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RESEARCH ARTICLE

Molecular Detection and Clinical Aspects of Feline Herpesvirus-1, Feline Immunodeficiency Virus and Feline Leukemia Virus in Cats in Istanbul, Turkey

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ABSTRACT

The aim of this study was molecular detection of FIV, FeLV and FHV-1 and determination of frequency of these infections in cats and evaluation of clinical signs and status of the animals. For this, 30 household cats and 30 stray cats were clinically examined and blood samples and eye-swabs were taken. Samples were analysed by ELISA and PCR. In ELISA, 4 of 60 cats were positive for FIV and 6 of 60 for FeLV. FIV proviral-DNA was detected in 6 of 60 cats and FeLV proviral-DNA in 11 of 60 cats by PCR. Also, FHV-1-DNA was detected in eye swabs in 26 cats out of 60 cats. Clinically, eye disorders, ulcers in oral mucosa and respiratory disorders were observed in FIV, FeLV and FHV infected cats. Eye disorders were mostly seen in FHV-1 positive cats while fever was prominent in FeLV and FHV infected cats. The results show that FIV, FeLV and herpesvirus-1 are affecting the cat's health and can make them susceptible to other pathogens which increase the risk of cat's life.

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INTRODUCTION

Feline immunodeficiency virus (FIV), feline leukemia virus (FeLV) and feline herpesvirus-1 (FHV-1) cause serious diseases in cats. The co-infection of 3 viruses makes the cat health worse and the risk of developing lymphoma may increase (Chhetri *et al.,* 2015; McLuckie *et al.,* 2018). FIV and FeLV are lymphotropic retroviruses which suppress the immune system of cats resulting in a wide range of clinical signs like depression and secondary infections causing gingivitis, stomatitis and systemic infections causing death (Yilmaz *et al.,* 2000; Arjona *et al*., 2006; Chhetri *et al.,* 2015). Both viruses particularly FIV are transmitted mainly through bites and blood (Sivagurunathan *et al.,* 2018). FHV-1 is responsible 50-70% of the ocular disease and upper respiratory infections in cats. FeHV-1 replicates in the epithelial cells and cause depression, pyrexia, anorexia, sneezing, conjunctivitis, keratitis and oculonasal discharge. Lifelong latency may occur. Cats having acute infection shed virus by saliva, ocular and nasal discharges and transmit the virus to other cats by close contact (Nasisse *et al.,* 1998; Kucuk *et al*., 2017; McLuckie *et al.,* 2018).

FIV, FeLV and FHV-1 infections in cats have been reported worldwide including Turkey (Arjona *et al*., 2000; Yilmaz *et al.,* 2000; Chhetri *et al.,* 2015; Ertl *et al*., 2015; Tasker *et al*., 2016; Kucuk *et al.,* 2017; Sivagurunathan *et al.,* 2018). In these studies, ELISA and PCR are mostly used to detect infected cats and to determine disease association with other factors age, sex, breed, neutering status, outdoor access and multi-cat households have been recognized as risk factors associated with FIV and FeLV infections (Arjona *et al*., 2000; Yilmaz *et al*., 2000; Chhetri *et al.,* 2015; Sivagurunathan *et al.,* 2018). At present, limited information available for these 3 viruses infecting cats together in Turkey. Therefore, the aim of this study was molecular detection of FIV, FeLV and FHV-1 and determination of frequency of FIV, FeLV and FHV-1 in cats and evaluation of clinical signs and status of the animals.

MATERIALS AND METHODS

Study population and collection of samples: Two populations of cats (household and stray) were targeted to analyze for feline herpesvirus, feline immunodeficiency

virus and feline leukemia virus in Istanbul, Turkey. Cats were first clinically examined and any clinical signs of disease such as fever, lethargy, respiratory signs, eye disorders, gingivitis and ulcers in the mouth and tongue and discharges from the eye and nose were recorded. A total of 60 blood samples with and without EDTA and 60 eye swabs (Microbrush, MFA400) were taken consisting 30 blood and 30 eye swabs from each population. Eye swabs were taken into tubes containing 50µl of sterile distilled water. All samples were transferred to laboratory in a cold storage. All cats were above 1 year of age. 32 female and 28 male cats were sampled.

ELISA: ELISA was used to detect FIV p-24 antigen (PetCheck–FIV-Antigen-IDEXX) and FELV p-27 antigen (Snap-FeLV-Antigen-IDEXX) in the cat sera as described by the manufacturer.

DNA extraction: DNA was extracted by using commercial DNA extraction kit from eye swabs for FHV (GenElute-Sigma-No: G1N-70) and from blood for FIV and FeLV (GenElute- Blood-Sigma-No: N-2010) as described by the manufacturer.

Polymerase chain reaction: The primers used to detect FHV (322bp), FIV (139bp) and FeLV (101bp) were from previously published studies (Nasisse *et al.,* 1998; Tasker *et al.,* 2006; Pinches *et al.,* 2007). For the test and control samples, PCR master mix kit (PCR Core kit-Sigma-Core-1) was used. An optimized 50µl PCR mixture consisted of 1.5mM MgCl2, 50mM KCl, 10mM Tris-HCl (pH 8.3), 200µM each dNTP, 1.0 U Taq-polymerase (Sigma-D1804), 0.1 µM each primer and 10 µl template DNA for FIV and FeLV and 8 µl for FHV-1. Amplifications for FHV, FIV and FeLV were performed in a thermal cycler (Biometra) by following the protocols described previously (Nasisse *et al.,* 1998; Tasker *et al.,* 2006; Pinches *et al.,* 2007). For all PCR reactions, positive (Professor M. Ackermann, Zurich University, Switzerland; Dr. C Helps, Bristol University, UK) and negative controls were included. For the negative controls, nuclease free water was included in place of template DNA. PCR products were analysed by horizontal gel electrophoresis using 1.5% agarose gel.

RESULTS AND DISCUSSION

Clinical findings: Amongst 60 cats, 26 (43%) of them sick and 34 (57%) were found to be clinically healthy. In sick cats, 12 (46%) cats were stray cats while 14 (54%) cats had owners. In sick cats living indoors (had owners) 8 (57%) cats had conjunctivitis, 2 (14%) cornea ulcer, 5 (36%) ocular discharge and 10 (71%) fever, 2 (14%) other eye disorders, 4 (29%) inappetence, 3 (21%) respiratory problems and 4 (29%) with wounds in the mouth and nose. In sick stray cats, 12 (10%) cats had conjunctivitis, 4 (33%) cats cornea ulcer, 9 (75%) ocular discharge, 1 (8%) other eye problems, 7 (58%) fever, 4 (33%) respiratory problems, and 6 (50%) wounds in the mouth and nose (Table 1).

ELISA: In FIV ELISA, amongst 60 cats, FIV antigen was detected in 4 (8.3%) cats. 1 (3.3%) cat was living indoors while the other 3 (10%) cats living in the street. In positive cats, 2 (50 %) cats were male 2 (50%) cats were female (Table 2).

In FeLV ELISA, amongst 60 cats, FeLV antigen was detected in 6 (10%) cats. 4 (13.3%) cats were living indoors while the other 2 (6.7%) cats living in the street. In positive cats, 5 (83.3%) cats were female 1 (16.7%) cats were male (Table 2).

Polymerase chain reaction

FHV-1-DNA: A 322 bp product was seen on gel electrophoresis in positive control and positive test samples but not in negative control. Amongst 60 cats, FHV-1-DNA was detected in the eye of 26 (43.3%) cats. 8 (26.6%) cats were living indoors while the other 18 (60%) cats living in the street. In cats positive for FHV-1, 12 (46.2%) cats were female 15 (57.7%) cats were male (Table 2). 8 of the 26 FHV-1 positive cats were also found to be positive either for FIV or FeLV or both (Fig. 1).

Table 1: Symptoms seen in sick cats positive for FIV, FeLV and FHV by PCR

Symptoms	% FIV	%FeLV	%FHV
House cats (n=14)	7%	29%	57%
Conjunctivitis (n=8)	13%	13%	88%
Corneal ulser (n=2)	0%	0%	100%
Eye discharge (n=5)	0%	20%	100%
Symblefaron (n=2)	0%	50%	100%
Fever (n=10)	10%	30%	40%
Inappetence (n=4)	25%	75%	0%
Respiratory disorder (n=3)	33%	33%	33%
Ulcers in the mouth (n=4)	25%	50%	25%
Stray cats (n=12)	42%	50%	83%
Conjunctivitis (n=10)	40%	40%	90%
Corneal ulser (n=4)	25%	25%	100%
Eye discharge (n=4)	44%	56%	100%
Symblefaron (n=1)	0%	0%	100%
Fever (n=7)	19%	71%	86%
Respiratory disorder (n=4)	75%	75%	100%
Ulcers in the mouth (n=6)	83%	50%	67%

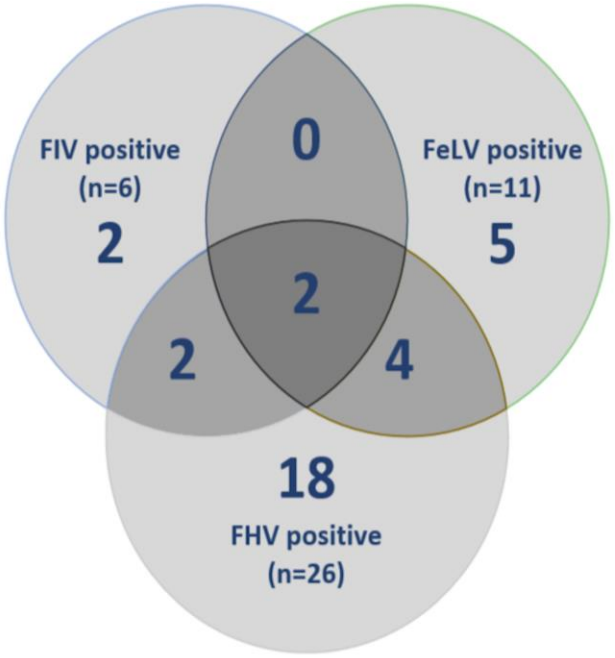

Fig. 1: Overlapping positivity of the PCR results for FHV, FIV and FeLV infected cats analysed in this study. The figure shows the frequency of the single, dual or triple infections in cats.

Table 2: Lifestyle and sex of PCR positive cats for FIV, FeLV and FHV (upper part) and seropositive cats for FIV and FeLV (lower part)

		PCR						
Lifestyle and sex of cats		% FIV positive	% FeLV positive	% FHV positive	% FIV and FeLV positive	% FIV and FHV positive	% FHV and FeLV positive	% FIV, FeLV and FHV positive
Stray cats	F (n=18)	17%	22%	61%	6%	11%	22%	6%
	M (n=12)	17%	25%	58%	8%	17%	8%	8%
House cats	F (n=14)	0%	7%	29%	0%	0%	0%	0%
	M (n=16)	6%	13%	25%	0%	0%	6%	0%
		ELISA						
Lifestyle and	sex of cats	% FIV seropositive	% FeLV seropositive	% FIV and FeLV seropositive				
Stray cats	F (n=18)	11%	0%	0%				
	M (n=12)	8%	17%	0%				
House cats	F (n=14)	0%	7%	0%				
	M (n=16)	6%	19%	0%				

FIV-proviral DNA: A 139 bp product was seen on gel electrophoresis in positive control and positive test samples but not in negative control. Amongst 60 cats, FIV proviral DNA was detected in 6 (10%) cats. 1 (3.3%) cats were living indoors while the other 5 (16.7%) cats living in the street. In cats positive for FIV-proviral DNA, 3 (50%) cats were female 3 (50%) cats were male (Table 2). 4 of the 6 FIV positive cats were also found to be positive either for FHV-1 or FeLV or both (Figure 1).

FeLV-proviral DNA: A 101 bp product was seen on gel electrophoresis in positive control and positive test samples but not in negative control. Amongst 60 cats, FeLV proviral DNA was detected in 11 (18.3%) cats. 4 (13.3%) cats were living indoors while the other 4 (23.3%) cats living in the street. In cats positive for FeLV-proviral DNA, 5 (45.5%) cats were female 6 (54.5%) cats were male (Table 2). 6 of the 11 FeLV positive cats were also found to be positive either for FHV-1 or FIV or both (Fig. 1).

Various prevalences have been reported for FIV and FeLV in cats in Turkey and other countries depending on life style, health status, age and sex (Arjona *et al.*, 2000; Yilmaz *et al.*, 2000; Chhetri *et al.*, 2015; Ertl *et al.*, 2015; Sivagurunathan *et al.*, 2018). In this study, 4 (8.3%) out of 60 samples examined by ELISA (8.3%) were found to be positive for FIV and 6 (10%) of them were positive for FeLV. In a previous study performed in Turkey, the seropositivity of FIV was 22.3% while it was 5.8% for FeLV (Yilmaz *et al.*, 2000).

In Malaysia, 10% were seropositive for FIV; 12% were seropositive for FeLV and 2.6% were seropositive for both (Sivagurunathan *et al.*, 2018). Factors associated with FIV seropositivity include adulthood, being male and having access to outdoors, while clinical illness was a stronger predictor for FeLV seropositivity (Chhetri *et al.*, 2015). The results of this study also indicate that frequency of FeLV and FIV were higher in sick cats and FIV in stray cats. In addition, both infections seem to be more frequent in male cats. Interestingly, the frequency of being positive for both viruses FIV and FeLV was very low. Similar results were obtained in Spain that in healthy cats, 15.6% were positive for FeLV, 8.3% were positive for FIV and 1.1% were positive for FIV and FeLV (Arjona *et al.*, 2000). In the present study, ELISA results indicate that cats living outdoors are at particular risk for FIV infection than cats living indoors. In contrast to FIV, 6 male cats and 1 female cat were positive for FeLV antigen indicating FeLV seropositivity was higher in male cats. The reason for that can be the result of aggressive behaviour in male cats during mating period. In contrast to FIV, amongst positive cats only 2 cats were living outdoors. The reason for that can be because FeLV positive outdoor cats might be dying earlier than the cats living indoors. Another possibility is that vertical transmission might be occurring in a higher rate in cats living indoors.

In this study, 2 tests, ELISA and PCR were used to determine FIV and FeLV infections. The prevalence (18.3%) of infected cats with FeLV detected in the PCR test was higher than that was found by ELISA (10%). Similar results were obtained in other study indicating that more positivity for FeLV was detected by PCR especially in the early phase of infection (Arjona *et al.*, 2000).

In the present study, PCR was performed and FHV-1-DNA in 26 (43.3%), FIV proviral DNA in 6 (10%) and FeLV proviral DNA in 11 (18.3%) cats were detected. In a previous study in Turkey, FeHV-1 DNA was found in 25.3% of cats. The presence of FeHV-1 infection was determined in 47.6% (10/21) of symptomatic and in 16.7% (9/54) of clinically healthy cats (Kucuk *et al.*, 2017). In a study in the USA, FHV-1 DNA was detected in 5.9% (1/17) of eyes from clinically normal cats and up to 76.3% of cats with eye problems (Nasisse *et al.*, 1998). Similar to above studies, in this study, FHV-1 was more frequent in cats with eye disorders.

It has been reported that, presence of FIV and/or FeLV increases the risk of other infections in cats (McLuckie *et al.*, 2018). This is the reason cats were also analysed for FHV-1 infections in this study. In the United Kingdom, FcaGHV1 DNA was detected in the blood of 11.56% of cats and most of those were also positive for haemoplasma (McLuckie *et al.*, 2018). The prevalence of GHV in pet cats from Germany and Austria was 16.2%. The GHV infection was high in FIV positive indicating the potential role as a co-factor in FIV-induced pathogeneses (Ertl *et al.*, 2015). In the present study, FHV-1 was found in 4 FeLV positive and in 2 FIV positive cats. Interestingly, 2 or 3 of these viruses together in one cat were not detected in house cats.

Conclusions: This study indicate that FIV, FeLV and FHV-1 infections are prevalent in Turkish cats, particularly in sick cats and the frequency of FHV-1 infection is high in cats with eye lesions. Therefore, cats with eye problems should be checked for FHV-1. Also, cats having lesions in their mouth should be analysed carefully for FIV and FeLV. Results of ELISA and PCR

results of FIV and FeLV show that FIV negative samples by ELISA should be analysed by PCR. Whereas, FeLV samples should be checked by both tests especially in the early phase of the FeLV infection.

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Authors contribution: HY was the supervisor of this research. He stated work plan and evaluated the work steps in the laboratory and field. He also contributed to the laboratory work, writing and editing the manuscript. EB has performed most of the laboratory and field work. He contributed to the writing of the manuscript.

REFERENCES

Arjona A, Escolar E, Soto I, *et al.,* 2000. Seroepidemiological survey of infection by feline leukemia virus and immunodeficiency virus in Madrid and correlation with some clinical aspects. J Clin Microbiol 38:3448-9.

Chhetri BK, Berke O, Pearl DL, *et al.,* 2015. Comparison of risk factors for seropositivity to feline immunodeficiency virus and feline leukemia virus among cats: a case-case Study. BMC Vet Res 11:1-7.

Ertl R, Korb M, Langbein-Detsch I, *et al.,* 2015. Prevalence and risk factors of gammaherpesvirus infection in domestic cats in Central Europe. Virol J 12:1-4.

Kucuk A, Sag N, Cakir C, *et al.,* 2017. Sağlıklı görünüşlü ve solunum sistemi problemli barinak kedilerinde feline herpesvirus tip 1 (FeHV-1) Enfeksiyonu. Erciyes Üni Vet Fak Dergi 14:25-30.

McLuckie AJ, Barrs VR, Lindsay S, *et al.,* 2018. Molecular Diagnosis of Felis catus Gammaherpesvirus 1 (FcaGHV1) infection in cats of known retrovirus status with and without lymphoma. Viruses 10-3:1-14.

Nasisse MP, Glover TL and Moore CP, 1998. Detection of feline herpesvirus 1 DNA in corneas of cats with eosinophilic keratitis of corneal sequestration. AJVR 59:856-8.

Pinches MD, Diesel G, Helps CR, *et al.,* 2007. An update on FIV and FeLV test performance using a Bayesian statistical approach. Vet Clin Pathol 36:141-7.

Sivagurunathan A, Atwa AM and Lobetti R, 2018. Prevalence of feline immunodeficiency virus and feline leukaemia virus infection in Malaysia: a retrospective study. J Feline Med Surg Open Rep 4:1-5.

Tasker S, Caney SM, Day MJ, *et al.,* 2006. Effect of chronic FIV infection, and efficacy of marbofloxacin treatment, on Mycoplasma haemofelis infection. Vet Microbiol 117:169-79.

Yilmaz H, Ilgaz A and Harbour DA, 2000. Prevalence of FIV and FeLV infections in cats in Istanbul. J Feline Med Surg 2:69-70.