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Epidemiology, pathological aspects and genome heterogeneity of feline morbillivirus in Italy

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ABSTRACT

Feline morbillivirus (FeMV) is an emerging morbillivirus first described in cats less than a decade ago. FeMV has been associated with chronic kidney disease of cats characterized by tubulointerstitial nephritis (TIN), although this aspect is still controversial and not demonstrated with certainty. To investigate FeMV prevalence and genomic characteristics, an epidemiological survey was conducted in a total number of 127 household cats originating from two Italian regions, Abruzzi and Emilia-Romagna. A total number of 69 cats originating from three feline colonies were also enrolled for the study. Correlation with TIN was investigated by employing a total number of 35 carcasses. Prevalence of FeMV RNA was higher in urine samples collected from cats of colonies (P = 31.8%, CI 95% 22.1–43.6) compared to household cats (P = 8.66%, CI 95% 4.9–14.9) and in young and middle-aged cats while prevalence of FeMV Abs was higher in old cats. Sequences obtained straight from infected biological samples, either partial or complete, cluster into two clades within FeMV genotype 1, distantly related to FeMV genotype 2. Immunohistochemistry analysis of kidney sections of FeMV RNA positive cats revealed immunoreactivity within epithelial cells of renal tubuli and inflammatory cells. However, statistically significant association between FeMV and renal damages, including TIN, was not demonstrated ($p = 0.0695$, Fisher exact test). By virus histochemistry performed with FeMV-negative feline tissues and a FeMV isolate, tropism for different cellular types such as inflammatory cells residing in blood vessels of kidney and brain, airway epithelial cells, alveolar macrophages and to a lesser extent, the central nervous system, was demonstrated. Additional studies are warranted in order to establish viral tropism and immune response during the early phases of infection and to disentangle the role of FeMV in co-infection processes.

1. Introduction

Morbilliviruses belong to the family *Paramoxyviridae*, subfamily *Paramyxovirinae*, genus *Morbillivirus*. This genus includes several enveloped, negative-sense single-stranded RNA viruses infecting humans (measles virus), terrestrial and aquatic animals (e. g. canine distemper virus, CDV) with severe and fatal systemic diseases (de Vries et al., 2015). Morbilliviruses have a linear single stranded negative RNA genome of nearly 16 kb encoding six structural proteins. One of these is the haemagglutinin protein (H) coded by the H gene. H protein is a major determinant for virus-host interaction and viral entry via attachment to signalling lymphocyte activation molecule (SLAM) (Tatsuo et al., 2001). The H protein binds the receptor resulting in cellular attachment and activation of the F protein by tissue-specific proteases leading to cellular infection.

Tubulo-interstitial nephritis (TIN) is the most frequent histopathological finding in feline chronic kidney disease (CKD) (Jepson, 2016) and CKD is one of the most commonly diagnosed diseases in old cats,

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affecting ≥30–40% of individuals over 10 years of age (White et al., 2006; Marino et al., 2014). In most cats, CKD is also a progressive disease and can be accompanied by a wide range of clinical and pathological changes, but the underlying aetiology of CKD is often undiagnosed. CKD could result, indeed, from a variety of potential factors that may include toxic insults, hypoxia, chronic glomerulonephritis, chronic pyelonephritis, upper urinary tract obstructions, viral infections, that lead to a final common pathway of irreversible, progressive kidney damage (Elliott and Barber, 1998). Idiopathic CKD such as pyelonephritis, glomerulonephritis and TIN due to unknown causes has been reported extensively (Elliott and Barber, 1998; Mayer-Roenne et al., 2007; Chakrabarti et al., 2013).

Morbilliviruses have been very recently discovered also in domestic cats. The new viral species so far denominated feline morbillivirus (FeMV) was first identified in Hong Kong in 2009 (Woo et al., 2012) and associated with tubulo-interstitial nephritis (TIN). Following the first report in Hong Kong, FeMV was identified and isolated in cell culture in Japan (Furuya et al., 2014; Sagaguchi et al., 2014; Park et al., 2016; Sutummaporn et al., 2019). Since then, several surveys have been conducted worldwide in order to unravel the epidemiology of this virus and the association with TIN. The virus was described in Germany, USA, South America, Turkey, UK and Malaysia (Sieg et al., 2015; Sharp et al., 2016; Darold et al., 2017; Yilmaz et al., 2017; McCallum et al., 2018; Mohd et al., 2019). In Italy, FeMV was described for the first time by our group in 2015 in the municipality of Teramo, Abruzzi region, Central Italy. A 15-years old domestic stray cat diagnosed with severe nephropathy was hospitalized and found positive for FeMV RNA in the urine (Lorusso et al., 2015). FeMV RNA was evidenced by quantitative real time PCR from the first day of observation up to cat's death which occurred 110 days later (De Luca et al., 2018). FeMV was also recently identified in Northern Italy (Stranieri et al., 2019). So far, a clear association between virus and TIN has been not yet demonstrated (Furuya et al., 2014; Sakaguchi et al., 2014; Park et al., 2016; Darold et al., 2017; Yilmaz et al., 2017; Stranieri et al., 2019).

2. Materials and methods

2.1. Field specimens

This study was performed from March 2016 to December 2017 in collaboration between the Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise (IZSAM) with two Italian Veterinary University Hospitals (VUHs) within the Faculty of Veterinary Medicine of Teramo (Abruzzi region) and the Department of Veterinary Medical Science of Bologna (Emilia-Romagna region). Cats included in the study were divided into two groups: group A and group B. Specifically, group A was composed by 143 and 53 individuals admitted to the VUHs of Teramo and Bologna, respectively; group B was composed by 35 carcasses admitted at IZSAM for necropsy. In detail, urine (n = 196), whole blood (n = 196) and serum samples (n = 196) were collected from 196 live cats of group A; from carcasses of group B tissue samples (n = 385) including brain, cerebellum, heart, lung, intestine, stomach, liver, kidney, urinary bladder, spleen and mesenteric lymph nodes were collected during necropsy. Cats of group A were further classified in random household cats, originating from the two Italian regions under investigation and admitted with different clinical histories at the VUHs (subgroup RC, n = 127), and cats from three independent colonies (subgroup C, n = 69, which includes C1, n = 52; C2, n = 14 and C3, n = 3). Colonies C1, C2 and C3 were in Tortoreto, Martinsicuro and Mosciano Sant'Angelo, respectively, in the province of Teramo, Abruzzi region (Fig. 1). As far as we know, cats from colonies were not vaccinated against the major feline infectious diseases. Urine samples from all cats were collected by cystocentesis. Cats from colonies were hospitalized in groups of 5 at the VUH of Teramo. Cats of group A that tested positive for FeMV underwent haemato-biochemical analysis, urinalysis, urinary protein/creatinine ratio and symmetrical dimethylarginine (data not included). Carcasses of group B were also classified in random carcasses of household cats (originating from different areas of Abruzzi region, subgroup RCC, n = 28), and in carcasses from colonies (n = 7). This latter subgroup, CC, was formed by 5/7 carcasses from C1 and 2/7 from C2, and named CC1 and CC2, respectively. Groups classification has been listed, for clarity, in Table1.

In order to analyse the association between the presence of FeMV RNA and age, a chi-squared test analysis was performed. A total number of 226 cats (196 live animals plus 30 carcasses) has been considered as five carcasses from subgroup CC of group B were counted only once as live animals. Cats were classified as follows: young cats, up to three years old 82/226; middle-aged cats, up to 8 years old, 91/226; old cats, more than 8 years old, 53/226.

2.2. Molecular detection of FeMV

RNAs were purified from EDTA-blood samples by means of the High Pure Viral Nucleic Acid Kit (Roche Life Science, Roche Diagnostics, Monza, Italy) and from urine and tissue samples by means of the QiAamp Viral RNA Mini kit (Qiagen, Hilden, Germany). RNAs were purified according to the manufacturer's recommendations and kept at −80 °C until testing. All samples were tested by a real-time RT-PCR for detection of FeMV RNA ($qPCR_{FeMV}$) previously established by the study group at IZSAM (De Luca et al., 2018).

2.3. Molecular detection of relevant feline pathogens in cats

Tissues samples of carcasses which resulted $qPCR_{FeMV}$ positive were further tested for feline infectious peritonitis virus (FIPV, Dual IPC-TaqVetTM, LSI, Lissieu, France), feline immunodeficiency virus (FIV, gag protein gene-genesig® Advanced Kit, Rownhams, UK) and feline leukemia virus (FeLV, U3 region LTR- genesig® Advanced Kit, Rownhams, UK). DNAs purified by means the BioSprint 96 One-For-All Vet Kit (Qiagen, Hilden, Germany) were tested for feline panleukopenia virus (FPLV, HotStartTaq Master Mix Kit, Qiagen, Hilden, Germany), for *Leishmania* spp. (Rodgers et al., 1990) and for *Leptospira* spp. (Stoddard et al., 2009).

2.4. Partial and whole genome sequencing

All positive samples (urine and tissues) by $qPCR_{FeMV}$ were also tested by a $RT\text{-}PCR_{Lgene}$ amplifying a 401-bp portion of the L protein encoding gene sequence of FeMV with primers FeMV fwd 5′- AAGTA TCCTTCAAACACCGAGT -3′, and FeMV rev 5′- TTGAGTAACTCCAAGA TGAGGG- 3′, designed *in house* by multiple alignments of the FeMV L encoding gene sequences available on line. The reaction was optimized using the QIAGEN OneStep RT-PCR kit as it follows: the 25 µl of reaction contained 5 µl of 5X QIAGEN OneStep RT-PCR Buffer, 1 µl of dNTPs Mix (10 mM each), 1 µl of QIAGEN OneStep RT-PCR Enzyme Mix, 1.5 µl of each primer (10 µM each), 10 µl of RNAase free water and 5 µl of purified RNA. cDNA was synthesized at 48 °C for 50 min with denaturation at 95 °C for 15 min. The amplification reaction was carried out for 40 cycles with denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s and elongation at 72 °C for 1 min. Amplicons were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced by the Big Dye Terminator v.3.1 kit (Applied Biosystems) in the 3130 XL Genetic Analyzer (Applied Biosystems) with both primers. In order to get sequence information straight from the infected biological specimens, all $qPCR_{FeMV}$ positive RNA samples were also processed on the NextSeq 500 (Illumina Inc., San Diego,CA) through a combination of sequence-independent, single-primer amplification (SISPA) and NGS according to a previous report of the study group at IZSAM (Marcacci et al., 2016). Either partial or complete sequences were deposited within the GenBank database (accession numbers are available in Table S1, Table S2 and Table S3).

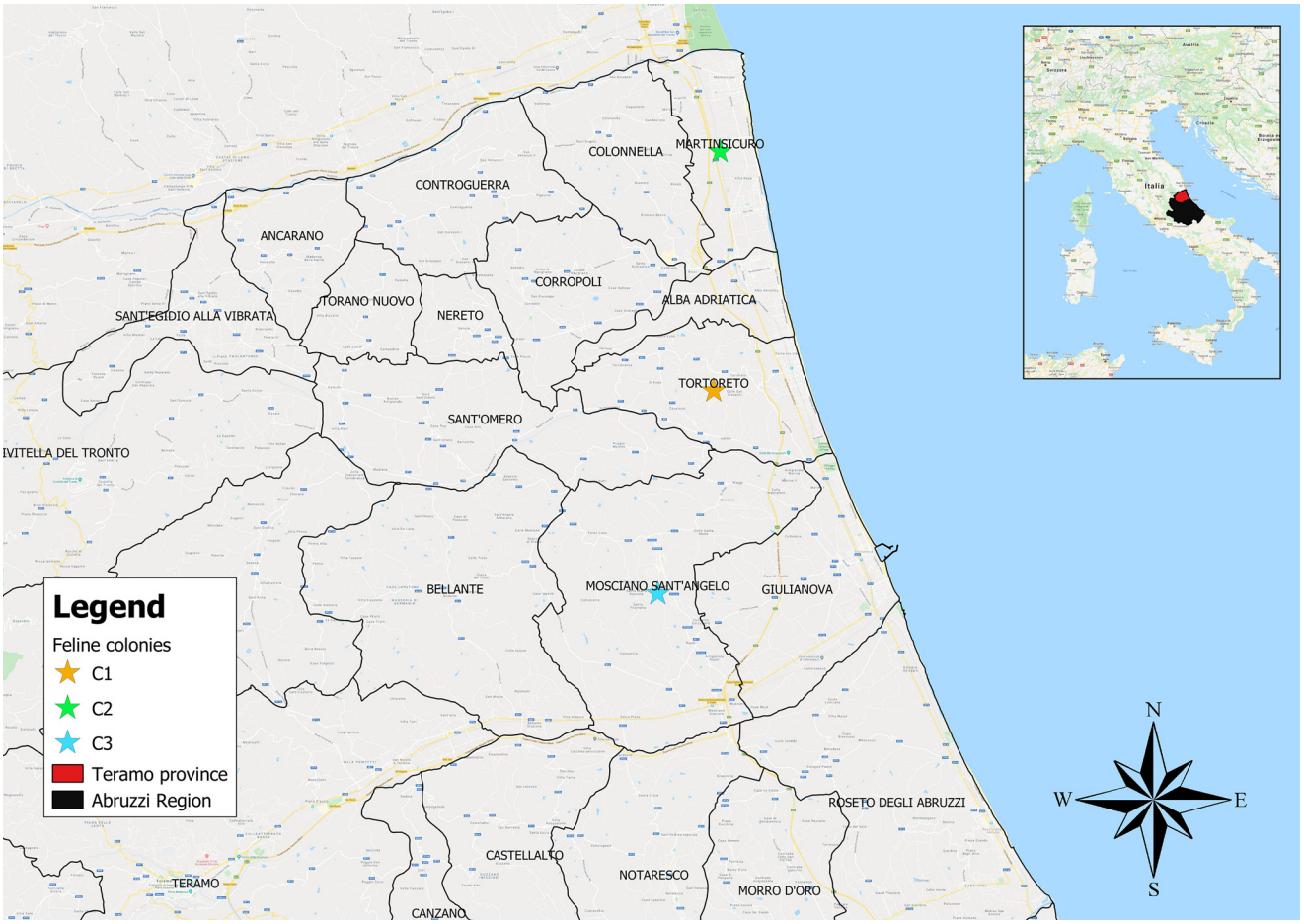

Fig. 1. Map showing the geographical localization of feline colonies included in this study (Abruzzi region, Central Italy).

Table 1
Groups classification.

Group A (alive cats)	
Subgroup RC (random household cats)	**127**
Subgroup C (cats from colonies)	**69**
C1	52
C2	14
C3	3
Group B (carcasses)	
Subgroup RCC (random carcasses of household cats)	**28**
Subgroup CC (carcasses from colonies)	**7**
CC1	5
CC2	2

2.5. Virus isolation

Virus isolation was attempted on feline embryonic fibroblast (FEA) cells from all $qPCR_{FeMV}$-positive urine samples following procedures recently described by our group (Donato et al., 2019). Serial blinded passages (up to the 5th) were performed every ten days.

2.6. Phylogeny

Phylogenetic analyses were performed by using the amplified portion of the L protein encoding genes obtained by RT-PCR_{Lgene} ($Phyl_{Lgene}$) and the complete genomes obtained by NGS ($Phyl_{complete}$). Phylogenetic analyses were performed recruiting all homologous publicly available FeMV sequences. $Phyl_{Lgene}$ was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). $Phyl_{complete}$ was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000). Both evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

2.7. Serological analysis

Indirect immunofluorescence (IIF) was performed to detect antibodies (Ab) in serum samples from cats of group A (n = 196). FeMV-*N*-expressing HeLa cells and rabbit polyclonal antibody against FeMV-N protein (kindly provided by Dr Shigeru Morikawa, National Institute of Infectious Diseases, Tokyo) were used for the IIF test as previously described (Park et al., 2016). Serum samples were serially diluted in phosphate-buffered saline (PBS) starting from a dilution of 1:40 to 1:20,480. Sera were applied to the wells of FeMV-*N*-expressing HeLa cells and let them react for 1 h at 37 °C in a humidified chamber. After washing in PBS, 1:32 diluted rabbit anti-cat-IgG FITC conjugated (Cappel) was applied and incubated for 1 h at 37 °C. Rabbit polyclonal antibody against FeMV-N protein and goat anti-rabbit-IgG (Sigma Aldrich) conjugated with FITC were used as the positive control. Uninfected cells were used as negative controls. After washing in PBS, the slides were examined under a Zeiss AxioVert.A1 fluorescence microscope and imaged using a Leica TCS SP5 II confocal laser scanning microscope. Sera showing Ab titres of less than 1:40 were considered negative.

2.8. Histological examination and immunohistochemistry

All tissues which resulted positive by $qPCR_{FeMV}$ and all kidney and bladder sections (regardless of the $qPCR_{FeMV}$ results) were evaluated by hematoxylin and eosin stain (HE) and immunohistochemistry (IHC). Formalin-fixed and paraffin-embedded samples were cut into 3-µm-thick sections, mounted on positive charged glass slides, dewaxed and rehydrated using standard procedures and stained by HE. For IHC, 3-µm-thick tissue sections were dewaxed and rehydrated and then incubated with 3% hydrogen peroxide diluted in absolute methanol for 30 min to inhibit endogenous peroxidase activity and 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 with 20% normal swine serum (Dako, Copenhagen, Denmark) diluted in TBS for 20 min, followed by a second incubation with 10% skimmed milk diluted in TBS for 20 min at room temperature. Then tissue sections were incubated overnight in a humidified chamber at 4 °C with the rabbit polyclonal antibody against the N protein of FeMV. After three washing in TBS, tissues sections were incubated with an HRP-labeled polymer conjugated with the secondary anti-rabbit antibody (REAL™ Envision detection system peroxidase Dako, Copenhagen, Denmark) for 30 min. Immune reactions were detected by means of a peroxidase technique (REAL™ Envision detection system peroxidase Dako, Copenhagen, Denmark) and visualized using 3-3′-diaminobenzidine as chromogen. 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. To analyse the association between FeMV and renal damage, two independent analyses were performed. In the first, FeMV was analysed in association with all observed renal lesions including TIN, whereas in the second, only the association between FeMV and TIN was investigated. All analyses were made by using Fisher test instead of a Chi-Squared test as we were managing a small sample size.

2.9. Virus histochemistry

To investigate the tissue tropism of FeMV, virus histochemistry (VHC) was performed with feline formalin fixed and paraffin embedded tissues including brain (cerebrum and cerebellum), lung and kidney. Published protocols established for influenza viruses (Eriksson et al., 2018; van Riel et al., 2006, 2007) were used with minor modifications (Di Teodoro et al., 2019). All feline tissues used in this experiment derived from three cats included in this study which tested negative for FeMV Abs and for FeMV and antigen by $qPCR_{FeMV}$ and IHC, respectively. Briefly, 3-µm-thick sections of the above mentioned paraffin embedded tissues were dewaxed and rehydrated using graded alcohols, blocked for endogenous peroxidase in 3% hydrogen peroxide diluted in absolute methanol for 30 min and incubated overnight at 4 °C with 200 µl FeMV/Pepito/2018 (10^5 $TCID_{50}$ mL; Donato et al., 2019, MK088517). The primary antibody anti-FeMV (rabbit polyclonal antibody against the N antigen of FeMV) diluted 1:1000 in 20% swine normal serum (Dako, Copenhagen, Denmark) was added and incubated 1 h at room temperature. Immune reactions were detected by means of a peroxidase technique (REAL™ Envision detection system peroxidase Dako, Copenhagen, Denmark) and visualized using 3-3′-diaminobenzidine as chromogen. Suitable positive and negative controls were also included in each run. The specificity of the immunolabeling was also verified by incubating homologous paraffin embedded tissue sections of different animal species (dog, cattle, sheep, swine and poultry) with the formalin inactivated FeMV/Pepito/2018 following procedures previously described.

3. Results

3.1. Prevalence of FeMV is higher in cats from colonies

Overall, a total number of 33 live cats tested positive for FeMV RNA. The 31.8% of urine samples (22/69 cats; IC 95% 22.1–43.6) of cats belonging to subgroup C (cats from colonies) tested positive for FeMV RNA, the 8.66% (11/127 cats; IC 95% 4.9–14.9) of subgroup RC (household cats). Within FeMV positive cats of subgroup RC, 2/11 were from the VUH of Bologna, 9/11 from the VUH of Teramo. Threshold cycle (C_T) values are reported in Table S1. The higher prevalence in subgroup C, compared to subgroup RC, was statistically significant (chi square test = 17.2, $p < 0.001$). FeMV RNA was not detected in blood samples. Within carcasses of group B, the 71.4% (5/7; IC 95% 35–91) of carcasses of subgroup CC (carcasses from colonies) tested positive for FeMV RNA in at least one tissue sample, the 10.71% (3/28; IC 95% 4–27) of subgroup RCC (random carcasses). Out of 8 positive carcasses, kidneys from 7 carcasses, urinary bladders (n = 4), spleen (n = 3), brain (n = 1), and mesenteric lymph nodes (1/1) tested positive by $qPCR_{FeMV}$ (for further details Table S2) titres generally low. One cat of subgroup RCC showed low FeMV RNA titres in the brain. All 5 FeMV-positive carcasses of subgroup CC were from cats which were demonstrated to shed FeMV RNA also *intra vitam* (group A, subgroup C). During necropsy of these 5 cats, approximately 8–10 months after the first molecular detection of FeMV *intra vitam*, a urine sample was collected from the urinary bladder. RNA was purified from it and tested by $qPCR_{FeMV}$ and $RT\text{-}PCR_{Lgene}$. C_T values and sequences of $RT\text{-}PCR_{Lgene}$ amplicons were identical to those obtained when the very first diagnosis of FeMV RNA was accomplished (data not shown). FeMV prevalence within age classes are showed in Fig. 2. Out of 226 (live cats plus carcasses) cats, the 17.0% of young cats (14/82 cats; IC 95%: 8.8–25.1), the 19.7% of middle-aged cats (18/91 IC 95%: 14.4–24.9) and 7.5% of older cats (4/53 IC 95%: 1.6–13,37) tested positive for FeMV RNA (Fig. 2).

3.2. Other feline pathogens were detected in $qPCR_{FeMV}$ positive carcasses

Three and four cats were found to be positive for FeLV RNA and FPLV DNA, respectively. One cat tested positive for feline coronavirus RNA and one cat for *Leishmania* spp. Three cats resulted simultaneously infected with more than one relevant pathogen (Table S5).

3.3. FeMV Sheryl/2018 Italy was isolated on cell culture

FEA cells inoculated with a urine sample tested positive for FeMV RNA (C_T value 31) showed cytopathic effect (CPE) at the 3rd passage on day 8. This CPE was characterized by cell rounding, detachment and lysis. At the 4th passage, small syncytia were evident at day 8. RNA purified from the supernatant and cell lysates of the 4th passage tested positive for FeMV RNA (C_T 28). However, when we tried to further propagate or titrate the virus, we had inconsistent results and CPE was rarely observed in further few attempts (data not shown). Though, whole genome sequence was obtained from nucleic acids purified from the supernatant of 4th cell passage (FeMV Sheryl/2018 Italy), deposited in GenBank (accession number MK188749) and used for $Phyl_{\text{-complete}}$.

3.4. Sequences of this study belongs to FeMV genotype 1

A total number of 36 partial L gene sequences were obtained by $RT\text{-}PCR_{Lgene}$ and used, together with homologous publicly available sequences, for $Phyl_{Lgene}$. According to the most inclusive partial L gene nt sequence analysis, sequences of this study cluster into two clades (clade 1a and 1b, Fig. 3a) composing FeMV genotype 1, distantly related to FeMV genotype 2 recently discovered in Germany. Italian sequences within the clade 1a and the 1b share the 95.4/100% and 98.1/100% of nt identity, respectively, whereas nt identity between the two clades

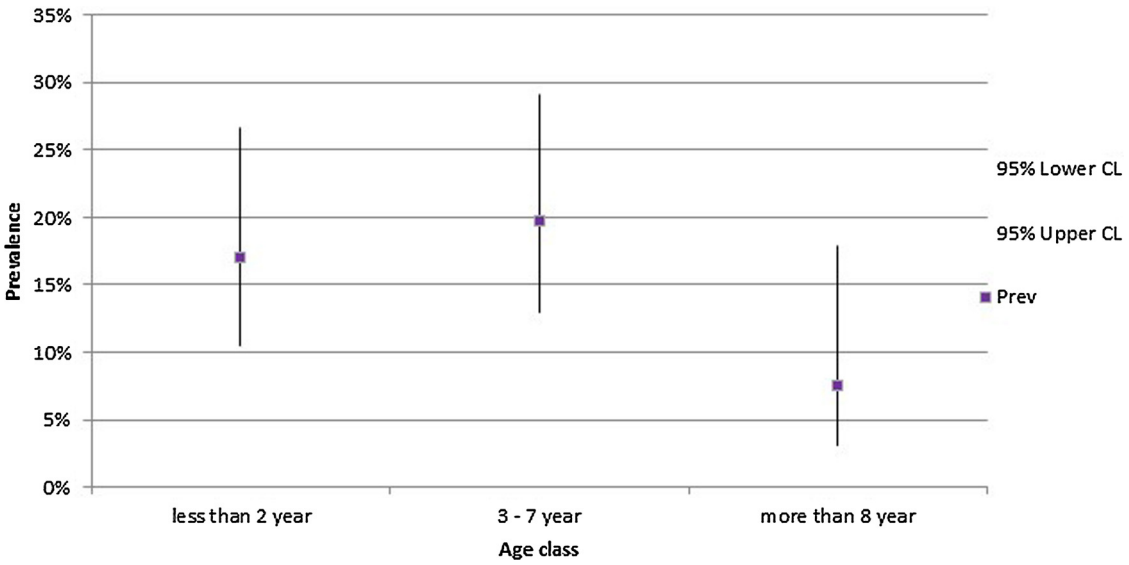

Fig. 2. qPCR$_{FeMV}$ positive cats according to age. Higher frequency of qPCR$_{FeMV}$ positive samples in middle-aged cats, compared to old cats, was statistically significant at $p < 0.05$ ($\chi 2 = 3,87$; p-value = 0,049; g.dl. = 1 ; $\alpha = 0,05$).

ranges from 87.8 to 91.1%. Italian sequences belonging to the first clade bear the highest nt sequence identity with FeMV strains 761U and 776U isolated in Hong Kong in 2009 (clade 1a, 95.4–97.3% and 94.3 to 96.2%, respectively) and with strain Piuma/2015 Italy (clade 1a, 95.4 to 100%). The lowest nt identity was evidenced with Japanese strains OtJP001 and SS1 (87.6–88.9 and 87.6–89.7%, respectively). Nt identity between sequences belonging to clade 1b and extant FeMV sequences ranges from 87.8 to 92.7%. This group shares only the 88.4–89.5% nt identity with the first discovered Italian FeMV Piuma/2015 strain. Sequences obtained from cats belonging to subgroup C1 cluster into both clades, showing an overall nt identity ranging from 87.8 to 89.2% between each other evidencing, apparently, the circulation in the same colony of two different viruses. Full-genome sequences were obtained from the FeMV isolate (FeMV Sheryl/2018 Italy) and from eight urine samples (group A, 8/9 and 1/9 from subgroup C1 and subgroup RC, respectively) and used for phylogenetic analysis (Phyl-$_{complete}$). The existence of two clades within FeMV genotype 1 evidenced in Phyl$_{-Lgene}$ is confirmed by the topology of Phyl$_{-complete}$ (Fig. 3b). Bioinformatic data from the NGS runs are available upon request. The nt identities (%) between full-genome sequences of this study and those available in GenBank database are reported in Table S4. Within the batch of whole genome sequences obtained from cats of subgroup C1, nt identity ranges from 98.7 to 100%; whereas 87.4–87.5% is the range of nt identity between them and with the unique whole genome sequence (Lilly/2018) obtained from subgroup RC, clade 1a.

3.5. 37 cats were positive for FeMV Abs, mostly middle-aged and old cats

In total, 37 cats were positive for FeMV Abs (Ab+). Urine samples of these cats were either positive (RNA+, n = 33) or negative (RNA-, n = 163) for FeMV RNA. IIF results were then combined with those obtained by qPCR$_{FeMV}$. Specifically, 21/33 and 16/163 live cats were Ab + for FeMV. Ab titres ranged between 1:80 to 1:10240. Four different patterns of infection were identified within the tested live cats of group A: pattern 1, RNA+/Ab+ (21 cats); pattern 2, RNA+/Ab- (12 cats); pattern 3 RNA-/Ab+ (16 cats) (Table S1), and pattern 4 RNA-/Ab- (147 cats). 15/21 RNA+/Ab + cats were from subgroup C (11/15 from C1; 3/15 from C2; 1/15 from C3) and 6/21 from subgroup RC. The remaining 16 RNA-/Ab + cats belonged to subgroup RC. 7/12 RNA+/Ab- cats were from C1, 5/12 from RC. An overall seroprevalence of 21.73% and 17.32% was demonstrated for subgroups C and RC, respectively. Data were also combined with the age of cats. FeMV serological prevalence was 17% in young cats (14/82 cats; IC 95%: 8.8–25.1), 25.27% in middle-aged cats (23/91 cats; IC 95%: 16.1–34.3) and 28% in older cats (15/53 cats; IC 95%: 15.9–40). Cats of pattern 1 and 2 were mostly young and middle-aged cats (11/21 young cats, 9/21 middle-aged cats, 1/21 old cats; 3/12 young cats, 7/12 middle-aged cats, 2/12 old cats, respectively), whereas cats of pattern 3 were mainly old cats (5/16 middle-aged cats and 11/15 old cats). Moreover, RNA+/Ab + cats of pattern 1 showed Ab titres higher (up to 1: 10240) than RNA-/Ab+ cats of pattern 3 (up to 1: 2560) (Fig. 4). These observations are consistent with the origin of cats. Pattern 1 and 2 were mainly composed by cats of colonies where the promiscuity enhances the chances of cats to get infected. Pattern 3 was composed by cats of subgroup RC, living indoor.

3.6. Association between FeMV and renal lesions and between FeMV and TIN was not evidenced

To analyse the relationship between virus infection and renal damage, histopathology was performed on all kidney tissues screened by qPCR$_{FeMV}$. According to the HE analysis, carcasses were divided in three patterns: RNA+/HE+ (7 cats), RNA-/HE+ (16 cats), RNA-/HE- (12 cats) (Table S3). Multifocal areas of subacute-to-chronic lymphoplasmacytic interstitial nephritis,tubular degeneration and necrosis were observed in 4/7 cats (Fig. 5a). Oxalate nephropathy with intratubular crystal deposits, microlithiasis, glomerular hyalinosis, glomerulo-nephrosis and tubulo-nephrosis were observed in 3/7 cats (Fig. 5b). As for the qPCR$_{FeMV}$-positive organs, 7/7 kidneys, 1/4 urinary bladders and 1/3 spleen, tested also positive by IHC for FeMV (Table S2).

IHC analysis of kidney sections of RNA + cats, revealed immunoreactivity within epithelial cells of renal tubuli and inflammatory cells (lymphoid and plasma cells) infiltrating the tubular and interstitial areas (Fig. 5c and Fig. 5d). In the RNA-/HE + pattern (n = 16 cats), microlithiasis, tubular and glomerular nephrosis and tubular lipidosis were detected; in one cat, nodular renal lymphoma was also observed. 10/16 cats showed multifocal lympho-plasmacytic interstitial nephritis. No lesions were observed in 12 cats, which tested also negative for FeMV RNA (RNA-/HE-). HE and IHC analyses were also performed on all qPCR$_{FeMV}$ positive tissues. The qPCR$_{FeMV}$ positive spleen showed hyalinosis, megakariocytosis and lymphocytic depletion (Fig. 6a) and FeMV immunoreactivity was observed within the cytoplasm of mononuclear cells, presumably lymphocytes (Fig. 6b). A qPCR$_{FeMV}$ positive

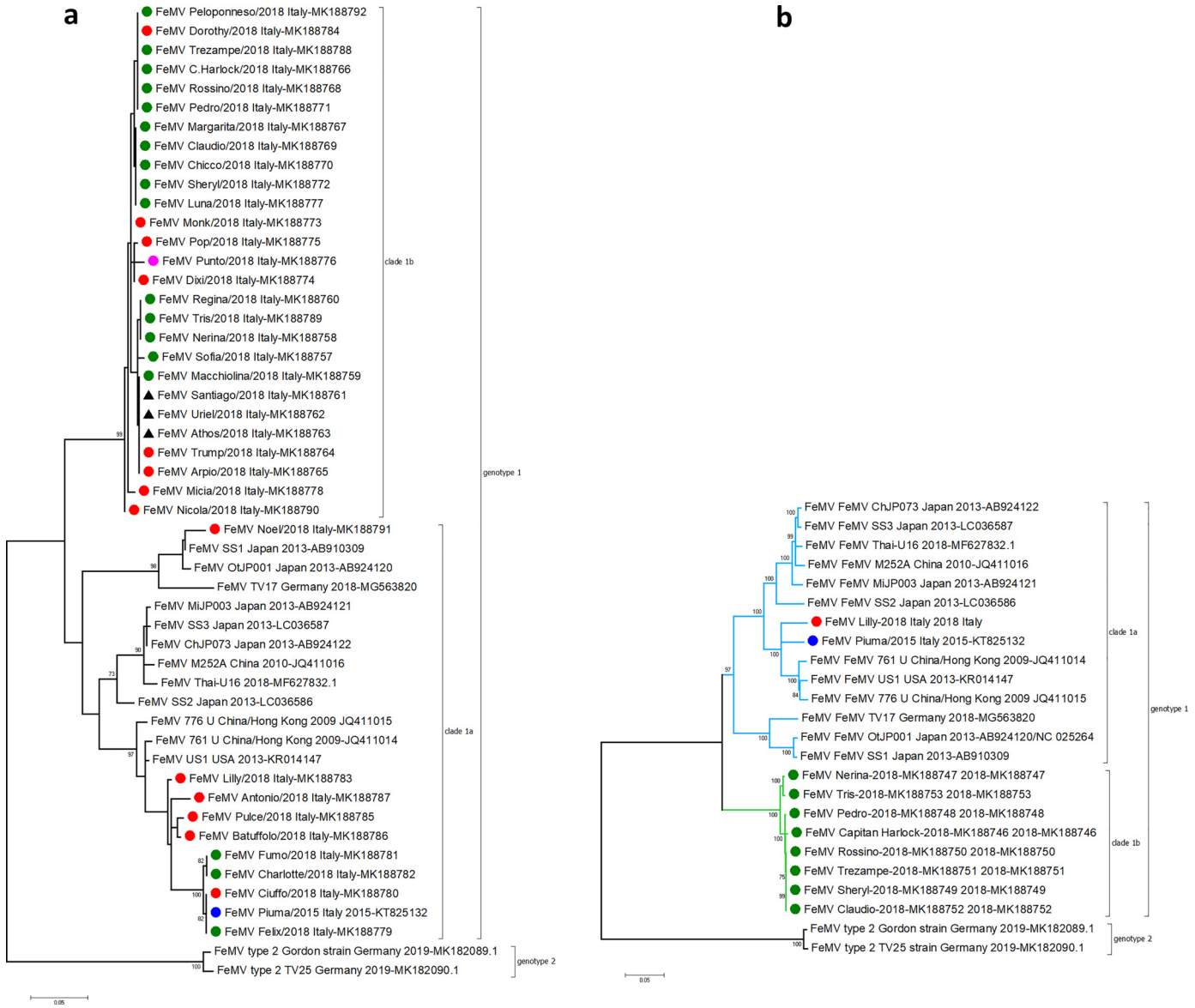

Fig. 3. (a) Phylogenetic analysis based on partial L nucleotide sequences ($Phyl_{Lgene}$) of 36 FeMV infected cats in this study. The analysis involved a total number of 51 nucleotide sequences. The tree with the highest log likelihood (-1519.1240) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3356)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 370 positions in the final dataset (b) Phylogenetic analysis based on full-genome sequences ($Phyl_{complete}$), obtained in this study and extant publicly available sequences. The analysis involved a total number of 24 nucleotide sequences. The tree with the highest log likelihood (-63742.7853) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5136)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 32.0329% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 15200 positions in the final dataset. Information upon strain names and accession numbers is listed in Tables S1-S3. Legend: green, subgroup C1; black, subgroup C2; pink, subgroup C3; red, subgroup RC; blue, FeMV Piuma/2015 Italy (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

urinary bladder without evidence of microscopic lesions (Fig. 6c), showed marked FeMV mucosal immunoreactivity (Fig. 6d). Although the evidence of renal lesions in all RNA + cats (Table 2), no statistically significant correlation was found between FeMV infection and renal lesions ($p = 0.0695$; Fisher exact test). TIN lesions were observed only in 4/7 RNA + cats and in 10/28 cats without evidence of FeMV infection (Table 3). No statistically significant correlation was found between FeMV infection and TIN ($p = 0.4007$; Fisher exact test). No immunoreactivity was observed in all tested $qPCR_{FeMV}$-negative urinary bladders (Fig. S1).

3.7. FeMV genotype 1 has in vitro tropism for epithelial cells of bronchioles and alveolar macrophages

VHC revealed specific immunoreactivity in lungs, kidneys and brain sections. FeMV particles were able to bind epithelial cells of bronchioles

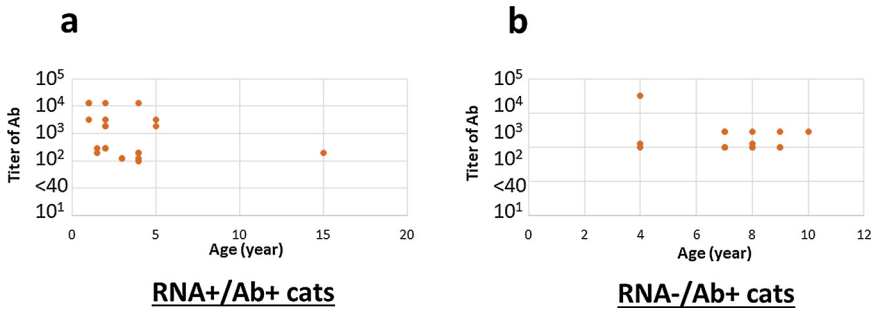

Fig. 4. Relationship between ages of FeMV infected cats and Ab titres. (a) RNA + /Ab + cats; (b) RNA-/Ab + cats.

and alveolar macrophages in lungs (Fig. 7a,b). In kidneys, FeMV antigens were attached on inflammatory cells in the lumens of tubuli and in correspondence of glomeruli (presumably inflammatory cells) (Fig. 7c,d). In brain tissues immunoreactivity was weak and occasionally seen in cerebellar granule cells and in inflammatory cells within blood vessels (Fig. 7e,f). Immunoreactivity was not evidenced in negative controls and non-feline animal species derived tissues (data not shown).

4. Discussion

In this study, we investigated the prevalence of FeMV in cats originating from two Italian regions, Abruzzi and Emilia-Romagna, and the association between FeMV and renal lesions, including TIN, the most frequent histopathological finding in feline CKD. Analyses were also performed from cats belonging to three different colonies located in the Abruzzi region. The prevalence of FeMV RNA positive urine samples was significantly higher ($p < 0.001$) in cats from colonies, compared to household cats. Reasonably, this difference is likely due to the higher probability of successful transmission occurring in cats of colonies. In this regard, young and middle aged cats of colonies are also the ones which mainly compose pattern 1 and 2, RNA + /Ab + and RNA + /Ab-, respectively, when virological and serological results were combined and analyzed together. On the other hand, pattern 3 (RNA-/Ab +) was mainly composed by old cats of subgroup RC, living indoor.

Phylogenetic analyses showed that all sequences of this study belong to the FeMV genotype 1 and within this genotype, they segregate into two clades. FeMV sequences of this study share indeed only the 78.2–78.6% of nt identity with FeMV GT2 sequences, a new genotype recently discovered in Germany. Results of this study also confirm the viral heterogeneity of FeMV circulating strains in accordance with other studies (Sakaguchi et al., 2014; Sieg et al., 2018). Genetic heterogeneity was remarkably demonstrated in cats from colony C1, apparently linked to the regular introductions of novel (infected) individuals to that colony from different sources.

This work has certainly some technical pitfalls. First, *in silico* analysis showed that forward primer and probe sequences of $qPCR_{FeMV}$ have one and two mismatches, respectively, with FeMV GT2, therefore we cannot exclude that $qPCR_{FeMV}$ negative samples were instead positive for FeMV GT2. This highly speculative scenario may have been responsible for the underestimation of FeMV prevalence in cats. All samples were indeed tested by this molecular assay developed prior to the discovery of FeMV GT2. Second, we did not perform NGS with nucleic acids purified from all $qPCR_{FeMV}$ negative samples, thus divergent viruses, including feline paramyxovirus (FPaV, Sieg et al., 2015) or potentially novel FeMV genotypes may have been missed.

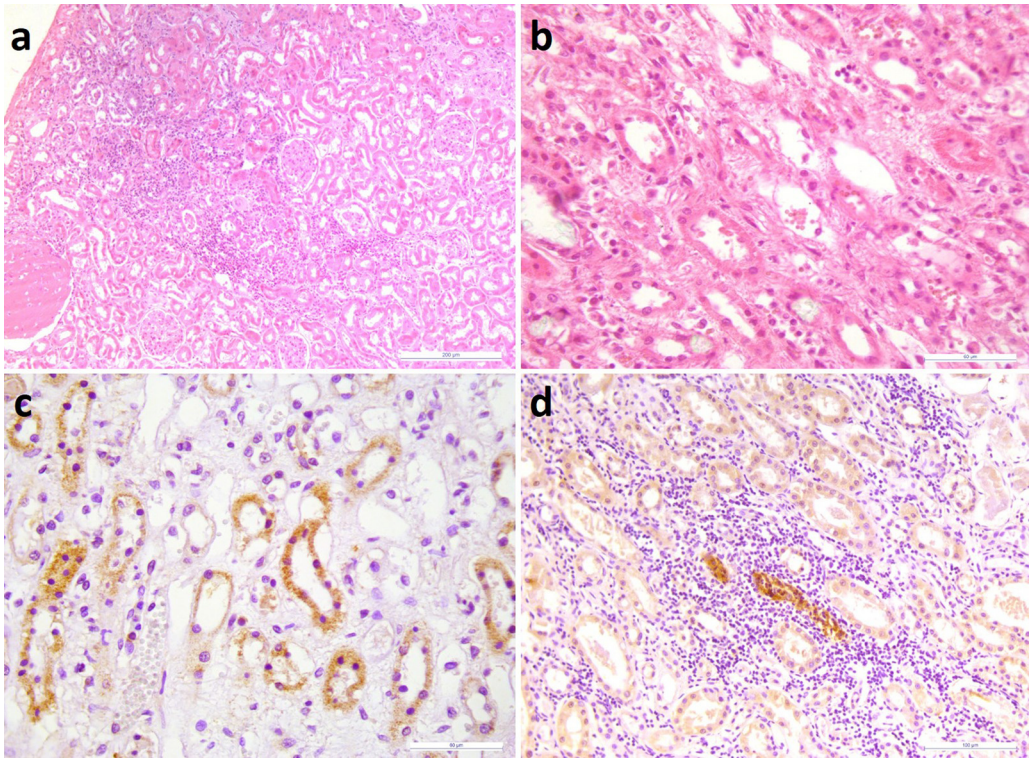

Fig. 5. Histological and immunoistochemical investigations, cat, kidney. (a) Tubulointerstitial nephritis with aggregates of lympho-plasmacytic cells around tubules of the cortex ("Batuffolo 9216/2016", see table S2 for further details). (b) Oxalate nephropathy with intratubular crystalline deposits, ("Monck9257/2016", see table S2 for further details); (c) FeMV antigen is visible in the tubular epithelial cells ("Monck 9257/2016", see table S2); (d) FeMV antigen is observed in necrotic residual tubular cells intermingled with a prominent inflammatory infiltration ("Batuffolo 9216/2016", see table S2). Haematoxilin and eosin stain (HE), final magnification X100 (a) and X400 (b) . Immunohistochemistry (IHC), Mayer's haematoxylin counterstain, final magnification X400 (c) and X200 (d).

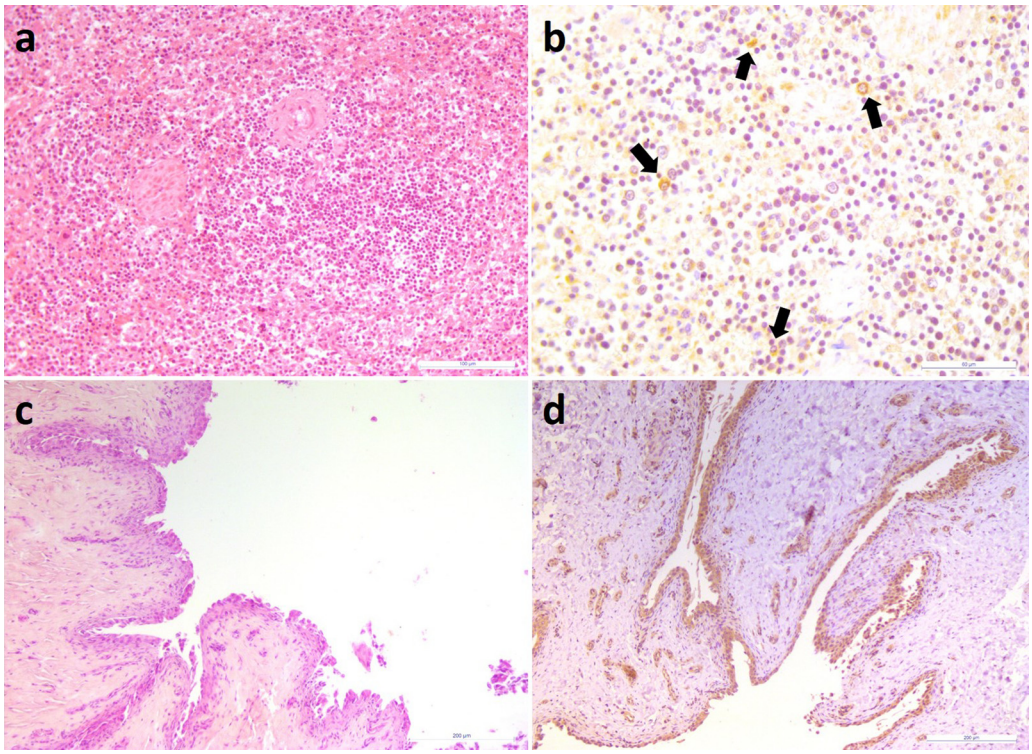

Fig. 6. Histological and immunoistochemical investigations, cat, spleen (a, b) and urinary bladder (c, d). (a) A moderate lymphocytic depletion is observed; and (b) immunoreactivity for FeMV is visible in mononuclear cells of spleen (Batuffolo 9216/2016, see table S2) (c) Urinary bladder without histopathological lesions (Chicco 29045/2016); (d) FeMV antigen in the mucosal layer (Chicco ID: 29045/2016). Haematoxilin and eosin stain (HE), final magnification X200 (a) and X100 (c). Immunohistochemistry (IHC), Mayer's haematoxylin counterstain, final magnification X400 (b) and X100 (d).

Table 2
Association between renal lesions and FeMV infection.

	Renal Lesions +	Renal lesions -	Total
FeMV RNA +	7	0	7
FeMV RNA -	16	12	28
Total	23	12	35

Table 3
Association between TIN and FeMV infection. TIN: Tubulointerstitial nephritis.

	TIN +	TIN -	Total
FeMV RNA +	4	3	7
FeMV RNA -	10	18	28
Total	14	21	35

Third, a complete virological follow up of cats from colonies was not performed for several reasons, including adoptions, spontaneous leaving and deaths by accidents with cars. A follow up was possible only from the 5 cats of colonies which succumbed 8–10 months later after the evidence of FeMV RNA *intra vitam*. Urine samples were still positive for FeMV RNA, confirming the long lasting shedding of FeMV (Sharp et al., 2016; De Luca et al., 2018).

Isolation of FeMV in cell cultures has been confirmed, in this study, to be, in general, difficult and time consuming (Sagaguchi et al., 2014; Woo et al., 2012). Although in a recent study we described the isolation of two FeMV strains (Donato et al., 2019) showing clear viral syncytia at the first cell passage, CPE characteristic of all morbilliviruses, in this occasion it was possible to observe CPE (cell rounding, detachment and lysis) in FEA cells only from 1/33 FeMV RNA positive urine sample after two blind passages of ten days each. Syncytia were visible only at the 4th passage but then CPE was not observed in the following passages. This apparent inefficiency in virus isolation may depend either from the biological characteristics of the virus possibly combined with the absence of a proper viable cell line, or from the timing of sample collection or from inappropriate sample storage. In general, in Morbillivirus diagnostics, viral isolation is still considered the "gold standard", even though efficient *in vitro* isolation and propagation of live virus is difficult to achieve. Hence, the chance that FeMV was an uncultivable virus, characteristics of many other viruses affecting domestic carnivores (Lorusso et al., 2008; Decaro and Buonavoglia, 2008; Decaro et al., 2014; Zaccaria et al., 2016), was unlikely, even more in light of the successful isolation of FeMV performed by our study group and other worldwide. In this regard, isolation and propagation in cells of a FeMV close relative, CDV have been for decades difficult to be achieved (Appel et al., 1992; Metzler et al., 1984; Seki et al., 2003; Shin et al., 1997). However less than twenty years ago, a Vero cell line stably expressing the canine signalling lymphocyte activation molecule (SLAM; CD150) was established and since then the efficiency of isolation and propagation of wild type CDVs strains greatly improved (Seki et al., 2003). However, we were not able to observe CPE or to rescue live FeMV from Vero cells expressing canine SLAM (data not shown), unlikely other morbilliviruses such as dolphin morbillivirus which was successfully isolated on these cells (Peletto et al., 2018). Thus, it would be beneficial to establish a homologous cell system stably expressing the feline SLAM and attempt isolation and propagation of FeMV RNA positive biological samples, including tissues (eg kidneys). Timing of sample collection may be also responsible for failure of virus isolation taking into account for the apparent chronic nature of FeMV infection evidenced by the long-lasting RNA shedding (De Luca et al., 2018; Sharp et al., 2016) and importantly by the low viral titres which are constantly detected by the used molecular assay. Low viral titres may account, *per se*, for failure of virus isolation. As evidenced for other viruses such as canine parvovirus type 2, virus isolation may give positive results only for few days post infection despite the high DNA viral titres present in the feaces of infected dogs likely due to antibodies in the intestinal lumen (and so the faeces) which may bind virions and prevent viral attachment to cell receptors (Desario et al., 2005; Decaro and Buonavoglia, 2017). All blood samples tested negative for FeMV RNA including those of the 12 cats of pattern 2 (RNA+/Ab-) supposedly to be individuals in the early phase of infection as suggested by the absence of FeMV antibodies yet with very low viral titres in the urine. One could reasonably argue that successful isolation was

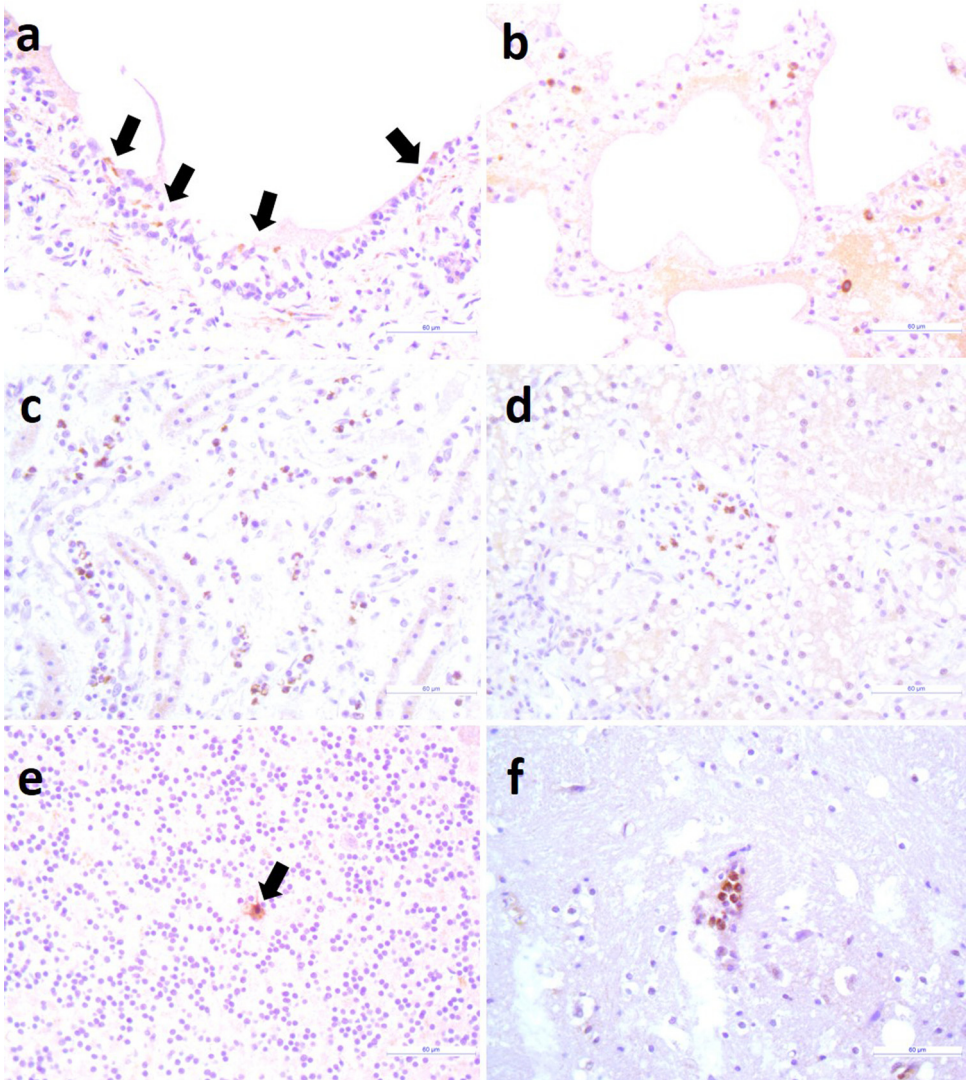

Fig. 7. Attachment (brown color) of FeMV for feline tissues. Virus particles were evidenced on the surface of epithelial cells of bronchioles (black arrows, a) and adhered on the alveolar macrophages (b). Extensive attachment was observed in inflammatory cells residing within kidney tubuli and glomeruli (c, d respectively). A weak and scattered FeMV attachment was detected in granule cells (e) and in inflammatory cells in the lumen of blood vessels (f) in feline brain tissues. Virus histochemistry (VHC). Mayer's haematoxylin counterstain, final magnification X400.

achieved, by our group, at the first cell passage from two fresh urine samples which also showed low RNA titres (Donato et al., 2019). Truth to be told, we do not have any explanations for this phenomenon. It is known that cell-to-cell spread of morbilliviruses in tissue culture was related to the capability of the virus to induce membrane fusion. H protein determines the extent and efficiency of cell- cell fusion, resulting in decisive influence on the CPE. We can only hypothesize the existence of FeMV strains with different *in vitro* properties and that each FeMV strain has different efficacy in fusion to host cells presenting a possible rationale why marked CPE is induced by some FeMV strains but not others as already speculated for different strains of CDV (von Messling et al., 2001; Lan et al., 2005). In this regard, the two FeMV strains which were isolated (genotype 1, clade 1a; Donato et al., 2019) are almost identical within each other and more distantly related to FeMV Sheryl/2018 Italy (87% of nt identity). Proper sample storage may also play a role for successful isolation, with particular emphasis for morbilliviruses. As for this, we attempted isolation from fresh urine samples diluted 1:8 in MEM or after refrigeration at 4 °C for a few days. This temperature was demonstrated to keep FeMV stable for at least twelve days (Koide et al., 2015).

Although performed with a limited number of carcasses, no statistically significant correlation was found between FeMV infection and renal lesions, including TIN. TIN was evidenced also in FeMV-negative cats. This finding, in line with recent reports from other study groups (Furuya et al., 2014; Sakaguchi et al., 2014; Park et al., 2016; Darold et al., 2017; Yilmaz et al., 2017; Stranieri et al., 2019; Mohd et al., 2019), apparently seems to undervalue the importance of FeMV in feline medicine. FeMV RNA was also revealed in the urinary bladder of FeMV-positive carcasses as also evidenced by IHC in Fig. 6d. This suggests that FeMV may have also tropism for receptors (e.g. nectin4) present in transitional epithelium of the bladder, scenario already described for CDV (Pratakpiriya et al., 2017). FeMV RNA could not be detected in the blood samples collected in this study, indicating that the viraemia might not be persistently observed in the infected cats or the viraemia duration was short. However, as suggested by the VHC, FeMV exhibits tropism for inflammatory cells residing in blood vessels of kidney and brain, airway epithelial cells, alveolar macrophages and, with a lesser extent, the central nervous system. In this regard, FeMV-GT2 was also demonstrated to replicate in primary feline epithelial cells from different organs and was also able to infect primary feline T and B cells, monocytes and brain tissues (Sieg et al., 2019). This aspect combined with the evidence of one cat showing low FeMV RNA titres in

the brain tissue, may suggest a role of FeMV in pathological processes in other organs or districts during the acute phase of infection or in individuals with compromised immune system triggered by other pathogens whose presence was evidenced in carcasses analyzed of this study. Considering that only few organs collected from carcasses tested positive for FeMV RNA, generally kidneys, a likely evidence of chronic infection, *in vivo* experimental infection of the natural host or of a susceptible animal model is warranted in order to examine the pathogenesis of FeMV, viral tropism and immune response during the early stages of infection. The analysis of pathogenesis and viral kinetics may be also beneficial for the development of a targeted sampling in field conditions. Moreover, in order to disentangle the role of FeMV in co-infection processes, we strongly believe that FeMV specific molecular tests are needed to be included in the diagnostic workflow of cats in clinical practice.

Declaration of competing interest

The authors declare that they have no competing interests. The funding body has no specific role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.108484.

References

Appel, M.J., Pearce-Kelling, S., Summers, B.A., 1992. Dog lymphocyte cultures facilitate the isolation and growth of virulent canine distemper virus. J. Vet. Diagn. Invest. 4, 258–263.

Chakrabarti, S., Syme, H.M., Brown, C.A., Elliott, J., 2013. Histomorphometry of feline chronic kidney disease and correlation with markers of renal dysfunction. Vet. Pathol. 50, 147–155.

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, 469–475.

Decaro, N., Buonavoglia, C., 2008. An update on canine coronaviruses: viral evolution and pathobiology. Vet. Microbiol. 132 (3–4), 221–234. https://doi.org/10.1016/j.vetmic.2008.06.007.

Decaro, N., Martella, V., Desario, C., Lanave, G., Circella, E., Cavalli, A., Elia, G., Camero, M., Buonavoglia, C., 2014. Genomic characterization of a circovirus associated with fatal hemorrhagic enteritis in dog, Italy. PLoS One 9 (8), e105909. https://doi.org/10.1371/journal.pone.0105909.

Decaro, N., Buonavoglia, C., 2017. Canine parvovirus post-vaccination shedding: interference with diagnostic assays and correlation with host immune status. Vet. J. 221, 23–24. https://doi.org/10.1016/j.tvjl.2017.01.020.

De Luca, E., Crisi, P.E., Di Domenico, M., Malatesta, D., Vincifori, G., Di Tommaso, M., Di Guardo, G., Di Francesco, G., Petrini, A., Savini, G., Boari, A., Lorusso, A., 2018. A real-time RT-PCR assay for molecular identification and quantitation of feline morbillivirus RNA from biological specimens. J. Virol. Methods 258, 24–28.

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

Desario, C., Decaro, N., Campolo, M., Cavalli, A., Cirone, F., Elia, G., Martella, V., Lorusso, E., Camero, M., Buonavoglia, C., 2005. Canine parvovirus infection: which diagnostic test for virus? J. Virol. Methods 126, 179–185.

Di Teodoro, G., Bortolami, A., Teodori, L., Leone, A., D'Alterio, N., Malatesta, D., Rosamilia, A., Colaianni, M.L., Petrini, A., Terregino, C., Savini, G., Bonfante, F., Lorusso, A., 2019. Replication kinetics and cellular tropism of emerging reoviruses in sheep and swine respiratory ex vivo organ cultures. Vet. Microbiol. 234, 119–127.

Donato, G., De Luca, E., Crisi, P.E., Pizzurro, F., Masucci, M., Marcacci, M., Cito, F., Di Sabatino, D., Boari, A., D'Alterio, N., Pennisi, M.G., Lorusso, A., 2019. Isolation and genome sequences of two Feline Morbillivirus genotype 1 strains from Italy. Vet. Ital. 55, 179–182.

Elliott, J., Barber, P.J., 1998. Feline chronic renal failure: clinical findings in 80 cases diagnosed between 1992 and 1995. J. Small Anim. Pract. 39, 78–85.

Eriksson, P., Lindskog, C., Engholm, E., Blixt, O., Waldenstrom, J., Munster, V., Lundkvist, A., Olsen, B., Jourdain, E., Ellstrom, P., 2018. Characterization of avian influenza virus attachment patterns to human and pig tissues. Sci. Rep. 8, 12215.

Furuya, T., Sassa, Y., Omatsu, T., Nagai, M., Fukushima, R., Shibutani, M., Yamaguchi, T., Uematsu, Y., Shirota, K., Mizutani, T., 2014. Existence of feline morbillivirus infection in Japanese cat populations. Arch. Virol. 159, 371–373.

Jepson, R.E., 2016. Current understanding of the pathogenesis of progressive chronic kidney disease in cats. Vet. Clin. North Am. Small Anim. Pract. 46, 1015–1048.

Koide, R., Sakaguchi, S., Miyazawa, T., 2015. Basic biological characterization of feline morbillivirus. J. Vet. Med. Sci. 77 (5), 565–569.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Lan, N.T., Yamaguchi, R., Kai, K., Uchida, K., Kato, A., Tateyama, S., 2005. The growth profiles of three types of canine distemper virus on Vero cells expressing canine signaling lymphocyte activation molecule. J. Vet. Med. Sci. 67 (5), 491–495.

Lorusso, A., Decaro, N., Schellen, P., Rottier, P.J., Buonavoglia, C., Haijema, B.J., de Groot, R.J., 2008. Gain, preservation, and loss of a group 1a coronavirus accessory glycoprotein. J. Virol. 82 (20), 10312–10317. https://doi.org/10.1128/JVI.01031-08.

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, 160–163.

Marino, C.L., Lascelles, B.D., Vaden, S.L., Gruen, M.E., Marks, S.L., 2014. Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies. J. Feline Med. Surg. 16, 465–472.

Mayer-Roenne, B., Goldstein, R.E., Erb, H.N., 2007. Urinary tract infections in cats with hyperthyroidism, diabetes mellitus and chronic kidney disease. J. Feline Med. Surg. 9, 124–132.

Metzler, A.E., Krakowka, S., Axthelm, M.K., Gorham, J.R., 1984. In vitro propagation of canine distemper virus: establishment of persistent infection in Vero cells. Am. J. Vet. Res. 45, 2211–2215.

McCallum, K.E., Stubbs, S., Hope, N., Mickleburgh, I., Dight, D., Tiley, L., Williams, T.L., 2018. Detection and seroprevalence of morbillivirus and other paramyxoviruses in geriatric cats with and without evidence of azotemic chronic kidney disease. J. Vet. Intern. Med. 32, 1100–1108.

Mohd, I.N.H., Selvarajah, G.T., Khor, K.H., Tan, S.W., Manoraj, H., Omar, N.H., Omar, A.R., Mustaffa-Kamal, F., 2019. Molecular detection and characterisation of feline morbillivirus in domestic cats in Malaysia. Vet. Microbiol. 236, 108382. https://doi.org/10.1016/j.vetmic.2019.08.005.

Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Park, E.S., Suzuki, M., Kimura, M., Mizutani, H., Saito, R., Kubota, N., Hasuike, Y., Okajima, J., Kasai, H., Sato, Y., Nakajima, N., Maruyama, K., Imaoka, K., Morikawa, S., 2016. Epidemiological and pathological study of feline morbillivirus infection in domestic cats in Japan. BMC Vet. Res. 12, 228.

Peletto, S., Caruso, C., Cerutti, F., Modesto, P., Biolatti, C., Pautasso, A., Grattarola, C., Giorda, F., Mazzariol, S., Mignone, W., Masoero, L., Casalone, C., Acutis, P.L., 2018. Efficient isolation on Vero.DogSLAMtag cells and full genome characterization of Dolphin Morbillivirus (DMV) by next generation sequencing. Sci. Rep. 8 (1), 860. https://doi.org/10.1038/s41598-018-19269-2.

Pratakpiriya, W., Ping, T.A.P., Radtanakatikanon, A., Pirarat, N., Thi Lan, N., Takeda, M., Techangamsuwan, S., Yamaguchi, R., 2017. Expression of canine distemper virus receptor nectin-4 in the central nervous system of dogs. Sci. Rep. 7, 349. https://doi.org/10.1038/s41598-017-00375-6.

Rodgers, M.R., Popper, S.J., Wirth, D.F., 1990. Amplification of kinetoplast DNA as a tool in the detection and diagnosis of Leishmania. Exp. Parasitol. 71, 267–275.

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, 1464–1468.

Seki, F., Ono, N., Yamaguchi, R., Yanagi, Y., 2003. Efficient isolation of wild strains of canine distemper virus in Vero cells expressing canine SLAM (CD150) and their adaptability to marmoset B95a cells. J. Virol. 77, 9943–9950.

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.

Shin, Y.S., Mori, T., Tomonaga, K., Iwatsuki, K., Kai, C., Mikami, T., 1997. Expression of the nucleocapsid protein gene of the canine distemper virus. J. Vet. Med. Sci. 59, 51–53.

Sieg, M., Busch, J., Eschke, M., Bottcher, D., Heenemann, K., Vahlenkamp, A., Reinert, A., Seeger, J., Heilmann, R., Scheffler, K., Vahlenkamp, T.W., 2019. A new genotype of feline morbillivirus infects primary cells of the lung, kidney, brain and peripheral blood. Viruses 11.

Sieg, M., Heenemann, K., Ruckner, A., Burgener, I., Oechtering, G., Vahlenkamp, T.W.,

2015. Discovery of new feline paramyxoviruses in domestic cats with chronic kidney disease. Virus Genes 51, 294–297.
Sieg, M., Vahlenkamp, A., Baums, C.G., Vahlenkamp, T.W., 2018. First complete genome sequence of a feline morbillivirus isolate from Germany. Genome Announc. 6.
Stoddard, R.A., Gee, J.E., Wilkins, P.P., McCaustland, K., Hoffmaster, A.R., 2009. Detection of pathogenic Leptospira spp. Through TaqMan polymerase chain reaction targeting the LipL32 gene. Diagn. Microbiol. Infect. Dis. 64, 247–255.
Stranieri, A., Lauzi, S., Dallari, A., Gelain, M.E., Bonsembiante, F., Ferro, S., Paltrinieri, S., 2019. Feline morbillivirus in Northern Italy: prevalence in urine and kidneys with and without renal disease. Vet. Microbiol. 233, 133–139.
Sutummaporn, K., Suzuki, K., Machida, N., Mizutani, T., Park, E.S., Morikawa, S., Furuya, T., 2019. Association of feline morbillivirus infection with defined pathological changes in cat kidney tissues. Vet. Microbiol. 228, 12–19.
Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.
Tatsuo, H., Ono, N., Yanagi, Y., 2001. Morbilliviruses use signaling lymphocyte activation molecules (CD150) as cellular receptors. J. Virol. 75, 5842–5850.
van Riel, D., Munster, V.J., de Wit, E., Rimmelzwaan, G.F., Fouchier, R.A., Osterhaus, A.D., Kuiken, T., 2006. H5N1 virus attachment to lower respiratory tract. Science 312, 399.
van Riel, D., Munster, V.J., de Wit, E., Rimmelzwaan, G.F., Fouchier, R.A., Osterhaus, A.D., Kuiken, T., 2007. Human and avian influenza viruses target different cells in the lower respiratory tract of humans and other mammals. Am. J. Pathol. 171, 1215–1223.
von Messling, V., Zimmer, G., Herrler, G., Haas, L., Cattaneo, R., 2001. The hemagglutinin of canine distemper virus determines tropism and cytopathogenicity. J. Virol. 75, 6418–6427.
White, J.D., Norris, J.M., Baral, R.M., Malik, R., 2006. Naturally-occurring chronic renal disease in Australian cats: a prospective study of 184 cases. Aust. Vet. J. 84, 188–194.
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, 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. 19, 1206–1214.
Zaccaria, G., Malatesta, D., Scipioni, G., Di Felice, E., Campolo, M., Casaccia, C., Savini, G., Di Sabatino, D., Lorusso, A., 2016. Circovirus in domestic and wild carnivores: an important opportunistic agent? Virology 490, 69–74. https://doi.org/10.1016/j.virol.2016.01.007. In this issue.