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A real-time RT-PCR assay for molecular identification and quantitation of feline morbillivirus RNA from biological specimens

Eliana De Luca[a,b], Paolo Emidio Crisi[a], Marco Di Domenico[b], Daniela Malatesta[b], Giacomo Vincifori[b], Morena Di Tommaso[a], Giovanni Di Guardo[a], Gabriella Di Francesco[b], Antonio Petrini[b], Giovanni Savini[b], Andrea Boari[a], Alessio Lorusso[b,*]

[a] Faculty of Veterinary Medicine, University of Teramo, Italy
[b] Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise (IZSAM), Teramo, Italy

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ABSTRACT

The aim of this study was to develop a real-time RT-PCR to detect and quantitate feline morbillivirus (FeMV) RNA in biological samples. Primers and probe were targeted on a conserved region of FeMV P/V/C gene. To validate the assay with field samples, a total number of specimens of cats have been recruited including 264 urine and blood samples and compared with a generic RT-PCR targeting the L protein encoding gene of morbilliviruses. In addition, 385 tissue samples from 35 carcasses of cats have been also employed. RNA titres were low in all tested samples. Results also indicated the absence of cross-reaction with related morbilliviruses and existing pathogens of cats. In tissues with low levels of FeMV RNA, the presence of viral antigen was also evidenced by immunohistochemistry targeting the N viral protein. This newly described assay allows for a rapid, accurate and reliable quantitative detection of FeMV RNA that can be applied for diagnostics and research studies.

1. Introduction

According to the International Committee on Taxonomy of Viruses (ICTV, https://talk.ictvonline.org), the family *Paramyxoviridae* are divided into seven genera, including *Respirovirus, Rubulavirus, Morbillivirus, Henipavirus, Avulavirus, Aquaparamyxovirus* and *Ferlavirus*. The genus *Morbillivirus* includes several enveloped, negative-sense single-stranded RNA viruses infecting humans and animals with severe and sometimes fatal systemic diseases (de Vries et al., 2015). Some of them are also associated with zoonotic potential (Bieringer et al., 2013; Qiu et al., 2011; Sakai et al., 2013), or with significant economic impact for livestock (Kumar et al., 2014). Morbilliviruses have been very recently discovered also in domestic cats. The new viral species so far denominated *feline morbillivirus* (FeMV) was first reported in Hong Kong in 2012 (Woo et al., 2012) and associated with tubulo-interstitial nephritis (TIN) and idiopathic chronic kidney disease (CKD) (McLeland et al., 2015). To investigate the presence of FeMV infection in domestic cats several surveys were conducted. The presence of the virus was evidenced in cats from Japan, Germany, Turkey, USA and South America (Furuya et al., 2015; Park et al., 2014; Sakaguchi et al., 2014; Sieg et al., 2015; Sharp et al. 2016; Darold et al., 2017; Yilmaz et al., 2017). In Italy, FeMV has been described for the first time in 2015 in the municipality of Teramo, Abruzzo region, Central Italy. A 15-years-old domestic stray cat, named Piuma, diagnosed with severe nephropathy was found positive for FeMV RNA (Lorusso et al., 2015) by sequencing the amplicon obtained by a RT-PCR assay (Woo et al. 2012) targeting a portion of the L protein-encoding gene of morbilliviruses. The whole genome sequence of the FeMV strain (Piuma/2015) was obtained by next-generation sequencing (NGS) directly from RNA purified from a urine sample (Marcacci et al., 2016). The cat died 4 months after diagnosis. To date, Furuya et al., 2015 described a real-time TaqMan RT-PCR designed by recruiting Japanese FeMV strains. However, most of the new sequences that were published on public databases after the development of this assay, were found to be unsuitable, at least *in silico*, for this method. Indeed, the genetic diversity of FeMV circulating strains has been evidenced (Sakaguchi et al., 2014; Sieg et al., 2015; Marcacci et al., 2016). Aim of this study was to develop a quantitative and specific real time PCR assay based on TaqMan technology ($qPCR_{FeMV}$) for diagnosis of FeMV RNA from biological specimens.

* Corresponding author.
E-mail address: a.lorusso@izs.it (A. Lorusso).

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Table 1
NCBI Nucleotide Accession numbers of the FeMV sequences used to design primers and probe.

Origin	Strain	NCBI Accession Number
U.S.A.	US1	KR014147
Italy	Piuma/2015	KT825132
Japan	OTJP001	NC_025264
Japan	CHJP073	AB924122
Japan	MiJP003	AB924121
China	M252A	JQ411016
China	776U	JQ411015
China	761U	JQ411014
Japan	SS3	LC036587
Japan	SS2	LC036586
Japan	SS1	AB910309

2. Materials and methods

2.1. qPCR design

All available FeMV full-length genome sequences were retrieved from GenBank and aligned with the multiple sequence editor CLUSTALX (Thompson et al., 1997). GenBank accession numbers of 11 complete genomes are listed in Table 1. The P/V/C gene was selected to design primers and probes by using the Primer Express Software Version 3.0 (Applied Biosystem). Primer FeMVrt-F sequence was 5′- GGG ATCCAGAGGGTAACCT -3′ (position 2061-2079, KT825132), and primer FeMVrt-R sequence was 5′-CCGGCCATTAATCTCTGAA -3′ (position 2119-2137, KT825132). FeMVrt TaqMan probe (5′-TATTCGAA AGCGATGATGATGAAAACCATTA-3′) was dual-labelled with 6-carboxyfluorescein (FAM) at the 5′ end and with tetramethylrhodamine (TAMRA) at the 3′ end (position 2088-2118, KT825132). Quantitect Probe RT-PCR Kit (Qiagen, Hilden, Germany) has been employed according to the manufacturer's recommendations. The reaction was optimized as follow: the 25 µl reaction volume contained 5 µl of purified RNA, 12.5 µl of 2× QuantiTect Probe RT-PCR Master Mix, 1 µl of Armored RNA West Nile Virus (HNY1999) (Asuragen) as non-competitive exogenous internal amplification control (EIAC), a final concentration of 200 nM for each EIAC primer (NS5-2-F, GAAGAGACCTGCGGCTC ATG; NS5-2-R, CGGTAGGGACCCAATTCACA), 160 nM for EIAC probe (NS5-2-P, CCAACGCCATTTGCTCCGCTG), 0.25µL of QuantiTect RT Mix, a final concentration of 600 nM and 250 nM for each FeMV primer and probe, respectively, and nuclease-free water up to final volume. The assay was carried out on a 7900HT Fast Real Time System cycler (Applied Biosystem) using the following thermal profile: 1 cycle of reverse transcription at 50 °C for 30 min, 1 cycle of PCR initial activation step at 95 °C for 15 min followed by 45 cycles of 94 °C for 30 s and 55 °C for 1 min.

2.2. Standard RNA

The $qPCR_{FeMV}$ target sequence was sub-cloned into a pGEM plasmid vector (pGEM®-T Easy Vector, Promega, Madison-WI, USA) according to the manufacturer's instructions, and used for the generation of standard RNA. Briefly, after linearization, transcription was carried out with the RiboMAX™ Large Scale RNA Production System-T7 kit (Promega), from the T7-promoter DNA according to the manufacturer's guidelines. After DNase treatment to remove residues of plasmid DNA, the transcripts were purified using a commercial column (RNeasy kit, Qiagen, Germantown, MD, USA) and quantified by spectrophotometric analysis. The copy number of cRNA calculated was 1.53×10^{11} μL^{-1}. Ten-fold serial dilutions of RNA transcript which contained from 10^8 to 10° copies of cRNA μL^{-1} were used to plot the standard curve.

2.3. qPCR performances evaluation

To evaluate the assay's performances the coefficient of determination (R^2) and the amplification efficiency (*E*) were calculated. Three replicates of each dilution were used. The analytical specificity was evaluated by adding to the analysis purified viral RNA from FeMV-related viruses including *canine distemper virus* (CDV), *peste des petits ruminants virus* (PPRV), and nucleic acids purified from isolates (or infected tissues) of other pathogens of cats such as *feline calicivirus* (FCV), *feline herpesvirus* (FHV-1), *feline leukemia virus* (FeLV), *feline immunodeficiency virus* (FIV), *feline infectious peritonitis virus* (FIPV), *feline parvovirus* (FPV), *Leishmania* spp., *Leptospira* spp., *Bartonella henselae*, *Mycoplasma* spp., *Rickettsia* spp. and *Toxoplasma* spp. All isolates or infected tissues were available at IZSAM. RNAs of three selected positive samples were purified and amplified three times in the same run for the assessment of the intra-assay repeatability, and in three different runs to evaluate the inter-assay repeatability. The C_T values obtained from each assay were considered to determine the standard deviation (SD) and coefficient of variation (CV%), which were used to assess the reliability of the $qPCR_{FeMV}$ protocol.

2.4. Field specimens

Urine (n = 264) and blood samples (n = 264) from 264 live cats and tissue samples (n = 385) including brain, cerebellum, heart, lung, intestine, stomach, liver, kidney, bladder, spleen and mesenteric lymph nodes from 35 dead cats were used in this study. Viral nucleic acids were purified from EDTA- blood samples by means of the High Pure Viral Nucleic Acid Kit (Roche Life Science, Roche Diagnostics, Monza, Italy) whereas from urine and tissue samples by means of the QiAamp Viral RNA Mini kit, Qiagen, Hilden, Germany). RNAs were purified according to the manufacturers recommendations and kept at −80 °C until testing.

2.5. RT-PCR_{Lgene}

All samples were also tested with a RT-PCR targeting a 155-bp portion of the L protein encoding gene sequence of morbilliviruses with primers (LPW12490, 5′-CAGAGACTTAATGAAATTTATGG-3′; and LPW12491, 5′-CCACCCATCGGGTACTT-3′) designed by multiple alignments of L sequences of morbilliviruses (RT-PCR_{Lgene}, Woo et al., 2012). Amplicons were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced by Big Dye Terminator v.3.1 (Applied Biosystems) in the 3130 XL Genetic Analyzer (Applied Biosystems).

2.6. Shedding of Piuma/2015 RNA

Urine samples collected at different time points from Piuma (Lorusso et al., 2015) were also tested by $qPCR_{FeMV}$ to evaluate and quantitate viral RNA shedding within a 4 month-time span, starting from July 2015 up to the death of the cat. Accordingly, also RNA samples purified from brain, cerebellum, heart, lung, intestine, stomach, liver, kidney, bladder, spleen and mesenteric lymph nodes of Piuma were tested by $qPCR_{FeMV}$.

2.7. Immunohistochemistry on $qPCR_{FeMV}$-positive tissues

In order to evidence viral antigen in tissues showing very low FeMV RNA titres by $qPCR_{FeMV}$, formalin-fixed, paraffin-embedded samples of kidneys from two cats (Table 3) were cut into 3–5 µm-thick sections for light microscopy and immunohistochemistry (IHC) investigations. A specific antibody anti-FeMV (1:500 rabbit polyclonal antibody against the N antigen of FeMV, kindly provided by Dr Shigeru Morikawa, National Institute of Infectious Diseases, Tokyo) was applied. Deparaffinized and rehydrated 5 µm-thick sections were incubated in 3% hydrogen peroxide in absolute methanol for 30 min to inhibit

endogenous peroxidase activity then rinsed in 0.05 M Tris-buffered saline (TBS), pH 7.6, for 5 min. Antigen retrieval was performed by heat treatment in citrate buffer 0.01 M pH 6.0 at 121 °C for 5 min. To reduce nonspecific binding, slides were incubated in 20% normal swine serum (Dako, Copenhagen, Denmark) in TBS for 20 min, followed by a second incubation with 10% skimmed milk in TBS for 20 min at room temperature followed by overnight incubation with primary antibody in a humidified chamber at 4 °C. Immune reactions were visualized by means of a peroxidase technique (REAL™ Envision detection system peroxidase Dako, Copenhagen, Denmark). Positive and negative controls were included in each IHC run. The specificity of the immunolabeling was verified with an irrelevant antibody directed against an unrelated antigen.

2.8. Isolation attempts from $qPCR_{FeMV}$ positive samples of Piuma

Five hundred microliters of urine samples which resulted positive by $qPCR_{FeMV}$ were centrifuged at 3000 rpm for 5 min to remove debris and filtered through 450 nm disc filters (Millipore). TPCK trypsin (Sigma–Aldrich, Zwijndrecht, The Netherlands) was then added to a final concentration of 1 μg ml^{-1}. Samples were incubated at 37 °C for 15 min. The mixture was then inoculated into feline embryonic fibroblast (FEA) cells in 24-well plates serum-free Minimum Essential Medium Eagle (MEM) (Sigma–Aldrich) supplemented with penicillin (100 units ml^{-1}) and streptomycin (100 μg ml^{-1}) (Invitrogen). After 8 h, inocula were replaced with MEM supplemented by 3% heat-inactivated FCS and antibiotics. Cells were incubated at 37 °C in a humidified atmosphere with 5% of CO_2 and observed daily for cytophatic effect (CPE) by microscopy. Each passage consisted of two weeks of incubation. Up to 10 blind passages using FEA cells were performed.

3. Results

3.1. $qPCR_{FeMV}$ evaluation

Nucleic acids from CDV, PPRV, FIV, FeLV, FIPV, FPV, *Leishmania, Leptospira,Bartonella henselae*, *Rickettsia* and *Toxoplasma* did not produce any amplification by $qPCR_{FeMV}$. Moreover, the no-template controls and FeMV-uninfected cat specimens did not produce any detectable fluorescence signal endorsing the high specificity of the test (100%). The standard curve was found to be linear with a wide dynamic range from 1×10^1 to 1×10^8 cRNA μL^{-1}. Therefore, the LoQ, the lowest concentration of the analyte that could be quantified with acceptable accuracy, was defined as 10 cRNA copies μL^{-1}. Moreover, the efficiency calculated was 100%; the coefficient of determination was 0.994. Intra- and inter-assay data is reported in Table 2.

Table 2
Repeatability data of the assay.

Sample	C_T Mean[c]	C_T Range[d]	S.D.[e]	CV % [f]
NRG 9216 Spleen[a]	24,96	(24,74–25,11)	0,194	0,70%
NRG 9216 Bladder[a]	33,32	(33,12–33,63)	0,270	0,80%
NRG 10704_1 Urine[a]	33,60	(33,41–33,94)	0,290	0,80%
NRG 9216 Spleen[b]	24,22	(23,70–24,96)	0,658	2,70%
NRG 9216 Bladder[b]	32,53	(31,30–33,32)	1,081	3,30%
NRG 10704_1 Urine[b]	33.90	(33,44–34,68)	0,674	1,90%

[a] Intra-assay variability was measured by performing a single qPCR assay from three different aliquots of each sample.
[b] Inter-assay variability was measured using three separate qPCR assays performed in three different days.
[c] Mean constant threshold values.
[d] Range for mean constant threshold values.
[e] Standard deviation of constant threshold values.
[f] Coefficient of variation.

Table 3
Results of $qPCR_{FeMV}$ with RNA purified from biological samples. [#]tested by IHC.

Sample	$RT\text{-}PCR_{Lgene}$	Sequence	$qPCR_{FeMV}$
Urine	+	FeMV	$1{,}52 \times 10^2$
Urine	+	FeMV	$5{,}75 \times 10^1$
Urine	+	FeMV	$1{,}52 \times 10^2$
Urine	+	FeMV	$1{,}17 \times 10^2$
Urine	+	FeMV	$3{,}32 \times 10^2$
Urine	+	FeMV	$7{,}64 \times 10^2$
Urine	–	na	$2{,}43 \times 10^1$
Urine	+	FeMV	$3{,}32 \times 10^2$
Urine	+	FeMV	$7{,}60 \times 10^2$
Urine	+	FeMV	$3{,}40 \times 10^2$
Urine	–	na	$3{,}11 \times 10^1$
Urine	+	FeMV	$3{,}11 \times 10^2$
Urine	+	FeMV	$2{,}58 \times 10^2$
Urine	+	FeMV	$9{,}93 \times 10^2$
Urine	+	FeMV	$3{,}28 \times 10^2$
Urine	+	FeMV	$3{,}35 \times 10^2$
Urine	–	na	$4{,}83 \times 10^1$
Urine	–	na	$9{,}83 \times 10^1$
Urine	–	na	$1{,}68 \times 10^1$
Urine	+	FeMV	$3{,}28 \times 10^2$
Urine	–	na	$2{,}93 \times 10^1$
Urine	–	na	$7{,}67 \times 10^1$
Urine	+	FeMV	$5{,}46 \times 10^1$
Urine	+	FeMV	$7{,}91 \times 10^2$
Urine	+	FeMV	$4{,}38 \times 10^2$
Urine	+	FeMV	$1{,}94 \times 10^2$
Urine	–	na	$1{,}14 \times 10^1$
Urine	+	FeMV	$7{,}67 \times 10^1$
Urine	–	na	$2{,}95 \times 10^1$
Urine	+	FeMV	$3{,}36 \times 10^2$
Urine	+	FeMV	$3{,}30 \times 10^2$
Urine	+	FeMV	$7{,}28 \times 10^2$
Urine	+	FeMV	$2{,}98 \times 10^3$
Urine	+	FeMV	$6{,}38 \times 10^2$
Urine	+	FeMV	$7{,}23 \times 10^2$
Urine	–	na	$5{,}28 \times 10^1$
Blood	–	na	$3{,}35 \times 10^1$
Urine	+	FeMV	$3{,}45 \times 10^2$
Urine	+	FeMV	$1{,}77 \times 10^2$
Urine	+	FeMV	$8{,}35 \times 10^2$
Urine	–	na	$4{,}76 \times 10^1$
Kidney	–	na	$5{,}5 \times 10^1$
Bladder	+	FeMV	$2{,}36 \times 10^2$
Spleen	+	FeMV	$2{,}6 \times 10^3$
Kidney[#] NRG 9216	+	FeMV	$1{,}38 \times 10^2$
Bladder	+	FeMV	$6{,}93 \times 10^2$
Kidney[#] NRG 12,807	+	FeMV	$5{,}73 \times 10^2$
Bladder	+	FeMV	$9{,}93 \times 10^2$
Spleen	+	FeMV	$2{,}56 \times 10^2$
Mesenteric Lymph nodes	+	FeMV	$6{,}49 \times 10^2$
Kidney	+	FeMV	$5{,}36 \times 10^2$
Kidney	+	FeMV	$8{,}72 \times 10^2$
Bladder	+	FeMV	$3{,}35 \times 10^2$
Kidney	+	FeMV	$1{,}03 \times 10^2$
Bladder	+	FeMV	$4{,}52 \times 10^2$
Kidney	+	FeMV	$6{,}91 \times 10^2$

3.2. Field specimens and comparison with $RT\text{-}PCR_{Lgene}$

Forty urine samples, one blood sample and 15 tissue samples from 8 carcasses turned out to be positive by $qPCR_{FeMV}$ (Table 3). Positive samples showed C_T values ranging from 26 to 35 corresponding to titres ranging from 2.98×10^3 to 1.14×10^1 cRNA μL^{-1} (Table 3). All field specimens were also tested by $RT\text{-}PCR_{Lgene}$. Thirteen out of 56 $qPCR_{FeMV}$-positive field samples were not positive by $RT\text{-}PCR_{Lgene}$. In general, all these samples showed the lowest titres of FeMV RNA (Table 3).

3.3. Shedding of Piuma/2015 RNA

Piuma/2015 RNA was detected at very low titres at all different

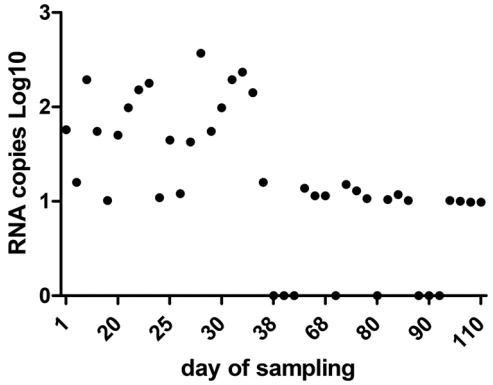

Fig. 1. Shedding of FeMV RNA from Piuma.

time points of observation. Titres ranged from 3.69×10^2 to 1.03×10^1 cRNA μL^{-1} (Fig. 1). Viral RNA was constantly detected from the first day of observation up to day 38. From that day onward, viral RNA was detected intermittently with an apparent plateau up to cat's death which occurred after 110 days. All internal tissues of Piuma were negative for FeMV RNA.

3.4. Immunohistochemistry

Histologically, moderate to severe aspects of chronic tubulo-interstitial nephritis with multifocal tubular atrophy or degeneration, associated with infiltration of mononuclear inflammatory cells were observed in both cats. Strong and diffuse immunoreactivity for FeMV were observed in some degenerate tubular structures of renal cortex (Fig. 2A) as well as in non-degenerate tubular structures of medulla (Fig. 2B).

3.5. Isolation attempts

All isolation attempts from $qPCR_{FeMV}$-positive urine samples of Piuma were unsuccessful.

4. Discussion

In this study we developed a sensitive, specific and reliable $qPCR_{FeMV}$ assay targeting a 76-bp portion of the P/V/C gene of FeMV genome. FeMV is a novel morbillivirus of cats suspected to be associated with feline renal diseases, including tubulo-interstitial nephritis (TIN), one of the leading causes of death in old housed cats (Sparkes et al., 2016). Although several reports of FeMV circulation worldwide evidencing the presence of TIN lesions in FeMV-infected cats, the association between virus and disease has not been yet demonstrated. Furthermore, a constraint hampering FeMV studies is the isolation procedure, which has been so far demonstrated to be difficult and very time consuming (Koide et al., 2015; Sakaguchi et al., 2014). Also, in this study, all isolation attempts from Piuma's specimens were unsuccessful. Accordingly, the development of a fast diagnostic molecular tool directly from biological specimens is recommended. The $qPCR_{FeMV}$ developed in this study can be successfully applied to quantitate viral loads in biological samples of cats, mainly urine. The specificity of $qPCR_{FeMV}$ was assessed against other morbilliviruses and pathogens of cats. Cross-reaction with extant feline pathogens was not revealed proving evidence for the exclusive detection of FeMV RNA. Furthermore, $qPCR_{FeMV}$ had higher sensitivity with respect to $RT\text{-}PCR_{Lgene}$ enabling the detection of FeMV RNA even in samples with low viral concentration. Considering that viral titres obtained in this study were demonstrated to be low, scenario already described in similar studies, the availability of a sensitive test is certainly welcome. Importantly, we also demonstrated by IHC the presence of FeMV antigen in two low-titres $qPCR_{FeMV}$ positive kidney sections. One could reasonably argue

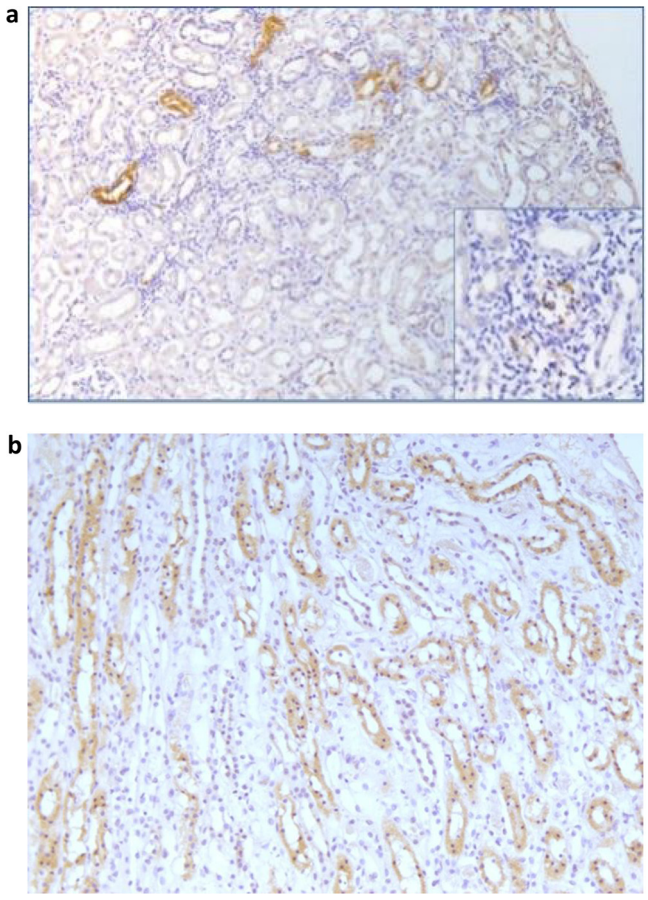

Fig. 2. A. Cat, Kidney. Viral antigen was demonstrated in cortical tubules with small clumps of inflammatory mononuclear cells. IHC, Mayer's haematoxylin counterstain, 10X Ob. *Inset:* Viral antigen immunostaining was observed in necrotic tubular cells surrounded by inflammatory response. IHC, Mayer's haematoxylin counterstain, 40X Ob. (NRG 9216); B. Cat, Kidney. Tubular structures of medulla showed strong and diffuse immunoreactivity with mild inflammatory mononuclear cells infiltration. 20×. IHC, Mayer's haematoxylin counterstain (NRG 12807).

that the main pitfall of this study is the absence of FeMV strains originating from other areas of the world to be tested by the newly developed assay. However, *in silico* analysis demonstrated that designed primers and probe are suitable for the molecular detection of all publicly available FeMV sequence. In conclusion, the use of this assay is certainly crucial for the assessment of several studies including epidemiological investigations and pathogenesis studies in different animal models. Further investigations to demonstrate FeMV prevalence and relationship between virus and kidney lesions, coupled with an extensive analysis of clinical parameters of FeMV-infected cats are currently in progress.

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References

Bieringer, M., Han, J.W., Kendl, S., Khosravi, M., Plattet, P., Schneider-Schaulies, J., 2013. Experimental adaptation of wild-type canine distemper virus (CDV) to the human entry receptor CD150. PLoS One 8 (3), e57488.

Darold, G.M., Alfieri, A.A., Muraro, L.S., Amude, A.M., Zanatta, R., Yamauchi, K.C., Alfieri, A.F., Lunardi, M., 2017. First report of feline morbillivirus in South America. Arch. Virol. 162 (2), 469–475.

de Vries, R.D., Duprex, W.P., de Swart, R.L., 2015. Morbillivirus infections: an introduction. Viruses 7, 699–706.

Furuya, T., Wachi, A., Sassa, Y., Omatsu, T., Nagai, M., Fukushima, R., Shibutani, M., Yamaguchi, T., Uematsu, Y., Shirota, K., Mizutani, T., 2015. Quantitative PCR detection of feline morbillivirus in cat urine samples. J. Vet. Med. Sci. 77 (12), 1701–1703.

Koide, R., Sakaguchi, S., Miyazawa, T., 2015. Basic biological characterization of feline morbillivirus. J. Vet. Med. Sci. 77, 565–569.

Kumar, N., Maherchandani, S., Kashyap, S.K., Singh, S.V., Sharma, S., Chaubey, K.K., Ly, H., 2014. Peste Des petits ruminants virus infection of small ruminants: a comprehensive review. Viruses 6, 2287–2327.

Lorusso, A., Di Tommaso, M., Di Felice, E., Zaccaria, G., Luciani, A., Marcacci, M., Aste, G., Boari, A., Savini, G., 2015. First report of feline morbillivirus in Europe. Vet. Ital. 51, 235–237.

Marcacci, M., De Luca, E., Zaccaria, G., Di Tommaso, M., Mangone, I., Aste, G., Savini, G., Boari, A., Lorusso, A., 2016. Genome characterization of feline morbillivirus from Italy. J. Virol Methods 234 (August), 160–163.

McLeland, S.M., Cianciolo, R.E., Duncan, C.G., Quimby, J.M., 2015. A comparison of biochemical and histopathologic staging in cats with chronic kidney disease. Vet. Pathol. Vol. 52 (3), 524–534.

Park, E.S., Suzuki, M., Kimura, M., Maruyama, K., Mizutani, H., Saito, R., Kubota, N., Furuya, T., Mizutani, T., Imaoka, K., Morikawa, S., 2014. Identification of a natural recombination in the F and H genes of feline morbillivirus. Virology 468–470, 524–531.

Qiu, W., Zheng, Y., Zhang, S., Fan, Q., Liu, H., Zhang, F., Wang, W., Liao, G., Hu, R., 2011. Canine distemper outbreak in Rhesus monkeys. China Emerg. Infect. Dis. 17 (August (8)) 1541.3.

Sakaguchi, S., Nakagawa, S., Yoshikawa, R., Kuwahara, C., Hagiwara, H., Asai, K., Kawakami, K., Yamamoto, Y., Ogawa, M., Miyazawa, T., 2014. Genetic diversity of feline morbilliviruses isolated in Japan. J. Gen. Virol. 95 (Pt. 7), 1464–1468.

Sakai, K., Yoshikawa, T., Seki, F., Fukushi, S., Tahara, M., Nagata, N., Ami, Y., Mizutani, T., Kurane, I., Yamaguchi, R., Hasegawa, H., Saijo, M., Komase, K., Morikawa, S., Takeda, M., 2013. Canine distemper virus associated with a lethal outbreak in monkeys can readily adapt to use human receptors. J. Virol. 87 (June (12)), 7170–7175.

Sharp, C.R., Nambulli, S., Acciardo, A.S., Rennick, L.J., Drexler, J.F., Rima, B.K., Williams, T., Duprex, W.P., 2016. Chronic infection of domestic cats with feline morbillivirus, United States. Emerg. Infect. Dis. 22, 760–762.

Sieg, M., Heenemann, K., Rückner, A., Burgener, I., Oechtering, G., Vahlenkamp, T.W., 2015. Discovery of new feline paramyxoviruses in domestic cats with chronic kidney disease. Virus Genes 51 (2), 294–297.

Sparkes, A.H., Caney, S., Chalhoub, S., Elliott, J., Finch, N., Gajanayake, I., Langston, C., Lefebvre, H.P., White, J., Quimby, J., 2016. ISFM consensus guidelines on the diagnosis and management of feline chronic kidney disease. J. Feline Med. Surg. 18, 219–239. http://dx.doi.org/10.1177/1098612X16631234.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Woo, P.C., Lau, S.K., Wong, B.H., Fan, R.Y., Wong, A.Y., Zhang, A.J., Wu, Y., Choi, G.K., Li, K.S., Hui, J., Wang, M., Zheng, B.J., Chan, K.H., Yuen, K.Y., 2012. Feline morbillivirus, a previously undescribed paramyxovirus associated with tubulointerstitial nephritis in domestic cats. Proc. Natl. Acad. Sci. U. S. A. 109 (14), 5435–5440.

Yilmaz, H., Tekelioglu, B.K., Gurel, A., Bamac, O.E., Ozturk, G.Y., Cizmecigil, U.Y., Altan, E., Aydin, O., Yilmaz, A., Berriatua, E., Helps, C.R., Richt, J.A., Turan, N., 2017. Frequency, clinicopathological features and phylogenetic analysis of feline morbillivirus in cats in Istanbul, Turkey. J. Feline Med. Surg. 1–10.