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
Vancat, Conjunctivitis, Felid herpesvirus-1 (FeHV-1), PCR

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 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, 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 litters 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 Daşğalp and Akça, 2004). There is also only one study reported FeHV-1 infection in purebred Van cat in Turkey (Çabalar and Bilge Daşğalp, 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 (100 units/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 Vögtlin et al. (2002). The PCR mix, with a total volume of 30μL, contained 3μL of extracted DNA, 3μL of Taq buffer (10×750 mM Tris-HCl, 200 mM (NH₄)₂SO₄, 0.1% (v/v) Tween 20), 2μL of the MgCl₂ (25mM), 1μL of each primer (10mM), 1μL of the four deoxynucleoside triphosphates (10 mM) solution, 0.25 μL of Taq DNA polymerase (5U/μL), and 18.75μL 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 (Techno 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).

**RESULTS**

Accession numbers of positive amplicons were sequenced commercially. The DNA sequences of Turkish strains in the GenBank were recorded as KC529346, KC529347 and KC529348 respectively.

**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.

**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

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

1. Mother cats and their kittens; a vaccinated; b 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 Chlamydophila felis in shelters because some of clinically diseased cats were determined not to 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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REFERENCES


