

Full Length Research Paper

Molecular study for detection of *Feline Leukemia Virus* (FeLV) in Iranian cats

Farzad Shahrani^{1,2}, Abbas Doosti^{1,2*} and Asghar Arshi²¹Islamic Azad University, Shahrekord Branch, Young Researchers Club, Shahrekord, Iran.²Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

Accepted 21 January, 2019

Feline leukemia virus (FeLV) is a *retrovirus* that infects cats. This infectious disease is a form of cancer of blood cells called lymphocytes. FeLV occurs in nature not as a single genomic species but as a family of closely related viruses. The aim of present study was to detect FeLV in Iranian domestic cats Reverse transcription polymerase chain reaction (RT-PCR). Fifty six blood samples were tested for FeLV using molecular techniques and out of 56 samples overall frequency of FeLV infection was 2.2%. The results showed that FeLV is a specific infection and the other common feline infectious pathogens and FeLV seem to be endemic in Iranian cats Vaccination and testing programs have proven to be effective in decreasing FeLV infection in Iran and may potentially totally eliminate it at least in other countries.

Key words: *Feline leukemia virus*, PCR, Iranian domestic cats.

INTRODUCTION

Feline *leukaemia* is a chronic disease which is characterized by tumoural development in haematopoietic organs as a result of oncogenic, immunosuppressive and immune proliferative effects of viral infection. The agent of the disease is a replication non-defective *retrovirus* (*feline leukaemia virus*) (Hoover et al., 1991; Rojko et al., 1991). *Feline leukemia virus* (FeLV) is a horizontally transmitted oncogenic, myelosuppressive, and immunosuppressive *retrovirus* and represents the most important pathogen of domestic cats (Jackson et al., 1996). FeLV is a *retrovirus* found in domestic and some wild/exotic cats. It appears to be a specific viral infection of cats and their relatives and is disease causing virus in the worldwide. FeLV is particularly common in large populations of cats (catteries, feral cats) (Fromont et al., 1998). FeLV is shed mainly in saliva and nasal secretions, so bites from an infected animal and intimate grooming or contact with

infected cats may spread the disease (Cattori et al., 2009). The most recent studies report a prevalence of 2.3–3.3% in North America, 0–2.9% in Asia, and 3.5–15.6% in Europe (Levy et al., 2006; Muirden, 2002). Several methods to detect FeLV infection in cats are available and the most widely used sample for testing is blood. In most cats with a persistent infection, both infectious virus and free viral p27 antigen are present in the plasma and viral antigen is demonstrable in the neutrophils (Miyazawa et al., 1997). Hematological abnormalities such as anemia and thrombocytopenia may appear in FeLV infected cats (Gleich et al., 2009). The polymerase chain reaction (PCR), which has been used to detect *retroviruses* such as human immunodeficiency virus (Albert et al., 1990), human T cell leukaemia virus (Bhagavati et al., 1988) and feline immunodeficiency virus (Hodatsu et al., 1992), may also be useful for the detection of exogenous FeLV in field cats (Miyazawa et al., 1997). A unique region which distinguishes endogenous from exogenous FeLV is the U3 region of long terminal repeat (LTR) (Berry et al., 1988). The U3 portion 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 determine the presence, number, and location of horizontally acquired

*Corresponding author. E-mail: biologyshk@yahoo.com. Tel: +98-38-13361001. Fax: +98-38-13361001.

Abbreviations: FeLV, *Feline leukemia virus*; PCR, polymerase chain reaction.



Figure 1. Gel electrophoresis of PCR products for detection of FeLV U3 LTR region (Line 1: 100 bp DNA marker (Fermentas, Germany), lines 2 and 3: positive and negative controls, respectively, lines 4 and 5: positive samples, and line 6: negative sample).

FeLV proviruses (Casey et al., 1981). Many deterministic models have been constructed to predict the dynamics of FeLV in cat populations. These models showed that FeLV dynamics depend on the size of the host population and the relationship between host density and the pattern of contacts of individual cats. They predict the possibility of FeLV extinction in smaller populations (Fromont et al., 1998). The aim of present study was to determine the frequency of *Feline leukemia virus* (FeLV) among Iranian domestic cats using PCR method.

MATERIALS AND METHODS

Samples collection and RNA extraction

Fifty six blood samples were collected from the saphenous or jugular veins into tubes that contained EDTA. RNA was extracted from cat's blood using a Qiagen RNA extraction kit (Qiagen, Ltd., Crawley, UK). Total RNA was reverse transcribed to cDNA with a first strand cDNA synthesis kit (Fermentas, Germany) according to the manufacturer recommendation.

Gene amplification

The polymerase chain reaction (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). In order to amplify FeLV U3 LTR region cDNA, PCR was performed in a 25 l reaction volume containing 1 g of template cDNA, 1 M of each primers, 2 mM MgCl₂, 200 M dNTP, 2.5 l of 10X PCR buffer and 1 unit of *Taq* DNA polymerase (Fermentas, Germany). PCR amplification was performed using thermal cycle (Master Cycler Gradient, Eppendorf, Germany) as follows: initial denaturation step at 95°C for 5 min, then amplified for 30 cycles of denaturation at 94°C for 1 min, alignment at 52°C for 1 min, elongation at 72°C for 1 min and, final elongation step at 72°C

for 5 min. The PCR product was analyzed by electrophoresis in 1% agarose gel in 1X TBE buffer and visualized by ethidium bromide staining on UVIdoc gel documentation systems (UK).

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 as a whole. Cats used in the analyses only appeared once. Significance was set at P 0.05.

RESULTS

The results of electrophoresis for FeLV long terminal repeat (LTR) amplification by the RT-PCR (166 bp) were shown in Figure 1. The frequency of FeLV was shown to be 2.2%. This 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, all of the FeLV positive cats were old (4 to 8 years old). Positive and negative controls of known sequence were also run for each reaction.

DISCUSSION

Feline leukemia virus (FeLV) is transmitted by close contact with cat secretion fluids such as saliva, blood and other body secretion. FeLV is an envelope, positive sense, single-stranded RNA virus that, once released in the environment, is not able to survive long periods on dry surfaces (Gomes et al., 2006). The role of the cat flea (*Ctenocephalides felis*) has also been confirmed as a vector in transmission (Vobis et al., 2003). The virus does

not survive well in urine, feces or in the environments, 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. Cats older than 16 weeks are less likely to be infected, but cats of any age may acquire FeLV, particularly through prolonged contact. Indoor cats, which do not contact strange cats at all, are at minimal risk of infection (Cattori et al., 2009).

FeLV was first described by Jarrett et al. in 1964 (Jarrett et al., 1964) and is one of the most common fatal pathogens affecting cats worldwide. The Tufts Veterinary Diagnostic Laboratory in Germany, where approximately 2000 serum samples are tested yearly for FeLV antigen, reported a decrease from 8% in 1989 to 4% in 1995 (Cotter, 1997). This virus is very common in Iran, especially in old cities and small towns where cat owners often live in houses with courtyards and gardens (Akhtardanesh et al., 2010). The U3 region may be used as a probe for studying the number and location of exogenously acquired FeLV proviruses in infected cat tissues (Casey et al., 1981).

Researchers have compared enhancer duplication within the FeLV LTR in cells from FeLV-infected cats with neoplastic and nonneoplastic disease, by PCR amplification of the enhancer region followed by nucleotide sequencing (Jackson et al., 1993).

The reported prevalence for FeLV in cats were 0.2% in Australia (Malik et al., 1997), 2.9% in Japan (Maruyama et al., 2003), 3% in Turkey (Yukseket al., 2005), 3.2% in Germany (Gleich et al., 2009), 3.5% in England (Muirden, 2002), 8.4% in Italy (Bandeccchi et al., 2006) and 1.9% in Canada (Little, 2005). Overall, the results of this study showed that 2.2% of Iranian domestic cats were infected with FeLV. Prevalence of retroviral infection represents obvious regional patterns in some countries (Akhtardanesh et al., 2010). In 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 (Gibson et al., 2002). A recent study of 18038 cats tested at North American veterinary clinics and animal shelters found 2.3% of cats seropositive for FeLV antigen (Levy et al., 2006). Goldkamp et al also demonstrated that more than 8% of cats presented for fighting injuries were FeLV positive, a prevalence considerably higher compared to the normal cat population (Goldkamp et al., 2008). Akhtardanesh et al. (2010) reported overall infection rate for FeLV in Iran was 14.2% (Akhtardanesh et al., 2010). In another study in Iran (Tehran), among 103 healthy domestic and stray cats, 4.8% showed positive serologic result for FeLV by ELISA method (Jamshidi et al., 2008). In conclusion, the RT-PCR described here is a highly sensitive and specific test for the detection of exogenous FeLV. FeLV seem to be endemic in Iran and retroviral-associated immunosuppression may be a risk factor for active toxoplasmosis in infected cats. This study highlights the necessity of using rapid, accurate and cost-effective diagnostic methods for screening healthy and sick household cats referred to veterinary clinic.

ACKNOWLEDGMENT

The authors would like to thank all the staff members of the Biotechnology Research Center of Islamic Azad University of Shahrekord Branch in Iran for their sincere support.

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