

## Felid Herpesvirus - 1 Infection in Van Cats with Conjunctivitis

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### SUMMARY

Felid herpesvirus 1 (FeHV-1) infecting in felidae can cause severe upper respiratory tract disease with clinical symptoms including nasal discharge, sneezing, inappetence, pyrexia and conjunctivitis. In this study, 20 ocular swab samples were obtained from Van cats, in age from 9 days to 1 year, presented signs of FeHV-1 infection. Polymerase Chain Reaction (PCR) method was used for the detection of virus. FeHV-1 specific amplicon (737 bp) was detected in 9 (45%) cases. Sequence analysis revealed that there is no diversity between the amplicons detected in samples. In conclusion, more effective preventive precautions should also perform for continuity of Van cat lineage in addition to routinely vaccination.

### Key Words

*Van cat, Conjunctivitis, Felid herpesvirus-1 (FeHV-1), PCR*

## Konjunktivitli Van Kedilerinde Felid herpesvirus - 1 Enfeksiyonu

### ÖZET

Felid herpes virus -1 ( FeHV-1) kedilerde genellikle nasal akıntı, hapşırma, iştahsızlık, yüksek ateş ve konjunktivit ile seyreden üst solunum sistemi enfeksiyonuna neden olur. Bu çalışmada Türkiye'de, FeHV-1 ile uyumlu klinik enfeksiyon belirtileri gösteren ve yaşları 9 gün ile 1 yıl arasında değişen 20 adet Van kedisinden alınan göz sıvı örnekleri FeHV-1 varlığı yönünden incelendi. Virus tespiti için Polimerize Zincir Reaksiyonu (PZR) yönteminden yararlanıldı. İncelenen kedilerin 9 (45%)' undan alınan örneklerde FeHV-1 spesifik amplicon (737 bç) tespit edildi. Elde edilen ampliconların sekans analizinde örnekler arasında fark bulunamadı. Sonuç olarak; Van kedisi neslinin korunması için daha etkili tedbirler alınması ile beraber rutin aşılamaların yapılması kanaatine varıldı.

### Anahtar Kelimeler

*Van kedisi, Konjunktivit, Felid herpesvirus - 1 (FeHV-1), PZR*

### INTRODUCTION

Felid herpesvirus - 1 (FeHV-1) causes an upper respiratory tract infection, known as feline viral rhinotracheitis, characterized by symptoms such as sneezing, eye lesions, high temperature, anorexia, runny eyes and nose, and conjunctivitis in felidae (Cai et al. 2002; Di Martino et al. 2007). FeHV-1 is a member of the *Alphaherpesvirinae* subfamily of the family *Herpesviridae*, and is transmitted via aerosol droplets and direct contact with the ocular, nasal and oral secretions of infected cats (Pedersen, 1987; Davison et al. 2009). After acute infection, as in all infections of the *Herpesviridae* family, a lifelong latency develops, and the virus persists in the neurological tissues (trigeminal ganglia) as the main site of viral latency (Gaskell et al. 2007). The infection is characterized by a high rate of mortality in new-born cats or cats with a depressed immune system (Povey, 1979).

The virus was first isolated from kittens with upper respiratory tract disease in 1958 (Crandell and Maurer, 1958). Several researchers throughout the world reported the high prevalence of FHV1 in cats (Nakamura et al. 1999; Burns et al. 2011). In contrast to these reports there are only a few reports for the presence of FeHV-1 in Turkish cats (Bilge Dağalp and Akça, 2004). There is also only one

study reported FeHV-1 infection in purebred Van cat in Turkey (Çabalar and Bilge Dağalp, 2008)

The presented study, reported the detection of FeHV-1 by PCR in purebred Van cats with conjunctivitis, in Van-Turkey.

### MATERIALS and METHODS

#### Samples

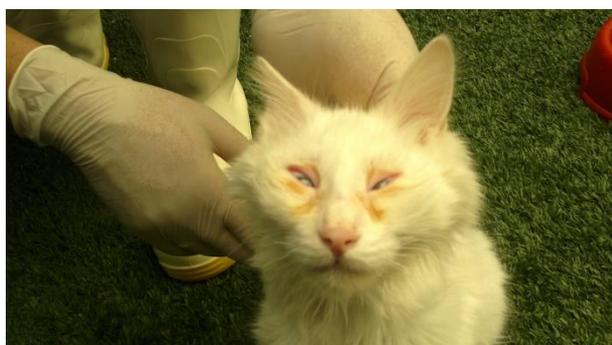
A total of 20 ocular swabs were obtained from cats showing symptoms of conjunctivitis (Figure 1).

The age of the infected cats ranging in 9 days to 1 year were kept in Van cat house of University of Yuzuncu Yil in Turkey. Excepting kitten younger than 2 months old, all cats were vaccinated regularly with inactive vaccine (Table 1). All ocular specimens were collected in 2 ml of Eagle's minimum essential medium (EMEM) containing penicillin (100units/mL) and streptomycin (0.1 mg/mL).

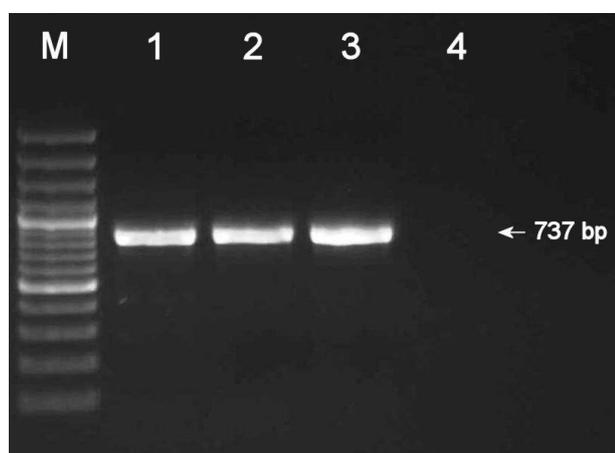
#### DNA Extraction and PCR

Viral DNA was extracted from 200 µL volumes of each sample using by High Pure Viral Nucleic Acid Extraction kit (Roche, Germany) according to manufacturer's instructions. A size of 737 bp for glycoprotein B (gB) gene

of FeHV-1 was amplified by PCR, using primer set as described by Vöggtlin et al. (2002). The PCR mix, with a total volume of 30  $\mu$ L, contained 3  $\mu$ L of extracted DNA, 3  $\mu$ L of Taq buffer (10 $\times$ , 750 mM Tris-HCl, 200 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% (v/v) Tween 20), 2  $\mu$ L of the MgCl<sub>2</sub> (25mM), 1  $\mu$ L of each primer (10mM), 1  $\mu$ L of the four deoxynucleoside triphosphates (10 mM) solution, 0,25  $\mu$ L of Taq DNA polymerase (5U/ $\mu$ L), and 18,75  $\mu$ L of molecular biology grade water (Thermoscientific, USA). Sterile molecular biology grade water was also used as a negative control. The PCR reactions were performed using a thermalcycler (Techne 3000G, BibbyScientific Ltd., UK) and using an initial denaturation at 95°C for 4 min followed by 27 cycles consisting of denaturation (95°C for 45 min), annealing (55°C for 1 min) and elongation (72°C for 1 min) as there action conditions. The PCR products were visualized using by a transilluminator after separation on a 2% agarose gel (Figure 2).



**Figure 1.** The ocular sign in a Van cat with conjunctivitis



**Figure 2.** PCR amplification products using FeHV-1 primers. Lane M: 100-bp DNA ladder marker (Thermoscientific); lane 1, 2 and 3: positive amplification PCR products; lane 4: negative control (distilled water)

#### DNA Sequencing

FeHV-1 specific DNA in these samples was confirmed by sequence analysis (Beckman Coulter CEQ 8000, USA). Positive amplicons were sequenced commercially. The accession numbers of *gB* gene sequences of Turkish FeHV-1 strains in the GenBank were recorded as KC529346, KC529347 and KC529348 respectively.

#### RESULTS and DISCUSSION

Recent studies indicated that FeHV-1 was detected in higher portion in cats, especially in shelter cats (Bilge-Dağalp and Akça 2004, Burns et al. 2011). Prevalence of FeHV-1 varied from 4.2% to 90.1% was reported in cats

with clinical symptoms such as upper respiratory disease and conjunctivitis (Harbour et al. 1991; Stiles et al. 1997; Bilge Dağalp and Akça 2004; Burns et al. 2011). However, FeHV-1 could also be detected in the healthy cats as high as in 63% (Harbour et al. 1991; Kang and Park, 2008). Furthermore, previous studies reported also a high seropositivity (58.3%) in Van cats with upper respiratory tract disease and conjunctivitis from the same Van cat house in Turkey, where the present study is performed (Çabalar and Bilge Dağalp 2008).

In the presented study, ocular samples from 20 cats with conjunctivitis were examined for FeHV-1 by PCR. FeHV-1 was detected in 9 (45%) cats with conjunctivitis. In the cathouse, new-born cats were kept in separate room together with their mothers for one month and then were moved to the common area. Two mother cats and their kittens marked with 1 and 4 were positive for FHV1. But another mother cat marked with 2 was found to be negative for FeHV-1, however two of her three kittens were positive for FeHV-1. Both mother cat and her kitten marked with 3 were negative for FeHV-1 (Table 1).

**Table 1.** Distribution of cats with conjunctivitis to their ages and to PCR results

No of cats	Age of cats	FeHV-1 DNA
1	1 year <sup>1,a</sup>	+
2	9 days <sup>1,b</sup>	+
3	9 days <sup>1,b</sup>	+
4	1 year <sup>2,a</sup>	-
5	15 days <sup>2,b</sup>	+
6	15 days <sup>2,b</sup>	-
7	15 days <sup>2,b</sup>	+
8	1 year <sup>3,a</sup>	-
9	10 days <sup>3,b</sup>	-
10	1 year <sup>4,a</sup>	+
11	10 days <sup>4,b</sup>	+
12	2 months <sup>b</sup>	+
13	2 months <sup>b</sup>	+
14	2 months <sup>b</sup>	-
15	2 months <sup>b</sup>	-
16	3 months <sup>a</sup>	-
17	10 months <sup>a</sup>	-
18	10 months <sup>a</sup>	-
19	10 months <sup>a</sup>	-
20	1 year <sup>a</sup>	-

<sup>1, 2, 3, 4</sup>Mother cats and their kittens; <sup>a</sup> vaccinated; <sup>b</sup> unvaccinated

It is likely that the source of infection is originated from their mothers which would be latently infected with FeHV-1. Latently infected cats may also be an important health risks for cat population in shelter as described elsewhere (Gaskell and Povey, 1982; Weigler et al. 1997; Bilge Dağalp and Akça 2004).

On the other hand, out of the 9 cats aged over 2 mounts without kittens, only two cats were found positive for FeHV-1, which may also be source of FeHV-1 infection for kittens or their mothers examined in this study (Table 1). Other studies suggested also that vaccination cannot protect cats from infection, but it may reduce the term and amount of virus shedding (Gaskell and Willoughby, 1999).

In conclusion, this result indicates that FeHV-1 infection has still been circulating in Van cat house in Turkey. Future studies should be performed to identify the presence of other viral and bacterial pathogens such as feline calicivirus and *Chlamydomphila felis* in shelters because some of clinically diseased cats were determined not be positive for FeHV-1. More effective preventive precaution in addition to routine vaccination should be performed to ensure the continuity of Van cat lineage.

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