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Detection and molecular characterisation of feline viruses from swab samples

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RESEARCH ARTICLE



ABSTRACT

Feline calicivirus (FCV), feline alphaherpesvirus 1 (FHV-1) and feline panleukopenia virus (FPLV) as well as retroviral agents such as feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are important viral pathogens of cats. The aim of this study was to detect and characterise FHV-1, FPLV, FeLV, FIV and feline foamy virus (FFV) in oropharyngeal, nasal and conjunctival swabs from 93 cats that had been screened for FCV previously. We wanted to determine the possible risk factors for infection with these viruses. The prevalence was found to be 12.9% for FHV-1 and 9.7% for FPLV. FIV was detected only in two samples and FeLV in one sample, whereas the presence of FFV was not demonstrated in any of the clinical samples. The statistical analysis of the results showed that breed, age, health status, and lifestyle are important predisposing factors to FHV-1 ($P < 0.05$). For FPLV, only clinically unhealthy animals were found to be at risk ($P < 0.001$). Sequence analysis revealed that the two FIV-positive samples in this study contained different (A and B) subtypes of the virus. This is the first report on the occurrence of subtype A FIV in Turkey.

KEYWORDS

feline, herpesvirus 1, panleukopenia, retroviruses

INTRODUCTION

Feline calicivirus (FCV), feline alphaherpesvirus 1 (FHV-1), and feline panleukopenia (FPLV) are among the major pathogens of domestic cats around the world. FPLV is a prototype of parvoviruses in dogs; its infection in kittens shows a fatal course, especially with leukopenia and diarrhoea (Battilani et al., 2011; Dall'Ara et al., 2019). FCV and FHV-1 play an important role in feline upper respiratory tract infections, also known as 'cat flu' (Monne Rodriguez et al., 2017). Additionally, ulcerative stomatitis in FCV infections and conjunctivitis in FHV-1 infections are among the remarkable findings (Fernandez et al., 2017). Across the globe, cats (especially young ones) who live together or in a shelter are at risk of coming into contact with these pathogens (Dall'Ara et al., 2019). Although vaccination is relatively successful in preventing FCV, FHV-1, and FPLV infections (Gore et al., 2006), latent infections caused by FHV-1 and genetic variations of FCV pose serious problems in fighting the disease (Kang and Park, 2008).

Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are significant retroviruses with global impacts on the health of domestic cats (Hartmann, 2012). Both are widespread, lymphotropic retroviruses that can settle in cells of the immune system (such as lymphocytes and granulocytes) and cause a wide range of clinical signs (Najafi et al., 2014).

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These two viruses make the affected cats more susceptible to other pathogens (Bande et al., 2012). Variations in the sequence of the envelope (*env*) gene encoding envelope proteins of FIV provide an advantage in the replication capacity of the virus. The *env* sequence contains nine highly variable regions referred to as V1 to V9. The domain V3–V5 is used to determine the subtype of FIV. As a result, 7 subtypes have emerged: A, B, C, D, E, F, and U-NZenv (Huguet et al., 2019; Szilasi et al., 2019, 2020). Similarly, it is possible to identify four subtypes of FeLV (A, B, C, T) by comparing sequences encoding FeLV *env* proteins (Coelho et al., 2008). Also, in recent years, FeLV-D has been introduced as a new subtype closely related to endogenous cat retroviruses (Anai et al., 2012).

Feline foamy virus (FFV) is a contact-dependent retrovirus that causes a chronic, largely asymptomatic infection in domestic and wild cat populations worldwide (Dannemiller et al., 2020). Occasionally, FFV may be associated with pathogenic retroviruses in cats, but it can also be isolated alone from healthy cats (Cavalcante et al., 2018). Lately, FFV has been frequently reported in feral cats (Dannemiller et al., 2020).

The aim of this study was to diagnose and characterise FeLV, FFV, FHV-1, FIV, and FPLV from oropharyngeal, nasal, and conjunctival swabs and determine possible risk factors for these viruses in the cat population in which FCV was previously investigated.

MATERIALS AND METHODS

Cat population, sample collection, and processing

This study included samples of 93 domestic cats from one veterinary hospital and two clinics ($n = 27$) and two animal protection shelters ($n = 66$) in Kayseri, Turkey. Samples were collected between December 2017 and March 2018. Samples from each cat were collected by nasal and conjunctival swab and oropharyngeal cytobrush. The cats in the sampled population were chosen randomly, regardless of the health status, sex or breed of the animals; particular attention was paid to making selections from different groups in the shelter. However, clinical samples were collected before any diagnostic treatment. Each sampling stick from the same cat was placed into a falcon tube containing 1 mL of sterile phosphate buffered saline. Samples were stored at 4 °C before being shipped to the laboratory. Then, they were thoroughly mixed and pooled just before RNA and DNA-RNA isolation. First, we examined clinical samples for the FCV virus by ELISA and reverse transcription polymerase chain reaction (RT-PCR) assay; these results were reported in our previous study (Abayli et al., 2020). The planned work for FeLV, FFV, FHV-1, FIV, and FPLV was carried out approximately 3 months after the samples had been collected.

Ethics statement

For this study, permission was obtained from the management of shelters in Kayseri province and the animal hospital

management of Erciyes University. In addition, approval was obtained from the Erciyes University Local Ethics Committee for Animal Experiments (approval number: 17/041) and from the animals' owners.

Epidemiological data and risk factors

A questionnaire that included queries regarding health/disease status, vaccination (against any viral agent), age, sex, neutering/spaying status, breed, and shelter status (lifestyle, living conditions) was created and used to collect data on each cat. It was filled out by cat owners or authorised veterinarians at the time of sampling. The questionnaire was also used to identify risk factors; variables such as aggression and the presence of an abscess or bite wound were ignored. Cats with conditions such as halitosis, oral ulcers, vomiting, diarrhoea, respiratory disease, conjunctivitis, nasal discharge, dehydration, loss or decrease of appetite, depression, or neurological disorders were described as unhealthy in the physical examination. Cats with a normal appearance that did not have any of these conditions were described as healthy.

Viral nucleic acid isolation and detection of viruses using polymerase chain reaction

DNA and RNA isolation from the oropharyngeal, conjunctival, and/or nasal swab sample pool of cats was performed using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). Extraction protocols were followed as recommended by the manufacturer. The attenuated vaccine (Nobivac Tricat Trio) for FHV and FPLV was used as a positive control. In each extraction step, a negative control consisting of 200 µL of PBS was used to monitor cross-contamination. As no positive control could be provided for every virus, all positive PCRs were repeated and the amplicons sequenced twice.

The target genes, along with the primer sequences, and the expected size of the amplicons as well as the references for the PCRs used for the detection of each viral agent are presented in Table 1. For the nucleic acid extraction control, the gene of the housekeeping enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used. The cycling conditions recommended in the corresponding publications referred to in Table 1 were used. The amplification products were analysed in 1.5% agarose gel electrophoresis with ethidium bromide, using the 100 bp DNA ladder (Cleaver Scientific, UK). Electrophoresis was performed in TAE buffer (40 mM Tris-acetate, 0.5 M EDTA) for 40 min at 120 V. PCR products were visualised under UV light.

Sequence analysis

Sequence analysis of all amplicons detected at the expected size after electrophoresis was performed at the Macrogen laboratory. The obtained sequence data were verified by testing with BLASTN (<http://www.ncbi.nlm.nih.gov>) and deposited to the GenBank under accession numbers from MT813093 to MT813116.

Table 1. Primer sets used in viral diagnosis and targeted DNA fragments

Viral agent	Nucleotide sequence (5'-3')	Region	Product (bp)	Reference
Feline leukaemia virus	AACCTAACCAATCCCCACGC AATGGCTGTCCCACTAGAG	<i>gag</i>	450	de Castro et al. (2014)
Feline foamy virus	AACAGCAACACTCTGATGTTCCCG TTGCTGCCTAACAGGTTCTTCTCC	<i>gag</i>	497	Winkler et al. (1999)
Feline alphaherpesvirus	GACGTGGTGAATTATCAGC CAACTAGATTTCCACCAGGA	TK	292	Kang and Park (2008)
Feline immunodeficiency virus	CTTCCTGAAGGGGATGAGTG CCTARTTCTTGCATAGCRAAAGC GAATGAGACTATAACAGGAC CAAGACCAATTTCCAGC AAT	<i>env</i>	831	Cano-Ortiz et al. (2017)
Proto-parvovirus	CGAAACAAATAGAGCATTGGGC TGGTGCATTTACATGAAGTCTTGG	VP2	625	Vannamahaxay et al. (2017)
GAPDH	CCTTCATTGACCTCAACTACAT CCAAAGTTGTCATGGATGACC		700	Martins et al. (2018)

GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

In addition to the sequences obtained in this study, 38 reference sequences consisting of 7 subtypes of FIV (subtype A to U-NZenv) were selected from GenBank. Similarly, 60 sequences from feline and canine parvovirus strains from different countries were selected from GenBank in addition to the sequences in this study. Alignments of these sequences were generated using Mega-X software (Kumar et al., 2018). The phylogenetic trees were generated by using the Maximum Likelihood method and the Tamura–Nei model (Tamura and Nei, 1993); bootstrap values were calculated in 1,000 replicates.

Statistical analysis

In the first step, descriptive statistics and frequency distributions were calculated and prevalence was determined as the number of cats with positive PCR tests divided by the total number of cats evaluated. Chi-square analyses were used to determine the association between the putative risk factors and the FHV-1 status or FPLV status. All statistical associations were considered significant at $P < 0.05$.

RESULTS

Polymerase chain reaction and sequence analysis

After agarose gel electrophoresis, sizes of the PCR products shown in Table 1 were obtained for each viral agent except FFV. No amplicons were detected in any of the negative control tubes. After sequencing, the identity of every PCR product was confirmed using the BLASTN algorithm. Sequence analysis of the amplicons obtained with the proto-parvovirus primers revealed that they belonged to FPLV. The phylogenetic analysis of the envelope protein gene of FIV revealed that the two FIV strains detected in this study belonged to subtypes A and B. Phylogenetic trees for FIV and FPLV, which compare the sequence data of strains

obtained from this study with other strains in GenBank, are shown in Figs 1 and 2, respectively.

Population profile, prevalence, and risk factors

The 93 cats included in this study were categorised as either vaccinated ($n = 13$; 14.0%) or unvaccinated ($n = 80$; 86.0%); male ($n = 42$; 45.2%) or female ($n = 51$; 54.8%); shelter cats ($n = 67$; 72.0%) or outdoor cats ($n = 26$; 28.0%); and healthy ($n = 67$; 72.0%) or unhealthy ($n = 26$; 28%). The ages of the cats varied from 3 weeks to 108 months. They were divided into groups based on age: ≤ 12 months ($n = 65$; 69.9%); 13–24 months ($n = 6$; 6.5%); 25–36 months ($n = 9$; 9.7%); and >36 months ($n = 13$; 14.0%). Finally, the cats were divided into groups according to their breed: Angora ($n = 4$; 4.30%); Siamese ($n = 2$; 2.2%); and mixed ($n = 87$; 93.5%). The profiles of animals for which positivity was determined for FeLV, FHV-1, FIV, or FPLV are summarised in Table 2.

The prevalence of infection was 12.9% (12/93) for FHV-1 [21.4% (9/42) male, 5.9% (3/51) female, 4.5% (3/67) healthy, 34.6% (9/26) unhealthy, and 7.5% (5/67) shelter cats, 26.9% (7/26) outdoor cats]. The prevalence of infection was 9.7% (9/93) for FPLV [11.9% (5/42) male, 7.8% (4/51) female, 0% (0/67) healthy, 34.6% (9/26) unhealthy, 10.4% (7/67) shelter cats, and 7.7% (2/26) outdoor cats]. The prevalence of FeLV and FIV infections was 1.1% (1/93) and 2.2% (2/93), respectively. FFV was not detected from clinical samples. The prevalence of cats positive for both FHV-1 and FIV or FPLV was 1.1% (1/93), while the prevalence of cats positive for both FCV and FHV-1 was 4.3% (5/93). The prevalence of cats positive for FCV, FHV-1, and FPLV was 1.1% (1/93). The numbers of cats carrying single or multiple viruses are shown in Fig. 3.

FHV-1 was more prevalent among cats aged 12–36 months and among Angoras (75.0%). At the same time, FHV-1 was more common in outdoor cats (26.9%, 7/26), while FPLV was more common in shelter cats (10.4%, 7/67).

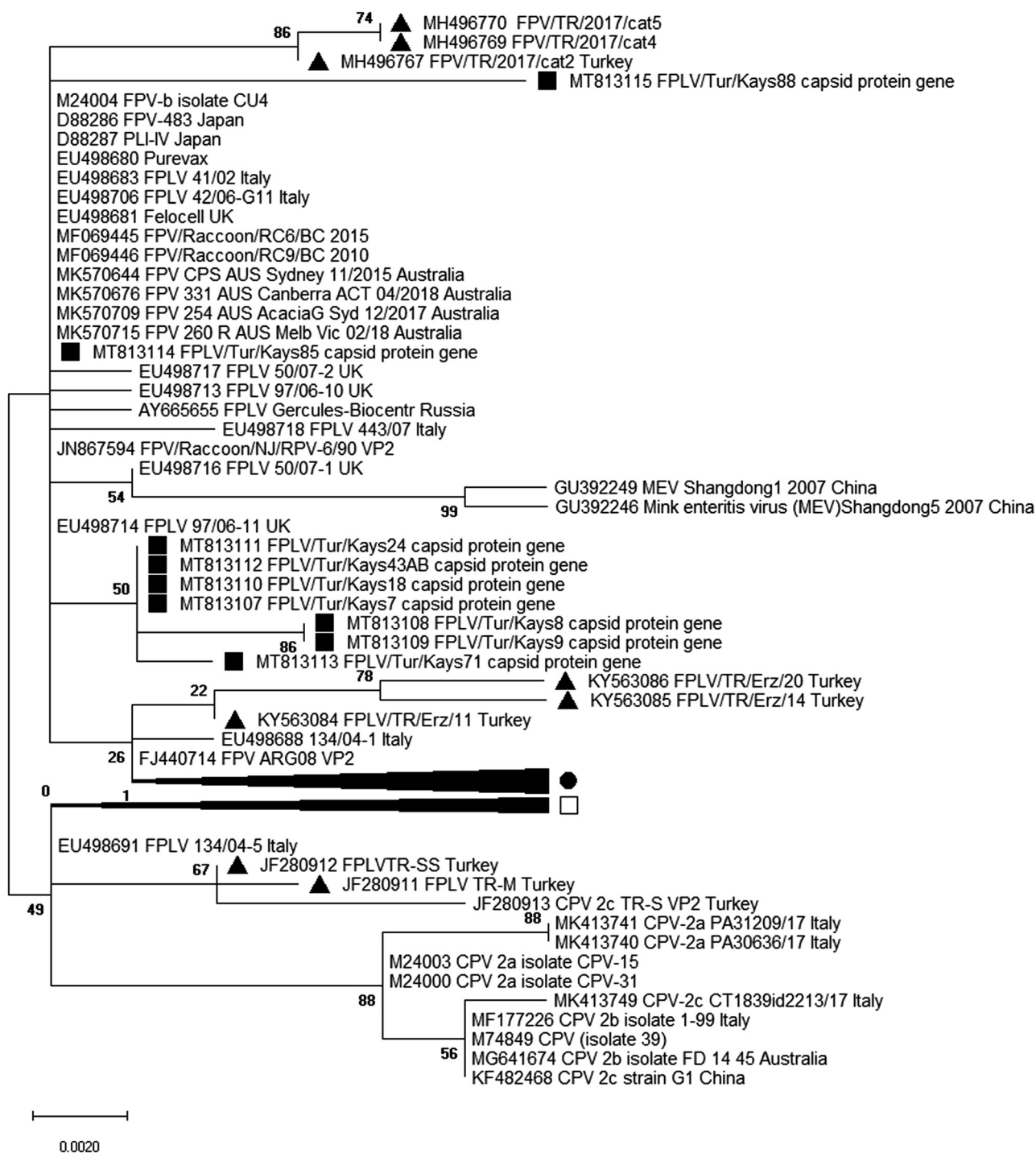


Fig. 1. Phylogeny of feline panleukopenia virus (FPLV) VP2 sequences. The tree was constructed using the maximum likelihood method. Bootstrap support values based on 1,000 replicates are given at the nodes. Black boxes represent the FPLV strains in the present study, the triangle symbols indicate strains obtained from other studies in Turkey. Filled circle represents some FPLV strains: ARG01, ARG03, 42/06-G2, 42/06-G8, 355/04, FPLV/TR/Erz/1, FPLV/TR/Erz/7, JF-3 FPLV, GT-2, and V211. The blank box represents some FPLV strains: 134/04-2, 22/06, 42/06-G1, 20/05, 300/03, FPLV/Tiger/PT06, and 103/02. Others are FPLV strains or isolates from different countries

According to the results of the statistical analysis, breed, age, health status, and lifestyle are important for predisposition to FHV-1 ($P < 0.05$). For FPLV, only clinically unhealthy animals were at risk ($P < 0.001$).

Although FPLV was more common in cats younger than 12 months (12.3%, 8/65), among shelter cats (10.4%, 7/67), male cats (11.9%, 5/42), and Angoras (25.0%, 1/4), the results were statistically insignificant ($P > 0.05$). The

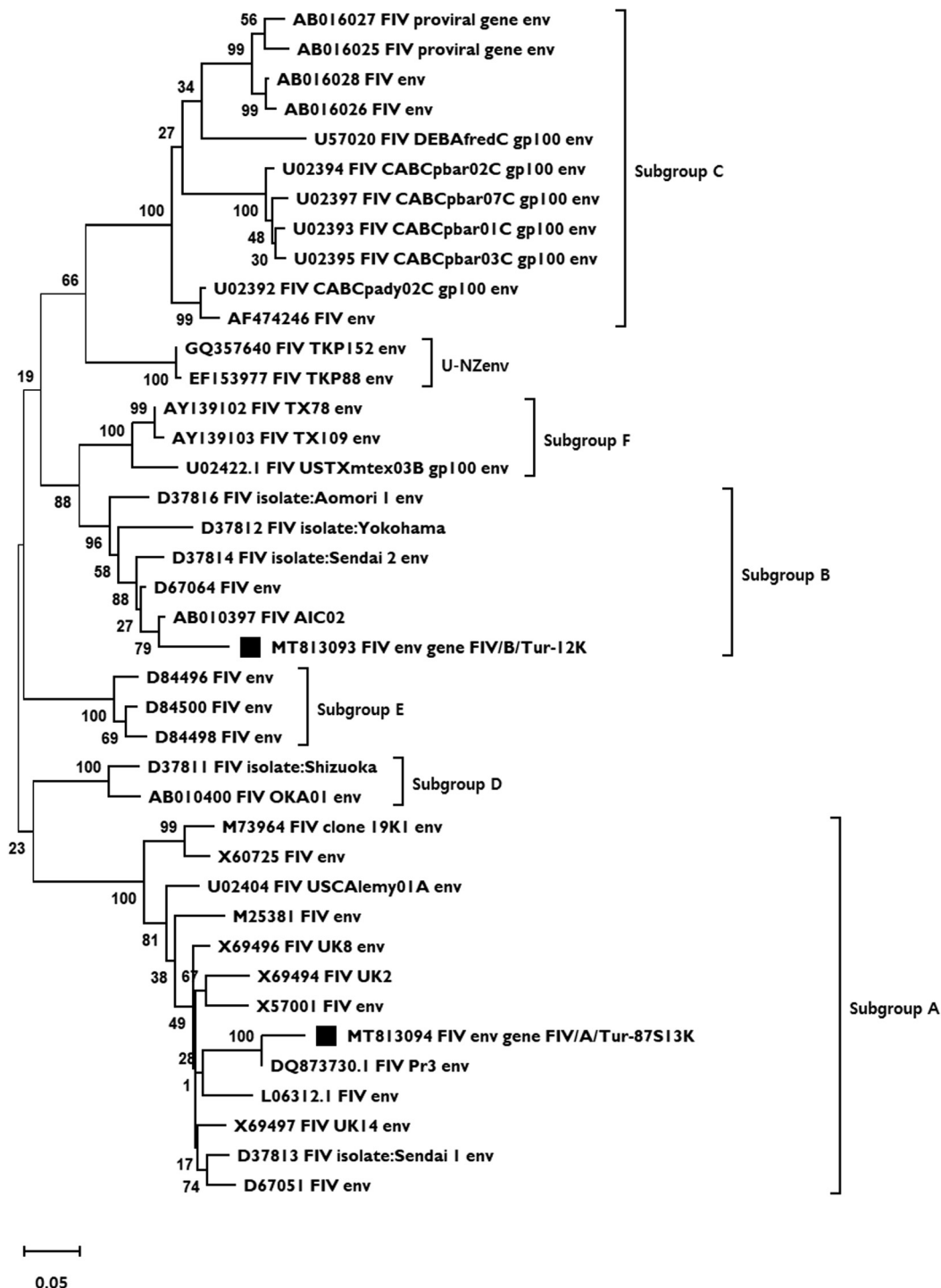


Fig. 2. Phylogeny of feline immunodeficiency virus (FIV) *env* sequences. The tree was constructed using the maximum likelihood method. Bootstrap support values based on 1,000 replicates are given at the nodes. Black boxes represent the FIV strains in the present study. Others are FIV strains or isolates from different countries

prevalence and risk factor analysis results for cats with FHV-1 and/or FPLV are summarised in Table 3.

Feline retroviruses such as FeLV, FFV, and FIV were not common among cats in this study. For this reason, risk factors for these viruses could not be evaluated.

FeLV and FIV were detected in unvaccinated cats aged nine months and older that lived outdoors.

DISCUSSION

FCV, FHV-1 and FPLV are among the most common pathogens in cats. The prevalence of FHV-1 varies from 3% to 31% according to PCR test results in different countries (Nguyen et al., 2019); we calculated its prevalence as 12.9%. According to the risk factor analysis, breed, age, lifestyle, and

Table 2. Data of the cats found positive for FeLV, FHV-1, FIV, or FPLV

Label	Clinical signs	Sampling	Sex	Lifestyle	Age (months)	Diagnosis	Breed	Strain name
7	Nasal discharge, halitosis, dehydration	O: N	M	Shelter	2	FPLV+	Mixed	FPLV/Tur/Kays7
8	Nasal discharge, halitosis	O: N	M	Shelter	2	FPLV+	Mixed	FPLV/Tur/Kays8
9	Ocular-nasal discharge, stomatitis	O: C	F	Shelter	2	FHV-1+ FPLV +	Mixed	FHV-1Tur/Kays9 FPLV/Tur/Kays9
12	Ocular-nasal discharge, cestodes	NP	F	Shelter	9	FIV	Mixed	FIV/B/Tur-12K
17	Ocular discharge	O: C	M	Shelter	18	FHV-1+	Mixed	FHV-1Tur/Kays17
18	Ocular-nasal discharge, vomiting	O: C	M	Outdoor	36	FHV-1 FPLV	Mixed	FHV-1Tur/Kays18 FPLV/Tur/Kays18
24	Depression, anorexia	OP	M	Shelter	3	FPLV	Mixed	FPLV/Tur/Kays24
33	Nasal discharge	N	M	Shelter	3 weeks	FHV-1	Mixed	FHV-1Tur/Kays33
35	Ocular-nasal discharge	N: OP	F	Shelter	1.5	FHV-1+	Angora	FHV-1Tur/Kays35
36	Fever, weight loss	OP	F	Outdoor	42	FeLV	Angora	FeLV/TurKays36
43	Weight loss, anorexia	O	M	Shelter	4	FPLV	Mixed	FPLV/Tur/Kays43AB
58	Healthy	O: N	M	Shelter	24	FHV-1	Mixed	FHV-1Tur/Kays58
*68	Healthy	O: N	M	Outdoor	4	FHV-1	Mixed	FHV-1Tur/KaysS9-68
71	Vomiting	OP	F	Outdoor	10	FPLV+	Angora	FPLV/Tur/Kays71
72	Ocular-nasal discharge, oedema	N: C	M	Outdoor	24	FHV-1+	Mixed	FHV-1Tur/KaysS2-72
*82	Conjunctivitis	N: C	M	Outdoor	3.5	FHV-1+	Siamese	FHV-1Tur/KaysS3-82
85	Anorexia, depression	O	F	Shelter	12	FPLV	Mixed	FPLV/Tur/Kays85
86	Conjunctivitis	C	F	Outdoor	72	FHV-1	Angora	FHV-1Tur/KaysS4-86
87	Ocular-nasal discharge, anorexia	O: C	M	Outdoor	36	FHV-1 FIV	Mixed	FHV-1Tur/KaysS13-87 FIV/A/Tur-87S13K
88	Dehydration, anorexia	O	F	Shelter	3	FPLV	Mixed	FPLV/Tur/Kays88
93	Healthy	O: N	M	Outdoor	36	FHV-1	Angora	FHV-1Tur/Kays93

The symbol (*) represents cats vaccinated against FCV, FHV and FPLV, while the plus (+) sign represents FCV co-infection in cats. F = female; M = male; C = conjunctival; N = nasal; O = oral; OP = oropharyngeal; NP = nasopharyngeal.

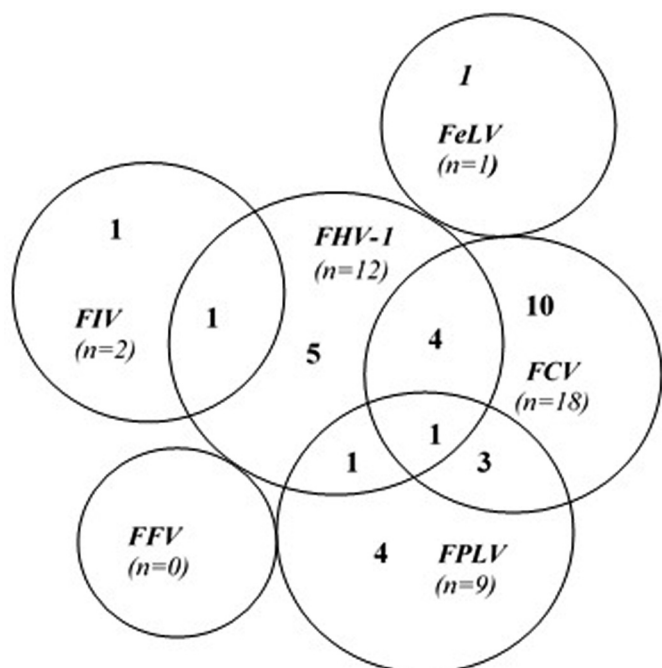


Fig. 3. Graphic representation of single infections and co-infections detected in 31 out of 93 cats in this study. Infections in cats are represented by numbers. FCV: Feline calicivirus; FeLV: Feline leukaemia virus; FFV: Feline foamy virus; FHV-1: Feline herpesvirus 1; FIV: Feline immunodeficiency virus; FPLV: Feline panleukopenia virus

health status are important with regard to predisposition to FHV-1. In previous studies, it has been reported that males are three times more susceptible to FHV-1 than females (Fernandez et al., 2017). Those results are similar to the results of our study. At least 80% of infected cats can remain latent after FHV-1 infection, and about 29% spontaneously scatter the virus. For this reason, some researchers think that FHV-1 infection is more common in crowded cat populations (Kang and Park, 2008). Stress-induced responses, especially in cats that are abandoned or in an unfamiliar environment, can lead to the reactivation of FHV-1; the virus can then spread to uninfected cats (Chvala-Mannsberger et al., 2009). In this study, the prevalence of FHV-1 was found to be higher in cats with outdoor access than in shelter cats, and the reason for this is unknown. Age is an important variable for FHV-1 (Dall'Ara et al., 2019). According to the results of a study in Brazil, the average age of FHV-1-infected cats was 29.9 months (Henzel et al., 2012), while in our study the average age was 21.4 months.

In our previous study, we reported that the prevalence of FCV in the same cat population was 19.4%. In that study, only health status was found to be a risk factor. As in previous studies, the presence of FCV was confirmed in FCV-vaccinated and healthy cats (Abayli et al., 2020).

FCV and FHV-1 infections are often reported together (Monne Rodriguez et al., 2018). This may pose a risk, especially for upper respiratory infection (Berger et al.,

Table 3. Data of prevalence and risk factor analysis in cats with FHV-1 and/or FPLV

Characteristics	Cat tested No.	FHV-1		FPLV		Characteristics	Cat tested No.	FHV-1		FPLV	
		No. Positive	%	No. Positive	%			No. positive	%	No. positive	%
Breed						Age (months)					
Angora	4	3	75.0	1	25	≤12	65	5	7.7	8	12.3
Mixed	87	8	9.2	8	9.2	13–24	6	3	50.0	0	0
Siamese	2	1	50.0	0	0	>36	13	0	0	0	0
<i>P</i> value		0.0001		0.519				0.0003		0.463	
Healthy						Vaccination					
Yes	67	3	4.5	0	0	Yes	13	2	15.4	0	0
No	26	9	34.6	9	34.6	No	80	10	12.5	9	11.3
<i>P</i> value		0.0004		0.001				0.874		0.443	
Lifestyle						Sex					
Shelter	67	5	7.46	7	10.4	Male	42	9	21.4	5	11.9
Outdoor	26	7	26.9	2	7.7	Female	51	3	5.9	4	7.8
<i>P</i> value		0.030		0.989				0.056		0.758	

2015). In this study, the prevalence of cats positive for both FCV and FHV-1 was 4.3%.

According to the PCR results, the prevalence of FPLV was 9.7%. According to the risk factor analysis, only health status was found to be an important risk factor of acquiring FPLV. It is noteworthy that FPLV-positive animals are usually younger than one year. In previous studies conducted in Turkey using PCR, the prevalence of FPLV was 39.0% and 10.0%, respectively (Oğuzoğlu et al., 2013; Aydin and Timurkan, 2018). FPLV and canine parvovirus are genetically close to each other. In another study conducted in Turkey, Muz et al. (2012) reported that canine parvovirus was detected in clinical samples from cats. In this investigation, no canine parvovirus from feline clinical samples was detected.

The prevalence of FeLV and FIV in developed countries was 2.3%–3.3% and 2.5%–5.2% in the United States, 3.4%–4.3% in Canada, 3.6%–3.2% in Germany, 15%–8.3% in Spain, and 8.4%–11.3% in Italy. The prevalence of co-infection with FeLV and FIV from two studies in Brazil was 0.25%–4.4%, while in North America it was 0.5% (Biezus et al., 2019). Most of the researchers who conducted previous studies in Turkey aimed to identify the antigens (p27 for FeLV) or antibodies (against gp70 for FeLV and p24 for FIV) against FeLV or FIV (Yilmaz et al., 2000; Oğuzoğlu et al., 2010a). According to the results of previous studies, the seroprevalence of FIV in cats in Turkey was 19.5%–23.0%. Using PCR, Oğuzoğlu et al. (2013) determined 20.5% and 9.5% positivity for FeLV and FIV in cats, and pointed out the importance of age and living conditions for these diseases. Bayraktar and Yilmaz (2020) detected a positivity rate of 10.0% and 18.3% (16.4% stray cats; 3.3% indoor cats), respectively, in the study targeting the proviral DNA of FeLV and FIV of cats in Istanbul Province of Turkey.

In this study, the prevalence of FeLV and FIV in shelter cats and outdoor cats was 1.1% and 2.2%, respectively. This prevalence rate was relatively low compared to other studies; but this is not clear enough as with all retrovirus studies. In many studies, in addition to PCR tests, serological evaluation

of the results is also recommended. In this study, only a PCR test was performed, so this may be a limitation that affected the prevalence rate. There may be different reasons for the low prevalence, but we do not think that it is related to test sensitivity or extraction. Molecular methods such as PCR (especially nested PCR) are considered more sensitive by researchers for detecting these viral agents (Arjona et al., 2007). Extraction controls were performed by PCR with housekeeping primers and clinical samples which were not kept in the freezer for a long time. The real cause may be low virus titre in the swabs taken for diagnosis; it could also be related to the infection period or a small cat population. In this study, clinical materials such as blood, which is commonly used in the diagnosis of cat retroviruses, was not preferred and all tests were performed from the cats' swabs. Similar studies have been reported in the past (Gomes-Keller et al., 2006; Westman et al., 2016, 2017; Studer et al., 2019; Victor et al., 2020). Because this method does not require invasive applications or sedation, the animal is not stressed and the sampling is easier. Normally it is necessary to determine positivity based on both blood samples and swabs, but this was not possible in our study. Future researchers might consider both methods.

The detection and characterisation of FeLV and FIV has not been studied extensively in Turkey. Only one study was found in which characterisation and phylogenetic analysis of FIV strains and/or isolates were performed. According to the results of this study conducted in Turkey, the two FIV strains were in subtype B and in a different cluster that has not yet been named (Oğuzoğlu et al., 2010b). By phylogenetic analysis we determined that one of the two FIV strains belonged to subtype A and the other to subtype B. This is the first study to indicate the presence of FIV subtype A in Turkey.

Globally, the most common subtypes of FIV are A, B, and C, which are found on all continents (Hayward and Rodrigo, 2010). While subtype A has been detected in Europe, the United States, and Australia, subtype B has been detected in Europe, South America, and Asia. Subtype D has

been detected in Japan, Vietnam, and Thailand, subtype E in Argentina and Russia, and subtype F in Europe and the United States. U-NZenv is only defined as existing in New Zealand. Recombinant sequences were also found among the subtypes, which occurred between A–B, B–D, and A–C (Samman et al., 2011; Perharić et al., 2016; Zhang et al., 2017; Martins et al., 2018).

In Turkey, until now, only B subtypes have been identified. In some studies, there are reports that the different subtypes of FIV led to differences in the clinical appearance of cats. It has been reported that FIV subtype A causes neurological findings and that fewer signs are shown with subtype B, while subtype C is the most pathogenic and causes strong immunosuppression (Huguet et al., 2019). However, in this study, no neurological disorder was found in the cat in which subtype A was detected.

FFV may be associated with single or multiple cat retroviruses. We have not detected this retrovirus in cats in Turkey (Koç and Oğuzoğlu, 2019).

As a result, this study shows that FeLV, FHV-1, FIV, and FPLV are still circulating in Turkey.

The presence of FIV subtypes A and B in the country has been identified in this study.

Vaccination should be continued against FCV, FHV-1, and FPLV, although the vaccines do not provide sterile immunity, and more prevalent strains should be used. Cats accepted by shelters should be vaccinated again, regardless of their vaccination history. In addition, cats in shelters should be tested at certain intervals and animals found positive should be housed in different places. Characterisations of the strains obtained from this study are thought to contribute to the choice of the vaccine strain. Unfortunately, research and preventive measures are insufficient in Turkey, especially regarding FeLV and FIV; it is recommended to expand research and tighten the preventive measures.

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