Iranian Journal of Microbiology
Volume 8 Number 5 (October 2016) 312-315

ORIGINAL ARTICLE

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

Nadi Maazi[1], Shahram Jamshidi[1*], Payman Kayhani[2], Hassan Momtaz[3]

[1]*Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
[2]*Department of Clinical Sciences, Faculty of Veterinary Medicine, ShahreKord Branch, Islamic Azad University, ShahreKord, Iran*
[3]*Department of Microbiology, Faculty of Veterinary Medicine, ShahreKord Branch, Islamic Azad University, ShahreKord, Iran*

Received: March 2016, **Accepted:** August 2016

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-

*Corresponding author: Shahram Jamshidi (DVM, DVSc), P.O. Box: 14155-6453, Tehran, Iran.
Tel: +98-21-66920035
Fax: +98-21-66438327
Email: shjamshidi@ut.ac.ir

ic ocular, oral and respiratory lesions. Clinical signs associated with these agents include nasal and ocular discharge, sneezing, coughing, oral ulcerations, 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 ($p \leq 0.05$).

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

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 sings	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

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.

REFERENCES

1. Di Martino B, Di Francesco CE, Meridiani I, Marsilio F. Etiological investigation of multiple respiratory infections in cats. *New Microbiologica* 2007; 30;455-461.
2. Gaskell RM, Dawson S, Radford A (2006). Feline Respiratory Disease. In: *Infectious Diseases of the Dog and Cat*. Ed, CE Green. 3rd ed. Saunders, Elsevier, St. Louis, Missouri, pp. 145-154.
3. Zicola A, Saegerman C, Quatpers D, Viandier J, Thiry E. Feline herpesvirus 1 and feline calicivirus infections in a heterogeneous cat population of a rescue shelter. *J Feline Med Surg* 2009; 11:1023-1027.
4. Bannasch MJ, Foley JE. Epidemiologic evaluation of multiple respiratory pathogens in cats in animal shelters. *J Feline Med Surg* 2005; 7:109-119.
5. Cai Y, Fukushi H, Koyasu S, Kuroda E, Yamaguchi T, Hirai K. An etiological investigation of domestic cats with conjunctivitis and upper respiratory tract disease in Japan. *J Vet Med Sci* 2002; 64:215-219.

6. Rampazzo A, Appino S, Pregel P, Tarducci A, Zini E, Biolatti B. 2003. Prevalence of *Chlamydophila felis* and feline herpesvirus 1 in cats with conjunctivitis in northern Italy. *J Vet Intern Med* 2003; 17:799-807.
7. Stiles J, McDermott M, Bigsby D, Willis M, Martin C, Roberts W, Greene C. 1997. Use of nested polymerase chain reaction to identify feline herpesvirus in ocular tissue from clinically normal cats and cats with corneal sequestra or conjunctivitis. *Am J Vet Res* 1997 Apr;58(4):338-342.
8. Sykes JE, Allen JL, Studdert VP, Browning GF. Detection of feline calicivirus, feline herpesvirus 1 and *Chlamydia psittaci* mucosal swabs by multiplex RT-PCR/PCR. *Vet Microbiol* 2001; 81:95-108.
9. Kang BT, Park HM. Prevalence of feline herpesvirus 1, feline calicivirus and *Chlamydophila felis* in clinically normal cats at a Korean animal shelter. *J Vet Sci* 2008; 9:207-209.
10. Mochizuki M, Kawakami K, Hashimoto M, Ishida T. Recent epidemiological status of feline upper respiratory infections in Japan. *J Vet Med Sci* 2000; 62;801-803.
11. Burns RE, Wagner DC, Leutenegger CM, Pesavento PA. Histologic and molecular correlation in shelter cats with acute upper respiratory infection. *J Clin Microbiol* 2011; 49:2454-2460.
12. Hartmann AD, Hawley J, Werckenthin C, Lappin M, Hartmann K. Detection of bacterial and viral organisms from the conjunctiva of cats with conjunctivitis and upper respiratory tract disease. *J Feline Med Surg* 2010; 12:775-782.