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A survey on feline leukemia virus infection in cats in Ahvaz district, Iran: Seroprevalence and risk factors

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Keywords

Serology, prevalence, feline leukemia virus (FeLV), cat, Ahvaz.

Abstract

The purpose of the present survey was to determine the seroprevalence rate of FeLV in cats in Ahvaz district, South-West of Iran, as well as, risk factors such as age, gender, breed, life style and clinical findings were evaluated. Blood samples were collected from 60 companion and 124 stray cats and antibody titers were measured against FeLV with ELISA kits. The seroprevalence was obtained 79.89% (95% CI: 74.1-85.68 percent). Chi-square test showed a significant relationship between age groups and infection ($p < 0.01$). Infection rate in cats with age below 2 years was significantly less than cats between 3-4 years ($p < 0.05$) and above 4 years old ($p < 0.01$). Also the seroprevalence was significantly higher in domestic short hair breed than Persian ($p < 0.01$). The seroprevalence was higher in stray cats than companion, nevertheless, the difference was not significant ($p > 0.05$). In conclusion, the seroprevalence was very high in cat's population of Ahvaz district and there was a significant difference between clinical findings and serological results.

Abbreviations

DSH: Domestic Short Hair
ELISA: Enzyme-linked immunosorbent assay
FeLV: Feline Leukemia Virus

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Introduction

Feline leukemia virus (FeLV) is a Gamma-retrovirus of domestic cats, a member of the oncornavirus subfamily of retroviruses. This virus is an exogenous agent with protein-core and single stranded covered RNA that can proliferate within many tissues including bone marrow, salivary glands and respiratory epithelium [8]. The three most important FeLV subgroups are FeLV-A, FeLV-B and FeLV -C, all immunologically closely related. Only FeLV-A is contagious and passes horizontally from cat to cat in nature [8,14]. Feline leukaemia is a chronic disease which is characterized by tumoural development in hematopoietic organs as a result of oncogenic, immunosuppressive and immune proliferative effects of viral infection [8, 16]. Feline leukemia virus has a worldwide distribution and it is very common in feline population. It is a contagious virus that spreads with direct contact and can transfer via saliva and blood [26]. Virus can enter to the tissues, fluids and excretions, but its spread via urine and feces is rare. Fleas are the potential source of transfer [8]. Infection has four stages including abortive, regressive, progressive and atypical infections [25]. Cats infected with FeLV may exhibit one or more of the following symptoms. Fever, stomatitis, gingivitis, lymphadenitis, cutaneous abscess, anemia, tumors and immunodeficiency are the most common signs [6,7,12,14].

Reported prevalence differs considerably depending on the geographical region and the cat population evaluated. Rate of infection in healthy stray cats differ from 1-8% [8]. If sick stray cats are only studied, prevalence rises up to 38%. Although, prevalence is decreasing because of vaccination and application of testing and removal in most places of the world [8], nevertheless, prevalence is increasing in some areas [1]. The prevalence is related to some factors such as age, gender, health condition, life style, and geographic location [8]. The seroprevalence of FeLV was reported to be 4.8% [11], 14.2% [1], 2.2% [24] and 12.22% [28] in cats from different areas of Iran.

Several laboratory methods have been developed to detect antigen or antibody in the serum of infected cats such as PCR, ELISA, LAT (Latex agglutination test), IHA (Indirect hemagglutination assay), VN (Virus neutralization) and FAT (Fluorescent antibody test). ELISA is one of the diagnostic methods that as a screening test is effective in the detection of antigen or antibody in blood or serum samples [10,15,16]. Specificity and sensitivity of these kits (Acrolab Ltd.) are more than 95%, according to the manufacturer instructions. The purpose of the present survey was to determine the seroprevalence rate of FeLV in companion and stray cats in Ahvaz district (South-West of Iran), as well as, risk factors such as age, gender, breed, life style (companion or stray) and the possible relationship between clinical findings and ELISA results were evaluated in different groups.

Results

Our results showed that 147 out of 184 samples (79.89% and 95% CI: 74.1-85.68 percent) were positive for the presence of antibody against Feline leukemia virus by ELISA method. Statistical analysis showed a significant relationship between different age groups and infection ($p < 0.01$). Infection rate

Table 1
Prevalence of feline leukemia virus antibodies based on gender, breed, age and life style in cats in Ahvaz district, Iran

Category	Groups	Prevalence	Odds Ratio	95% CI for OR	P-Value
Gender	Female	76.47% (65/85)	-	-	-
	male	82.83% (82/99)	1.48	0.72-3.06	0.67
Breed	Persian	37.5% (3/8)	-	-	-
	DSH	81.82% (144/176)	7.5	1.7-33	0.008
Age	0-2 years	62.8% (27/43)			
	3-4 years	81.9% (86/105)			
	>4 years	94.44% (34/36)	1.95	1.31-2.89	0.001
Life style	Companion	73.33% (44/60)	-	-	-
	Stray	83.06% (103/124)	1.74	0.85-3.74	0.13

Table 2
Odds ratio in multivariate logistic regression based on age and breed in cats in Ahvaz district, Iran

Category	Groups	Odds Ratio	95% CI for OR	P-Value
Age	-	1.86	1.25-2.77	0.002
Breed	Persian	-	-	-
	DSH	5.43	1.18-25.06	0.03

in cats with age below 2 years was significantly less than cats between 2-4 years ($p < 0.05$) and above 4 years old ($p < 0.01$). Logistic regression showed that the odds ratio of infection between the age based on year and disease is 1.95 (95% CI: 1.31-2.89) ($p < 0.001$), and odds of infection increased 95% with rising one year of age. Moreover, 11.1% of fluctuation in infection was justified by age (Table 1). Also, 11.1% of fluctuation in infection was justified by age (Table 1). The relative frequency of positive cases was higher in male cats than females, but this difference was not significant ($p > 0.05$). The odds of infection in male cats was 1.48 (95% CI: 0.72–3.06) in comparison with females and 1% of fluctuation of infection was justified by sexuality ($p > 0.05$) (Table 1). Also the seroprevalence was higher in domestic short hair breed than Persian and this difference was significant ($p < 0.01$). The odds of infection in DSH breed was 7.5 (95% CI: 1.7–33) in comparison with Persian and 6.1% of fluctuation in infection was justified by breed ($p < 0.01$) (Table 1). The seroprevalence was higher in stray cats than companion, nevertheless, the difference was not significant ($p > 0.05$). The odds of infection was 1.74 (95% CI: 0.85–3.74) in stray cats than companion and 2% of fluctuation in infection was justified by life style ($p > 0.05$) (Table 1). Multivariate logistic regression showed that age and breed were risk factors for infection (Table 2). Absolute Frequency of Feline leukemia virus is summarized in Table 3 based on clinical findings. Statistical analysis with McNemar test showed a significant difference between ELISA method and clinical findings ($p < 0.01$).

Table 3
Frequency distribution of Feline leukemia virus based on clinical findings in cats in Ahvaz district, Iran

Clinical findings	ELISA Positive	ELISA Negative
Positive	43	4
Negative	104	33
Total	147	37

Discussion

The present study revealed that 79.89 % of companion and stray cats were seropositive for FeLV by ELISA technique in Ahvaz district, Sout-West of Iran. ELISA is typically used to detect FeLV antigen or antibody within blood or serum. Consequently, use of highly sensitive methods may help to clarify the relatively high prevalence of FeLV infection. Antibody detection against FeLV is very important in cat population, because this virus is highly contagious and there are many stray cats in this area. The obtained results indicated that FeLV may be as a cause of mortality in cat's population of this region, because of the very high prevalence. These animals can be concerned in disease transmission to other cats, particularly companion cats. Originally, certain diseases, such as lymphoma, are associated with very high rates (up to 75%) of FeLV infection [8]. Cats of these regions may suffer from such diseases. The prevalence of infection is more necessary, due to the increasing tendency for individuals to keep pets such as cats in the house and bringing feral cats inside the house. Since the vaccine is not commercially available for FeLV in Iran, the only way for prevention of the disease is the principle of hygiene and avoidance of the contacts between cats, especially stray cats [1].

Recent studies have been shown that the seroprevalence of FeLV is different in parts of the world. Rate of infection has been reported 0-2% in Sidney [18], 18% in Italy [3], 0% in South Vietnam [22] and 1% in Finland [27]. In one study in USA, the presence of FeLV antigen in pet cats was 13% [23]. The prevalence has been declared 15.6-35% in Europe [20], 2.3-3.3% in North America [17], 0-2.9% in Asia [24], 6.5-7.5% in Australia [18], 2.9-9.8% in Japan [19], 3-4.5% in turkey [30] and 3.5-10.4% in U.K [20].

In the present study, the prevalence of the FeLV infection was found to be higher than other areas of Iran. In a survey in Tehran, on 103 stray cats and healthy domestic, 4.8% were positive for FeLV by ELISA technique [11]. In another survey by Akhtardanesh et al. (2010), overall infection rate was 14.2% for FeLV in Kerman district. Shahrani et al. (2011) detected FeLV infection in Iranian domestic cats by RT-PCR. Fifty six blood samples were tested using molecular methods and total frequency of FeLV infection was 2.2%. Infection rate was reported 12.22% and 65%, respectively by RT-PCR methods in other areas of Iran [21,28]. In

our study, the rate of infection was 79.89% which represents a serious danger for Province cat's population and shows the importance of testing and eradication of infected cats and more attention to the prevention methods such as vaccination, isolation of patient cats and sterilization. Higher prevalence than previous research probably goes back to used techniques in the diagnosis, so that the sensitivity and specificity of ELISA kits are more accurate than other methods such as agglutination and immunochromatography tests. In this research, diagnostic kits were designed to detect antibodies in blood samples. Furthermore the high rate of incidence may be due to different cut-off point. Another possible reason for the high percentage of prevalence is that we investigated the cats above one year as the prevalence is low in most cats less than 1 year. In the present survey, the rate of infection in cats with age below two years was significantly less than cats between 2-4 years and older. Cats older than 16 weeks are more likely to be infected, but cats of any age may acquire FeLV, particularly through prolonged contact [8,12]. In line with this research, Akhtardanesh et al. (2010) showed that the prevalence of infection was higher in older cats. According to the results of Alves et al. (2011) rate of infection was under the influence of age and older cats are more likely to be infected [2]. Beatty et al. (2011), Maruyama et al. (2003) and Yuksek et al. (2005) obtained similar results [4, 20, 31].

Several risk factors may affect the prevalence of FeLV infections. Age, breed, gender, life style and health status that has been discussed associated with the prevalence of viral infections in cat's population. In this study, the seroprevalence of positive cases was more in male cats than females, but this difference was not significant statistically. Probably because males roam in the environment, is likely to be more engaged (14]. Torkan et al. (2014) showed that 63.63 percent of infections were in males; nevertheless, Hitt et al. (1992) assessed serum samples and showed that the prevalence was more in females than males [9]. Unlike the above results, the prevalence of infection were not significantly different in males (9.4%) and females (8.3%) [13]. The prevalence of FeLV is higher in cats that are allowed to roam outside, because direct contact is necessary for transmission. In a study in the United States, the prevalence was clearly related to the time spent outdoors and the degree of exposure to other cats. Of cats in a study in Boston and Detroit, which many were allowed to roam outside, 63% and 47% had positive serum FeLV antibody test results, respectively, whereas only 5% of New York cats that were primarily confined to high-rise apartments had FeLV-specific antibodies. Although fighting, free-roaming, intact male cats are still considered mainly at risk for acquiring FIV infection, the same risk factors also facilitate FeLV infection [6,8].

In the present study, the ratio of positive cases was more in stray cats than companions. In a survey conducted by Goldcamp et al. (2008), the prevalence was 7.6% and 9% among domestic and stray cats respectively. Shahrani et al. (2011) showed that domestic cats which did not have contact with stray cats were exposed to the least risk of infection. Taking care of domestic cats (such as vaccination, sterilization and blood tests for the presence of viruses) is necessary. Cats that do not have access to the outside; have less contact with seropositive cats. Neutered cats are more likely to be kept at home and are less at risk of exposure to the retroviruses. These items can be effective on the prevalence of infection [8]. The role of the cat flea (*Ctenocephalides felis*) has been confirmed as a vector in transmission also. The virus does not survive well in urine, feces or in the environment, so cats will not be infected just because another cat with FeLV has lived in a house before them or comes into their garden or yard. Indoor cats, which do not have contact with stray cats at all, are at minimal risk of infection [5].

It is determined that there is a significant difference between clinical findings and ELISA results. Originally, certain diseases, such as lymphoma, are associated with very high rates of FeLV infection. Of course, clinical symptoms are not specific and a high prevalence of animals that seem to have FeLV infection, are negative [8]. Our study concludes that a significant percentage of cats infected with FeLV did not show any clinical signs. As the clinical signs, are not definitive for accurate diagnosis of FeLV and on the other hand the sick cats may have not symptoms, serologic testing is recommended for all suspected cats. Clinical symptoms can include weight loss, anemia, chronic diarrhea, lymphadenopathy, oral ulcers and mucosal congestion [29,30].

In conclusion, it can be stated that ELISA technique is a sensitive and specific method for the detection of exogenous FeLV. FeLV appears to be endemic in Iran. This study highlights the need

for fast, correct and cost effective diagnostic techniques for screening healthy and sick household cats referred to Veterinary Hospitals. Our results showed that FeLV is a specific infection and the different common feline infectious pathogens seem to be endemic in cats of Ahvaz district. Testing for contamination and then prevention of contact are the most effective preventative ways between sick and healthy cats [15]. Vaccination and testing programs have proven to be effective in reducing FeLV infection and may potentially eliminate it at least in some areas. The cats should be prohibited from going outside and social behaviors like sharing food dishes and using common grounds. Regressive and progressive infections can be distinguished by repeated testing for viral antigen in peripheral blood. Bite wound is more coomon in intact cats than sterile cats. This study highlights the necessity of using rapid, accurate and effective diagnostic methods for screening healthy and sick household cats.

Materials and Methods

Blood samples were randomly collected from 184 cats (60 companion and 124 stray), in Ahvaz district, the capital of Khuzestan province in South-West of Iran from April 2015 to September 2016. Ketamine (10 mg/kg) and Acepromazine (0.15 mg/kg) were injected for sedative effects. The samples were collected from jugular or femoral veins and allowed to clot and centrifugated for 5 min at 1800× g. Serum was removed and stored at −20°C until assayed. Investigated parameters included putative risk factors such as age, gender, breed, life style and clinical findings. All cats were grouped with regard to housing conditions (indoor or outdoor). The age of the studied cats was between 1-7 years. Age was estimated by owner's information and dental formulary. The companion cats owners were asked to fill out a questionnaire for further information about signalment. The studied cats were divided into three age groups [≤ 2 year (n=43), between 2-4 years (n=105) and ≥ 4 year (n=36)] and based on clinical findings into two groups (at least one of signs fever, stomatitis, gingivitis, cutaneous abscess, anemia, lymphadenopathy, periodontal diseases, abscess and cachexia). Eighty five of the studied cats were female and ninety nine were male. Eight cats were Persian breed and 176 were DSH (domestic short hair). Sixty cats were kept indoor and 124 outdoor. Forty seven cats had clinical symptoms but one hundred and thirty seven had no signs.

Laboratory methods

The test was carried out with a commercial indirect ELISA antibody test kit (manufactured by Acrolab Ltd.). In this way, microwells of kit were coated with specific antigens. If samples had specific antibodies against the virus, they could bind to the antigens. After addition of the substrate, a colorimetric reaction appeared which could be measured by spectrophotometer at a wavelength of 450 nm. The presence of color was interpretd as the presence of antibody against the virus in sera, and the absence of color revealed the absence of specific antibody. Four microwells were allocated for negative controls in each kit. Negative (less than 0.25) and positive samples (higher than 1) were determined according to the manufacture instructions.

Statistical analysis

Statistical analyses were performed using SPSS (Version 16.0; SPSS Inc., Chicago, USA). The association between age, sex, breed, life style and clinical findings were analyzed by Chi-square test, logistic regression and McNemar test. Differences were considered statistically significant when $p < 0.05$.

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Conflict of interest statement

The authors declare no conflict of interest.

Author Contributions statement

Conceived and designed the experiments: M.P., B.M. Performed the experiments: B.M., F.Z., M.R.S.A.S., M.P. Analyzed the data: M.P. Contributed reagents/materials/analysis tools: M.P., M.R.S.A.S., B.M. Wrote the paper: M.P., B.M.

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