Occurrence of Chlamydophila felis, feline herpesvirus 1 and calcivirus in domestic cats of Iran

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ABSTRACT

Background and Objectives: Feline herpesvirus-1, feline calicivirus and Chlamydophila felis are the main causes of feline upper respiratory tract disease. This study was conducted to identify of FeHV-1, FCV and C. felis infections in domestic cat population and also to estimate the prevalence of each specific infection in Iran.

Materials and Methods: The ocular conjunctiva and oropharyngeal specimens obtained from 80 cats were examined using PCR and reverse transcription PCR.

Results: FeHV-1 was detected in 23 (28.8%), FCV in 2 (2.5%) and C. felis in 16 (20%) cats. Twelve cats(15%) had co-infection with 2 or 3 of the mentioned pathogens. Ocular lesions were the most common clinical signs in the FeHV-1 and C. felis infections whereas respiratory lesions were more observed with the FCV infections. It seems that there is an age-related tendency in the infected cats, meaning that the age of the C. felis positive cats was less than those with FeHV-1 and FCV infections.

Conclusion: These results confirm the presence and show the prevalence of three major pathogens associated with upper respiratory tract disease for the first time in Iran.

Keywords: Chlamydophila felis, FeHV-1, FCV, URSD, Ocular lesions

INTRODUCTION

Upper respiratory tract disease (URTD) is a common disease in domestic cats with worldwide prevalence. Mainly three pathogens have been associated with URTD, a Gram negative, obligatory intracellular bacterium: Chlamydophila felis and two highly contagious viruses: feline herpesvirus-1 (FeHV-1) that is a member of the Varicellovirus genus of the subfamily Alphaherpesvirinae containing double-stranded DNA with a glycoprotein-lipid envelope and feline calicivirus (FCV) which is a small, unenveloped, single-stranded positive-sense RNA virus in the family Caliciviridae, genus Vesivirus (1, 2).

These three pathogens are responsible for acute illness and may also be the cause of recurrent or chron-
ic ocular, oral and respiratory lesions. Clinical signs associated with these agents include nasal and ocular discharge, sneezing, coughing, oral ulcersations, chronic gingivitis, acute or chronic conjunctivitis, blepharospasm and abortion (2, 3).

The prevalence of FeHV-1, FCV and *C. felis* in cats has been reported throughout the world and dual or multiple infections with FCV, FeHV-1 and *C. felis* have been identified (4-7). Despite the presence of clinical evidence of URTD in cats, the causative pathogens have not been yet identified in Iran. The aim of this study was to identify the FeHV-1, FCV and *C. felis* infections in domestic cat population and also to determine the occurrence of each specific infection in Iran.

**MATERIALS AND METHODS**

Samples were collected randomly from 80 domestic short hair cats (39 males and 41 females, age ≤ 6 month) attended at the teaching pet hospital of veterinary faculty of Tehran University from October 2009 until May 2010. Information about sex, age, vaccine status and observed clinical signs were recorded for each cat and then specimens were obtained from the conjunctiva and the oropharynx. Nucleic acids were extracted from specimens using RNeasy Total RNA Kit (Qiagen, Valencia, CA) and DNA isolation kit for cell and tissues (Roche Applied Science, Germany) according to the manufacturer’s instructions. Complementary DNA (cDNA) of the isolated RNA was produced using moloney murine leukemia virus reverse transcriptase (MMLV-RT, Fermentas) and random hexamer primers (Fermentas).

DNAs from *Chlamydia trachomatis* (D0015, Genekam Biotechnology AG, Germany), *Chlamydia pneumoniae* (D0015, Genekam Biotechnology AG, Germany) and the vaccine strains of FeHV and FCV (Fel-O-Vax® PCT, Fort Dodge, Iowa 50501, USA) were used as positive controls. Sterile distilled water was used as negative control. The extracted DNA and synthesised cDNA were amplified using three pairs of oligonucleotide primers which previously designed (8). The primer pair HerpF / HerpR was used to amplify a 292 base pair (bp) region in the thymidine kinase gene of FeHV-1. The primer pair CalcapF / CalcapR was used to amplify a 673 bp sequence of FCV capsid protein gene. Amplification of a 1069 bp fragment of the *C. felis* outer membrane protein gene was performed by using the ChlaF and ChlaR primers. PCR and RT-PCR was carried out according to protocols described elsewhere (8, 9). A Chi-square test and Fisher exact test was used to determine significant association between age, sex, clinical signs and each pathogen (p≤ 0.05).

**RESULTS**

Of the 80 examined cats, 23 (28.8%) were positive for FeHV-1, 16 (20%) for *C. felis* and 2 (2.5%) for FCV (Table 1). Overall, the percentage of positive cats was 33.75% (27/80). Single infections were detected in 15 cats of which 11 were related to FeHV-1 and 4 were related to *C. felis*. Dual infections (FeHV-1 and *C. felis*) were detected in 10 cats. Two cats had multiple infections with FeHV-1, *C. felis* and FCV. Overall 74% of positive cats had clinical signs. Six of 23 (26.1%) FeHV-1 positive cats had no clinical signs while 3 of 16 (18.7%) *C. felis* positive cats were without clinical signs (Table 1). Our data indicate that the prevalence of FeHV-1 and *C. felis* infections in clinically normal cats was 17.1% (6/35) and 8.6% (3/35) respectively. Data about sex and prevalence of infections is shown in Table 1. The median age of the *C. felis* positive cats were 2.5 months (range, 1 to 3 months), while median age of FeHV-1 and FCV infected cat were 4 months (range, 3 to 6 months). There was no statistical difference between age, sex and origin of infection (P> 0.05). Ocular discharge and conjunctivitis were the most common clinical signs in FeHV-1 and *C. felis* infections (37.1%, 10/27), although not statistically significant (P> 0.05). Sneezing was recorded in all FCV positive cats.

**DISCUSSION**

In this study, the prevalence of FeHV-1, FCV and *C. felis* were 28.8%, 2.5% and 20% respectively. Our findings is in accordance with those previous reports that stated the prevalence of FeHV-1, FCV and *C. felis* was in the range from 0 to 52, 1 to 29 and 3%, for clinically normal cats of breeding catteries or shelters in the USA and European countries (4, 7, 10), while cats with diseases in the same environments had the prevalence ranging from 0 to 41, 0 to 47 and 0 to 10% respectively (4, 5, 8, 10). This wide range may be due to several reasons: the stress of being captured.
or surrendered to a shelter, the concentration of cats and respiratory agents in shelters, the hygiene status in catteries and shelters, variable vaccination histories and immune status, differences in sensitivity of PCR assay, type and anatomical site of collected specimen and feature of the examined population.

The results of this study showed FeHV-1 infection was the more frequent. It is in accordance with those reported by Sykes et al. (2001), Di Martino et al. (2007) and Burns et al. (2011). Dual or multiple infections was recorded in 11.28% of examined cat and 33.3% of positive cats which the most concurrent infection was FeHV-1 and C. felis. These data consisted with other reports that showed both pathogens were more common in Italian and Japanese cat population (5, 6) whereas it was less frequent in USA and Australia (4, 8).

We found that there is no statically significant difference between sex and origin of infections (P> 0.05). This finding is in line with previous studies where they showed there was no sex predisposition for infections (3, 6). Several reports have shown high occurrence of infections among cats under one-year-old (1, 3). Although not statistically significant here we found that the age of FeHV-1 and FCV positive cats was greater than of C. felis positive cats (P> 0.05). These results could be related with their specific immunological condition.

Our results indicate that the ocular lesions like conjunctivitis and ocular discharge were the most common clinical signs associated with FeHV-1 and C. felis infections while respiratory lesions were most observed in FCV infections. In the case of infection with both viruses, the absence of lesions was rarely observed. Rampazzo et al. (2003) and Hartmann et al. (2010) reported the significant association of presence of C. felis with conjunctivitis. Almost a quarter of FeHV-1 and one-fifth of C. felis infected cats did not show any clinical signs. This situation may be due to existing carrier cats and subclinical form of the disease as shown by Kang and Park (2008). On the other hand, vaccination may be effective because it may reduce the severity of disease, although it could not prevent infection and viral shedding (8).

**CONCLUSION**

This study shows the presence of these pathogens among cat population in the examined area for the first time. The higher prevalence of FeHV-1 infection and concurrent infections with C. felis suggested FeHV-1 can be a main pathogen and C. felis has become a clinical concern. Therefore, it seems necessary to implement the routine vaccination programs. Future studies should be conducted to determine prevalence the disease, identify all pathogens involved in this disease and analysis of risk factors.

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Table 1. Prevalence of FeHV-1, C. felis and FCV in cats based on URTD signs and sex

| Infectious status | Cat with URTD sings | Cat without URTD signs | Sex | Total positive cat by PCR/RT-PCR assays |
|-------------------|---------------------|------------------------|-----|---------------------------------------|
|                   | #                   | #                      | M(#) | F(#) | #                          | %       |
| FeHV-1            | 17                  | 6                      | 11   | 12   | 23                         | 28.8    |
| C. felis          | 13                  | 3                      | 10   | 16   | 16                         | 20      |
| FCV               | 2                   | 0                      | 1    | 1    | 2                          | 2.5     |
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