Intrahost genetic diversity of Brazilian isolate of feline immunodeficiency virus

Brief Report

Teixeira, B.M.[1*]; Logan, N.[2]; Cruz, J.C.M.[3]; Reis, J.K.P.[3]; Brandão, P.E.[4]; Hagiwara, M.K.[1]; Willett, B.J.[2]; Hosie, M.J.[2]

[1]Department of Medical Clinics, College of Veterinary Medicine, University of São Paulo; [2]Retrovirus Research Laboratory, Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow; [3]Retrolab, Veterinary School, Department of Preventive Veterinary Medicine, Federal University of Minas Gerais; [4]Department of Preventive Veterinary Medicine and Animal Health, College of Veterinary Medicine, University of São Paulo

* bmteixeira@yahoo.com.br

Telephone: 0044 7503875363 / 0055 31 34981262

Fax number: 0055 11 30911287

Address: Av. Prof. Dr. Orlando Marques Paiva, 87, Sao Paulo, SP, Brazil, 05508-270

Summary. We isolated *Feline immunodeficiency virus* (FIV) **from three adult domestic cats, originating from two open shelters in Brazil**. Viruses were isolated from PBMC following co-cultivation with the feline T-lymphoblastoid cell line MYA-1. All amplified *env* gene products were cloned directly into $pGL8_{MYA}$. The nucleic acid sequences of seven clones were determined and then compared with those of previously described isolates. The sequences of all of the Brazilian virus clones were distinct and phylogenetic analysis revealed that all belong to subtype B. **Three variants isolated from one cat and two variants were isolated from each of the two other cats,** indicating that intrahost diversity has the potential to pose problems for the treatment and diagnosis of FIV infection.

*

Feline immunodeficiency virus (FIV), originally isolated in the Unites States in 1986 from a cat with chronic opportunistic infections [28], is a member of the *Retroviridae* family, genus lentivirus. FIV-infected cats can exhibit illnesses including gingivitis, stomatitis, lymphoma, neurological disorders and wasting [13, 28, 33]. The hallmark of FIV infection is a progressive reduction in the number of circulating $CD4^+$ lymphocytes which ultimately results in an impairment of immunity, similar to AIDS caused by human immunodeficiency virus (HIV) [38] . FIV is both a significant pathogen of domestic cats and a widely used model to investigate HIV pathogenesis and approaches to AIDS vaccination [5].

The viral genome consists of three major genes and several smaller regulatory genes. Like other lentiviruses, FIV exhibits extensive genetic variation [18,32]. The *pol* and *gag* genes encode viral enzymes and core proteins respectively, and are relatively highly conserved. The *env* gene encodes surface and transmembrane glycoproteins and is highly variable [8, 24, 36]. Within the *env* gene, nine variable regions have been defined, separated by more conserved regions [26]. On the basis of the analysis of envelope glycoprotein variable regions 3-5, FIV has been classified into five subtypes [17], [27, 35], a number that can be expected to increase as further studies reveal additional diversity. Recent studies identified distinct groups of FIV isolates from the Unites States and New Zealand [40, 10]. Although genetic subtyping, in general, is based on nucleotide sequences from *gag* [4], [12], the high varia bility of the lentiviral *env* gene makes it the preferred region for subtyping [18]. The *env* region contains determinants important for cell tropism, cytopathogenicity, and infectivity and prominent immunoreactive domains [21, 26, 34].

The rapid evolution of lentiviruses within an infected individual results in the formation of a viral quasispecies, a phenomenon well documented in HIV infection [7, 32]. Similar to all retroviruses, FIV has a relatively high evolutionary rate, attributed largely to substitution errors made during reverse transcription. The existence of multiple variants, or quasispecies, of FIV has been reported as well [19, 35] although information on intrahost sequence variation is sparse and limited [15, 16]. Without

molecular cloning, the existence of minor quasispecies would not have been detected [19].

FIV infection is prevalent worldwide [43]. Preliminary studies carried out suggested that FIV infection is widespread in the domestic cat population of Brazil [29, 1, 37, 22]. Prevalence rates of FIV infection in Brazil have not been well evaluated and regional variations are largely unexplored. Larger surveys of Brazilian isolates are required to determine whether FIV isolates in Brazil have evolved within a single subtype. Preliminary work has suggested that subtype B isolates are present in the domestic cat population of Brazil [1, 22], but a definitive identification of circulating subtypes is essential in order to develop strategies for molecular diagnosis, since the genetic diversity is high [30]. In this study, novel Brazilian strains of FIV were isolated and *env* gene products were amplified and then cloned directly into $pGL8_{MYA}$, a molecular clone of FIV-GL8. The nucleotide sequences of *env* of **seven** clones were determined. Phylogenetic analysis was conducted on the nucleotide sequences derived from these clones and other published FIV sequences and the Brazilian sequences were submitted to GeneBank.

FIV infected cats were identified using the polymerase chain reaction (PCR) to amplify the gag gene [11] and using the SNAP FIV/FeLV Comb Test, IDEXX™ – Westbrook, EUA. One cat, Leviano, originated from an open shelter in Minas Gerais, Brazil [37], and two other cats, Didi and Dengosa, originated from another open shelter in São Paulo, Brazil. All three cats were chronically infected. Virus were isolated by cultivation of the cats' PBMC [14]. PBMCs were fractionated from 5 ml of heparinised whole blood by centrifugation over a Ficoll-Paque solution with a density of 1.077 g/ml. The separated PBMCs were then co-cultivated with MYA-1 cells in the absence of mitogenic stimulation [23]. DNAs were prepared from positive viral isolations by column chromatography (QIAamp DNA maxiprep Kit; Qiagen) as soon as a positive ELISA for FIV p24 was recorded; thus, viruses had undergone minimal passage *in vitro*. Full-length viral envelope glycoprotein (*env*) genes were amplified from these replication-competent viruses using a high-fidelity (proofreading) PCR (High Fidelity PCR system; Roche) using primers corresponding to the 5' L-SU cleavage site (TAGACGCGTAAGATTTTTAAGGTATTC) and *Nde*I site 3' of the Rev responsive element (CCCTTTGAGGAAGATGTGTCATATGAATCCATT) incorporating *Mlu*I and *Nde*I restriction sites, respectively. Due to the inherent instability of the full-length *env* genes from primary isolates of FIV such as GL8, standard high-copy-number PCR

product cloning vectors could not be used; thus, all amplified *env* gene products were digested with *Mlu*I/*Nde*I and were cloned directly into $pGL8_{MYA}$, a molecular clone of FIV-GL8 in the low-copy-number plasmid pBR328 and in which an *Mlu*I site had been introduced at the L-SU junction.

The nucleic acid sequence of seven independent *env* clones from each cat was determined using IRD800-labeled oligonucleotides on an automated sequencer, LI-COR Biosciences, Lincoln, Nebr. [15]. DNA sequence alignments and analyses were conducted using BioEdit version 5.0.6 software [9]. The first analysis included 2375 bp of the envelope glycoprotein sequences analyses, from the V2 region to the end of the *env* open reading frame (orf), 791 amino acids (Fig. 1). The *env* gene V3-V5 regions encode neutralizing epitopes, such that mutations in these regions induce resistance to viral neutralizing antibodies. Analyses have reported that a major neutralizing epitope is present in the V3 region [21, 31]. In addition, V3-V5 region has been identified as having an important role in cell tropism, with mutations in V3 affecting potential sites for N-linked glycosylation which influence cell tropism [15] and therefore there are many V3-V5 sequences in GenBank, including those from other FIV sequences reported previously from Brazil. In addition, a second analysis was conducted with 473 bp of sequence encoding a region of 157 amino acids comprising the V3-V4 region (Fig. 2).

Comment [MJH1]: REFS?

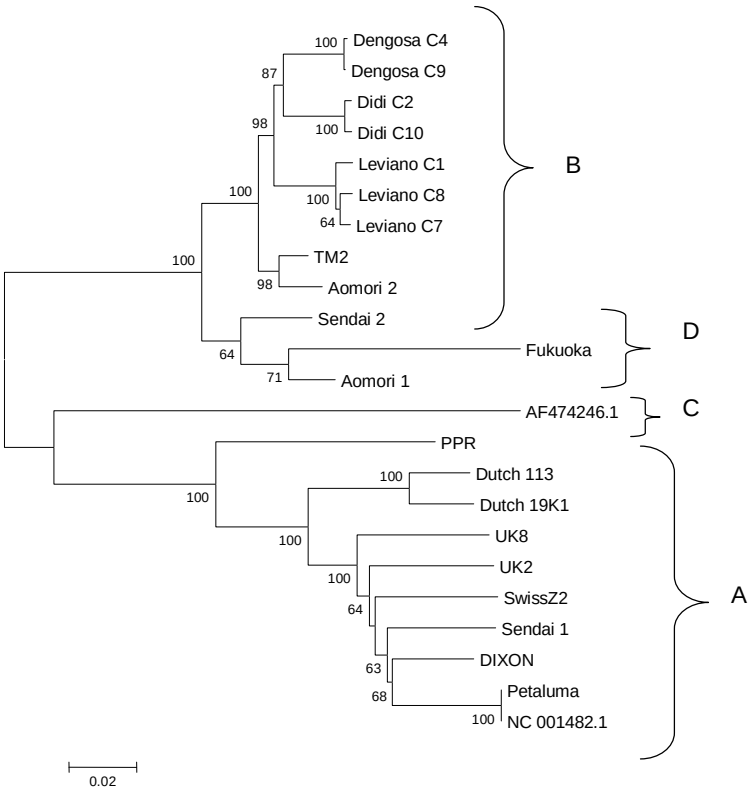

Fig. 1. Phylogenetic tree of 2375 bp sequences from the region of FIV *env*. The subtype of the obtained sequences was determined by phylogenetic analyses, using a rooted Neighbour-joining tree with Kimura 2-parameter genetic distances and bootstrap analysis with 1000 iterations to evaluate clade consistency.

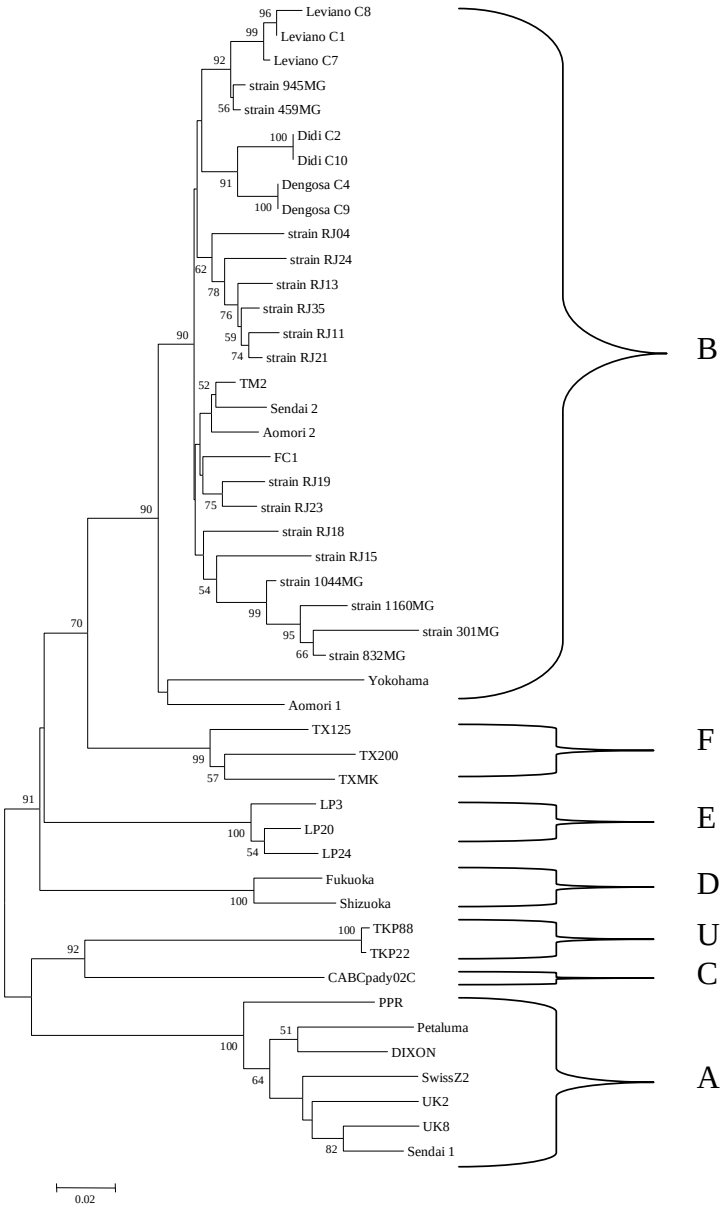

Fig. 2. Phylogenetic tree of 473 bp sequences from the V3-V4 region of FIV *env*. The subtype of the obtained sequences was determined by phylogenetic analyses, using a unrooted Neighbour-joining tree with Kimura 2-parameter genetic distances and bootstrap analysis with 1000 iterations to evaluate clade consistency.

Nucleotide sequence data from Leviano´s clones reported in this paper have been deposited in the GenBank database under accession numbers FJ374695, Leviano C8, FJ374696, Leviano C1 and FJ374697, Leviano C7. Others FIV sequences included in the first phylogenetic tree are as follows (the GenBank accession numbers, names of isolates, country and subtype for the FIV *env* sequences are listed): X60725.1, Dutch 113, Netherlands, A; X69494.1, Scotland, A; X57001.1, SwissZ2, Swiss, A; L00608.1, DIXON, United States, A; M73964.1, Dutch 19K1, Netherlands, A; M59418.1, TM2, Japan, B; M36968.1, PPR, United States, A; X69496.1, UK8, England, A; D37813.1; Sendai 1, Japan, A; D37814.1, Sendai 2, Japan, B; D37815.1, Fukuoka, Japan, D; D37816.1, Aomori 1, Japan, B; D37817.1, Aomori 2, Japan, B; D37811.1, Shizuoka, Japan, D; AF474246.1, Canada, C; NC_001482.1, refseq fiv, United States, A and M25381.1, Petaluma, United States, A. The NC_001413.1, Bovine immunodeficiency virus was used as an outlier.

For the second phylogenetic tree the GenBank accession numbers, names, country and subtype for the FIV *env* sequences included were: M25381.1, Petaluma, Unite Satates, A; L00608.1, DIXON, United States, A; X6075, FIV-UT 113, Netherlands, A; M59418.1, TM2, Japan, B; M36968.1, PPR, Unite States, A; X69496.1, UK8, England, A; X69494, UK2, Scotland, A; X57001, SwissZ2, Switzerland, A; AY621093, FC1, United States (Florida), B; U02392.1, CABCpady02C, Canada, C; D84498, LP20, Argentine, E; D84496, LP3, Argentine, E; D84500, LP24, Argentine, E; D37813.1, Sendai 1, Japan, A; D37816, Aomori 1, Japan, B; D37814.1, Sendai 2, Japan, B; D37812, Yokohama, Japan, B; D37815.1, Fukuoka, Japan, D; D37817.1, Aomori 2, Japan, B; D37811.1, Shizuoka, Japan, D; AY139094.1, TX125, United States (Texas), F; AY139096.1, TX200, United States (Texas), F; AY139097.1, TXMK, United States (Texas), F; EF153977.1, TKP88, New Zealand, U; EF153979.1, TKP22, New Zealand, U; EU375619, RJ35, Brazil, B; EU375617, RJ24, Brazil, B; EU375616, RJ23, Brazil, B; B; EU375614, RJ21, Brazil, B; EU375597.1, strain RJ04, Brazil, B; EU375604.1, strain RJ11, Brazil, B; EU375606.1, strain RJ13, Brazil, B; EU375608.1, strain RJ15, Brazil, B; EU375611.1, strain RJ18, Brazil, B; EU375612.1, strain RJ19, Brazil, B;

DQ248885.1, strain 1044MG, Brazil, B; DQ177159.2, strain 945MG, Brazil, B; DQ641681.1, strain 459MG, Brazil, B; DQ865447.1, strain 301MG, Brazil, B; DQ865449.1, strain 832MG, Brazil, B; DQ865454.1, strain 1160MG, Brazil, B.

Analyses of the viral sequences present of molecular clones showed that all clones grouped within subtype B and that those clones varied from each other **even the clones from the same cat (Fig. 1 and table 1)**. Neither the prevalence of FIV infection in Brazil nor all prevailing subtypes are known. Identification of circulating subtypes **from different areas of Brazil** is essential to develop strategies for molecular diagnosis, since the genetic diversity is high [18]. A recent publication declared that Brazilian FIV strains and their epidemic spread have not been deeply characterized [22]. In this study the analyses classified the clones as subtype B in accordance with other reports from Minas Gerais and also the Brazilian state of Rio de Janeiro [1, 22]. The isolation and characterization of FIV *env* genes from Minas Gerais **and São Paulo** states is the first of its kind. It is important to state that Minas Gerais, **São Paulo** and Rio de Janeiro are neighbouring states and Brazil is a huge country. To verify the difference between the clones from Brazilian isolates, HIV pseudotype assays were performed using the methods described previously [41]. In addition, the viral variants were compared for receptor usage. All clones were infected MCC cells expressing the native feline CD134 (FFF) and the FFHH chimaeric version of CD134 but the luciferase activity from all clones showed different values (data not shown).

We observed variability amongst the clones isolated from three infected cats and, in agreement with a recent study [16], our results confirmed that biological isolates contain heterogeneous viral quasispecies [16]. These results are consistent with the emergence of a quasispecies and the diversity of the viruses examined confirmed previous observations [19]. Quasispecies arise as the virus-encoded reverse transcriptase (RT) enzyme, an RNA-dependent DNA polymerase that is present in mature virions of all members of the *Retroviridae*, lacks a proofreading mechanism that corrects for erroneously incorporated nucleotides, resulting in extensive sequence variation, a typical feature of lentiviruses that includes base substitution, addition, and deletion [32]. Previously, intrahost sequence variation has been assessed based on sequence analyses of PCR amplicons obtained using DNA isolated from the PBMC of infected cats as a template, reflecting the most abundantproviral genomes rather than the complete repertoire of virus sequences in circulation [16]. In this study, FIV *env* was analyzed since this gene encodes the protein that is a target for the humoral response and is

known for its capacity to escape from recognition by antibodies by the accumulation of mutations in the variable regions especially. Sequence variation was most extensive in the V3-V6 regions, where none of the analyzed sequences were identical.

Knowing the prevalence and variability of FIV is of importance for designing and testing vaccines under field conditions. The sequence of the *env* gene is particularly important since the envelope glycoprotein is the major target for virus neutralization [21, 31] and it may be possible to establish potential associations between specific subtypes and the severity of clinical outcomes [30].

In this study we observed that FIV displays genetic variation amongst the variants identified within three biological isolates (Figures 1 and 2). Further studies will be required to identify the importance of the variability amongst Brazilian FIV isolates identified in this study. Preliminary data have shown variable cell tropism amongst the clones in their ability to infect MCC cell expressing CD134. Since the *in vivo* cell tropism of FIV expands as disease progresses [3, 6] and a single potential site for N-linked glycosylation in the envelope glycoprotein of FIV modulates the virus-receptor interaction [42], even minor variation in the *env* gene could have great significance. Sequence variations and conformational changes within the Env protein are responsible for determining the receptor usage of FIV [20]. Chemokine receptor tropism has been linked to specific sequence variations in the Env of primate lentiviruses [2]. For FIV, a mutation in V3 is sufficient to convert a non-CRFK tropic virus into a CRFK-tropic virus [39]. Other domains of SU distinct from V3 have also been identified as important determinants and/or co-determinants of cell tropism in HIV-1 [25]. The results of this work would provide further information for studies involving structure, diagnosis, vaccine development and phylogenetic analysis of FIV.

Acknowledgements

This work was supported by FAPESP (the São Paulo State research funding foundation), CNPq (Brazil's National Research Council) and Public Heath Service grant AI049765 to B.J.W and M.J.H from the National Institute of Allergy and Infectious Diseases.

References

1. Caxito FA, Coelho FM, Oliveira ME, Resende M (2006) Feline immunodeficiency virus subtype B in domestic cats in Minas Gerais, Brazil. Veterinary research communications 30: 953-956
2. Cho MW, Lee MK, Carney MC, Berson JF, Doms RW, Martin MA (1998) Identification of determinants on a dualtropic human immunodeficiency virus type 1 envelope glycoprotein that confer usage of CXCR4. Journal of virology 72: 2509-2515
3. Dean GA, Reubel GH, Moore PF, Pedersen NC (1996) Proviral burden and infection kinetics of feline immunodeficiency virus in lymphocyte subsets of blood and lymph node. Journal of virology 70: 5165-5169
4. Duarte A, Tavares L (2006) Phylogenetic analysis of Portuguese Feline Immunodeficiency Virus sequences reveals high genetic diversity. Veterinary microbiology 114: 25-33
5. Elder JH, Dean GA, Hoover EA, Hoxie JA, Malim MH, Mathes L, Neil JC, North TW, Sparger E, Tompkins MB, Tompkins WA, Yamamoto J, Yuhki N, Pedersen NC, Miller RH (1998) Lessons from the cat: feline immunodeficiency virus as a tool to develop intervention strategies against human immunodeficiency virus type 1. AIDS research and human retroviruses 14: 797-801
6. English RV, Johnson CM, Gebhard DH, Tompkins MB (1993) In vivo lymphocyte tropism of feline immunodeficiency virus. Journal of virology 67: 5175-5186
7. Goodenow M, Huet T, Saurin W, Kwok S, Sninsky J, Wain-Hobson S (1989) HIV-1 isolates are rapidly evolving quasispecies: evidence for viral mixtures and preferred nucleotide substitutions. Journal of acquired immune deficiency syndromes 2: 344-352
8. Greene WK, Meers J, del Fierro G, Carnegie PR, Robinson WF (1993) Extensive sequence variation of feline immunodeficiency virus env genes in isolates from naturally infected cats. Archives of virology 133: 51-62

9. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95-98
10. Hayward JJ, Taylor J, Rodrigo AG (2007) Phylogenetic analysis of feline immunodeficiency virus in feral and companion domestic cats of New Zealand. Journal of virology 81: 2999-3004
11. Hohdatsu T, Yamada M, Okada M, Fukasawa M, Watanabe K, Ogasawara T, Takagi M, Aizawa C, Hayami M, Koyama H (1992) Detection of feline immunodeficiency proviral DNA in peripheral blood lymphocytes by the polymerase chain reaction. Veterinary microbiology 30: 113-123
12. Hohdatsu T, Motokawa K, Usami M, Amioka M, Okada S, Koyama H (1998) Genetic subtyping and epidemiological study of feline immunodeficiency virus by nested polymerase chain reaction-restriction fragment length polymorphism analysis of the gag gene. Journal of virological methods 70: 107-111
13. Hosie MJ, Robertson C, Jarrett O (1989) Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom. The Veterinary record 125: 293-297
14. Hosie MJ, Dunsford T, Klein D, Willett BJ, Cannon C, Osborne R, Macdonald J, Spibey N, Mackay N, Jarrett O, Neil JC (2000) Vaccination with inactivated virus but not viral DNA reduces virus load following challenge with a heterologous and virulent isolate of feline immunodeficiency virus. Journal of virology 74: 9403-9411
15. Hosie MJ, Willett BJ, Klein D, Dunsford TH, Cannon C, Shimojima M, Neil JC, Jarrett O (2002) Evolution of replication efficiency following infection with a molecularly cloned feline immunodeficiency virus of low virulence. Journal of virology 76: 6062-6072
16. Huisman W, Schrauwen EJ, Rimmelzwaan GF, Osterhaus AD (2008) Intrahost evolution of envelope glycoprotein and OrfA sequences after experimental infection of cats with a molecular clone and a biological isolate of feline immunodeficiency virus. Virus research 137: 24-32
17. Kakinuma S, Motokawa K, Hohdatsu T, Yamamoto JK, Koyama H, Hashimoto H (1995) Nucleotide sequence of feline immunodeficiency virus: classification of Japanese isolates into two subtypes which are distinct from non-Japanese subtypes. Journal of virology 69: 3639-3646

18. Kann R, Seddon J, Kyaw-Tanner M, Meers J (2007) Co-infection with different subtypes of feline immunodeficiency virus can complicate subtype assignment by phylogenetic analysis. Archives of virology 152: 1187-1193
19. Kyaw-Tanner MT, Robinson WF (1996) Quasispecies and naturally occurring superinfection in feline immunodeficiency virus infection. Archives of virology 141: 1703-1713
20. Lerner DL, Elder JH (2000) Expanded host cell tropism and cytopathic properties of feline immunodeficiency virus strain PPR subsequent to passage through interleukin-2-independent T cells. Journal of virology 74: 1854-1863
21. Lombardi S, Bendinelli M, Garzelli C (1993) Detection of B epitopes on the p24 gag protein of feline immunodeficiency virus by monoclonal antibodies. AIDS research and human retroviruses 9: 141-146
22. Martins AN, Medeiros SO, Simonetti JP, Schatzmayr HG, Tanuri A, Brindeiro RM (2008) Phylogenetic and genetic analysis of feline immunodeficiency virus gag, pol, and env genes from domestic cats undergoing nucleoside reverse transcriptase inhibitor treatment or treatment-naive cats in Rio de Janeiro, Brazil. Journal of virology 82: 7863-7874
23. Miyazawa T, Furuya T, Itagaki S, Tohya Y, Takahashi E, Mikami T (1989) Establishment of a feline T-lymphoblastoid cell line highly sensitive for replication of feline immunodeficiency virus. Archives of virology 108: 131-135
24. Olmsted RA, Hirsch VM, Purcell RH, Johnson PR (1989) Nucleotide sequence analysis of feline immunodeficiency virus: genome organization and relationship to other lentiviruses. Proceedings of the National Academy of Sciences of the United States of America 86: 8088-8092
25. Palmer C, Balfe P, Fox D, May JC, Frederiksson R, Fenyo EM, McKeating JA (1996) Functional characterization of the V1V2 region of human immunodeficiency virus type 1. Virology 220: 436-449
26. Pancino G, Fossati I, Chappey C, Castelot S, Hurtrel B, Moraillon A, Klatzmann D, Sonigo P (1993) Structure and variations of feline immunodeficiency virus envelope glycoproteins. Virology 192: 659-662
27. Pecoraro MR, Tomonaga K, Miyazawa T, Kawaguchi Y, Sugita S, Tohya Y, Kai C, Etcheverrigaray ME, Mikami T (1996) Genetic diversity of Argentine isolates of feline immunodeficiency virus. The Journal of general virology 77 (Pt 9): 2031-2035

28. Pedersen NC, Ho EW, Brown ML, Yamamoto JK (1987) Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. Science (New York, NY 235: 790-793
29. Reche Jr A, Hagiwara MK, Lucas SRR (1997) Clinical study of acquired immunodeficiency syndrome in domestic cats in São Paulo. Brazilian Journal of Veterinary Research and Animal Science 34: 152-155
30. Reggeti F, Bienzle D (2004) Feline immunodeficiency virus subtypes A, B and C and intersubtype recombinants in Ontario, Canada. The Journal of general virology 85: 1843-1852
31. Rigby MA, Mackay N, Reid G, Osborne R, Neil JC, Jarrett O (1996) Immunogenicity of a peptide from a major neutralising determinant of the feline immunodeficiency virus surface glycoprotein. Vaccine 14: 1095-1102
32. Roberts JD, Bebenek K, Kunkel TA (1988) The accuracy of reverse transcriptase from HIV-1. Science (New York, NY 242: 1171-1173
33. Ryan G, Grimes T, Brankin B, Mabruk MJ, Hosie MJ, Jarrett O, Callanan JJ (2005) Neuropathology associated with feline immunodeficiency virus infection highlights prominent lymphocyte trafficking through both the blood-brain and blood-choroid plexus barriers. Journal of neurovirology 11: 337-345
34. Siebelink KH, Chu IH, Rimmelzwaan GF, Weijer K, Osterhaus AD, Bosch ML (1992) Isolation and partial characterization of infectious molecular clones of feline immunodeficiency virus obtained directly from bone marrow DNA of a naturally infected cat. Journal of virology 66: 1091-1097
35. Sodora DL, Shpaer EG, Kitchell BE, Dow SW, Hoover EA, Mullins JI (1994) Identification of three feline immunodeficiency virus (FIV) env gene subtypes and comparison of the FIV and human immunodeficiency virus type 1 evolutionary patterns. Journal of virology 68: 2230-2238
36. Talbott RL, Sparger EE, Lovelace KM, Fitch WM, Pedersen NC, Luciw PA, Elder JH (1989) Nucleotide sequence and genomic organization of feline immunodeficiency virus. Proceedings of the National Academy of Sciences of the United States of America 86: 5743-5747
37. Teixeira BM, Rajão DS, Haddad JPA, Leite RC, Reis JKP (2007) Occurrence of feline immunodeficiency virus and feline leukemia virus in Sheltered domestic cats of Belo Horizonte. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 59: 939-942

38. Torten M, Franchini M, Barlough JE, George JW, Mozes E, Lutz H, Pedersen NC (1991) Progressive immune dysfunction in cats experimentally infected with feline immunodeficiency virus. Journal of virology 65: 2225-2230
39. Verschoor EJ, Boven LA, Blaak H, van Vliet AL, Horzinek MC, de Ronde A (1995) A single mutation within the V3 envelope neutralization domain of feline immunodeficiency virus determines its tropism for CRFK cells. Journal of virology 69: 4752-4757
40. Weaver EA, Collisson EW, Slater M, Zhu G (2004) Phylogenetic analyses of Texas isolates indicate an evolving subtype of the clade B feline immunodeficiency viruses. Journal of virology 78: 2158-2163
41. Willett BJ, McMonagle EL, Ridha S, Hosie MJ (2006) Differential utilization of CD134 as a functional receptor by diverse strains of feline immunodeficiency virus. Journal of virology 80: 3386-3394
42. Willett BJ, McMonagle EL, Logan N, Samman A, Hosie MJ (2008) A single site for N-linked glycosylation in the envelope glycoprotein of feline immunodeficiency virus modulates the virus-receptor interaction. Retrovirology 5: 77
43. Yamamoto JK, Pu R, Sato E, Hohdatsu T (2007) Feline immunodeficiency virus pathogenesis and development of a dual-subtype feline-immunodeficiency-virus vaccine. AIDS (London, England) 21: 547-563