SHORT COMMUNICATION

Isolation and genome sequences of two Feline Morbillivirus genotype 1 strains from Italy

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Summary
Feline morbillivirus (FeMV) is a novel viral paramyxovirus detected in cats. FeMV is suspected to be associated to tubulointerstitial nephritis, but its pathogenic role is far to be clearly understood. In this short communication, we report the whole genome coding sequences of the first two FeMV strains isolated in Italy.

The genus Morbillivirus includes several enveloped negative-sense single-stranded RNA viruses infecting humans and animals. Feline morbillivirus (FeMV) is a novel morbillivirus infecting cats and first described in stray cats from Hong Kong nearly ten years ago (Woo et al. 2012). Soon after, FeMV circulation was detected worldwide (Furuya et al. 2014, Park et al. 2014, Sakaguchi et al. 2014, Lorusso et al. 2015, Sieg et al. 2015, Sharp et al. 2016, Yilmaz et al. 2017, Darold et al. 2017).

FeMV isolation on cell culture has been described to be difficult and time consuming (Sakaguchi et al. 2014) and a limited number of viral isolates and related whole genome sequences are, indeed, publicly available. Here, we describe the complete genome coding sequences of two FeMV isolates from Italy. Urine samples were taken in March 2018 from two male cats (Tremedino and Pepito, 1 and 7 year old, respectively), living in Reggio Calabria (Calabria region, Southern Italy). The two cats did not show clinical and laboratory signs of renal damage (Donato, manuscript in preparation). Briefly, the first cat (Tremedino, one year old, domestic short-air, male cat) showed stomatitis and an enlargement of popliteal and submandibular lymph nodes, whereas the second cat (Pepito, 7 year old, domestic long-hair male cat) was overweight and presented for stomatitis and bilateral otitis. In both cats, no abnormalities suggestive of renal disease
supplemented by 3% heat inactivated fetal calf serum and antibiotics. Cells were incubated at 37 °C in a humidified atmosphere with 5% of CO₂ and observed daily for cytopathic effect by microscopy. At the 1st cell passage, syncytia were evident at day 8. Cells were stained by May Grünwald-Giemsa (Figure 1a). RNA was purified (QIAamp® Viral RNA minikit, Qiagen, Germantown, MD, USA) from 140 µL of cell culture supernatants and tested by real time RT-PCR for FeMV (Cq 26 and 23 for Tremedino and Pepito, respectively).

FEA cells that tested positive by real time RT-PCR were also fixed in chilled acetone at -20 °C for 20 min. Fixed cells were incubated with 1:100 dilution of rabbit polyclonal antibody against the N protein of FeMV (kindly provided by Dr Shigeru Morikawa, National Institute of Infectious Diseases, Tokyo), followed by incubation with a FITC-goat anti-rabbit IgG (Sigma-Aldrich, Zwijndrecht, The Netherlands) 1:32 diluted. Cells were then examined under a fluorescence microscope and imaged using the Leica TCS SP5 II confocal laser scanning microscope. Uninfected FEA cells were used as negative control. Infected cells tested positive for FeMV (Figure 1b).

Isolates were named FeMV Tremedino/2018 Italy and FeMV Pepito 2018/Italy, further passaged and stored at -80°. Total RNA was purified from 300 µL of supernatant of the first passage by using the QIAamp® viral RNA minikit (Qiagen, Germantown, MD, USA). Sequencing was performed by using a combination of sequence-independent/single-primer amplification (SISPA) and next generation sequencing (NGS) as previously described (Marcacci et al. 2015). Library preparation was carried out by using the Nextera XT Library Prep kit (Illumina Inc., San Diego, CA) according to the manufacturer’s protocol. Sequencing was

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**Figure 1.** FEA cells infected by FeMV Pepito2018/Italy. Multinucleated syncytium was observed by May Grünwald-Giemsa staining (A); strong and specific cytoplasmic fluorescence (green color), nuclei are stained with DAPI (blue) (B). Scale bar = 100 µm (A), 75 µm (B).
performed on the NextSeq 500 (Illumina Inc., San Diego, CA) using the NextSeq 500/550 Mid Output Reagent Cartridge v2, 300 cycles and standard 150 bp paired-end reads. The resulting 3,539,382 and 1,421,904 reads for Tremedino and Pepito, respectively, were de novo assembled by SPAdes v3.8.0. A total number of 266,976 and 388,432 reads mapped on a reference FeMV sequence (GenBank accession number AB924120, strain OtJP001). The length of the final de novo assemblies were of 16,027 and 15,946 bp for FeMV Tremedino2018/Italy and FeMV Pepito2018/Italy, respectively. The obtained nucleotide (nt) genome sequences were compared to those of extant FeMVs available online and genetic distances were calculated by using MegAlign (Lasergene 15.0, Madison, WI, USA). The genome sequences of Tremedino2018/Italy and Pepito2018/Italy were found to be nearly identical as they share the 99.2% of nt identity. Nt identity between sequences obtained in this study and extant whole FeMV genome sequences ranges from 98.7% to 78.1%. Tremedino2018/Italy and Pepito2018/Italy showed the highest % of nt sequence identity with the Japanese strains SS1 (98.7%, AB910309) and OtJP001 (98.5%, AB924120); nt identity was lower with the early FeMV strains 761U and 776U (87.8%, JQ411014 and JQ411015) isolated in Hong Kong (Woo et al. 2012) and with strain US1 from USA (87.9%-87.8%, KR014147). Tremedino2018/Italy and Pepito2018/Italy strains share the 88.1% of nt identity with Piuma/2015 (Lorusso et al. 2015). Very recently, a new genotype of FeMV, tentatively named FeMV genotype 2 (FeMVGT2), was described in Germany (Sieg et al. 2019). FeMV sequences obtained in this study share the 78.1% of nt identity with FeMVGT2 sequences. Overall, our results confirm the viral heterogeneity existing between FeMV circulating strains and that the strains described in this study belong to the FeMV genotype 1. Further molecular analysis of FeMV strains circulating in Southern Italy is currently underway (Donato, manuscript in preparation) as well as the assessment of a serum-neutralization assay to quantify specific FeMV antibodies in cat serum.

**Nucleotide sequence accession numbers**

Nucleotide sequences of Tremedino2018/Italy and Pepito2018/Italy have been deposited in GenBank with accession numbers MK088516 and MK088517, respectively.

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References


