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Short Communication

Antibody Detection to *Feline Immunodeficiency virus (FIV)* in stray cats in Ahvaz, southwestern Iran

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ABSTRACT

The present study was performed to determine the prevalence of FIV in stray cat's population of Ahvaz different area. Serum samples were collected from 90 cats from 2005 to 2007. The studied cats were divided into two age groups (<3 years and >3 years) and based on clinical signs (such as lymphadenopathy, periodontal diseases, gingivitis, abscess and cashecsi) into two groups also. The results were analyzed using Fischer's exact test and Chi-square analysis. Prevalence to FIV antibodies in these cats was 15.55% (14 of 90) by means of ELISA Test Kit, indicating that this virus is present in the ecosystem. The infection had more prevalence in cats above 3 years (78.6%; 11 of 14) compared with cats less than 3 years (21.4%; 3 of 14). Statistical analysis showed significant difference between different age groups ($P<0.05$). Prevalence of infection was 17.31% (9 of 52) in males and 13.16% (5 of 38) in females, nevertheless the infection was not significant between different genus ($p>0.05$). Three out of 12 cases (25%) which had clinical signs and 11 out of 78 cases (14.1%) which hadn't clinical signs were seropositive. There was no significant difference between the two groups also ($P>0.05$). This study showed that FIV exist among cat's population of Ahvaz area and separation of companion and stray cats is very important for prevention of disease transmission to companion cats.

Keywords: *Feline Immunodeficiency virus*, ELISA, cat, Ahvaz

INTRODUCTION

Feline immunodeficiency virus (FIV) is a lentivirus that belongs to the Retroviridae family. FIV differs from *feline leukemia virus* (FeLV) and *feline foamy virus* (FFV) and is more closely related to *human immunodeficiency virus* (HIV). FIV is an important viral pathogen worldwide in cats. Five different subtypes have been recognized for FIV (subtypes A, B, C, D, and E) (Sodora *et al* 1994, Uema *et al* 1999, Sellon & Hartmann 2006). In natural settings, FIV is transmitted primarily by parenteral inoculation of virus present in saliva or blood, presumably by bite and fight wounds, accounting for the higher prevalence in male cats (Elder *et al* 2008). Clinical signs are generally manifest in middle age cats (4-7 years) that have harbored the virus for an extended period, but cats

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of any age may contract the disease. These signs may include chronic gingivitis, periodontal disease, chronic anemias and leukopenias, pustular dermatopathies, chronic upper respiratory syndrome and generalized lymphadenopathy (Gabor *et al* 2001, Sellon & Hartmann 2006). A large number of different approaches have been taken in attempts to create FIV vaccines (Uhi *et al* 2002). Rapid diagnosis of FIV infection is especially important in order to isolate infected cats and prevent secondary infections of susceptible animals. Several laboratory techniques have been developed to detect FIV in the samples (saliva and blood) of infected cats such as PCR, ELISA, Rapid immunomigration-type assays, Virus isolation and Monoclonal antibodies. These tests are sensitive, specific and reproducible (Bienzle *et al* 2004). In our study, ELISA Test Kit (VIRACHEK/FIV) was used to investigate the presence of *Feline immunodeficiency* antibodies in stray cats in Ahvaz area. Sensitivity and specificity of these kits were above 98 percent according to the manufacture's instructions. The present study is the first report on prevalence of FIV in cats of this area. This survey provides preliminary information about FIV endemic to the Ahvaz region.

MATERIALS AND METHODS

Sample collection and preparation. Serum samples were selected randomly from 90 stray cats from 2005 to 2007, in Ahvaz area, southwestern Iran. The studied cats were divided into two age groups (<3 years and >3 years) and based on clinical signs (such as lymphadenopathy, periodontal diseases, gingivitis, abscess and cashecsi) into two groups. Fifty two male and 38 female cats were studied. Two ml blood was collected from jugular vein of cats to determine CBC. Ketamine (10 mg/kg) and acepromazine (0.15 mg/kg) were injected for sedative effects. The age of cats ranged between 1 to 7 years. Age was estimated by dental formulary. Most of the studied cats were domestic short hair (DSH). Three long hair cats were seen too.

ELISA Test Kit and its principles. VIRACHEK/FIV Kit (Lyon–France) was used in our survey. It contains a highly specific peptide to quickly identify antibodies to FIV in infected cats. It had been optimized for whole blood, plasma or serum specimens. The plastic wells were coated with Protein A, an antibody-capturing protein. A highly specific peptide of FIV was labled with horseradish peroxidase (HRP). The specimen (serum) was simultaneously incubated with the enzyme-labeled antibody. Antibodies to FIV were bound to the well and enzyme-linked FIV peptide at the same time. The free enzyme-linked peptide was washed away and a chromogenic substrate was added. The development of a distinctly blue color indicated the presence of antibody to FIV. In the absence of FIV antibody, no color change would be observed (according to the manufacture's instructions)**.**

Test procedure. There was one well for positive control, 1 well for negative control and other wells for samples. We placed 3 drops of HRP FIV conjugate into each well and 1 drop of positive control into the first well. Then, we followed 1 drop of negative control into the second well. In continue we used one collibrated sample loop for each sample and added one loopfull of sample to appropriate wells (3, 4, etc). Finally we placed 1 drop of chromogen into each well and followed by adding 1 drop of Substrate Buffer, then we mixed by gently tapping the holder and incubated for 5 minutes. Presence of FIV antibody was indicated by the formation of a blue color in the sample well and the absence of FIV antibody by the lack of a blue color (according to the manufacture's instructions).

Statistical analysis. Test results and potential association with age, sex, CBC and clinical signs were analyzed using SPSS 10.0 for windows and by Fishers exact test and Chi-square analysis. Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Prevalence to FIV antibodies in these cats was 15.55% (14 of 90) by ELISA Test Kit, indicating that this virus is present in the ecosystem. The infection had more prevalence in cats above 3 years (78.6%; 11 of 14) compared with cats less than 3 years (21.4%; 3 of 14). Also, statistical analysis showed significant difference between different age groups ($P<0.05$). The age of FIV-positive pet cats ranged from 1 to 7 years. The median age of FIV-positive pet cats (3.6 years) was significantly greater than the median age of FIV-negative pet cats (2.1 years). Three out of 12 cases (25%) which had clinical signs and 11 out of 78 cases (14.1%) which had not clinical signs were seropositive. The difference was not significant for clinical signs ($p>0.05$). In relation to sex, 17.31% (9 of 52) of males and 13.16% (5 of 38) female carried the FIV infection. Although the prevalence of FIV infection in males was more compared with female, but the difference was not significant. Results are summarized in Table 1: CBC was as leukopenia (less than 5500 cells/ml), lymphopenia and neutropenia in most of cats that were affected to infection (71.43%; 10 of 14). Nine of 76 (11.84%) of healthy cats had leukopenia. The difference was significant for leukopenia ($P<0.05$). The present study that is the first report on the prevalence of Feline immunodeficiency virus in cats in Ahvaz area (southwestern Iran) using ELISA kits revealed that 15.55% of cats were seropositive for FIV. The results indicated that FIV can be as a cause of mortality in cat's population in Ahvaz area.

Table 1. Prevalence of *Feline immunodeficiency virus* infection in cats of different age and sex in Ahvaz district, Iran by ELISA Test Kits, 2005-2007.

Age / Sex	< 3 years		> 3 years	
	Neg.	Pos.	Neg.	Pos.
Male	19	2	24	7
Female	12	1	21	4
Total = 90	31	3	45	11

Antibody detection against FIV is very important in cat population in Ahvaz area, because FIV is highly contagious and there are many stray cats in this area. These animals can be concerned in disease transmission to other cats, particularly companion cats. The first study on FIV was carried out by Malmasi (2002) in Tehran. Two out of 131 samples were positive against FIV by western blot technique. Akhtar Danesh et al (2010) showed 19.2% infections in cat's population in Kerman area. Seventy companion and seventy stray cats were studied by immunochromatography assay. Cats between 4 – 7 years old are in high risk (Sellon & Hartmann 2006). Our study showed that the seroprevalence of infection was more in cats above 3 years, and the difference was significant by statistical analysis ($P<0.05$). Cats with high age have more contact with infected animals. Male cats are affected to FIV more than females due to more contention between males, but our results did not show significant difference. In stray cat's population, both sexes are similarly in exhibition of infection. It is suggested that leukopenia is an important paraclinical feature for suspicious cats to FIV, because ten out of 14 ill cats had leukopenia, so complete blood count is useful, particularly in concurrent infections. The prevalence of infection varies in different countries. Some countries report few infected cats, and others, such as Italy and Japan, with large populations of free-roaming cats have prevalence rates that can approach 30% (Sellon & Hartmann 2006). Seroprevalence of FIV infection was 3.6% among cats exported from the Gulf Coast hurricane disaster area. It was significantly higher in adult cats than in juveniles and in males than in females (Levy *et al* 2007). Evidence of FIV was seen in feral cats on Mauna Kea, Hawaii. Six of 68 (8.8%) cats were antibody positive to FIV (Danner *et al* 2007). In other survey in Germany, antibodies were identified in 5.3 % of the animals of population (Adler *et al* 2007). Serum

samples from 340 pet cats presented to three inner city clinics in Sydney Australia, 68 feral cats from two separate colonies in Sydney, 329 cattery-confined pedigree and domestic cats in eastern Australia, were collected and tested for antibodies directed against FIV using ELISA methods. The FIV prevalence in the two feral cat populations was 21% and 25%. The FIV prevalence in cattery-confined cats was nil. The prevalence of FIV in the pet cat sample population was 8% with almost equal prevalence in healthy cats. The median age of FIV-positive pet cats (11 years) was significantly greater than the median age of FIV-negative pet cats (Norris *et al* 2007). In another study on 168 serum samples, prevalence was 7.4% for FIV in Spain. FIV antibodies were associated with illness and cats older than 2 years (Solano-Gallego *et al* 2006). Seroprevalence of FIV was evaluated in 3 groups of cats in Ottawa. Seventy-four unowned urban strays were tested, as well as 20 cats from a small feral cat colony, and 152 client-owned cats. Seroprevalence for FIV was 23% in the urban strays, 5% in the feral cat colony, and 5.9% in the client-owned cats (Little 2005). Seroprevalence of FIV infection among cats in North America was 2.3% (Levy *et al* 2006). Forty-five wildcats (Felis silvestris), 17 sand cats (Felis margarita), and 17 feral domestic cats were captured in central west Saudi Arabia. Serologic prevalence in wildcats, sand cats, and feral domestic cats were 6%, 0% and 8% for FIV, respectively (Ostrowski *et al* 2003). Infection with opportunistic pathogens of viral, bacterial, protozoal, and fungal origin have been reported in FlV-infected cats (Glaus *et al* 1997, Sellon & Hartmann 2006, Maruyama *et al* 1998, Levy *et al* 2003, Harrus *et al* 2008, Macieira *et al* 2008). Cats may seroconvert in as little as 3 weeks or as long as 10 months after becoming infected with FIV. Also, care must be taken when interpreting test results in kittens less than four months of age as maternal FIV antibodies may be present. So cats selected in our study were above 1 year. Our data indicates that chronically infected carriers without clinical symptoms are frequent in the investigated cat population. In conclusion, we emphasis that prevention of contact with stray cats is important for the prevention of FIV infections to companion cats. Unfortunately, vaccination against FIV is not applied in Iran and many countries. Further epidemiological and biological surveillance are needed to control the disease in stray and domestic cats. FlV is a feline pathogen, and no demonstrated evidence has been found that it can infect people, even veterinarians who are at greater risk of exposure (Xiang *et al* 2005, Jill *et al* 2008).

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