FELINE IMMUNODEFICIENCY VIRUS: A REVIEW

ABSTRACT

Feline immunodeficiency virus is a typical lentivirus, like human immunodeficiency virus, exhibits up to 26% sequence variation between strains and phylogenetic analysis of env gene sequences defined five unevenly geographically distributed subtypes. Five clinical stages are usually described in FIV infection: acute stage, asymptomatic seropositivity period, persistent lymphadenopathy, AIDS Related Complex and AIDS. Serologic and virus isolation studies have shown that FIV is enzootic worldwide. Its prevalence, however, varies greatly depending upon in geographical location and other variables of the cat populations surveyed. FIV infection diagnostic depends on detection of FIV-specific antibodies in serum, plasma, whole blood or detection of FIV provirus in genomic DNA of peripheral lymphocytes or tissues by a sensitive nested PCR procedure. To our knowledge there are few studies that demonstrated the presence of FIV in Brazil. Therefore there is an urgent need for more information that could provide new insights towards the distribution of the FIV among cats with clinical signs of disease and high-risk cats to learn more about the prevalence of infection and expand our understanding of the epidemiology of FIV infection in Brazil.

INTRODUCTION

Feline immunodeficiency virus (FIV) was isolated in 1986 from a feline leukaemia virus (FeLV) negative cat with chronic opportunistic infections (Pedersen et al. 1987). FIV belongs to the Retroviridae family and is a typical lentivirus that resembles the human and simian immunodeficiency viruses in its morphologic features, genomic and protein structures (Olmsted et al. 1989).

FIV infects the members of the Felidae family and the infection in domestic cats (Felis catus) is associated with immunodeficiency-like diseases similar to those seen in human immunodeficiency virus type 1 (HIV-1) infection. The biological and immunological similarities between FIV and HIV-1 make FIV infection of cats a useful animal model system for studies on pathogenesis, vaccine development and antiviral chemotherapy (Yamamoto et al. 1988, Ishida et al. 1989, Yamamoto et al. 1989, Ishida & Tomoda 1990, Pedersen et al. 1990).

FIV genomic structure.

The mature FIV virions are enveloped, have 100-125 nm in diameter, and consist of a unique three-layered structure. Innermost is the genome-
nucleoprotein complex and has helical symmetry. This structure is enclosed within an icosahedral capsid, which in turn is surrounded by a host cell membrane-derived envelope. FIV genome is diploid; consisting of an inverted dimer of two molecules of linear positive sense, single stranded RNA. Each monomer is 9,200 nucleotides in size and has a 3'-polyadenylated tail and 5'-cap (Bendinelli et al. 1995).

The genome of FIV contains three major genes from 5' to 3' gag, pol, and env flanked at both sides by long terminal repeat (LTR) sequences comprised of untranslated 3'(U3, promoter/enhancer elements), repeat (R, from the RNA start site, containing the polyadenylation signal) and untranslated 5'(U5) regions (Bendinelli et al. 1995).

The gag gene generates the structural proteins: the matrix (MA), the capsid (CA) and the nucleocapsid (NC) proteins (Pancino et al. 1993). The pol gene encodes very important enzymes: the reverse transcriptase (RT), the dUTPase (DU), the protease (PR) and the integrase (IN) (Bendinelli et al. 1995). Among lentviruses, only FIV, equine infectious anemia virus (EIAV) and Visna/Maedi virus encode a deoxyuridine pyrophosphatase (dUTPase), which is responsible for deoxyuridine triphosphate (dUTP) degradation, which may improve viral replication by reducing or avoiding misincorporation of dUTP in the viral genome and also providing the substrate (dUMP) for deoxycytidine triphosphate (dTTP) synthesis (Tye et al. 1977). Protease is essential for virus maturation and infectivity. FIV and HIV protease are structurally similar, but amino acid sequence and mutational analyses have shown differences in enzymatic activity; FIV protease are more efficient at cleaving at sites with hydrophobic residues (Lin et al. 2000). This pattern of preferences is also typical of HIV proteases found in strains resistance to current protease inhibitor drugs, and FIV protease contains residues identical to those associated with resistance in HIV protease at six different homologous locations (Kanzaki & Looney 2004). The viral integrase catalyses the viral DNA integration in the cat genome, and several of such molecules become integrated as provirus at different sites in the cellular DNA (Miyazawa 2002).

The env gene encodes the virion envelope proteins, the surface (SU) and the transmembrane (TM) proteins. Proper folding and modification of the envelope is necessary for receptor-mediated tropism of the virus, fusion activity, and recognition by host cell immune responses to infection (Bendinelli et al. 1995).

In addition to encoding gag, pol, and env genes, FIV encodes several other genes, referred to accessory genes. These include three short ORFs located between pol and env, within env and at 3' end of the FIV genome, which encode a number of auxiliary proteins (Miyazawa et al. 1994). The two ORFs, which are located in the region between pol and env, are called vif (or ORF-1) and ORF-A (or ORF-2). The ORF-A of FIV is located between the vif and env genes and appears to have only a low, if any, transactivation activity. The vif gene of FIV produces a protein that is required for some step in virion morphogenesis (Waters et al. 1996). A short 3' ORF that overlaps the LTR is called ORF-B (or ORF-4) (Olmsted et al. 1989). The ORF-B of FIV is at least a part of the rev gene. The Rev protein is crucial for the expression of genes encoded by the partially spliced and unspliced viral mRNAs (Kiyomasu et al. 1991). 

**FIV replication.**

FIV replication is unique and complex and starts with reverse transcription of virion RNA into double-stranded DNA (dsDNA) by reverse transcriptase. These dsDNA intermediates are circularized and integrated into the cat chromosomal DNA. This integrated dsDNA is used for transcription of full-length genomic RNA and various subgenomic mRNAs (Miyazawa 2002). FIV attaches to a number of different specific cellular receptors via their envelope glycoproteins and infects a variety of cells types such as CD4+, CD8+ T cells, macrophages and microglia. Hosie et al. 1993 generated a monoclonal antibody, which blocked FIV infection and recognized feline CD9. It was proposed that CD9 might be a receptor for FIV. However, subsequent studies showed that the inhibitory effects of the antibody impacted FIV infection at a stage after viral entry (Willett et al. 1997, DeParseval & Elder 1998).

Recently, it was shown that CRFK-adapted-cells FIV uses the chemokine receptor CXCR4 for cell fusion and viral entry. The use of CXCR4 as a receptor by some FIV strains is intriguing, given that destruction of CD4 cells in HIV disease seems to be accelerated in individuals with strains utilizing CXCR4 (syncytium inducing or SI strains). However, immunodeficiency also occurs without use of this coreceptor in both FIV and HIV induced disease (Willett et al. 1997, Poeschla & Looney 1998, Saphire et al. 2001).
In the cytoplasm, but still within the viral capsid, a dsDNA copy of the virion RNA is synthesized by the virion-associated reverse transcriptase. The FIV RNA reverse transcribed product has bases added to end of each genomic RNA molecule (LTRs). Transcription by cellular RNA polymerase II initiated at 5'LTR and ending at 3'LTR, generates new virus RNA (Miyazawa 2002).

The FIV proviral genome generates 6 viral mRNA classes of 9.8 kb, 5.0 kb, 4.4 kb, 2.0/1.9 kb and 1.2 kb which can be grouped in 3 classes: the unspliced full-length mRNA (9.8 kb), the singly spliced medium sized mRNAs (5.0 and 4.4 kb) and the small commonly multiple spliced mRNA (2.0/1.9 kb doublet and 1.2 kb), which represent the gag/pol, vit/env, env, ORF-2/rev and rev transcripts respectively (Philips et al. 1992; Tomonaga & Mikami 1996). The gag gene encodes the 15 kDa matrix (MA), 25 kDa capsid (CA) and 7/10 kDa nucleocapsid (NC) proteins and the pol gene encodes the 13 kDa protease, 61 kDa reverse transcriptase, 14 kDa dUTPase and 13 kDa integrase, while the env gene encodes 120/100 kDa surface (SU) and 44 kDa transmembrane (TM) envelope glycoproteins. The vit gene encodes a 23-29 kDa protein and the ORF-2/lat like gene encodes a 9 kDa protein. The rev gene overlaps the 5'env gene and the 3'LTR encoding a 23 kDa protein. Additional 2 mRNAs derived from the env region were identified coding for Rev-related proteins of 11 and 15 kDa (Philips et al. 1992, Tomonaga & Mikami 1996, DeParseeval & Elder 1998). Also, an antisense open reading frame is transcribed antisense to the rev responsive element at the 3'terminus of the env sequence, producing a theoretical product of 11.4 kDa molecular mass, designated ASP (Briquet et al. 2001).

**FIV molecular characterization.**

FIV strains isolated from domestic cats have been classified into five subtypes, designated A, B, C, D and E, by comparing 684 nucleotides sequences comprising the variable regions V3 through V5 of the env gene (Sodora et al. 1994, Kakinuma et al. 1995, Pecoraro et al. 1996).

Sequences from gag, pol and env genes have been used to evaluate phylogenetic relationships and evolution of any FIV isolates. Multiple subtypes have been found in cats from the same continent; however geographic clustering of subtypes is evident. Subtype A viruses are distributed worldwide but predominate in the western United States, northern Japan, Germany and South Africa (Sodora et al. 1994, Bachman et al. 1997, Nishimura et al. 1998). Subtype A includes the prototype virus FIV-A-Petaluma is less diverse than subtype B, and contains viruses with fewer synonymous site mutations suggesting more recent and less host-adapted strains (Bachmann et al. 1997). Subtype B viruses are also distributed worldwide but have been more consistently identified in eastern Japan, Italy, Portugal, central and eastern United States and Brazil (Sodora et al. 1994, Bachmann et al. 1997, Duarte et al. 2002, Caxito et al. 2003).

Except in northern Taiwan, detection of subtype C FIV is uncommon and has otherwise been confined to single animals or small clusters of cats from Vancouver, Munich, and Japan (Sodora et al. 1994, Bachmann et al. 1997, Inada et al. 1997). Several subtype D viruses have been characterized; all from Japan, primarily from the western areas (Kakinuma et al. 1995, Bachmann et al. 1997, Nishimura et al. 1998), and the subtype E comprise just two Argentine strains (Pecoraro et al. 1996).

Significant subtype-based diversity has been identified in ORF-2 and FIV subtypes can be distinguished also by evaluation of gag sequences suggesting that strain differences may extend to the more conserved areas of lentiviral genome (Hohdatsu et al. 1998, Pistello et al. 1999, Chatterji et al. 2002).

**FIV infection and course of disease.**

FIV infection occurs mainly through biting among cats fighting for territorial demarcation and during mating. The virus has been isolated from saliva, blood, serum, plasma, and genital secretions (Courchamp & Pontier 1994, Jordan et al. 1996, Hartmann et al. 1999). Horizontal transmission appears to be predominantly mediated by biting, but the presence of FIV has also been demonstrated in semen (O'Neil et al. 1995) and vaginal washings in experimentally infected queens; therefore male and female reciprocal FIV transmission in the wild may possibly proceed by sexual routes as well (O'Neil et al. 1995, Rogers et al. 1998). Vertical transmission has been reported only from queens experimentally infected during pregnancy. In the latter experiments it was impossible to establish whether transmission had occurred in utero (the epitheliochorial placenta of cats is considered an efficient barrier to be crossed) or through colostrum, milk, and maternal grooming (Yamamoto et al. 1989, Sellon et al. 1994, Rogers et al. 1998, Hartmann et al. 1999).
Clinical and epidemiological studies revealed that a wide range of clinical signs is seen in FIV infected cats. Infection in cats can be staged in five phases: acute phase, asymptomatic carrier phase, the persistent lymphadenopathy phase, the ARC (for AIDS Related Complex) and the AIDS phase (Pedersen et al. 1989, Ishida & Tomoda 1990). The first phase is marked by lymphadenopathy, fever and usually goes unnoticed. The second phase is asymptomatic and characterized by a prolonged persistence of variable duration up to the appearance of clinical signs. In the third phase occurs the persistent generalized lymphadenopathy (PGL) that lasts around one year and may be linked to anorexia (Ishida et al. 1992). The fourth phase is characterized by PGL and general manifestations like progressive weight loss, chronic wasting and fever of unknown origin. Cats in this phase generally present chronic infections, in particular of the oral cavity, of the upper respiratory tract and of other body locations but there are no opportunistic infections. The fifth phase is marked by loss of immune defenses and specific opportunistic infections, encephalopathy and neoplasias (Ishida et al. 1992).

Signs most frequently observed in clinical cases of FIV infection are relatively abnormal physical findings in the oral cavity or around face and ears, such as stomatitis, naso-ocular discharge, and dermatitis (Yamamoto et al. 1989, Hartmann et al. 1999). Opportunistic bacterial and fungal infections are especially common in the mouth cavity, periodontal tissue, cheeks and tongue. About 25% of cats have chronic respiratory disease, and a lesser number have chronic enteritis, urinary tract infection, dermatitis and neurological signs. Terminally about 5% of cats have serious neurological disease, although higher proportions have central system lesions at necropsy (Ishida et al. 1992, Hartmann et al. 1999).

Pathogenesis of FIV infection.

The incubation period of FIV infection may last for several years and the progress of disease follows parallels the decline in CD4+ T lymphocytes (Yamamoto et al. 1989). The hallmark of infection is depletion of CD4+ peripheral T-cells and reduction of CD4/CD8 ratios. The mechanism by which FIV produces selective CD4 depletion and immunodeficiency, despite infection of both CD4 and CD8 lymphocytes as well as other cell types, is currently unknown (Tompkins et al. 2002). During the early acute phase of FIV infection, the inversion of the CD4/CD8 ratio is compounded by a sharp increase in CD8+ T cells expressing low levels of CD8 and increased levels of major histocompatibility complex (MHC) class II molecules (Willet et al. 1994). This activated T-cell subpopulation persists throughout the course of infection, suggesting that the early interaction between the virus and the immune system may ultimately determine the outcome of the infection. CD4+ T lymphocytes support FIV replication only when dividing, therefore FIV–cat interaction results in long-standing subclinical infection that may take years to progress to clinical stage. T cell proliferation stimulated by mitogens, cytokines, antigenic stimulation, unrelated viral infection are of critical importance in determining the level of FIV replication and hence the onset and progression of disease (Willet et al. 1997).

The onset of a functional immunodeficiency is marked by reduced responsiveness of feline peripheral blood mononuclear cells (PBMCs) to in vitro mitogenic stimulation. Longitudinal studies have revealed that the degree of immune impairment correlates with the decline in CD4+ T-cell numbers (Novotney et al. 1990). The data suggest that, as with HIV infection in humans, the primary lesion in the FIV-infected cat is an impairment of helper T-cell function (Willet et al. 1997).

FIV replication in cells of monocyte/macrophage lineage is thought to be required for neurovirulence, but it is not sufficient, as both neurovirulent and nonneurovirulent strains can infect macrophages and mixed glial cell cultures with equal efficiency. Some have hypothesized that central nervous system (CNS) surveillance by T lymphocytes may cause release of factors inducing FIV replication in microglial cells or macrophages in the brain and/or which are directly toxic to neurons (Hein et al. 2001, Johnston et al. 2002). FIV envelope may also directly produce neurotoxicity, through alterations in glutamate uptake and calcium signaling. Glutamate uptake by FIV infected astrocytes is decreased, while glutamate levels are high in the brain of cats with neurological disease. In the presence of low concentrations of glutamate, FIV envelope protein disturbs cellular calcium flux when infused into the CNS of affected cats, about 5% have central nervous system lesion that are associated with behavior abnormalities, dementia and convulsions (Hein et al. 2001, Sanders et al. 2001).
Epidemiological features.
Serologic and virus isolation studies have shown that FIV is enzootic worldwide. Its prevalence, however, varies greatly depending in geographical location and other variables of the cat populations surveyed. Among apparently healthy domestic cats, the lowest seroprevalence rates (1% or less) were observed in central European countries and the United States and highest (up to 30%) were seen in Japan and Australia (Hosie et al. 1989). Age and gender also markedly affect FIV prevalence. Infection is acquired most commonly after 1 year of age, and its prevalence increase up to 10 years of age and then remains stable or tends to decline (the mean lifespan of domestic cats is about 15 years), most likely as a result of a negative balance between FIV-induced mortality and new infections (Ishida et al. 1989, Pedersen et al. 1989). Seropositivity rates in male cats are two or more times higher than in females. It is generally accepted that this and other variations in FIV distribution are mainly the result of differences in social behavior and lifestyle of cats, which influence the likelihood of FIV transmission. Another important variable that has been seen to affect the results of serosurveys is the health status of the animals. The seroprevalences rates observed were usually several times higher in sick cats than in their healthy counterparts, thus providing a persuasive, though indirect indicator of disease-inducing potential of FIV (Spargher et al. 1989, Yamamoto et al. 1989, Lutz 1990, O’Connor et al. 1991, Pedersen et al. 1991, Bandecchi et al. 1992, Pedersen et al. 1993).

Epidemiological surveys have shown an overall seroprevalence of 11.04% (9,750 out of 85,529) worldwide, among both healthy and ill felines screened in North America, Asia, Europe and Oceania. It is estimated that approximately 44 million cats are FIV infected, as total cat population in the world is around 400 million (Kanzaki & Looney 2004). This figure is likely to be underestimate since it does not take into account the fact that 10 to 15% of FIV infected cats are seronegative (Courchamp & Pontier 1994). The highest FIV prevalence is found among ill cats, ranging from 47% in England (Carpenter et al. 1998), 30% in Japan (Ishida et al. 1989), 21% in Australia (Zimmerman et al. 2002), 19% in Canada (Sheehy et al. 2002). In Brazil the few studies about FIV occurrence have demonstrated a prevalence of 2.66% to 37.5% (Table 1).

Serological surveys of 27 nondomestic feline species have revealed that at least 17 display antibodies cross-reactive with FIV antigens (Barr et al. 1989, Barr et al. 1995, Olmsted et al. 1992, Brown et al. 1993, Brown et al. 1994, Carpenter et al. 1996). Genetic characterization of puma, lion, and pallas cat isolates has determined that these viruses are distinct from each other and related to the lentivirus of the domestic cat (Olmsted et al. 1992, Brown et al. 1994, Barr et al. 1995, Carpenter et al. 1996). The pathogenic nature of these viruses in their natural hosts has been fully characterized; while one report was demonstrated the association with lymphoma in a captive lion with a FIV-like lentivirus, other analyses have not correlated specific disease has been identified in association with the infection. (Olmsted et al. 1992, Brown et al. 1994, Poli et al. 1995).

Table 1 - Review about the epidemiological studies of FIV in Brazil.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>State</th>
<th>Diagnostic test</th>
<th>Feline species</th>
<th>Nr. of tested animals</th>
<th>% of positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hagihara et al.</td>
<td>1993</td>
<td>SP</td>
<td>ELISA</td>
<td>Felis catus</td>
<td>401</td>
<td>11.7%</td>
</tr>
<tr>
<td>Caldas et al.</td>
<td>2000</td>
<td>RS</td>
<td>PCR</td>
<td>Felis catus</td>
<td>40</td>
<td>37.5%</td>
</tr>
<tr>
<td>Souza et al.</td>
<td>2002</td>
<td>RJ</td>
<td>ELISA</td>
<td>Felis catus</td>
<td>126</td>
<td>16.66%</td>
</tr>
<tr>
<td>Taniwaki et al.</td>
<td>2002</td>
<td>SP</td>
<td>PCR</td>
<td>Felis catus</td>
<td>500</td>
<td>13.4%</td>
</tr>
<tr>
<td>Filoni et al.</td>
<td>2003</td>
<td>SP</td>
<td>ELISA</td>
<td>Leopardus pardalis, L. tigrinus, L. wiedii, Herpailurus yaguarondo, Oncifelis geoffroyi</td>
<td>104</td>
<td>0%</td>
</tr>
<tr>
<td>Caxio et al.</td>
<td>2003</td>
<td>MG</td>
<td>PCR</td>
<td>Felis catus</td>
<td>450</td>
<td>2.66%</td>
</tr>
</tbody>
</table>
FIV diagnostic and treatment.

FIV diagnostics used to rely on the detection of FIV antibodies in the peripheral blood (Hartmann et al. 2001). FIV infection does not release sufficient levels of virus in circulation for conventional enzyme immunoassays (EIA) to detect viral antigens consistently. The serologic detection of FIV infection usually depends on the detection of specific antibodies in serum, plasma or whole blood. Three basic techniques are employed in FIV antibody tests, the enzyme-linked immunosorbent assay (ELISA), the indirect immunofluorescence assay (IFA), and the Western blot or immunoblot assay. Moreover, it is important to retest in two months for confirmation of result from cats with a known, recent exposure, if the initial result is negative. False-positive enzyme immunoassay results range from 2 to 20%, probably because the use of cat vaccine that have been produced in feline cell cultures (Ishida & Tomoda 1990).

Virus isolation and detection of FIV proviral DNA or viral RNA by polymerase chain reaction (PCR) or reverse transcriptase PCR is also now shown to be more sensitive, faster and easier to perform than the standard serological test and may be easily used for routine diagnosis (Vahlenkamp et al. 1996, Klein et al. 1999, Klein et al. 2001, Pedersen et al. 2001).

The extensive genetic variation observed in FIV also has a direct impact on PCR-based methods, which are increasingly used for diagnosis and monitoring of FIV infection (Leutenegger et al. 1999, Hosie et al. 2002). Furthermore, PCR assays to distinguish vaccinated from infected cats will gain importance with the advent of the first commercially available FIV vaccine (Uhl et al. 2002).

The FIV treatment at present is largely supportive although many HIV anti-retroviral drugs, including the nucleoside analogues such as azidothymidine (AZT), have been tested in experimental FIV-infected cats and have been used by veterinarians for treating naturally infected pet cats with modest success (Hart & Noite 1995). The use of new nucleoside analogues such lamivudine (3TC and ABC combined with AZT has been shown to be synergistically inhibitory in vitro (Bisset et al. 2002).

FIV control and vaccine development.

An appropriate test and removal programs and certification at the point of sale can be applied to control the infection in order to achieve minimal risk of FIV infection. There should be as little contact as possible with cats outside the house, and particularly it is important to avoid contact with stray and feral cats. To reduce the tendency for a cat to socialize and fight with the other cats its advisable to neuter the male’s cats. Infected cats should ideally be kept indoors to prevent risk of spread to other cats in the neighborhood. The risk of transmission to other non-infected cats in the household is possible but remote because neither closes contact nor maternal grooming appears to be significant modes of transmission. Euthanasia of known infected FIV positive cats in a household is not considered necessary and is only justified if they are clinically ill or are known to bite other cats (Hopper et al. 1991).

FIV vaccine development has had its successes and failures similar to those encountered in human immunodeficiency virus (HIV) vaccine research. As the success of a vaccine could be hampered by the occurrence of highly divergent virus variants, the genetic diversity of FIV field strains circulating in all regions where vaccination is planned should be determined (Pistello et al. 1997). A wide range of experimental vaccines have been developed, including products made from whole inactivated virus, a variety of recombinant DNA-produced antigens and a fully infections DNA clone. Some of several single-subtype vaccines protected against challenge with homologous or slightly heterologous virus but failed to protect against more distantly related strains (Elyar et al. 1997). In contrast, a double-subtype virus vaccine has been proven to elicit considerable protection against challenge with virus of a third subtype, not included in the vaccine (Pu et al. 2001). The USDA has now approved this vaccine; however, its efficacy still remains to be demonstrated under field conditions (Uhl et al. 2002).

CONCLUSIONS

The interest in the study of feline immunodeficiency virus has expanded in recent years, and can bring major information in several domains. The most evident interest is in Veterinary Science. In addition to cat sanitary aspect, FIV could be an interesting model of lentiviral epidemiological studies. Moreover, presence of antibodies to a specific FIV strain in non-domestic species of felids implies another interest of lentivirus studies, for biodiversity conservation. FIV strain and their feline host could be a good model to
study host/parasite coevolution, especially concerning lentivirus virulence evolution. Finally, FIV is a particularly well-fitted study model of its human homologous, the HIV, on many domains.

REFERENCES


on of feline immunodeficiency virus with semen from seropositive cats. J. Reprod. Immunol. 41: 341-357.


