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RAPID COMMUNICATION

Feline immudeficiency virus subtypes B and A in cats from São Luis, Maranhão, Brazil

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Abstract Feline immunodeficiency virus (FIV) is a retrovirus of the genus *Lentivirus* that is distributed worldwide, with prevalence rates varying between 2.5% and 44%. FIV causes immunosuppression, with depletion of $TCD4^+$ lymphocytes, with the majority of clinical signs caused by secondary and opportunistic infections. Blood samples were collected from nine domestic cats (*Felis catus domesticus*) from the city of São Luís, Maranhão State, Brazil. All samples were positive in a rapid immunochromatographic test (SNAP® Combo FeLV Ag/FIV Antibody Test) and in a polymerase chain reaction (PCR) assay. Phylogenetic analysis showed that six samples clustered within subtype B, one within subtype A, and two did not cluster with any known subtype. Five unique haplotypes (Hap-1, Hap-2, Hap-3, Hap-5 and Hap-6) and a shared haplotype (Hap-4) were found, this last one being the most frequent. This is the first report on the genetic diversity of FIV in the city of São Luís and the first report of subtype A in Brazil. New variations of the virus are also reported.

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Introduction

Feline immunodeficiency virus (FIV) is a lentivirus of the family *Retroviridae* that has structural, morphological, biochemical and genetic characteristics similar to those of human immunodeficiency virus (HIV). Based on this similarity, domestic cats can be a valuable model for infection and pathogeny studies of retroviruses [1].

FIV infects mainly $TCD4^+$ lymphocytes [2]. Infected felines produce specific neutralizing antibodies against the p15 and p24 proteins of the virus between two and four weeks postinfection [3]. Due to the persistence of the viral infection, high antibody titers are produced, which gradually increase in a few weeks [4].

Serological surveys show that the worldwide prevalence of FIV infection varies from 2.5% to 44% [5]. However, when using molecular tools, the prevalence decreases from 4% to 2% of the world feline population [6]. Differences can be attributed to factors such as gender, age, housing, population density, reproductive status, and territorial behavior [7].

The FIV proviral genome has two long terminal repeats, which regulate viral replication, and three structural genes [8]. The *gag* gene encodes the structural proteins, which are produced as a polyprotein that is then cleaved to form the capsid (CA), matrix (MA) and nucleocapsid (NC) proteins [9]. The *pol* gene encodes enzymatic proteins present inside the capsid: protease (PR), reverse transcriptase (RT), integrase (IN), and dUTPase (DU), which are necessary for the maturation, reverse transcription, integration, and genomic repair, respectively, of the virus, [10, 11]. The *env* gene encodes the envelope, surface (gp 120), and transmembrane (gp 41) proteins of the virion [11].

Currently, FIV is classified into five subtypes. Subtypes A, B and C are found on all continents. Subtype A isolates are common in Australia, Europe and the United States [12];

subtype B, in Europe, Japan and the United States [12, 13] and subtype C, which is considered the most pathogenic, in Canada, Europe, Taiwan and Vietnam [14, 15]. Subtype D has been isolated only in Japan and Vietnam; and subtype E, in Argentina. Most isolates obtained until now have been of subtypes A or B. In addition, recombinant sequences of subtypes A and B, B and D, and A and C have also been identified, classified as "between subtypes" [12]. Studies based on molecular analysis have revealed great genetic diversity within the different FIV subtypes [16, 17].

In Brazil, subtype B is the one most frequently identified [14, 15]. It has been identified based on restriction fragment length polymorphism (RFLP) [18] in the state of Minas Gerais. In the state of São Paulo, genetic sequencing revealed the existence of subgroups within subtype B, as indicated by high bootstrap values in phylogenetic analysis [19]. In the city of São Luís, Maranhão State, 18.33% of the animals tested were positive in both PCR and indirect enzyme-linked immunosorbent assay (ELISA) [20].

The most commonly used serological tests for FIV diagnosis in veterinary clinics are immunoenzymatic assays (ELISAs) that detect specific antibodies against the viral proteins p15 and p24, using samples such as blood, serum or plasm [21]. The detection of virus in cell culture and virus isolation is possible from 10 to 14 days after infection, but this is not practical for routine laboratory tests [22]. Molecular tools such as polymerase chain reaction (PCR) can be used to detect viral genome particles in infected blood cells after five days of infection [23].

The aim of this study was to identify the subtypes of FIV in cats of the city of São Luís, Maranhão State, Brazil, and to investigate the phylogenetic relationships and genetic variation among the subtypes.

Materials and methods

Blood samples from nine domestic cats (*Felis catus domesticus*) from the city of São Luís, Maranhão State, Brazil, that were collected and analysed previously [20] were used in this study. All samples were positive in a rapid immunochromatographic test (SNAP® Combo FeLV Ag/FIV Antibody Test) and in a PCR assay. This study was approved by the Ethical Committee on Animal Experimentation of the Universidade Estadual do Maranhão (protocol number 041/2012).

Proviral DNA was extracted using a commercial QIAamp DNA Blood Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. To verify the quality and the integrity of the extracted DNA, the samples were subjected to PCR to confirm the presence of the *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) gene, using the primers 5′-GGTGATGCTGGTGCTGAGTA-3′ (forward) and 3′-CCCTGTTGCTGTAGCCAAAT-5′ (reverse) [24]. PCR was performed in a final volume of 25 µL, with the following reagents: 5 µL of 5x Green GoTaq Flexi Buffer (Promega, USA), 2 µL of each primer (10 pmol/µL, Invitrogen, USA), 0.5 µL of dNTPs (10 mM, Promega, USA), 1.5 µL of $MgCl_2$ (10 mM, Promega, USA), 0.1 µL of Go Taq Flexi DNA Polymerase (500 U, Promega, USA), and DNAse/RNAse-free ultrapure water (Invitrogen-Life Technologies, USA). The amplification conditions were 4 minutes at 95 °C, followed by 35 cycles at 95 °C for 30 seconds, 54 °C for 30 seconds, and 72 °C for 50 seconds, and a final extension at 72 °C for 7 minutes. After amplification, PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide (0.5 µg/mL).

For the amplification of the *gag* gene, were used the primers 5′-CTAGGAGGTGAGGAAGTTCA-3′ (forward) and 5′-CTGCTTGTTGTTCTTGAGTT-3′ (reverse) [25]. The PCR reaction mixture was as follows: 5.1 µL of 5x Green GoTaq Flexi Buffer (Promega, USA), 1.9 µL of each primer (5 pmol/µL, Sigma), 0.5 µL of dNTPs (10 mM, Promega, USA), 1.5 µL of $MgCl_2$ (10 mM, Promega, USA), 0.26 µL of Go Taq Flexi DNA Polymerase (500 U, Promega, USA), 2 µL of DNA template, and DNAse/RNAse-free ultrapure water (Invitrogen, Life Technologies, USA), to a final volume of 25 µL. PCR master mix plus 2 µL of water was used as a negative control. The amplification parameters were one cycle of 5 min of 94 °C, followed by 35 cycles at 94 °C for 1 min, 2 minutes at 55 °C, and 72 °C for 2 min, and a final extension step at 72 °C for 5 min. The amplified products were visualized on 1.5% agarose gel stained with ethidium bromide (0.5 µg/mL).

The PCR amplicons of the nine samples were extracted from the gel and purified using a commercial PureLinkT-MQuick Gel Extraction Kit following the manufacturer's instructions. The amount and quality of the recovered amplicons were estimated by measuring optical density at 260/280 nm wavelength in a NanoVue spectrophotometer (GE Healthcare, USA). Nucleotide sequencing of the purified amplicons was performed in an ABI310 Automated Sequencer using the reagent from a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to standard laboratory operating procedures.

The sequences were edited using the program BioEdit 7.0 [26] and aligned using the program ClustalW 1.4 [27]. Phylogenetic analysis was carried out using the software MEGA 5.0 [28].

To build phylogenetic trees, the evolutionary model of Tamura and Ney [29] and the Neighbor-Joining (NJ) method were used. The best evolutionary model was determined by the likelihood ratio test in MEGA 5.0. The significance of the groups was estimated by bootstrap analysis with 1,000 pseudoreplications [30]. The haplotype network was built using the software Network 4.6 [31].

Nineteen FIV *gag* gene sequences from Brazil were randomly selected from the GenBank database, to identify FIV subtypes., These included the isolates Petaluma (M25381) [32], Wo (L06311) [9], San Diego (M36968) [33], Sendai (D37821) [34], Aomoro (D37823) [34], MG (AY747073; AY747069) [35], Botucatu (DQ407172) [19], São Paulo (DQ407184) [19], Jau (DQ407177) [19], Mogi das Cruzes (DQ407188) [19], T1/T2/T4 (AB027298, AB027299 and AB027301) [36], Fukuoka (D37822) [34], Shizuoka (AY679785) [37], and LP3/LP20/LP24 (AB027302, AB027303 and AB7304) [36].

Results

Samples tested with the GAPDH-Foward and GAPDH-Reverse primers, which amplify part of the glyceraldehyde-3-phosphate dehydrogenase gene, demonstrated that the in DNA extractions and amplifications were successful, yielding a 709-bp fragment. A 244-bp product was obtained in the PCRs for the *gag* gene [20].

Sequencing of the *gag* gene, of samples from nine cats from the city of São Luís, Maranhão and nineteen *gag* sequences from the GenBank database (28 analyzed samples in total) resulted in a fragment of 224 bp. Phylogenetic analysis indicated that six samples from São Luís (90, 93, 79, 63, 23 and 74) are genetically similar to samples from Jaú, Botucatu and São Paulo, grouping with 83% bootstrap support, suggesting that they belong to subtype B (Fig. 1).

A single sample from São Luís (MA) (117) a phylogenetic relationship with 90% bootstrap support to subtype A FIV samples from San Diego and Petaluma (USA) and Wo (France). Samples 41 and 44, however, formed an independent subclade with 100% bootstrap support and showed no similarity to any of the analyzed subtypes (Fig. 1).

Of the 28 sequences analyzed, 21 haplotypes were obtained. Six haplotypes were found in São Luís (MA), one of which (h4) is shared with Jaú (SP), while the others were unique (H1-3, H5 and 6), demonstrating that FIV is

Fig. 1 Phylogenetic tree constructed by the neighbor-joining method and the Tamura and Nei model with 1000 bootstrap replicates, based on nine nucleotide sequences of FIV gag genes from São Luís, MA. The scale bar represents 10% divergence between sequences

genetically variable (Fig. 2). A haplotype network showed a dispersion center from the most frequent haplotype, H4, indicating that it is the oldest of the haplotypes found. The H4 haplotype brings together samples from São Luís (MA) of subtype B, and it can be inferred that it is one of the first subtypes to affect the feline population in that state. Haplotypes 2 and 3, which included samples for which the FIV subtype was uncertain, and the haplotype 6, composed of subtype A samples, were unique among the others (they were not fixed in other populations or shared) suggesting that they appeared more recently in the cat population in the state of Maranhão.

Discussion

Nested PCR is a sensitive molecular method that uses a set of homologous primers to make the reaction specific. The first tests using the nested PCR for the detection of FIV provirus were described in the 1990s [20, 38]. Studies have shown that it is possible to perform genetic classification of FIV virus using nucleotide sequence of the p17-p24 region of the *gag* gene [39, 40]. For this reason, that gene region was used in this study, showing strong indications that new subtypes or haplotypes occur in São Luís, MA, which need to be better characterized.

Two FIV subtypes (A and B) that are possibly affecting the cat population were detected in the city of São Luís, MA, and an unknown subtype was reported for the first time in Maranhão State. FIV samples 41 and 44 formed an independent subclade (100% bootstrap) support and did not cluster with any of the subtypes used for comparison. This independent clade was probably formed due to the genetic variability of the virus, resulting from mutation and/or recombination of the variant strains circulating in the city of São Luis. The high degree of molecular heterogeneity reported for FIV subtypes identified around the world and their ability to acquire mutations under immunological, pharmacological or environmental pressures are inherent characteristics of lentiviruses. Under conditions of high population density, there is the possibility of genetic recombination of the infecting virus, and the chances are increased by coinfections or superinfection of felines with variants of the same subtype or different virus subtypes [41].

Sequencing of the p17-p24 region of the *gag* gene showed that 23 of the isolates belonged to subtype B of FIV, and three subgroups (1, 2 and 3) were consistently identified within subtype B. Subgroup 1 was composed of 15 strains

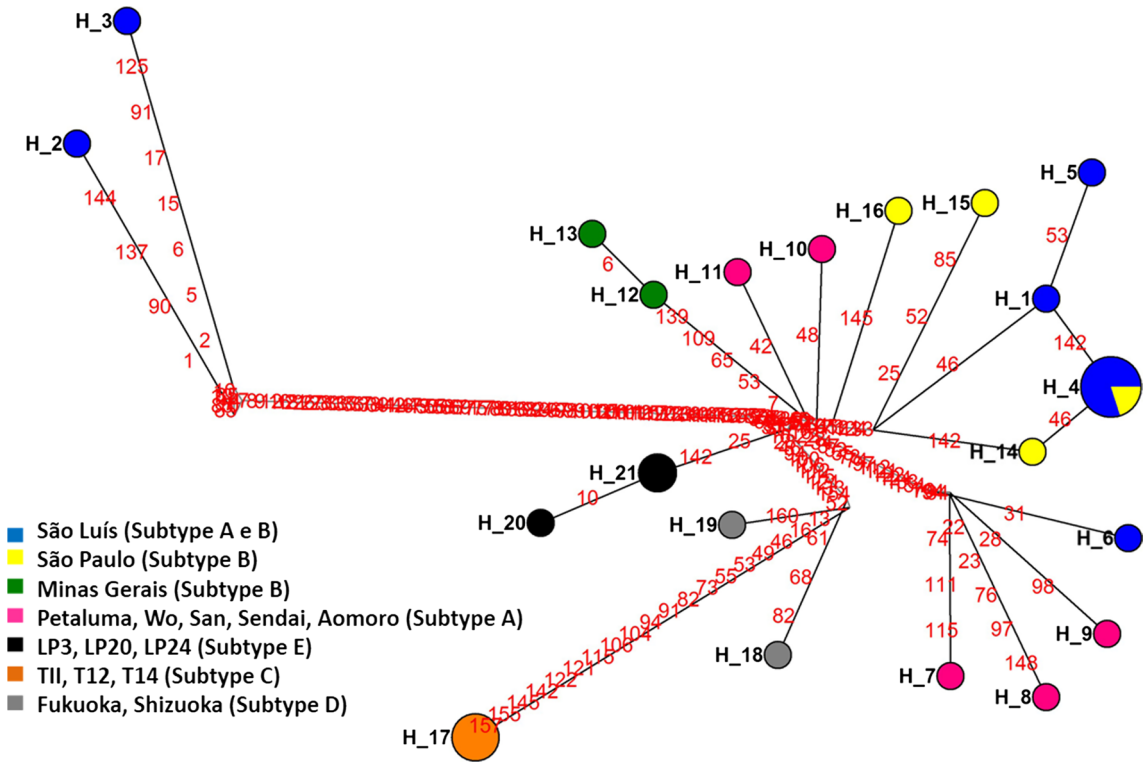

Fig. 2 A network of haplotypes for FIV populations. The size of the circle is proportional to the frequency with which the haplotypes occur in the population. H_1, São Luis; H_2, São Luis; H_3, São Luis; H_4, São Luis and SP; H_5, São Luis; H_6, São Luis; H7, H8, H9, H10, and H11, samples from other countries; H_12 and H_13, Minas Gerais; H_14, H15, and H16, samples from São Paulo; H_17 to H_21, samples from other countries. The numbers in red represent the mutation sites among the haplotypes

from São Paulo State, subgroup 2 included two studied strains, and subgroup 3, included only one of the samples (Mogi das Cruzes 283) and five strains from Japan. The other five strains also belonged to subtype B but did not form subgroups [19].

Caxito et al. [35] also studied the p17-p24 region of the *gag* gene in 10 samples from the state of Minas Gerais, and those also belonged to subtype B. They observed that most of them belonged to a subgroup within subtype B, which suggests that they might have had a common ancestor. In the present study, the samples did not form a subgroup within the same subtype, with six samples belonging to subtype B. The feline population harboring FIV is very small, with most of the viruses corresponding to subtype B; however, one sample formed a group that was distinct from all other known subtypes, suggesting that it belongs to a new subtype [42].

Haplotype network analysis demonstrated that subtype B was probably one of the first subtypes to infect felines in São Luís, MA, and that subtype A was recently introduced to that state. The data also suggest the emergence of a new FIV subtype or variation.

The identification of the predominant FIV subtype in the northern region of Brazil and knowledge about the genetic diversity of the circulating strains are fundamental for the development of immunization strategies and the production and validation of diagnostic tests, especially those that are based on the detection of genetic material from the vírus. In general, molecular methods are strongly influenced by variations in the target sequences where the primers bind. Molecular studies have been carried out to identify and to characterize the Brazilian strains of FIV in other regions [19, 42–45]. Those studies have higher importance if we consider that Brazil has large territorial dimensions and occupies roughly half of South America.

Additional studies of FIV isolates in the state of Maranhão are of fundamental importance for achieving a better understanding of the circulation of different strains of the virus. In addition to improving diagnosis, further research is needed to develop and introduce a vaccine against FIV in Brazil.

Conclusion

The feline population in the city of São Luís, MA, is affected by FIV subtypes B and A, and new variations of this virus have been introduced into this state. The results of this study are important for establishing prevention and treatment goals for infected cats and can serve as the basis for further studies related to FIV. There is no effective treatment for FIV, and therefore there is a need for accurate diagnosis for better control and prevention of infection. The development of a vaccine for FIV is difficult and presents a major challenge because of the high mutation rate and high variability in the *env* gene observed in virus isolates worldwide. The success of a vaccine in providing adequate protection will depend on knowledge about the genetic diversity of the circulating FIV strains in the region where the vaccination deployment is planned, because the vaccination program may fail if the strain used for vaccination differs significantly from the circulating field strains.

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Compliance with ethical standards

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Conflict of interest The author declares that she has no competing interests and no conflict of interest.

Ethical approval The present study was submitted to and approved by the Ethics Committee and Animal Testing (CEEA) of the Veterinary College of Maranhão State University UEMA, under the protocol 041/2012.

References

1. Goff SP (2007) Retroviridae: the retroviruses and their replication fields virology, vol 2, 5th edn. Lippincott Williams and Wilkins, Philadelphia, pp 1999–2069
2. Paillot R, Richard S, Bloas F, Piras F, Poulet H, Brunet S, Andreoni C, Juillard V (2005) Toward a detailed characterization of feline immunodeficiency virus-specific T cell immune responses and mediated immune disorders. Vet Immunol Immunopathol 106(1–2):1–14
3. English RV, Johnson CM, Gebhard DH, Tompkins MB (1993) In vivo lymphocyte tropism of feline immunodeficiency virus. J Virol 67(9):5175–5186
4. Yamamoto JK, Sparger E (1988) Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats. Am J Vet Res 49:1246–1258
5. Lara VM, Taniwaki SA, Junior JPA (2008) Occurrence of feline immunodeficiency virus infection in cats. Ciência Rural 38:2245–2249
6. Hayward JJ, Taylor J, Rodrigo AG (2010) Phylogenetic analysis of feline immunodeficiency virus in feral and companion domestic cats of New Zealand. J Virol 81:2999–3004
7. Norris JM, Bell ET, Hales L et al (2007) Prevalence of feline immunodeficiency virus infection in domesticated and feral cats in eastern Australia. J Feline Med Surg 9:300–308
8. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ (1999) Veterinary virology, 3rd edn. Academic Press, California, pp 363–389
9. 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(2):659–662

10. Miyazawa T, Tomonaga K, Kawaguchi Y, Mikami T (1994) The genome of feline immunodeficiency virus. Arch Virol 134:221–234
11. Bendinelli M, Pistello M et al (1995) Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen. Clin Microbiol Rev 8:87–112
12. Duarte A, Tavares L (2006) Phylogenetic analysis of portuguese feline immunodeficiency virus sequences reveals high genetic diversity. Vet Microbiol 114:25–33
13. Kusuhara H, Hohdatsu T, Okumura M, Sato K, Suzuki Y, Motokawa K, Gemma T, Watanabe R, Huang C, Arai S, Koyama H (2005) Dual-subtype vaccine (Fel-O-Vax FIV) protects cats against contact challenge with heterologous subtype B FIV infected cats. Vet Microbiol 108:155–165
14. Elder et al (2010) Feline immunodeficiency virus (FIV) as a model for study of lentivirus infections: parallels with HIV. Curr HIV Res 8:73–80
15. Hartmann K (2012) Clinical aspects of feline retroviruses: a review. Viruses 4:2684–2710
16. Sodora DL et al (1994) Identification of three feline immunodeficiency virus (FIV) env gene subtypes and comparison of the FIV and human immunodeficiency virus type 1 evolutionary patterns. J Virol 68:2230–2238
17. Teixeira BM, Jr Reche, Hagiwara MK (2010) Feline immunodeficiency virus—an update. Vet Clin 88:54–66
18. Caxito FA (2003) Detecção da subtipagem do vírus da imunodeficiência felina em Minas Gerais. 90 p. Dissertação (Mestrado em Microbiologia)-Instituto de Ciências Biológicas. Universidade de Minas Gerais, MG
19. Lara VM, Taniwaki AS, Araújo Júnior JP (2007) Phylogenetic characterization of feline immunodeficiency virus (FIV) isolates from the state of São Paulo. Pesquisa Veterinária Brasileira 27:467–470
20. Martins NS, Sousa-Rodrigues AP, Gonçalves SA, Abreu-Silva AL, Oliveira RA, Reis JKP (2015) Occurrence of feline immunodeficiency virus (FIV) and leukemia (FeLV) in São Luís-MA. Am J Anim Vet 10(3):187–192
21. Tonelli QJ (1991) Enzyme-linked immunosorbent assay methods for detection of feline leukemia virus and feline immunodeficiency virus. J Am Vet Med Assoc 199:1336–1339
22. Jarrett O, Pacitti AM, Hosie MJ et al (1991) Comparison of diagnostic methods for feline leukemia virus and feline immunodeficiency virus. J Am Vet Med Assoc 199:1362–1364
23. Caldas APF, Leal ES et al (2000) Detection of feline immunodeficiency provirus in domestic cats by polymerase chain reaction. Pesquisa Veterinária Brasileira 20:20–25
24. Pinheiro de Oliveira TF, Fonseca JRAA, Camargos MF et al (2013) Detection of contaminants in cell cultures, sera and trypsin. Biologicals 41:407–414
25. 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. Vet Microbiol 30(2–3):113–123
26. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
27. Thompson JD, Higgins DJ, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
28. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739
29. Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526
30. Felsenstein J (1985) Confidence limits on phylogenies: an using the bootstrap. Evolution 39:783–791
31. Bandelt HJ, Forster P, Rohl A (2008) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48
32. Olmsted RA, Hirsch VM et al (1989) Nucleotide sequence analysis of feline immunodeficiency virus: genome organization and relationship to other lentiviruses. Proc Natl Acad Sci 86:8088–8092
33. Phillips TR, Talbott RL, Lamont C, Muir S, Lovelace K, Elder JH (1990) Comparison of two host cell range variants of feline immunodeficiency virus. J Virol 64:4605–4613
34. 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. J Virol 69(6):3639–3646
35. Caxito FA, Coelho FM, Oliveira ME, Resende M (2006) Feline immunodeficiency virus subtype B in domestic cats in Minas Gerais. Braz Vet Res Commun 30:953–956
36. Kurosawa K, Ikeda Y, Miyazawa T et al (1999) Development of restriction fragment-length polymorphism method to differentiate five subtypes of feline immunodeficiency virus. Microbiol Immunol 43:817–820
37. Coleman JK, Pu R, Martin M, Sato E, Yamamoto JK (2005) HIV-1 p24 vaccine protects cats against FIV. AIDS 19(14):1457–1466
38. Rimstad E, Ueland K (1992) Detection of feline immunodeficiency virus by a nested polymerase chain reaction. J Virol Methods 36(3):239–248
39. Hohdatsu T, Motokawa K et al (1998) Genetic subtyping and epidemiological study of feline immunodeficiency virus by nested polymerase chain reaction-restriction fragment length polymorphism analysis of the gag gene. J Virol Methods 70:107–111
40. Duarte A, Tavares L (2005) Phylogenetic analysis of Portuguese feline immunodeficiency virus sequences reveals high genetic diversity. Vet Microbiol 114:25–33
41. Roberts JD, Bebenek K, Kunkel TA (1988) The accuracy of reverse transcriptase from HIV-1. Science 242(4882):1171–1173
42. Marçola TG, Gomes CPC, Silva PA, Fernandes GR, Paludo GR, Pereira RW (2013) Identification of a novel subtype of feline immunodeficiency virus in a population of naturally infected felines in the Brazilian Federal District. Virus Genes 46:546–550
43. 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. J Virol 82:7863–7874
44. Teixeira et al (2010) Genetic diversity of Brazilian isolates of feline immunodeficiency virus. Arch Virol 155(3):379–384
45. Teixeira et al (2011) Isolation and partial characterization of Brazilian samples of feline immunodeficiency virus. Virus Res 160(1–2):59–65