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Prevalence, Molecular Characterization, and Risk Factors of Feline Morbillivirus Infection in Shelter Cats in Erzurum Province

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Feline morbillivirus (FeMV) has been detected with an increasing incidence in cats in the last decade. The molecular characterization and the risk factors of FeMV may help to understand the nature of this emerging disease. The objectives of this study were to determine the prevalence and genotypes of FeMV and possible risk factors in cats. Samples of blood, urine and swabs were collected from 28 shelter cats. Clinical findings have been recorded, and complete blood counts, serum and urine chemistry analyses have been performed. The analysis of urine samples was performed to detect the presence of FeMV RNA, using molecular techniques. The presence of FeMV RNA was detected in 6 (21.4%) of 28 cat urine samples. The virus in positive cases was identified by phylogenetic analysis as belonging to the FeMV-1C subtype. No relationship was found between FeMV positivity and age or gender. Concurrent infections with feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) and feline herpesvirus-1 (FHV-1) were detected in all FeMV-positive cases. In conclusion, FHV-1, FeLV, and FIV infections may be related to the presence of the FeMV infection. Further studies are needed to evaluate the existence of the concurrent infections and possible risk factors in cats.

Key Words: *Epidemiology, FeMV, genotype 1C, paramyxovirus, prevalence*

Erzurum İli Barınak Kedilerinde Feline Morbillivirus Enfeksiyonunun Yaygınlığı, Moleküler Karakterizasyonu ve Risk Faktörleri

Kedi morbillivirüsü (FeMV), son on yılda kedilerde artan bir sıklıkla tespit edilmiştir. FeMV'nin moleküler karakterizasyonu ve risk faktörleri, bu ortaya çıkan yeni hastalığın doğasını anlamaya yardımcı olabilir. Bu çalışmanın amacı, kedilerde FeMV prevalansını ve genotiplerini ve olası risk faktörlerini belirlemektir. 28 barınak kedisinden kan, idrar ve sürüntü örnekleri alındı. Klinik bulgular kaydedildi, tam kan sayımı, serum ve idrar kimyası analizleri yapıldı. Moleküler teknikler kullanılarak FeMV RNA varlığını tespit etmek için idrar örneklerinin analizi gerçekleştirildi. 28 kedi idrar örneğinin 6'sında (%21.4) FeMV RNA varlığı tespit edildi. Filogenetik analizle pozitif vakalarda virüsün, FeMV-1C alt tipine ait olduğu tespit edildi. FeMV pozitifliği ile yaş veya cinsiyet arasında herhangi bir ilişki bulunmadı. Tüm FeMV pozitif vakalarda kedi lösemi virüsü (FeLV), kedi immün yetmezlik virüsü (FIV) ve kedi herpesvirüs-1 (FHV-1) ile eşzamanlı enfeksiyonlar tespit edildi. Sonuç olarak, FHV-1, FeLV ve FIV enfeksiyonları FeMV enfeksiyonunun varlığıyla ilişkili olabilir. Kedilerde eşzamanlı enfeksiyonların varlığını ve olası risk faktörlerini değerlendirmek için daha fazla çalışma yapılması gerekmektedir.

Anahtar Kelimeler: *Epidemiyoloji, FeMV, genotip 1C, paramiksovirus, prevalans*

Introduction

Paramyxoviruses represent a diverse group of viruses associated with important diseases in both animals and humans. According to the classification established by the International Committee on Taxonomy of Viruses (ICTV), Morbilliviruses are classified within the *Paramyxoviridae* family and the *Orthoparamyxovirinae* subfamily (1). In 2012, a new feline morbillivirus (FeMV) was identified in stray cats in Hong Kong and China (2). FeMV is an enveloped, helically symmetric RNA virus (3, 4). The genome of the virus contains codes for six structural proteins (nucleocapsid N, large protein L, phosphoprotein P, matrix protein M, fusion protein F, and hemagglutinin protein H) and two nonstructural proteins (V and C). In particular, glycoproteins F and H have been demonstrated to determine tissue tropism, host range and pathogenesis (5, 6). FeMV is classified into two genotypes, genotype 1 and 2. Genotype 1 is divided into 4 subtypes (FeMV-1A, FeMV-1B, FeMV-1C, FeMV-1D) and is thought to be globally circulated (2, 5, 7, 8). The L gene region in the genome is known to be highly conserved, meaning it is protected against mutation, according to many studies. Consequently, the L gene region is frequently used in molecular diagnostic methods (9).

Morbilliviruses possess a wide host spectrum, including various mammals, birds and reptiles, and are capable of causing systemic infections in the animals, with respiratory, intestinal, neurological, reproductive, and cutaneous clinical signs (5). The initial case-control study indicated a correlation between FeMV infection and chronic tubulointerstitial nephritis (TIN), the most prevalent histopathological finding in feline chronic kidney disease (2). FeMV has been predominantly identified in the kidneys

(3, 10) and has also been detected in other organs, such as lymph nodes, lungs, liver, spleen, and brain (7, 11-13). In most of the previous studies, FeMV has been detected in urine samples by reverse transcriptase polymerase chain reaction (RT-PCR) indicating a high prevalence in the urine (2, 6, 12, 14, 15).

In high-density cat populations, such as animal shelters, in addition to the increased risk of direct transmission of infectious agents, chronic stress enhances susceptibility to disease and facilitates both viral replication and shedding. Deaths have been reported in cats younger than four months, particularly from feline parvovirus (FPV); in middle-aged cats, from feline leukemia virus (FeLV) and urinary tract diseases; and in cats over eight years old, from tumours (16-18). Infections caused by FeLV, feline immunodeficiency virus (FIV), feline herpesvirus-1 (FHV-1), and feline calicivirus (FCV) are among the most frequently reported viral pathogens, especially in densely populated feline colonies (19). However, data investigating their association with FeMV remain limited (3, 12, 20).

This study aimed to determine the prevalence of FeMV in urine samples collected from naturally infected cats, to perform molecular characterization and phylogenetic analysis of the virus, and to identify risk factors in FeMV-positive cats.

Materials and Methods

Research and Publication Ethics: This study was conducted with the approval (Protocol No: 2021/122, Date: May 27, 2021) of the local ethics committee for animal research of Atatürk University, Faculty of Veterinary Medicine.

The study material consisted of cats housed in an animal shelter in Erzurum from 2022 to 2023. A total of 78 cats were initially included in the study both with or without clinical signs; however, the analyses were conducted on 28 cats, as urine samples were successfully obtained only from these individuals. Signalment data (gender, age, and breed) and clinical signs were recorded for each cat, and blood, urine, and conjunctival and oropharyngeal swab samples were subsequently collected for analysis. Urine samples were investigated for FeMV, and dipstick analysis was performed. In addition, conjunctival and oropharyngeal swab, and blood samples obtained from FeMV-positive cats were used to identify possible co-infections. Blood samples were also utilized for hematological and serum biochemical assessments. The cats were classified as kittens (under 1 year old), young adults (aged 1–6 years), mature adults (aged 7–10 years) or seniors (over 10 years old) (21).

Sample Collection, Blood Analysis and Urinalysis: Conjunctival and oropharyngeal swab samples were obtained from the cats using sterile swabs. Five milliliters of blood were collected from the cat's *vena cephalica antebrachii*. Blood count analyses were conducted on samples collected in EDTA vacutainers (BD Vacutainer®, K2E 3.6 mg, BD Plymouth, UK) and analyzed using a hematology analyzer (Abacus Junior Vet5, Diatron, Hungary). The reference values for the hematological analysis were based on the set-up reference ranges of the hematology analyzer.

To avoid invasive procedures, urine samples were collected by manual bladder compression from cats in shelter conditions. However, insufficient urine could be obtained from some cats due to stress and behavioral factors, and it was impossible to safely collect samples from others. Consequently, urine samples could only be obtained from 28 animals. To evaluate the impact of FeMV on kidney function, samples were analysed immediately using a dipstick test (Combur 9 test strips, Roche Diagnostics, USA). An aliquot of each urine sample was stored at +4 °C for FeMV RNA detection. Normal urinalysis values were considered to be a pH range of 5-7, with negative results for protein, glucose, and ketones (22).

Blood samples were collected in serum separator vacutainers (BD Vacutainer®, SST II Advance, BD-Plymouth, UK) and centrifuged at 3,000 rpm for 10 minutes to prepare them for biochemical analysis. The serum samples were stored at -80 °C until they were analyzed. Serum albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamate dehydrogenase (GGT), total protein, blood urea nitrogen (BUN), creatinine, iron, and total iron binding capacity were analyzed using an automated biochemistry analyzer (Beckman Coulter® AU5800, Inc., USA). Serum AST, ALT, GGT, total protein, BUN, iron, and total iron-binding capacity reference values were derived from Tvedten (23), Klaassen (24) and Gest, et al. (25). The upper limit for blood creatinine levels was considered at 1.7 mg/dL (26).

Virological Analyses: The leukocyte layer (buffy coat) of the blood samples was separated by centrifugation and washed twice with phosphate-buffered saline (PBS). The conjunctival and oropharyngeal swab and urine samples were also centrifuged, and the precipitated layer at the bottom was collected and transferred to 2 ml sterile tubes, and all samples were kept at -80 °C until use. Viral RNA was extracted from leucocyte and urine samples using a viral nucleic acid extraction kit (GF-1 Viral Nucleic Acid Kit, Vivantis, Malaysia) according to the manufacturer's instructions. A first-strand cDNA synthesis kit (First strand cDNA synthesis kit, Thermo Scientific, USA) was used to obtain complementary DNA (cDNA), and the obtained cDNAs were used as templates in the nested RT-PCR analysis (Taq DNA Polymerase Kit, Thermo Scientific, USA). For the detection of FeMV, we used L gene-specific primers (Table 1) and thermal conditions reported by Furuya et al. (27). Additionally, PCR analysis was performed for FHV-1, FeLV, FIV, and FCV, which are important viral infections in cats. The primers and conditions used in the study were the same as those in the articles on which the study was based (Table 1) (28, 29).

The PCR amplicons were analyzed by electrophoresis on a 1% agarose gel, and those ~401 bp in size were sequenced for FeMV. Sequence analysis was performed using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, CA, USA). The obtained nucleotide sequences were aligned with the reference FeMV sequences in Gen-Bank using the BioEdit v.7.2.5 software (30). Phylogenetic analyses were carried out using the maximum likelihood method with 1,000 bootstrap replicates based on the Tamura 3-parameter model using MEGA X software to analyze feline morbilliviruses and some other paramyxoviruses (31). Local FeMV sequences were compared with GenBank reference strains from Germany, Japan, Italy, Brazil, and Türkiye. For phylogenetic reconstruction, canine distemper virus (CDV; KJ466106) and measles virus (MV; OR453544), members of the family *Paramyxoviridae*, were designated as outgroup taxa.

Statistical Analysis: Analyses were conducted using SPSS version 20.0 (IBM, Chicago, IL, USA). The prevalence rate of FeMV and the rate of co-infections were presented as percentages. The distribution characteristics of the continuous variables were assessed using the Shapiro–Wilk test. Data that did not follow a normal distribution were expressed as the median and interquartile range (IQR). The Mann–Whitney U test was used to determine whether there were statistically significant differences in the median values of categorical variables that showed an abnormal distribution. Biochemical parameters and urine dipstick analysis for FeMV-positive cats could not be analyzed comparatively due to insufficient valid observations in the FeMV-negative group. Therefore, only descriptive statistics (median and interquartile range) for the FeMV-positive group are presented. The chi-square test (Fisher's Exact Test) was used to evaluate the relationship between FeMV positivity and categorical variables such as age group and gender.

Results

PCR analysis revealed that six (21.4%) of the urine samples were positive for FeMV, while twenty-two (78.6%) samples were negative. Of the cats that tested positive for FeMV, two (33%) also tested positive for FHV-1, two (33%) for FIV and four (66.7%) for FeLV (two of which were co-infected with FIV). Co-infection with FCV was not detected in any of the cats. The sequences from this study of FeMV strains have been deposited under accession numbers PQ438791-PQ438796 (Table 2). In the phylogenetic tree, sequences from this study and previously reported FeMV sequences formed a distinct cluster, separating from the other members of the Morbillivirus genus. Based on the phylogenetic tree of the FeMV partial L gene, the FeMV strains were divided into two genotypes based on the phylogenetic tree. All the FeMV strains obtained in our study were clustered in FeMV- 1C together with the isolates reported from Italy, Brazil, Germany and Japan (Figure 1). The FeMV strains included in this study exhibited 99.6-100% nucleotide similarity and 100% amino acid similarity among themselves. When compared to FeMV strain previously identified in Türkiye, the strains from our study showed 98.5% nucleotide similarity and 100% amino acid similarity. In terms of FeMV subgroup 1C, the strains displayed 93.5-99.6% nucleotide similarity and 98.9-100% amino acid similarity with other strains. Phylogenetic analysis revealed 90.2-91% nucleotide similarity with FeMV subgroup 1D, 88.4-90.2% with subgroup 1B, and 88.1-88.4% with subgroup 1A. The Turkish strains showed a distant similarity of 82.7-83% with FeMV genotype 2 strains (Figure 1).

Signalment and Co-infections of the FeMV-Positive Cats: The cats included in the study were young adults between one and five years of age, comprising 16 males and 12 females. Of these, six young adult mixed breed cats—three males and three females—were positive for FeMV. FeMV-positive cats were consecutively numbered (Cat 1– Cat 6). All cats that tested positive for FeMV were co-infected with other viruses, including FHV-1, FeLV and/or FIV (Table 2). No statistically significant relationship was found between FeMV positivity and the variables of age (p=1.00) and gender (p=0.062) (Table 3).

The clinical manifestations observed in FeMV-positive cats varied and included conjunctival hyperemia, ocular discharge, conjunctivitis, purulent nasal discharge, unilateral lymph adenomegaly, stomatitis, dehydration, lethargy, coughing, and sneezing. Among the six FeMV-positive cats, five exhibited one or more clinical signs, whereas one cat remained completely asymptomatic. All cases testing positive for FeLV or FIV were clinically asymptomatic, or presented with non-specific findings. No pathognomonic clinical signs specific to these infections were identified.

Urine and Blood Analysis Findings in FeMV-Positive Cats: Urine dipstick analysis was only evaluated in the urine of five cats. Analyses showed proteinuria in five cats (5/5) that were FeMV positive and blood was detected in the urine of four cats (4/5). Other parameters were within the normal reference range. No statistically significant differences were found between the FeMV-positive and FeMV-negative groups in any of the hematological parameters examined (p>0.05) (Table 4). In terms of biochemical parameters, one cat had low albumin levels, three had low total protein levels, two had high GGT, one had high BUN, and four had low iron-binding capacity, while the albumin/globulin ratio (A/G ratio), AST, ALT, BUN, creatinine, iron levels were within normal limits in FeMV-positive cats (Table 5).

Table 1. Specific PCR primers and product sizes in the study

Gene Region	Primers	Sequence	Product sizes	Reference
FeMV L-Gene	FMoRV-F	GGAACATGGCCTCCTGTAGA	480 bp	Furuya et al. (27)
	FMoRV-R	CTCCATTGGCAATCAGGTTT		
	FMoRV-nF	CCAAATCATGCATCTGCTGT	401 bp	
	FMoRV-nR	GCGAACAATCGACCTACCTC		
FCV VP1 A-B	Cali 1 F1	AACCTGCGCTAACGTGCTTA	924 bp	Acar et al. (28)
	Cali 2 R1	CAGTGACAATACACCCAGAG		
	Cali 3 F2	TGGTGATGATGAATGGGCATC	467 bp	
	Cali 4 R2	ACACAAGAGCCAGAGATAGA		
FHV TK	FHT F	TTGCCGCACCATACCTTCTT	287	Acar et al. (28)
	FHT R	CTCCGGTTCTTGAGCGTCCC		
FeLV Env	Env Fw	TAY TGG GCC TGT AAC ACY G	508	
	Env Rv	CGC TGT TTT AGT CTT TCT CTT A		
FIV Env	VE1S	GAG TAG ATA CWT GGT TRC AAG	1211	Aydin and Yildirim (29)
	VE1R	CAT CCT AAT TCT TGC ATA GC		
	nVE2S	CAA AAT GTG GAT GGT GGA AY	859	
	nVE2R	ACC ATT CCW ATA GCA GTR GC		

Table 2. Signalment and viral co-infections in FeMV-positive cats

FeMV+Cats	Access. No	Age	Sex	Clinical signs	FHV-1	FCV	FeLV	FIV
Cat 1	PQ438791	Young adult	Male	Hyperemic mucosa, eye discharge	-	-	+	+
Cat 2	PQ438792	Young adult	Male	Purulent nasal discharge, unilateral enlarged lymph nodes	-	-	+	-
Cat 3	PQ438793	Young adult	Male	No clinical signs	+	-	-	-
Cat 4	PQ438794	Young adult	Female	Stomatitis, dehydration, lethargy, eye discharge, conjunctivitis	+	-	-	-
Cat 5	PQ438795	Young adult	Female	Stomatitis, unilateral enlarged lymph nodes, cough, sneezing	-	-	+	+
Cat 6	PQ438796	Young adult	Female	Eye and nasal discharge	-	-	+	-

Table 3. Evaluation of the relationship between FeMV positivity and age and gender

Parameters	FeMV (+) n (%)	FeMV (–) n (%)	Total	Test	*p* value
Age					
Kitten	0	10 (45.5)	10	Fisher's Exact	1.00
Young adult	6 (100)	12 (54.5)	18		
Sex					
Male	3 (50)	9 (40.9)	12	Fisher's Exact	0.062
Female	3 (50)	13 (59.1)	16		

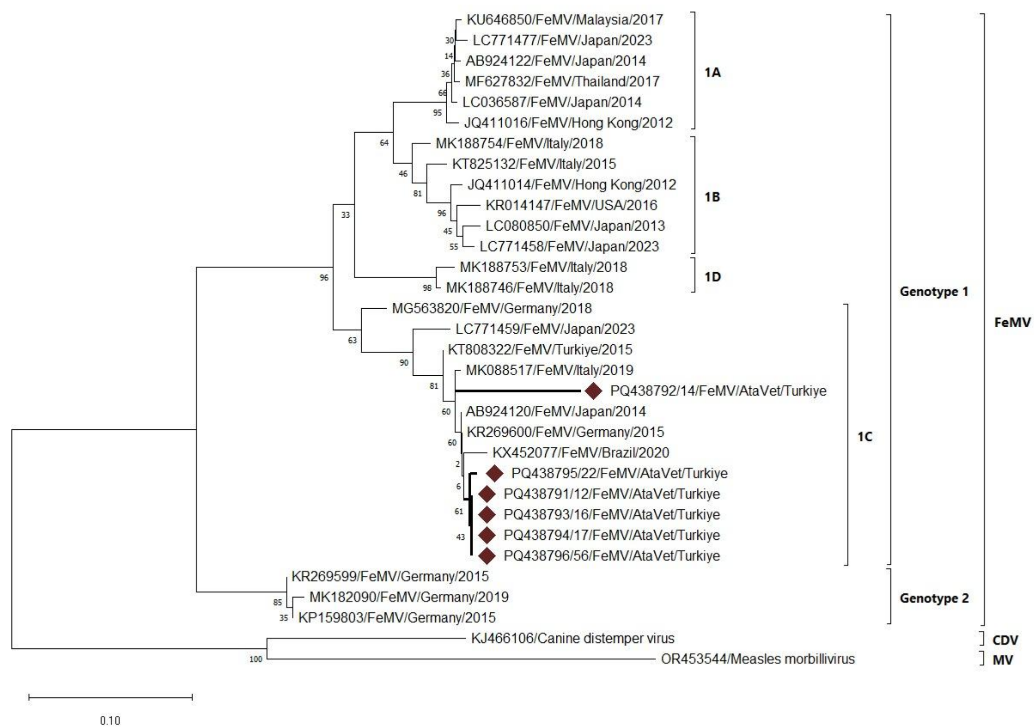

Figure 1. A phylogenetic tree depicting the relationship between FeMV strains encoding the partial L gene. The analysis was performed using the Maximum Likelihood method, with bootstrap values calculated from 1000 replicates. The Tamura 3-parameter model was applied, and the analysis was conducted using MEGA X. Canine distemper virus and measles virus sequences obtained from GenBank were used as outgroups. The diamond (◆) symbols in the phylogeny represent the study samples.

Table 4. Comparison of hematological parameters in FeMV-positive and FeMV-negative cats

Parameters	FeMV Positive (n=6) Median (IQR)	FeMV negative (n=13) Median (IQR)	*p* value
WBC (x10⁹/L)	25.41 (12.41-38.73)	13.83 (9.94-19.42)	0.062
LYM (x10⁹/L)	7.90 (3.33-12.3)	3.71 (0.98-5.02)	0.075
NEU (x10⁹/L)	15.75 (7.36-25.65)	9.44 (7.85-14.16)	0.268
PLT (x10⁹/L)	205.5 (103.5-234.75)	175 (125-366)	0.448
RBC(x10¹²/L)	8.69 (7.11-10.16)	9.10 (6.85-11.31)	0.771

Data are presented as the median (interquartile range, IQR). Comparisons between groups were performed using the Mann–Whitney U test. WBC: White blood cell; LYM: Lymphocyte NEU: Neutrophil; PLT: Platelet; RBC: Red blood cell

Table 5. Biochemical findings in FeMV-positive cats

Variables	RI	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5	Cat 6
Albumin (g/dL)	2.5-3.9	**L** (22)	**N** (28)	**N** (25)	**N** (35)	**N** (31)	**N/A**
Total Protein (g/dL)	5.5-7.7	**L** (47.59)	**L** (50)	**L** (42.33)	**N** (61.68)	**N** (58.81)	**N/A**
A/G ratio	0.80–1.68	**N** (0.86)	**N** (1.27)	**N** (1.44)	**N** (1.31)	**N** (1.11)	**N/A**
GGT (U/L)	1-10	**N** (3)	**N** (2)	**H** (13)	**H** (12)	**N** (6)	**N/A**
BUN (mg/dL)	14-36	**N** (32.9)	**N** (24.3)	**N** (23.5)	**H** (39.1)	**N** (29.1)	**N/A**
Creatinine (mg/dL)	<1.7	**N** (1.01)	**N** (1.39)	**N** (0.81)	**N** (1.35)	**N** (0.79)	**N/A**
Iron Binding Capacity (µg/dL)	196-464	**L** (64.6)	**L** (42.6)	**L** (147.4)	**N** (309.7)	**L** (124.6)	**N/A**

N/A: Not Available; **+**: low; **++**: medium; **+++**: high; **-**: negative. GGT: Gamma-glutamyl transferase; BUN: Blood urea nitrogen; A/G Ratio: Albumin/globulin ratio.

Discussion

This study detected FeMV RNA in six of the 28 urine samples (21.4%) collected in Erzurum, Türkiye, using nested RT-PCR. In studies on FeMV, L and N genes are generally preferred in molecular and bioinformatic analyses. However, in some molecular studies, the N gene could not be detected in FeMV L gene-positive samples (32). Therefore, we used specific primers for the L gene of FeMV in our study to avoid false negativity. Sequence analysis was performed on six positive samples obtained from the analysis of the FeMV L gene by nested RT-PCR. Bioinformatics analyses of the FeMV sequences obtained in this study and GenBank reference sequences revealed that the FeMV strains were divided into two different branches as Genotype 1 and Genotype 2. Genotype 1 FeMV strains were divided into four different subgroups, including FeMV-1A to FeMV-1D. When examining the strains included in the phylogeny and their geographic distributions, it is noteworthy that Genotype 2 strains were detected only in Germany (33), while Genotype 1 exhibited a broad geographical distribution across countries.

The recently discovered FeMV-1D is limited only in Italy, according to a more thorough analysis of Genotype 1 (34). FeMV-1B was discovered to be more extensively distributed, whereas FeMV-1A was determined to consist of strains from Asia (Malaysia, Thailand, Japan, and Hong Kong). All FeMV strains obtained in this study were grouped in subgroup 1C together with strains obtained from Brazil, Italy, Germany, Türkiye and Japan. FeMV-1C has been recorded in many nations across the world, according to the phylogenetic tree, indicating a wide geographic distribution. Genotype 2, on the other hand, is still uncommon. With 99.6–100% nucleotide and 100% amino acid identity, the FeMV strains found in this investigation showed a remarkable level of genetic conservation. When compared with the FeMV strain previously reported in Türkiye, these strains showed 98.5% nucleotide and 100% amino acid similarity. Furthermore, within FeMV subgroup 1C, they shared 93.5–99.6% nucleotide and 98.9–100% amino acid similarity with other reference strains, indicating close phylogenetic relatedness despite minor genomic variations. The sequences obtained from this study were found to be very similar to those previously reported from Türkiye (9).

In the present study, the molecular prevalence of FeMV was found to be 21.4%. Although studies analyzing FeMV RNA in cat urine reported relatively low prevalence rates in Japan [6.1% (27), 15.1% (35), and 13.7% (36)], Brazil (11.4%) (37), the United Kingdom (12.5%) (26), and Italy (7.3%) (34), a considerably higher prevalence rate has been reported in Malaysia (50.8%) (32). These differences in prevalence rates might be associated with various factors, such as the lifestyle of the cats included in the studies (household, stray, shelter, or access to the street), their habitat (urban, suburban, or rural), and the testing methodologies employed. To date, only one study has been conducted in Türkiye (Istanbul), reporting a prevalence of 3.13% in urine (9). In that study, samples were collected from cats presented to veterinary clinics, the majority of which had access to the street. In contrast, the present study revealed that 21.4% prevalence rate in shelter cats.

All FeMV-positive cats were found to be concurrently infected with additional viral agents such as FHV-1, FeLV, and FIV. There is still a lack of comprehensive knowledge regarding the relationship between FeMV infection and other feline pathogens. One study reported that cats positive for FIV antibodies and concurrently shedding FeMV RNA in their urine had higher viral loads than FIV antibody negative cats (20). Other studies have reported a concurrent association between FeMV-positive cats and both FIV and FeLV infections (9, 12). FIV and FeLV co-infection in cats has been documented in multiple studies (38-40). In this study, consistent with previous reports, simultaneous detection of FeMV, FIV, and FeLV was observed in two cats, while co-detection of FeMV and FeLV was observed in another two cats. Additionally, two FeMV-positive cats were found to be concurrently infected with FHV-1, whereas FCV was not detected in any of the FeMV-positive cats. The presence of co-infections in all FeMV-positive cats may prevent the interpretation of clinical findings. Donato, et al. (12) reported that cats positive for both FeMV and FIV were more affected by lymph node enlargement compared to cats positive for FeMV alone. Zahro, et al. (41) detected FeLV in the kidneys of four FeMV-positive cats and noted that FeLV, through systemic infection, can impact renal function, potentially confounding the interpretation of FeMV-specific pathology. Further studies are required to identify other factors (viral, bacterial, parasitic, etc.) that may cause co-infections with FeMV infection, and to evaluate the findings.

Data on potential risk factors for FeMV exposure remain limited; however, male gender, young age, and multicat households have been reported to be associated with increased FeMV positivity rates (3, 8, 20, 32, 42). In contrast to previous studies reporting a higher prevalence of FeMV infection in male cats, FeMV RNA was detected in urine samples from three male and three female cats in the present study (p=0.062). All FeMV positive cats were young adults (p=1.00). Transmission is reported to occur readily among cats in multicat environments, where the virus can spread chronically, resulting in higher prevalence in these populations (32). The high prevalence observed in this study is thought to be due to the sampling of cats living in shelter conditions, consistent with previous studies.

FeMV-positive cats showed positive reactions for protein (5/5) and blood (4/5) in their urine dipstick analyses. Chronic kidney disease is a long-term condition, particularly prevalent in older cats, characterised by the irreversible loss of kidney function, which usually develops without an identifiable cause (43). Therefore, it is difficult to distinguish the true cause of proteinuria clearly (44). For this reason, diagnosing chronic kidney disease requires more advanced techniques. However, when detected by dipstick in

individuals at risk of chronic kidney disease, proteinuria is a clinically significant screening finding that may indicate the possibility of underlying renal disease. Dipstick results are therefore considered indicative of cases requiring further evaluation rather than a definitive diagnosis. In addition, some hematological and biochemical parameters were found to be above, within, or below the reference ranges. The results of this study are consistent with those reported by Muratore, et al. (34), which suggested a urine pH of 5-7 and the presence of proteinuria, as well as findings by Darold, et al. (20), which demonstrated that FeMV RNA shedding primarily occurs in the urine of cats without clinical, biochemical or ultrasonographical signs. Yilmaz, et al. (9) reported that urine, hematological, and biochemical parameters in morbillivirus positive cats did not differ from those in negative cats. However, Donato, et al. (12) demonstrated a relationship between anemia, neutrophilia, basophilia, eosinopenia, monocytosis, thrombocytosis, and FeMV infection. In contrast, Ito et al. (7) reported significant leukopenia (7 out of 32) and thrombocytopenia (6 out of 32) in FeMV-infected cats. These differences in hematological and biochemical findings in FeMV-positive cats may be due to co-infections alongside FeMV. The relationship between the presence of a viral disease and clinical / biochemical / hematological findings needs further investigation, which may support to determine challenging treatment options for viral diseases in small animal practice (45, 46).

Viruses are constantly evolving. Viral genomes can alter in ways that improve their capacity to infect new hosts through processes like genetic mutation, recombination, and reassortment. Further modifications could improve the virus's ability to replicate more effectively, escape host immune responses, or spread between hosts (47). Therefore, more thorough research on the evolutionary dynamics and host adaptability of the FeMV, a relatively recently discovered RNA virus, is necessary. This study is the second detection of FeMV in the Türkiye and the first in the eastern region, as FeMV has only been detected in the western region of Türkiye (Istanbul) thus far (9). The finding of the virus indicates the existence and possible circulation of FeMV in the area, despite the fact that its detection was restricted to a single geographic location. To understand the potential therapeutic significance of FeMV, it is necessary to examine its genetic characteristics and epidemiological distribution. This study provides an important foundation for future research.

One of the main limitations of the study was that urine samples could only be obtained from a relatively small number of cats. Only twenty-eight of the seventy-eight cats included in the study provided urine samples. Additionally, statistical analysis could not be performed on the biochemical and urine analysis results due to the small quantity of blood and urine obtained from the cats. This reflects the difficulty of collecting urine samples under ideal conditions, particularly from uncooperative or asymptomatic cats.

In conclusion, the findings of this study reveal the presence and molecular characterization of FeMV in urine samples from cats and provide information about risk factors. The results of the virologic analysis, performed using RT-PCR, provide information on the distribution of four different phenotypes and demonstrate that FeMV subtype 1C is the most prevalent in the region. Furthermore, this study confirmed the presence of FeMV RNA in 6 (6/28) of the cat urine samples, indicating that the virus was actively circulating within the local cat population. No association was found between FeMV positivity and age or gender. It is also noteworthy that all cats infected with FeMV tested positive for one or more of the known immunosuppressive viruses FHV-1, FeLV, and/or FIV. These findings indicate that such viral agents may improve the spread of FeMV by predisposing cats to infection. The data obtained in this study provide valuable information about FeMV, a relatively new virus, and may contribute to directing future research in this field. FeMV infection should be investigated in larger populations through more comprehensive analyses to further elucidate the role of concurrent infections in its transmission and pathogenesis.

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