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Feline morbillivirus in northwestern Italy: first detection of genotype 1-B

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1 **Genotype 1-B of Feline Morbillivirus in north-western Italy without evident relationship**
2 **with kidney diseases**

3

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24 **SUMMARY**

25 **Objectives:** A novel Morbillivirus was recently described in stray and domestic feline population
26 of Asia, America and Europe. Most cats infected with Feline Morbillivirus (FeMV) are affected by
27 low urinary tract or kidney's disease. However, although association of FeMV infection with
28 kidney diseases in cats has been suggested, the pathogenicity of the virus remains unclear.
29 The present study aimed to investigate the distribution of FeMV infection as well as the
30 relationship between FeMV infection and kidney diseases in cats of north-Western Italy.

31 **Methods:** A total of 153 samples of urine (150 individuals and 3 pools) and 50 samples of
32 kidneys were collected and included in the present study; total RNA was extracted and a reverse
33 transcription-quantitative PCR (rt-qPCR) was performed in order to identify FeMV. Kidneys were
34 also submitted to anatomopathological investigations. Phylogenetic analysis and isolation
35 attempts were carried out on positive samples. In FeMV positive cats' urinalysis and blood
36 analysis were performed.

37 **Results:** FeMV RNA was detected in 7.3% of urine samples and in 8% of the kidneys both in
38 healthy cats and in cats with clinical signs or post-mortem lesions compatible with kidneys'
39 disease. At histopathological examination kidneys showed tubule-interstitial nephritis (TIN) in
40 three out of four positive samples, but a clear relationship between FeMV infection and TIN was
41 not observed. Isolation attempts resulted unsuccessful, although the urine sample of one
42 castrated male cat hosted in a cattery showed a positive signal in rt-qPCR until the fourth cell
43 passage. Interestingly, in the same cattery a second variant was detected from a urine pool.
44 Urinalysis showed proteinuria in all cats while blood analysis reported altered creatinine levels
45 in two positive cats.

46 **Conclusion and Relevance:** Data reported suggests the presence of a FeMV sub-cluster well
47 distinct from the strain previously isolated in Italy whose role in renal disorders remains
48 uncertain.

49 **INTRODUCTION**

50 The genus *Morbillivirus* includes a consistent number of pathogens that cause some of the most
51 devastating viral diseases of humans and animals (i.e. Measles virus, Canine Distemper virus,
52 Rinderpest virus)¹. In 2012 a novel Morbillivirus has been discovered in stray cats' population of
53 Hong Kong and named Feline Morbillivirus (FeMV)². FeMV is a negative sense not-segmented
54 single stranded RNA virus whose genome of 16.050 bp was recently characterized³. It follows
55 the "rule of six", typical for morbilliviruses: 6 genes (N, P/V/C, M, F, H, L) coding for six structural
56 and two non-structural proteins. A not negligible number of FeMV infected cats in Japan
57 showed lower urinary tract diseases (LUTD) with typical tubulointerstitial nephritis (TIN)² but
58 possible correlations between renal diseases and FeMV infection remain still unclear⁴. In recent
59 years, different epidemiological studies were carried out in Japan, United Kingdom, Brasil,
60 Turkey, and Italy^{3,5-8}. From a diagnostic point of view, several biomolecular approaches were
61 developed and optimised for investigation purposes^{5,6,8-10}. Previous studies demonstrated that
62 urine and kidney represent the best targets for biomolecular detection of FeMV by rt-qPCR^{11,12}.
63 Concerning isolation procedures, FeMV was demonstrated to replicate into Crandell-Rees feline
64 kidney cells (CRFK)¹³, and into feline embryonic fibroblast (FEA) cells¹⁴ causing a discrete
65 cytophatic effect characterised by syncytia formation. However, long incubation periods for the
66 infected-CRFK and biological features of FeMV make isolation attempts time-consuming and
67 often unsuccessful¹². Indeed, Koide et al., (2015)¹³ demonstrated that viral titre increases
68 exponentially between the 18th and the 30th hour post-inoculation on CRFK. Conversely, no
69 increasing in virus titre was observed after 63 hours post-infection¹³. However, only few studies

70 on FeMV reported information about cat anamnesis or clinico-pathological features related to
71 FeMV and data regarding the correlation between kidney diseases and FeMV infection are
72 often discordant. The aim of this study was to investigate the presence of FeMV as well as the
73 relationship between FeMV infection and kidney disease in cats of North-Western Italy
74 performing biomolecular analysis on kidney and urine samples and histopathological
75 examination of kidney. Phylogenetic analysis was finally carried out to investigate genetic
76 correlations with other field strains from different countries.

77

78 **MATERIALS AND METHODS**

79 **SAMPLES COLLECTION**

80 **Urine**

81 A total of 150 individual urine samples were collected from cats hospitalised in a veterinary
82 clinic or hosted in a private cattery. Particularly, 127 client-owned cat and 23 cats from the
83 cattery were sampled. The private cattery hosted a total of 55 cats at the time of the study
84 divided in 4 rooms with an adequate number of food and water bowls, litter boxes and cat beds.
85 All the rooms had access to an external fenced area where the cats were free to move. No cats
86 were kept in cages. Moreover, in the same cattery, three pooled urine samples were collected in
87 order to rapidly screen the hosted population for FeMV as some cats of the cattery are feral cats
88 and cannot be manipulated without sedation. Urine pools were collected confining 10 cats per room
89 and placing different litter boxes in each room. All individual cats (n=150) included in the study were
90 clinically examined and sex, breed and age were recorded (Table 1). Concerning sex ratio, 68%
91 of the cats were male, mainly neutered, while 32% were female, mainly spayed. Urine samples
92 were collected from animals of ten different breeds (Table 1). Across the animals included in
93 this study, 21% of cats were affected by LUTD; acute and chronic urinary failures were detected

94 in 3% and 25% of cats, respectively. The remaining cats (51%) were healthy or affected by non-
95 urinary diseases. Urine samples were kept frozen at -80°C until the extraction of total RNA.

96 **Kidneys**

97 Fifty renal tissue samples were collected from cats necropsied at the Department of Veterinary
98 Science (University of Turin, Italy). Particularly, 40 kidney samples were from client-owned cats
99 and 10 kidney samples were from cattery cats. Sex, breed, age and available anamnestic
100 information were gathered (Table 1). Six different breeds were included. Concerning sex ratio,
101 50% were male, mainly and 50% were female, mainly unspayed. Both gross and
102 histopathological investigations were performed in order to describe *post-mortem* features of the
103 tested kidneys. Moreover, kidney samples were kept frozen at -80°C until total RNA extraction.

104 **BIOMOLECULAR ASSAYS**

105 Total RNA was extracted from 350µl of urine or 1 mg of renal tissue by using Trizol RNA
106 isolation reagents (Life Technologies, Carlsbad, CA) following the manufacturer's instructions
107 with a final elution in 30 µl DNase/RNase-free water. The extracted RNA was tested by One
108 Step rt-qPCR according to De Luca et al., (2018)¹², with minor modifications. The diagnostic
109 assay target was represented by a fragment of the P/V/C gene (74 bp) and the original protocol
110 was optimized for use with Superscript III Platinum One Step rt-qPCR System (Invitrogen,
111 Carlsbad, CA). The reaction volume of 25µl contained 5µl of purified total RNA, 12.5 µl of
112 Superscript III One-Step rt-qPCR Invitrogen Reaction Mix, a final concentration of 0.6 µM for
113 each primer (FeMV-rt Forward 5'- GGGATCCAGAGGGTAACCT -3' and FeMVrt Reverse 5'-
114 CCGGCCATTAATCTCTGAA -3'), 0.225 µM of FeMVrt TaqMan Probe (5'-
115 CCGGCCATTAATCTCTGAA -3'), 4 µM of MgSO₄, 0.5µl of Taq mix and nuclease-free water up
116 to the final volume. The assay was carried out on a CFX96 Touch™ rt-PCR Detection System
117 (Bio-Rad Laboratories Inc., Hercules, CA), setting the following thermal conditions: 1 cycle of

118 reverse transcription at 50°C for 30 min, 1 cycle of PCR initial activation step at 95°C for 15 min
119 followed by 45 cycles at 95°C for 30 s, 55 °C for 40 s and 60°C for 30 s.

120 **FeMV ISOLATION ATTEMPTS**

121 From each rt-qPCR positive sample, FeMV isolation was carried out according to Woo et al.
122 (2012)². Urine samples were diluted 1 to 10 into Minimum Essential Medium Earle (MEM-Earle,
123 Biowest, Riverside, MO) supplemented with a solution 5X of a stock 1000X of penicillin (100
124 units ml⁻¹) and streptomycin (100 µg ml⁻¹) (Sigma–Aldrich, St. Louis, MO). The mixture was
125 filtered through 450 nm disc filters (Millipore), further diluted 1 to 2 in MEM Earle supplemented
126 with 1µg/ml of Polybrene Infection Reagent (Sigma-Aldrich, St. Louis, MO) and inoculated into a
127 flask of 25cm² of CRFK cells. After 1 hour of incubation in gentle agitation at 37°C, the mixture
128 was decanted and the CRFK were washed two times with PBS 1X. Seven ml of MEM
129 supplemented with 0,1 µg/ml of *L*-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK)-
130 treated trypsin and with 2% heat-inactivated fetal bovine serum (Gibco, Thermofisher scientific,
131 Waltham, Massachusetts) and antibiotics (see above) were added directly on the monolayer.
132 The CRFK were incubated at 37 °C in a humidified atmosphere with 5% of CO₂ and observed
133 daily for cytophatic effect (CPE) by microscopy. Each passage consisted of twelve days of
134 incubation then the cell lysate was tested by FeMV rt-qPCR protocol¹² and further cells passages
135 were performed until the cell lysate stayed positive in biomolecular investigations. The Cq mean
136 values of cell passages were compared in order to semi-quantitatively detect a potential
137 increase in virus titre, indicative of efficient viral replication.

138 **MORPHOLOGICAL AND HISTOPATHOLOGICAL INVESTIGATIONS**

139 Systemic post-mortem examination was performed on the cats, and kidneys were grossly
140 evaluated. All the kidneys also underwent histopathological investigation in order to confirm
141 macroscopic diagnosis. Cross-sections of renal cortex, medulla and pelvis were collected, fixed

142 in 10% buffered formalin solution, paraffin embedded, sectioned using a microtome, and stained
143 with hematoxylin and eosin (HE). The samples were observed by means of light microscopy.
144 The histopathological evaluation was mainly focused on the presence of acute or chronic TIN
145 with associated glomerulopathies, sclerosis, calcifications, granulomas or tumor lesions.

146 **URINE AND BLOOD ANALYSIS**

147 Urinalysis was performed in rt-qPCR positive samples in order to try to correlate FeMV with
148 urinary tract infections and subsequent altered urine composition as reported by Yilmaz et al.,
149 (2017)⁸. Samples were obtained by cystocentesis or spontaneous urination. Each sample was
150 placed in a sterile universal tube and processed for physical and chemical analysis within 6
151 hours after sampling. Urinalysis was performed using dipstick tests (Multistix-10-SG, Siemens
152 Healthcare Diagnostics, Tarrytown, NY) read by Clinitek Status analyzer (Siemens Healthcare
153 Diagnostics, Tarrytown, NY). Urine specific gravity (USG) was measured by refractometer.
154 Microscopic examination of the urine sediment on 10 field at 100x magnification was also
155 performed in order to identify casts, crystals or epithelial cells. Moreover, two blood samples
156 were collected in positive cats: the first one was collected with urine samples (T0) and the
157 second one was collected one month later (T30). Blood samples were placed in sterile tubes to
158 evaluate blood creatinine (mg/dl) as a marker of renal function according to IRIS (International
159 Renal Interest Society) guideline¹⁵.

160 **PHYLOGENETIC ANALYSIS**

161 The total RNA of PCR positive samples was reverse transcribed by First Strand cDNA
162 Synthesis Kit (Roche, Basilea, Switzerland). The cDNA was subsequently used for the
163 amplification of a fragment (401 bp) of L gene using a double-step nested PCR, as described by
164 Furuya et al. (2014)⁵.

165 An agarose gel electrophoresis allowed to detect nested-PCR products with GelGreen® Nucleic
166 Acid Gel Stain (Biotium Inc., Fremont, CA, USA) using Uvitec technology for image capture. The
167 amplified fragment of 401 bp was sequenced after DNA purification from agarose gel, performed
168 by High Pure PCR Product Purification Kit (Roche, Basilea, Switzerland). The Sanger
169 sequencing was conducted using the BigDye® Terminator v3.1 Cycle Sequencing Kit on a 3130
170 Genetic Analyzer (Applied Biosystems, Forster City, CA, USA). Two software were used for
171 sequence analysis of the detected basis (Sequencing Analysis) and for the manual correction
172 (FinchTV, Geospiza, Inc), respectively. From the obtained sequences of the amplified products,
173 the primer sequences were removed and the software Clustal Omega¹⁶ allowed the alignment
174 with 30 sequences of FeMV genotypes available in the public database NCBI, and 3 sequences
175 from Lombardy¹⁷, kindly provided by Dr. Stefania Lauzi (Università degli Studi di Milano Statale,
176 Milan, Italy). For phylogenetic inference, the best nucleotide substitution model was estimated
177 by jModelTest²¹⁸. The phylogenetic tree was constructed by MrBayes v. 3.2.6¹⁹ with the
178 following settings: 2 parallel runs of 4 chains each for 5,000,000 generations and TPM1uf+Ias
179 nucleotide substitution model, as selected by jModelTest2, and a 10% burn-in to summarize
180 parameter and tree log files. Convergence and effective sample size (ESS) were controlled with
181 Tracer v1.7.1. The tree was then visualized with Figtree v1.4.4.

182

183 **RESULTS**

184 **Biomolecular assays**

185 Eleven out of 150 individual urine samples (5 client owned cats and 6 cats from the cattery) and
186 two urine pools resulted positive for FeMV RNA according to the cut-off level established by
187 Lorusso and colleagues (2015)¹⁰. As for kidney samples, 4 out of 50 (3 client owned cats and 1
188 cat from the cattery) were positive for FeMV RNA (Table 1).

189 **FeMV isolation attempts**

190 The entire panel of RT-Real-Time positive samples were used for isolation attempts;
191 nevertheless, just the urine sample of “cat Totò” showed a promising biomolecular outcome for
192 isolation purposes. The first two cell passages, tested by rt-qPCR showed Cq mean values
193 (23.48 and 24.22 respectively) higher than the starting urine sample (29.78). However, no
194 FeMV-associated cytophatic effect was observed during 13 days of daily observations.
195 Conversely, isolation attempts from the remaining positive samples (4 kidney and 11 urine
196 samples) were unsuccessful from the first cell passage.

197 **Morphological and histopathological investigations**

198 At gross examination, 20 cats showed no macroscopic detectable lesions (40%), while 30 cats
199 showed different renal injuries (60%). In particular, pale firm hypertrophic/hypotrophic kidneys,
200 multifocal cortical granulomas and renal infarcts were detected.

201 At histological examination, TIN was detected in 50% of processed samples (Figure 1A), while
202 granulomatous nephritis (Figure 1B) and tumor lesions were detected in 14% and 4% of
203 examined cats, respectively. Finally, 16% of the analyzed kidney samples showed other lesions
204 (tubular calcification, hyperemia, tubular steatosis, metastasis)and 16% did not present any
205 histopathological lesions (Table 1).

206 Among the four rt-qPCR positive kidneys, one cat showed no detectable lesions at gross
207 examination, one cat had pale firm hypotrophic kidneys while two cats presented bilateral
208 hypertrophy and multifocal cortical granulomas. Histopathological findings revealed the
209 presence of TIN in three out of four animals, while the last cat was affected by a systemic
210 lymphoma with evident renal metastatic lesions.

211 **Urine and blood analysis**

212 Among the 11 cats with rt-qPCR positive urine, 9 samples were available for urinalysis (4 cats
213 from the cattery and 5 client owned cats). In particular, 8 cats showed normal urine pH (6-7)
214 while one showed acidic urine (pH 5); 6 showed normal USG (\geq 1035) while 3 presented lower
215 USG (1014-1027). Three cats presented proteinuria and 7 showed urine sediment with crystals,
216 epithelial cells and lipid droplets. Regarding blood creatinine levels, the two blood samples were
217 used to classify cats creatinine levels according to the IRIS guidelines¹⁵: 7 cats were classified
218 as IRIS stage 1 (<1.6 mg/dL), 1 cat was IRIS stage 3 (3.61 mg/dL) and 1 cat was IRIS stage
219 4 (12.38 mg/dL) (Supplementary Table S1).

220 **Phylogenetic analysis**

221 Across the entire set of rt-qPCR positive samples, sequencing of a portion of FeMV L gene (401
222 bp) was possible only for two of them. In more details, the first sequenced sample was obtained
223 by urine of cat Totò collected by cystocentesis (GenBank Acc. Num. MT561453); the second
224 sequenced sample was obtained by a pool of urine collected from cats cohabitant with Totò
225 (GenBank Acc. Num. MT561453). The percentage of sequence similarity between the two
226 samples was calculated as 97.9%. Moreover the two sequences belong to the clade of the
227 German isolates Schmusi and Moj, but they are phylogenetically distant from the first Italian
228 isolate of cat Piuma and all the other Italian strains^{3,17}. Focusing on the Italian samples, they are
229 present in three out of four sub-clusters (B, C, and D) Sub-cluster D is composed by Italian
230 samples only. Ours are the only Italian samples in sub-cluster B (Figure 2).

231

232 **DISCUSSION**

233 FeMV represents a novel negative single-stranded RNA virus with still few information about
234 dynamic of infection, host susceptibility in target population, time and ways of excretion by
235 infected animals. After its first discovery in Hong Kong², FeMV was reported in many other

236 countries with different prevalence in urines ranging from 3% to 23% in the US⁹ and Japan⁴,
237 respectively.

238 In 2015, FeMV was identified in Italy from the urine of a 15-years old stray cat with suspected
239 chronic kidney disease (CKD)¹⁰, and recently Stranieri et al. (2019)¹⁷ investigated the presence
240 of FeMV in Northern Italy reporting a lower prevalence (1.23%) compared with previously
241 published studies.

242 According to in-vitro studies, the ability of the virus to cross species barrier and to infect humans
243 seems to be remote²⁰. However, new epidemiological, histopathological and phylogenetic
244 investigations could improve field strains' characterization by increasing current knowledge
245 about this new member of the genus Morbillivirus. Our data show that at least three different
246 sub-clusters of FeMV are circulating in Italy, and the samples collected in our study do not
247 share the same clade with the other Italian strains. Hence, a multiple introduction of the virus
248 may have occurred in Italy, but further analyses, especially at genomic level, are needed.

249 In this scenario, this study focused on different diagnostic aspects of FeMV infection:
250 biomolecular detection in biological samples, isolation attempts from rt-qPCR positive matrices
251 (both urine and kidney samples) and sequencing of target genes in order to reveal phylogenetic
252 relationships.

253 Considering the group of cats involved in the present study, the rate of infection appears
254 relatively low (7.3 %) but quite similar to the prevalence data reported in the literature,
255 particularly in stray cats, believed more easily infected than client-owned². The case of “cat
256 Totò” and of the cohabitant cats from the private cattery (mostly negative for FeMV detection)
257 leads to speculate that just few animals can develop an effective infection and/or an effective
258 excretion of FeMV. Moreover, according to the detected sequence data, a second FeMV strain,
259 different from Totò's strain, was present in the cattery. Even though other 5 cats cohabiting with

260 Totò resulted positive to the biomolecular assays, it can be hypothesized that cat-to-cat
261 transmission is not a common occurrence, or that FeMV infection could have a long incubation
262 period before viremia and viral shedding⁸.

263 A serological investigation could clarify how many cohabitant cats have developed at least a
264 humoral response against FeMV but, unfortunately, no commercial serological ELISA is
265 currently available for this purpose. Moreover, the development of an “in house ELISA” would
266 require a considerable number of antibody-positive FeMV sera, which are actually not available
267 as well. Concerning clinical features of FeMV infection, cat Totò showed no signs of renal
268 disease or LUTD and was healthy for the entire study. Regarding urine positive samples, two
269 cats were affected by CKD, while the remaining FeMV positive cats were negative to renal
270 disease and all cats presented proteinuria. These findings are in agreement with a recent study
271 reporting not clear correlation between chronic urinary disease and FeMV infection⁸.

272 The authors investigated also a possible association between FeMV infection and TIN. TIN was
273 detected in three out of four RT-Real Time positive kidney samples, and this feature could
274 support the hypothesis of a co-existence of FeMV infection with renal disease.

275 On the other hand, the presence of FeMV viral RNA in one kidney affected by lymphoma could
276 suggest that FeMV is possibly able to cause lesions other than TIN. Clinically only one positive
277 cat was affected by CKD. Moreover, in the present study, TIN was detected in the 54% of all
278 processed kidneys, and granulomatous nephritis was observed in the 14%. Therefore, a clear
279 relationship between the presence of FeMV infection and kidney diseases in cats was not
280 observed according to recent studies^{5,7,8,17,21}.

281 Concerning host susceptibility in cats, few information is currently available about viral
282 absorption or mechanisms involved in feline viremia but it is likely that just certain animals
283 expose optimal receptors involved in viral infection and/or in virus excretion. A panel of

284 candidate cell-receptors should be evaluated at molecular level in order to identify sequence
285 mutations associated with more efficient FeMV membrane transport²².

286

287 **CONCLUSION**

288 In conclusion, this is the first study reporting FeMV genotype 1-B in North-western Italy
289 suggesting that at least three different sub-clusters of FeMV are circulating in Italy (subcluster
290 B, C and D). Further studies are required to better investigate the potential role of FeMV in the
291 pathogenesis of kidney disease.

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300 **Conflict of interest**

301 The authors declared no potential conflicts of interest with respect to the research, authorship,
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303 freedom of research.

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307 **Ethical approval**

308 This work involved the use of non-experimental animals only (including owned or unowned
309 animals and data from prospective or retrospective studies). Established internationally
310 recognised high standards ('best practice') of individual veterinary clinical patient care were
311 followed. Ethical approval from a committee was therefore not necessarily required.

312 **Informed consent statement**

313 Informed consent (either verbal or written) was obtained from the owner or legal custodian of all
314 animal(s) described in this work (either experimental or non-experimental animals) for the
315 procedure(s) undertaken (either prospective or retrospective studies). No animals or humans
316 are identifiable within this publication, and therefore additional informed consent for publication
317 was not required.

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370

371 **Figure Legend**

372 **Figure 1.** A) Cat, kidney. Multifocal moderate interstitial nephritis. Haematoxylin and Eosin (H-e),
373 100x. B) Cat, kidney. Multifocal moderate granulomatous nephritis. H-e, 100x.

374 **Figure 2.** Bayesian phylogenetic tree based on a 339 bp long fragment of L gene of the Feline
375 morbillivirus. Node support is reported as posterior probability for the node. The strains
376 characterized in this study are marked as bold. Genotype and sub-cluster of the clades is
377 reported as described by Sieg et al (2019) and Stranieri et al (2019). The novel clade described
378 by Stranieri et al (2019) has been named here as genotype 1-D, in order to continue the
379 previous nomenclature.

Table 1. Signalment, clinical signs and test results of urine samples analyzed in this study

	URINE					
	N	rt-qPCR positive	Clinical diagnosis			
			FLUTD	CKD	AKD	Other ¹
	150					
Age (years)						
< 2	5	0	3	0	0	2
2-9	93	8	21	22	3	47
>10	52	3	8	14	2	28
Sex						
Castrated male	88	7	21	23	3	41
Uncastrated male	14	0	5	0	1	8
Spayed female	44	3	6	12	1	25
Unspayed female	4	1	0	1	0	3
Breed						
Cross	122	11	28	27	4	63
Pure	28	0	4	9	1	14
Birman	4	0	1	2	0	1
British short hair	4	0	0	4	0	0
Chartreux	3	0	0	0	1	2
Exotic short hair	1	0	0	1	0	0
Maine Coon	2	0	0	0	0	2
Norwegian Forest	4	0	0	0	0	4
Ragdoll Seal Point	3	0	2	0	0	1
Persian	4	0	1	1	0	2
Siamese	3	0	0	1	0	2
Siberian	0	0	0	0	0	0

FLUTD: feline lower urinary tract disease; CKD: chronic kidney disease; AKD: acute kidney disease; ¹ pathology not related to urinary tract;

Table 2. Signalment, histopathological findings and test results of kidney samples analyzed in this study

	KIDNEY						
	N	rt-qPCR positive	Histological findings				
			Lymphoma	TIN	GN	Other ¹	ALS
	50						
Age (years)							
< 2	10	0	0	2	3	3	2
2-9	25	3	2	10	4	3	6
>10	15	1	0	13	0	2	0
Sex							
Castrated male	12	1	0	8	2	0	2
Uncastrated male	13	0	0	7	3	1	2
Spayed female	4	2	0	2	0	1	1
Unspayed female	21	1	2	8	2	6	3
Breed							
Cross	42	3	2	22	5	8	5
Pure	8	1	0	3	2	0	3
Birman	0	0	0	0	0	0	0
British short hair	0	0	0	0	0	0	0
Chartreux	3	0	0	2	0	0	1
Exotic short hair	0	0	0	0	0	0	0
Maine Coon	2	0	0	0	2	0	0
Norwegian Forest	1	1	0	1	0	0	0
Ragdoll Seal Point	0	0	0	0	0	0	0
Persian	1	0	0	0	0	0	1
Siamese	0	0	0	0	0	0	0
Siberian	1	0	0	0	0	0	1

TIN: Tubulo-interstitial nephritis; GN: granulomatous nephritis; ALS: absence of alterations. ¹tubular calcification, hyperemia, tubular steatosis, metastasis.