Short Paper

Feline herpesvirus 1 infections in a domestic cat population in Ahvaz, Iran

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Summary

Feline herpesvirus 1 (FeHV-1) and feline calicivirus (FCV), associated with upper respiratory tract disease, are highly prevalent in cats worldwide. With the aim of investigating the importance of FeHV-1 in a population of cats, samples were taken in a hospital in Ahvaz, south-west Iran, between June 2007 and June 2008. Oropharyngeal, nasal and ocular swabs were collected from 65 domestic cats, including 52 stray and 13 household animals and were tested for the presence of FeHV-1 DNA by polymerase chain reaction (PCR). The overall prevalence rate of FeHV-1 was 35.38%. There was a statistically significant association between the prevalence rate of FeHV-1 and the presence of respiratory signs. High prevalence of FeHV-1 infection strengthens the importance of applying hygienic and preventive measures in cats in the study area.

Key words: Feline herpesvirus 1, Upper respiratory tract disease, PCR

Introduction

Feline herpesvirus 1 (FeHV-1) and feline calicivirus (FCV) are the main agents involved in the feline upper respiratory tract disease (URTD) (Bannasch and Foley, 2005; Gaskell *et al*., 2005). The prevalence of FeHV-1 and FCV infections in cats has been studied and reported from several countries (Coutts *et al*., 1994; Stiles *et al*., 1997; Sykes *et al*., 2001; Cai *et al*., 2002; Rampazzo *et al*., 2003; Bannasch and Foley, 2005; Helps *et al*., 2005; Holst *et al*., 2005; Byeong-Teck and Hee-Myung, 2008; Zicola *et al*., 2009). Such studies have been mostly carried out by virus isolation, serology or amplification of viral nucleic acids by polymerase chain reaction (PCR) on diseased as well as normal cats. Application of PCR to study the prevalence of FeHV-1 and FCV in cat population is due to the fact that both viruses can establish a carrier state and be shed periodically (Gaskell *et al*., 2005; Radford *et al*., 2007).

In Iran, many cases of respiratory disease in cats are frequently observed clinically, but so far there has been no research to determine the presence and prevalence of the viral etiologic agents. However, as well as many other parts of the world, a combined FeHV-1, FCV and feline panleukopenia virus vaccine (NOBIVAC, Intervet, Holland), although limited, has been in use in the country during recent years. As a first attempt to identify the viral respiratory pathogens of cats in Iran, the present investigation was performed in Ahvaz and focused on FeHV-1.

Materials and Methods

Animals and sampling

From June 2007 through June 2008, a total of 65 cats (33 males and 32 females)

were included in the study. The household cats were presented to the Veterinary Hospital of Shahid Chamran University of Ahvaz for vaccination or health care as requested by the owners. Forty eight cats (73.85%) were clinically healthy, while the remaining (n=17, 26.15%) were considered as diseased animals. Health was determined based on general physical examination and the presence or absence of clinical signs of URTD (including marked sneezing, pyrexia, serous nasal discharge and cough), and also ocular disease (conjunctivitis or keratitis) and oral ulcers (Gaskell *et al*., 2007).

In order to estimate the age of animals, history was taken from the owner and also dental formula (Riis *et al*., 1997) was used. The age of the cats ranged from 1 month to 5 years (mean 15 months). According to their age, the animals were divided into two age groups of less than 6 months (13 cats) and more than 6 months (52 cats) old. Vaccination history (against FCV, FeHV-1, Feline panleukopenia virus and Rabies virus) was only available for 8 animals among the household cats. During this study, animals were treated humanely and tranquilized by an intramuscular injection of ketamine hydrochloride (Rotexmedica Co., Germany) (5 mg/kg) prior to subject for sampling. Animal samples from the conjunctival sac of eyes, nose and oropharynx were taken using three sterile cotton tipped swabs.

DNA extraction

Three swabs of each animal were pooled by immersing and shaking them in a 1.5 ml microtube containing 0.5 ml cell culture Dulbecco's modified eagle's medium (DMEM). After shaking for about 1 min the swabs were squeezed and removed. The extraction of DNA was performed using a commercial DNA purification kit (CinnaGen Inc, Iran) according to the manufacturer's instructions. All samples were stored at -70°C until tested.

PCR assays

Detection of FeHV-1 by PCR was carried out with a primer pair, FeHV-F (5´- GCG AAG TAC CTG GTC AGA GC-3´) and FeHV-R (5´- TAG TGG GCG GTG ATA TAG GC -3´), expected to amplify a 182-base pair (bp) fragment of the thymidine kinase gene of FeHV-1 (Rouhizadeh, 2009). The primers have been designed by the program Primer3 and analysed by the BLAST program of the National Center of Biotechnology Information (NCBI) of the USA and sequencing of the PCR product for their specificity. The reaction of PCR consisted of 5 µl 10 X PCR buffer, 1.5 µl $MgCl_2$ (50 mM), 1 µl dNTPs mix (10 mM), 1 µl (50 pmoles) of each primer, 0.5 µl (2.5 U) *Taq* DNA polymerase (CinnaGen Inc, Iran), 35 µl water and 5 µl of template DNA. The mixture was overlaid with 50 µl mineral oil and subjected to a thermal program of 5 min at 95°C, 40 cycles of 1 min at 95°C, 1 min at 54°C, and 1 min at 72°C, followed by a final extension at 72°C for 5 min.

A laboratory strain of FeHV-1, propagated in the feline embryo fibroblast (FEA) cell line and the cell culture medium were, respectively, used as the positive and negative controls in PCR. To verify the results, 10 µl of each PCR product was electrophoresed in a 1.5%-2% agarose gel, stained with ethidium bromide and visualized on a UV transilluminator. Finally, the results were analysed with respect to sex, age, history of vaccination and clinical signs, using the Chi-square test.

Results

A total of 65 cats were included in the study, of which 17 were classified as affected and 48 as healthy cats. Affected cats had evidence of both upper respiratory tract and ocular disease.

Electrophoresis of PCR products and analysis of the results are presented in Fig. 1 and Table 1, respectively. Overall, FeHV-1 was detected in 35.38% of the samples. Following statistical analysis, positive result in PCR was only significantly related to the presence of clinical respiratory signs ($P<0.001$). Cats presenting the clinical signs of URTD and ocular disease showed a prevalence of 70.58%, compared to 22.91% prevalence in healthy cats. There was no significant association between FeHV-1 infection and age, sex, lifestyle as well as

vaccination history in the cats studied.

Table 1: Prevalence of FeHV-1 in domestic cats in Ahvaz, Iran

Variable	Category	No. of FeHV-1 positive cats/No. of tested cats (positive %)
Age	<6 months	4/13 (30.7)
	>6 months	19/52 (36.5)
Sex	Male	13/33 (39.3)
	Female	10/32 (31.2)
Lifestyle	Household	5/13 (38.4)
	Stray	18/52 (34.6)
Vaccine	Vaccinated	3/8 (37.5)
	Unvaccinated	20/57 (35)
Health status	Affected[1]	12/17 (70.5)
	Healthy	11/48 (22.9)
Total		23/65 (35.4)

[1]Cats with evidence of upper respiratory tract and ocular diseases

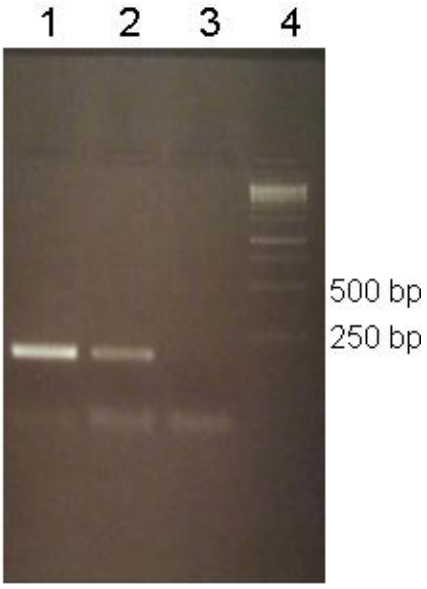

Fig. 1: Agarose gel electrophoresis of FeHV-1 PCR products. Lanes 1 to 4 represent, FeHV-1 positive control, a field sample, the negative control and 1 kb ladder, respectively

Discussion

In the present study, a population of healthy cats showed a prevalence of 22.91% for FeHV-1. With regard to the fact that among the persistently infected animals, only about 29% shed the virus spontaneously (Gaskell *et al*., 2005), the real prevalence of FeHV-1 in the region might be estimated as high. This conclusion can also be supported by data from the diseased cats (17 animals), of which 70.58% were positive in PCR. Therefore, the results suggest that FeHV-1 is an important and prevalent pathogenic agent of cat in the region.

So far, several studies have reported the prevalence of FeHV-1 infections of cat from different countries. Based on the studies in the USA and some European and Asian countries, FeHV-1 infections have been found to be prevalent among healthy cats from 0 to 63% (Stiles *et al*., 1997; Burgesser *et al*., 1999; Nakamura *et al*., 1999; Binns *et al*., 2000; Pedersen *et al*., 2004; Helps *et al*., 2005; Holst *et al*., 2005; Byeong-Teck and Hee-Myung, 2008; Edwards *et al*., 2008). However, data from Japan and England indicates that the prevalence of FeHV-1 in diseased cats has decreased during recent years, compared to past decades (Cai *et al*., 2002; Mochizuki *et al*., 2002). In Japan, two recent studies found the prevalence of FeHV-1 to be 3.6% (Mochizuki *et al*., 2002) and 16.7% (Cai *et al*., 2002) among diseased cats, while in the 1970s, the virus was present at a much higher rate (Mochizuki *et al*., 1977). In contrast, for FeHV-1, an increase has been observed in the role of FCV in URTD in recent years (Mochizuki *et al*., 2002).

FCV is a highly infectious pathogen of cats with a widespread distribution in the feline population. The virus typically causes moderate, self-limiting acute oral and upper respiratory tract disease. However, some strains induce lameness and recently, more virulent strains have evolved, particularly in the USA (Radford *et al*., 2007). FCV and FeHV-1 are probably responsible for the majority of cases of URTD in cats. Co-infections with both viruses have also been reported (Zicola *et al*., 2009).

The shift in the involvement of FeHV-1 and FCV in URTD could be related to the application of attenuated vaccines. All isolates of FeHV-1 constitute a single serotype and the current vaccines, although not completely protective against infection, can prevent the disease and reduce the overall rate of infections induced by FeHV-1. In contrast to FeHV-1, the ability of FCV to mutate can play a major role in virus perpetuation and dispersion. In fact, field isolates of FCV make a heterogeneous population and the vaccine may not cover all of them. In Iran, there is no data on the

prevalence of FeHV-1 and FCV in the past, but the present situation of FeHV-1 seems to be similar to that in Japan and some European countries in the past. Additional studies on the prevalence of both viruses are necessary to gain a better knowledge of the epidemiology of FeHV-1 and FCV in Iran.

Based on the results of this study, between the sexes, the male presented a higher prevalence of infection, though the difference was not statistically significant. Lack of sex predisposition for infection with FeHV-1 has been reported in other works (Harbour *et al*., 1991; Ostrowski *et al*., 2003).

In accordance with previous investigations in Northern Italy (Rampazzo *et al*., 2003) and Saudi Arabia (Ostrowski *et al*., 2003), cats of more than 6-month-old were also at a greater risk of infection by FeHV-1, than cats of less than 6-month-old, but the differences were not singnificant. Moreover, the results of this research, like a previous study of Marsilio *et al*. (2004), showed that the prevalence of FeHV-1 in vaccinated and non-vaccinated cats was not significantly different.

In conclusion, the present study provides the first data on prevalence of FeHV-1 in Iran. The results indicate the presence of FeHV-1 in the region and that FeHV-1 is highly implicated in URTD of cats. However, further studies are necessary to determine the prevalence of FeHV-1 and FCV in other parts of the country and to isolate and characterize the circulating viruses. Due to a high prevalence of FeHV-1 in the region, regular use of vaccine is recommended.

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