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Research article

MOLECULAR STUDY OF THE PREVALENCE OF FELINE LEUKEMIA VIRUS (FELV) IN IRANIAN DOMESTIC CATS FROM BLOOD SAMPLES BY REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR) IN IRAN

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ABSTRACT: Feline leukemia virus (FeLV) is a retrovirus that infects cats. This disease is a form of cancer of blood cells called lymphocytes. FeLV happens in nature not as one genomic species however as a family of closely related viruses. Cats living by infected cats or by cats of unknown infection status, people allowed outdoors unsupervised, where they may be bitten via an infected cat and Kittens born to infected mothers are major groups which are at greatest risk of Feline leukemia virus (FeLV) infection. This investigation was carried out to study the prevalence of FeLV in Iranian domestic cats using Reverse transcription polymerase chain reaction (RT-PCR) method. Totally, 90 cats were collected randomly. From each cat, 1 mL blood sample was taken and all samples were transferred to the laboratory in a cooler with ice-pack. DNA was extracted and PCR was developed to detect the U3 LTR region of FeLV. Totally, 11 out of 90 samples (12.22%) were positive for presence of FeLV. The FeLV had the higher prevalence in male cats (63.63%) than female (36.36%). Cats which were lower than 3 years had the highest prevalence of FeLV. The results showed that FeLV is a specific infection and the different common feline infectious pathogens and FeLV seem to be endemic in Iranian cats. Vaccination and testing programs have proven to be effective in reducing FeLV infection in Iran and may potentially totally eliminate it at least in different countries.
Key words: Feline leukemia virus, PCR, Clinical signs, Risk factor, Iranian domestic cats.

INTRODUCTION

Feline *leukaemia* is a chronic disease that is characterised via tumoural development in haematopoietic organs as a results of immunosuppressive, myelosuppressive, oncogenic and immune proliferative effects of infection. The agent of the disease is a replication non-defective *retrovirus* (feline leukaemia virus), they are distributed global and induce determined infections in domestic cats and other small felids and Feline leukemia virus (FeLV) is a horizontally transmitted [1, 2, 3, 4]. FeLV is a *retrovirus* found in domestic and several wild/exotic cats. It seems to be a specific viral infection of cats and their relatives and is disease causing virus in the global. FeLV is mostly common in large populations of cats (catteries, feral cats). FeLV infection prevalence rates differ from 1.0- 38.0% [2, 5]. FeLV is shed typically in saliva and nasal secretions, therefore bites from an infected animal and intimate grooming or contact by infected cats could spread the disease [3, 6]. The most new studies report a prevalence of 3.5– 15.6% in Europe, 2.3–3.3% in North America and 0–2.9% in Asia [3, 7, 8]. The saliva of infected cats is the chief form of transmission of FeLV. The transmucosal infection via vaginal and rectal route can also occur, as well as through milk [2]. The most common clinical signs of FeLV infection are anemia, immune suppression, and Lymphoma [2].

FeLV infected cats may stay asymptomatic for years; though, finally succumb due to direct viral effects or, more commonly, to secondary infections resulting from virus-induced immunosuppression [2]. Several techniques to detect FeLV infection in cats are available and the most commonly used sample for testing is blood. In most cats by a persistent infection, each infectious virus and free viral p27 antigen are present in the plasma and viral antigen is demonstrable in the neutrophils [3, 9]. Diagnosis of FeLV infection is based on clinical history and viral p27 protein detection in serum, plasma, or saliva of allegedly positive animals. Indirect immunofluorescense (IFA) and immunoenzimatic assays are the most used diagnostic techniques [10] and PCR is presently being used [11].

The rate of FeLV infection is influenced by age (older cats are more likely to be infected); gender (males greater than females due to roaming behavior); and health (sick cats are more likely to be infected, due to an impaired immune system) [2]. Hematological abnormalities for instance thrombocytopenia and anemia may appear in FeLV infected cats [12]. The polymerase chain reaction (PCR), which has been used to detect *retroviruses* e.g. human T cell leukaemia virus [13], human immunodeficiency virus [14] and feline immunodeficiency virus [15], may too be suitable for the detection of exogenous FeLV in field cats [9]. A unique region which distinguishes endogenous from exogenous FeLV is the U3 region of long terminal repeat (LTR) [16]. The U3 part of the long terminal repeat (LTR) of infectious (exogenous) FeLV is not endogenous to domestic cats, and it may be used as a probe to find the occurrence, number, and place of horizontally acquired FeLV proviruses [17].

A number of deterministic models have been created to predict the dynamics of FeLV in cat populations. These models obtainable that FeLV dynamics depend on the size of the host population and the relationship amid host density and the pattern of contacts of each cat. They predict the possibility of FeLV extinction in smaller populations [5]. The goal of the current study was to find the prevalence of Feline leukemia virus (FeLV) among Iranian domestic cats using Reverse transcription polymerase chain reaction (RT-PCR) technique.

MATERIALS AND METHODS

Samples collection and RNA extraction

A total of the 90 blood samples were collected randomly from February 2011 to August 2012 with permission of the cats' owners from the saphenous or jugular veins into tubes that contained EDTA. From each cat, 1 mL blood sample was taken and all samples were transferred to the laboratory in a cooler with ice-pack. The sampled cats had been brought to veterinary clinics to vaccine application, routine controls or with complaints associated to some clinical signs (chronic gingivitis or any mouth ulcerations, chronic gastrointestinal, urinary tract and respiratory symptoms). All rules and protocols used in this study were approved by the Universidade Federal de Minas Gerais Animal Experimentation Ethics Committee (101/09). RNA was extracted from cat's blood using a Cinagen RNA extraction kit (Cinnagen, Tehran, Iran). Total RNA was reverse transcribed to cDNA with a first strand cDNA synthesis kit (Cinnagen, Tehran, Iran) according to the manufacturer recommendation. Extracted DNA of each sample was kept frozen at -20°C until used, and delivered to Biotechnology Research Center of Islamic Azad University of Shahrekord.

Gene amplification

The PCR is a molecular technique which can be applied to the amplification of viral RNA. Primers targeting a 166 bp segment of the FeLV U3 LTR region. The primers sequences were as follows: U3-F: 5'-TTACTCAAGTATGTTCCCATG-3' and U3-R: 5'- CTGGGGAGCCTGGAGACTGCT-3' (accession number: GU731413.1). Amplification of FeLV U3 LTR region cDNA, was done in thermocycler (Eppendorf, Hamburg, Germany). PCR was performed in a 25 µL reaction volume containing 1 µg of template cDNA, 1 µM of each primers, 2 mM Mgcl2, 200 µM dNTP, 2.5 µL of 10X PCR buffer and 1 unit of *Taq* DNA polymerase (Fermentas, Germany). And PCR reaction was performed as follows: first denaturation step at 94°C for 3 min, then amplified for 35 cycles of denaturation at 94°C for 1 min, alignment at 52°C for 1 min, elongation at 72°C for 2 min and, final elongation step at 72°C for 5 min.

Analysis of PCR Products

The PCR product was run using electrophoresis in 2% agarose gel in 1X TBE buffer at 80V for 30 min, and stained with ethidium bromide on UVIdoc gel documentation systems (Uvitec, UK). The PCR products were identified by 100 bp DNA size marker (Fermentas, Germany). Show band including 166 bp.

Statistical analysis

Analysis of data was performed using the SPSS version 17.0 computer software (SPSS, Chicago, IL). Unadjusted seroprevalence estimates of FeLV infection was calculated for the study population. Cats used in the analyses only appeared once. Significance was set at $P<0.05$.

RESULTS

The frequency of FeLV was shown to be 12.22%. Our results showed that 11 out of 90 samples (12.22%) were positive for presence of FeLV in cats. The associations between the virus prevalence's and gender and age are reported in Table 1 and Table 2. Also, the results of electrophoresis for FeLV long terminal repeat (LTR) amplification via the RT-PCR (166 bp) were shown in Fig. 1. In the current study. Evaluated the usefulness of blood RNA detection by RT-PCR. The PCR technique allows detection of amounts of viral RNA in blood samples. In this study, more of the FeLV positive cats were young and male (less than 1 year old). The youngest cats of this study (less than 3 years old) were much more often infected with FeLV than the other age groups. Positive and negative controls of known sequence were also run for each reaction.

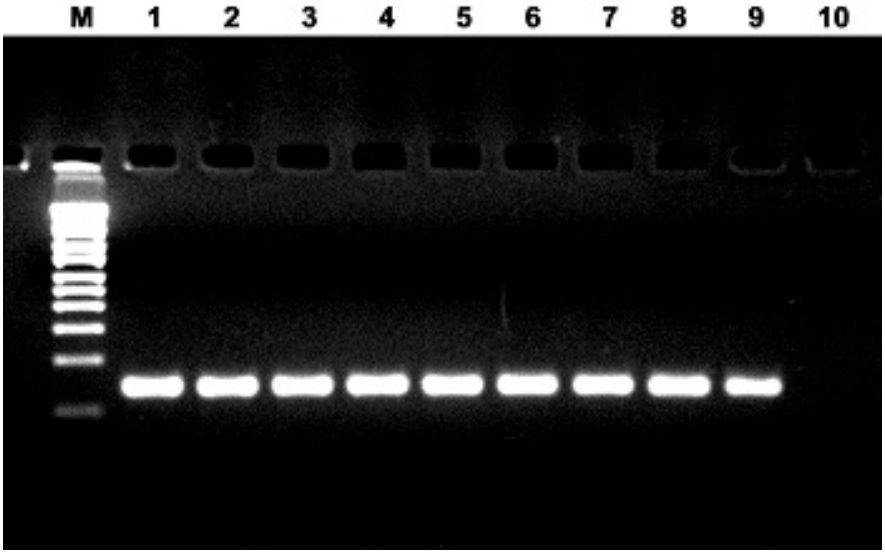

Figure 1: Gel electrophoresis of PCR products for detection of FeLV U3 LTR region (M: 100 bp DNA marker (Fermentas, Germany); lines 1: positive controls; lines 2- 9: positive samples (166 bp); and line 10: negative controls.

Table 1: PCR results-occurrence of FeLV infection in cats with different ages.

Total samples	Positive samples (%)	Results-occurrence with different ages (%)			
		Less than 1 year	1 to 2 years	2 to 3 years	More than 3 years
90	11 (12.22%)	8 (72.72%)	2 (18.18%)	1 (9.09%)	0 (0%)

Table 2: PCR results-occurrence of FeLV infection in cats with different gender.

Total samples	Positive samples (%)	Results-occurrence with different gender (%)	
		Male	Female
90	11 (12.22%)	7 (63.63%)	4 (36.36%)

DISCUSSION

While no useful database about how many domestic cats live in Iran is available, the cat population in Iran can be described as free-roaming stray cats and owned cats. This article describes the prevalence of FeLV infection among domestic cats in Iran. The current results showed that the FeLV infections may also constitute a risk for the cat population. Several risk factors may affect the prevalence of FeLV infections. Age, breed, sex, lifestyle, health status have been discussed associated with the prevalence of viral infections in cat population [3, 18]. FeLV is transmitted via close contact with cat secretion fluids like blood, saliva and other body secretion. FeLV is an envelope, positive sense, single-stranded RNA virus that, once released in the environment, isn't able to survive long periods on dry surfaces [3, 19]. The role of the cat flea (*Ctenocephalides felis*) has also been confirmed as a vector in transmission [3, 20]. FeLV doesn't survive well in urine, feces or within the environments, thus cats won't be infected simply because another cat by FeLV has lived during a house within the past them or comes into their garden or yard. Cats older than 16 weeks are less seemingly to be infected, however cats of any age could acquire FeLV, significantly through prolonged contact. Indoor cats, which do not contact strange cats at all, are at minimal risk of infection [3, 6]. FeLV was first described via Jarrett et al. in 1964 [19] and is one of the most common fatal pathogens affecting cats global. The Tufts Veterinary Diagnostic Laboratory in Germany, where about 2000 serum samples are tested yearly for FeLV antigen, reported a decrease from 8% in 1989 to 4% in 1995 [21]. This disease is very common in Iran, specially in old cities and small towns where cat owners often live in houses by courtyards and gardens [22]. The U3 region may be used as a probe for finding out the number and place of exogenously acquired FeLV proviruses in infected cat tissues [17]. Researchers have compared attention repetition in the FeLV LTR in cells from FeLV-infected cats by neoplastic and nonneoplastic disease, via PCR amplification of the enhancer region followed via nucleotide sequencing [3, 23]. The reported prevalence for FeLV in cats were 0.2% in Australia [24], 1.9% in Canada [25], 2.9% in Japan [26], 3% in Turkey [27], 3.2% in Germany [28], 3.5% in England [7], and 8.4% in Italy [29]. In the main, the results of this study displayed that 12.22% of Iranian domestic cats were infected with FeLV. Prevalence of retroviral infection represents obvious regional patterns in some nations [22]. During a study of a trap, neuter, and release program for feral cats on Prince Edward Island, 6.5% (12/185) of cats were seropositive for FeLV antigen [30]. A new study at North American veterinary clinics and animal shelters found 2.3% of cats seropositive for FeLV antigen [8]. Goldkamp et al additionally established that over 8% of cats presented for fighting injuries were FeLV positive, a prevalence significantly higher compared to the regular cat population [31]. The prevalence of the FeLV infection in present study was found to be higher than among cats in Iran and other study [3], similarly to studies conducted in the Italy, and Turkey [18, 29]. The prevalence of FeLV infections in Iran has been reported by some researchers [3, 22, 32]. The molecular detection of FeLV in Iran via PCR was first described in this study. Enzyme-linked immunosorbent assay (ELISA) was earlier and typically used to detect FeLV antigens, although detection of the FeLV proviral DNA by PCR was accomplished in the current study. RT-PCR is based on detection of the viral RNA, and does not give the data as proviral DNA specially in persistently infected cats without clinical signs [19]. PCR based on detection of proviral DNA may be more suitable, sensitive and specific to clarify the false negative results and not FeLV antigen ELISA. Furthermore, purpose of the proviral DNA in blood cells allows identification of the virus independently from antibodies or viremia [33]. Consequently, use of highly sensitive methods for viral nucleic acid detection from whole blood samples may probably help to clarify the relatively high prevalences of FeLV infection in this study. Study by Akhtardanesh et al. (2010), overall infection rate for FeLV was 14.2% in Iran [22]. In another study in Iran, between 103 stray cats and healthy domestic, 4.8% showed positive for FeLV by ELISA technique [32]. The aim of study by shahrani et al., (2011) was to detect FeLV in Iranian domestic cats Reverse transcription polymerase chain reaction (RT-PCR). From 56 blood samples were tested for FeLV using molecular methods and out of 56 samples total frequency of FeLV infection was 2.2%. The results presented that FeLV is a specific infection and the other common feline infectious pathogens [3].

CONCLUSION

In conclusion, the RT-PCR designated here is a sensitive and specific check for the detection of exogenous FeLV. FeLV appear to be endemic in Islamic Republic of Iran. This study highlights the need of with fast, correct and cost effective diagnostic techniques for screening healthy and sick household cats referred to veterinary hospital. The results showed that FeLV is a specific infection and the different common feline infectious pathogens and FeLV seem to be endemic in Iranian cats. Vaccination and testing programs have proven to be effective in reducing FeLV infection in Iran and may potentially totally eliminate it at least in different countries.

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