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Short communication

Genome characterization of feline morbillivirus from Italy

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A B S T R A C T

Feline morbillivirus (FeMV) has been recently identified by RT-PCR in the urine sample of a nephropathic cat in Italy. In this report, we describe the whole genome sequence of strain Piuma/2015 obtained by combination of sequence independent single primer amplification method (SISPA) and next generation sequencing (NGS) starting from RNA purified from the infected urine sample. The existence in Germany and Turkey of FeMVs from cats divergent from Piuma/2015, suggests the presence of FeMV heterogeneity in Europe as it has been described previously in Japan and China.

The genus *Morbillivirus* of the family *Paramyxoviridae* comprises several viral species with a non-segmented single stranded negative RNA genome. They cause severe and often fatal infections of humans and animals including measles, distemper and the emerging pest of small ruminants (Lamb and Parks, 2013). Morbilliviruses have been very recently identified also in cats. The new viral species so far denominated feline morbillivirus (FeMV) was detected for the first time in Hong Kong (China) and apparently associated with tubule-interstitial nephritis (TIN) (Woo et al., 2012). TIN involves primary injury to renal tubules and interstitium and is the most common cause of chronic kidney disease (CKD) and one of the leading causes of deaths in housed old cats. Further studies demonstrated the presence of FeMV in Japanese domestic cats (Sakaguchi et al., 2014; Furuya et al., 2014), although without any clear clinical association with CKD. By the end of June 2015, FeMV was identified by RT-PCR in the urine sample of a stray cat suffering from CKD in Teramo, Abruzzi region, Central-Italy (Lorusso et al., 2015). Interestingly, the cat did not show other known pathogens related with kidney disease. The presence of FeMV in Europe was already evidenced in 2013 in Germany (Sieg et al., 2015) and recently also described in Turkey (acc. nos KT808322 and KT021480; Presence of feline morbillivirus-RNA in cats in Istanbul, Turkey, unpublished). FeMV has also been recently detected in USA from healthy cats and from cats suffering from CKD (Sharp et al., 2016). In this short report, it is described how the whole genome sequence of FeMV Piuma/2015 has been obtained from an infected urine sample and the genetic relation of this strain with extant FeMVs.

Urine sample was centrifuged at 3000*g* for 5 min to remove cell debris. Total RNA has been purified from 300 microliters (μl) of urine by using the High Pure Viral Nucleic Acid kit (Roche) following manufacturer's guidelines. Purified total RNA was treated with RNase-free DNaseI (New England Biolabs, Ipswich, MA) at 37 °C for 10 min and quantified with Qubit® RNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA). A total quantity of 60 nanograms (ng) of RNA was used for the assessment of the sequence-independent single primer amplification (SISPA, Allander et al., 2005) with some modifications. cDNA was obtained by reverse-transcription (RT) with 200 Unit of the SuperScript® IV Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA) in presence of 1X SSIV Buffer, 50 μM of the random primer FR26RV-N, 10 mM of dNTPs mix, 100 mM of DTT, 40U of RNAse OUT RNase Inhibitor (Thermo Fisher Scientific, Waltham, MA). The reaction was incubated at 23 °C for 10 min and 50 °C for 50 min. After an inactivation step at 80 °C for 10 min, 5 units of Klenow Fragment (3′ → 5′ *exo*-) (New England Biolabs, Ipswich, MA) was directly added to the reaction to perform the second strand cDNA synthesis. The incubation was carried out at 37 °C for 1 h and 75 °C for 10 min. Next, 5 μl of ds cDNA was added to PCR master mix containing 1 × PfuUltra II reaction buffer, 1 μl of PfuUltra II Fusion HS DNA Polymerase (Agilent Technologies Santa Clara, CA), 10 mM of

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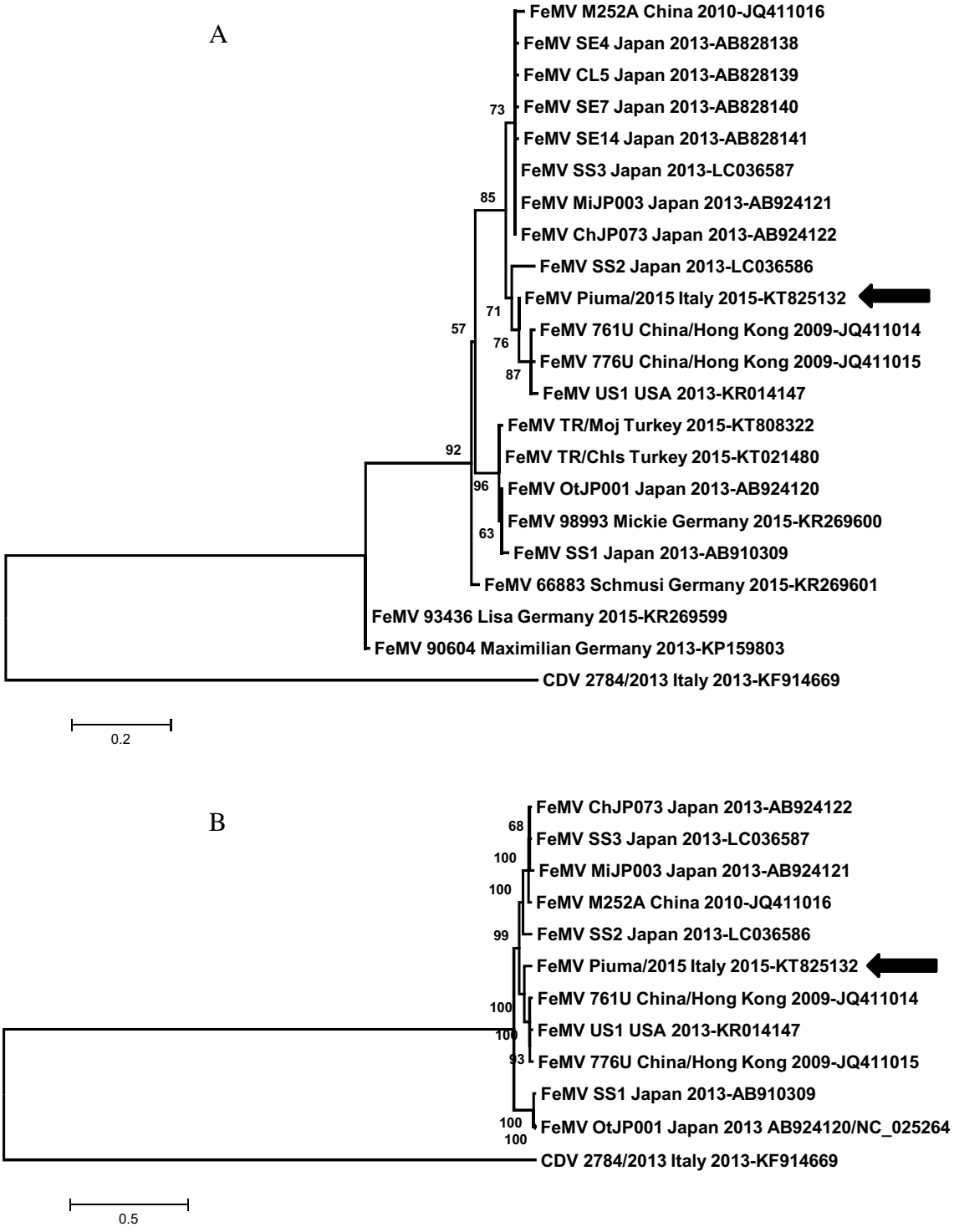

Fig. 1. Phylogenetic analyses were inferred from partial (approximately 400 nt) L protein encoding gene of all existing FeMVs ($ML_{partial}$, 1A) and complete FeMV genomes available on line ($ML_{complete}$, 1B). Analyses were conducted using the Maximum Likelihood (ML) method in Mega 6 (Tamura et al., 2013). The best-fit model of nucleotide substitution was identified by the Find Best DNA/protein Model available in Mega 6 as the T92 + I model for $ML_{partial}$, whereas the GTR + G + I for $ML_{complete}$. To assess the robustness of individual nodes on the phylogenetic trees, we performed a bootstrap resampling analysis (1000 replications) using the neighbor-joining method, incorporating the ML substitution model. Canine distemper virus (CDV, Marcacci et al., 2014) served as outgroup. Bars indicate the estimated number of nt substitutions per site.

dNTPs mix and 40 μM of FR20RV primer. The incubation was performed with the following thermal conditions: 95 °C for 1 min, 40 cycles of 95 °C for 20 s, 65 °C for 20 s and 72 °C for 2 min and a final extension step of 72 °C for 3 min. The PCR product was purified by using Expin™ PCR SV (GeneAll Biotechnology CO., LTD Seoul, Korea) and then quantified by using the Qubit® DNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA). The sample was diluted to an initial concentration of 0.2 ng/μl and then 1 ng was used for library preparation by using the Nextera XT Library Prep kit (Illumina Inc., San Diego, CA) according to the manufacturer's protocol with the only exception regarding the quantity of the transposome. We indeed added 3 μl of the tagmentation enzyme instead of the 5 μl recommended in order to avoid over fragmentation of the viral genome which would have caused quantitative and qualitative