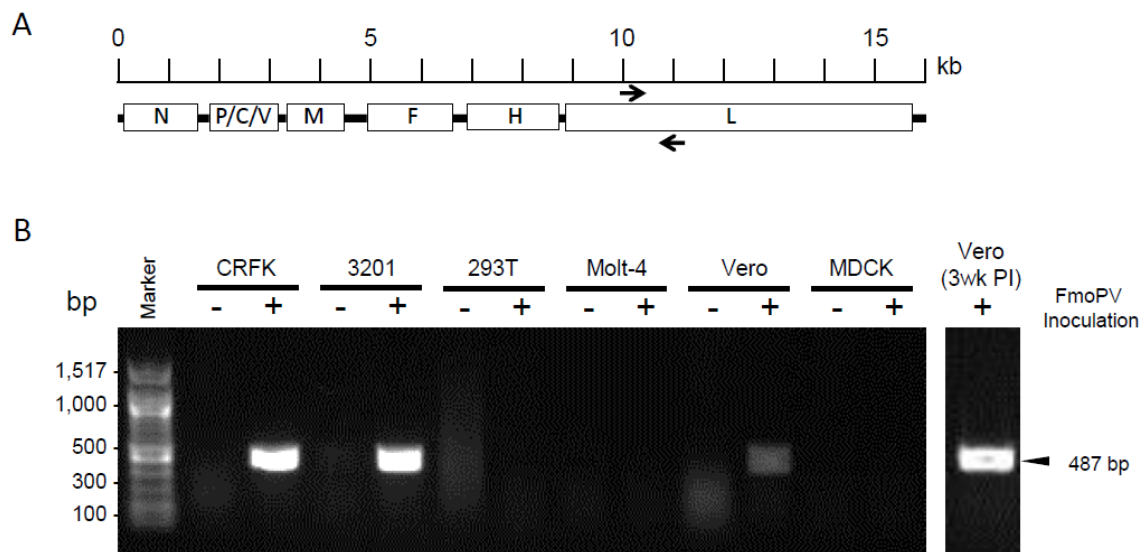
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197 Fig. 1. The PCR amplification targeting a 487 bp fragment of the L gene (A) and gel
 198 electrophoresis analysis of representative samples (B). Only cell lines originated from African
 199 green monkeys and cats were positive for FmoPV. Three weeks after inoculation, the band of
 200 FmoPV-infected Vero cells became stronger. Both kidney and lymphocyte cell lines derived
 201 humans were insensitive to FmoPV.

Table 1. Infectivity of feline morbillivirus in cell lines of 13 animals.

Cell line	Host	Origin	RT-PCR (Infectivity)*
293T	Human	Embryonic kidney	-
TE671	Human	Rhabdomyosarcoma	-
HT1080	Human	Fibrosarcoma	-
Hela	Human	Adenocarcinoma	-
MT-4	Human	T cell leukaemia	-
Molt-4	Human	lymphoblastic leukemia	-
Vero	African green monkey	Kidney	+
Cos-7	African green monkey	Kidney	+
CRFK	Cat	Kidney	++
FEA	Cat	Embryonic fibroblast	++
G355-5	Cat	Astrocyte	++
AH927	Cat	Fibroblast	++
FER	Cat	Embryonic fibroblast	++
QN10s	Cat	Fibroblast	++
fcwf-4	Cat	Fetus	++
3201	Cat	Thymic lymphoma	++
MCC	Cat	Large granular lymphoma	++
FL-4	Cat	T cell	++
FL-74	Cat	lymphoma	++
MDCK	Dog	Kidney	-
Cf2Th	Dog	Thymus	-
MDTF	Mouse	Fibroblast	-
NIH3T3	Mouse	Fibroblast	-
HSN	Rat	Sarcoma	-
C6	Rat	Glioma	-
SIRC	Rabbit	Cornea	-
MPf	Ferret	Brain	-
Mv1Lu	Mink	Lung	-
QT6	Quail	Fibrosarcoma	-
MDBK	Cattle	Kidney	-
FHK-Tcl 3.1	Horse	Kidney	-
PK15	Pig	Kidney	-

* + and ++ indicate weak and strong bands, respectively.