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**Communication**

[*Comunicação*]

# Phylogenetic analysis of feline immunodeficiency virus strains from State of Minas Gerais, Brazil

[*Análise filogenética de amostras do vírus da imunodeficiência felina detectadas no estado de Minas Gerais*]

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Feline immunodeficiency virus (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 was isolated in 1986 from a feline leukemia virus (FeLV) negative cat with chronic opportunistic infections (Pedersen et al., 1987) and is a major pathogen of domestic cats throughout the world (Bendinelli et al., 1995).

Previous studies based on phylogenetic analysis of *env* gene sequences defined five, unevenly geographically distributed FIV subtypes. Multiple subtypes have been found in cats from the same continent; however geographical 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; Bachmann et al., 1997; Nishimura et al., 1998). 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 Canada, Germany, 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 with just two Argentine strains (Pecoraro et al., 1996). Similar results were also obtained when the nucleotide (nt) sequences of the *gag* gene were analyzed, indicating that this gene has the prerequisites needed for classifying virus isolates (Kakinuma et al., 1995).

A *gag* gene region of FIV strains from Minas Gerais, Brazil, was previously examined by a RFLP analysis (Hohdatsu et al., 1998) to distinguish the subtype circulating in this state and the results showed that all belong to the subtype B (Caxito et al., 2003). The aim of this survey was to study the genetic diversity of these strains and their relationship to previously published sequences.

Peripheral mononuclear blood cells (PBMC) of 10 FIV positive cats were isolated from EDTA-fresh blood by brief exposure of cell pellet to buffered ammonium chloride (Toth et al., 1992). PBMC were disrupted by addition of 40.0µl of 50mM NaOH to the pelleted cells followed by a heating to 95ºC for 5 min. Then, 15.0µl of 0.5M Tris-HCl pH 8.0 was added to neutralize the pH total DNA sample (Richards et al., 1993).

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A 329 bp nested-PCR product from the p17-p24 *gag* gene region was amplified using the conditions and primer pairs previously described by Hohdatsu et al. (1998). A clone of FIV pet14 strain for PCR was used as a positive control. The PCR product was cloned in the plasmid vector TOPO TA[1] according to the manufacturer's instructions. Three clones of each strain were sequenced using the dideoxynucleotide chain terminator method and an automated DNA sequencer[2] with M13 primers using a Big Dye Terminator Sequencing Kit for MegaBace[3].

The nucleotide sequence data were deposited at the NCBI Nucleotide Sequences Databases with the following accession numbers: 2MG (AY747073), 10MG (AY747069), MG35 (AY500850), 36MG (AY747072), 37MG (AY747071), 301MG (AY772943), 310MG (AY747070), 459MG (AY772942), 832MG (AY747074), 884MG (AY772941).

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.0 (Kumar et al., 2004). All the other *gag* nucleotide sequences included in this phylogenetic analysis were obtained from GenBank, and were representative of FIV subtypes A, B, C, D and E. For each subtype, the following strains were used: subtype A: Petaluma (M25381), Wo (L06311), PPR (M36968), CaONA14 (AY220059), Sendai1 (D37820), CAONA05 (AY220050), CaONA09 (AY220054) and FIV-113 (X68019); subtype B: USgaB03 (AY220073), Yokohama (D37818), Sendai2 (D37821), Aomori1 (D37823), Aomori2 (D37824), TM2 (M59418), USIL2489_7B (U11820), CaONB02 (AY220067), ITTOOO2PIU (M2) (Y13867), USgaB01 (AY220071), TX77 (AY139108), TX78 (AY39107) and TX84 (AY139108); subtype C: TI1 (AB027298), TI2 (AB027299) and TI4 (AB027301); subtype D: Fukuoka (D37822) and Shizuoka (AY679785); subtype E: LP20 (AB027303), LP24 (AB027304), LP3 (AB027302) and PP2 (AJ304961).

FIV *gag* nucleotide sequences clustered the Minas Gerais strains with the subtype B isolates in the nucleotides phylogenetic tree (Fig. 1). These results are in agreement with the previously results obtained by RFLP analysis and suggest a common phylogenetic origin for these strains.

Table 1 shows the similarity between the nucleotide sequence of Minas Gerais strains and the nucleotide sequences representative of A, B, C, D and E subtypes previously reported. The highest similarity values (95–98%) were observed in the TM2 and Aomori-1 subtype B strains, both Japanese strains. Despite the geographic distance between Brazil and Japan, in the present study it was clear the remarkable genetic similarity of these strains.

Almost all Minas Gerais strains formed a clear subcluster inside the subtype B, except for the 884MG strain. This pattern suggests a high similarity between these strains and the existence of a common ancestry. The sequence of the segment of *gag* gene of 10 strains proved the occurrence of FIV subtype B in Minas Gerais.

Keywords: cat, feline immunodeficiency, phylogeny, *gag* gene

## RESUMO

*A região p17-p24 do gene gag de 10 amostras do vírus da imunodeficiência felina detectadas no estado de Minas Gerais (Brasil) foi seqüenciada com o objetivo de determinar a sua classificação molecular e a sua relação com seqüências de amostras previamente descritas. As amostras pertenciam ao subtipo B, entretanto foi possível observar que a maioria delas encontra-se em um subgrupo dentro do subtipo B, o que indica presença de um possível ancestral comum entre elas.*

*Palavras-chave: gato, imunodeficiência felina, filogenia, gene gag*

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[1] Invitrogen Corp., Carlsbad, CA, USA
[2] MegaBace - Amersham Pharmacia Biotech, Piscataway, NJ, USA
[3] Amersham Pharmacia Biotech

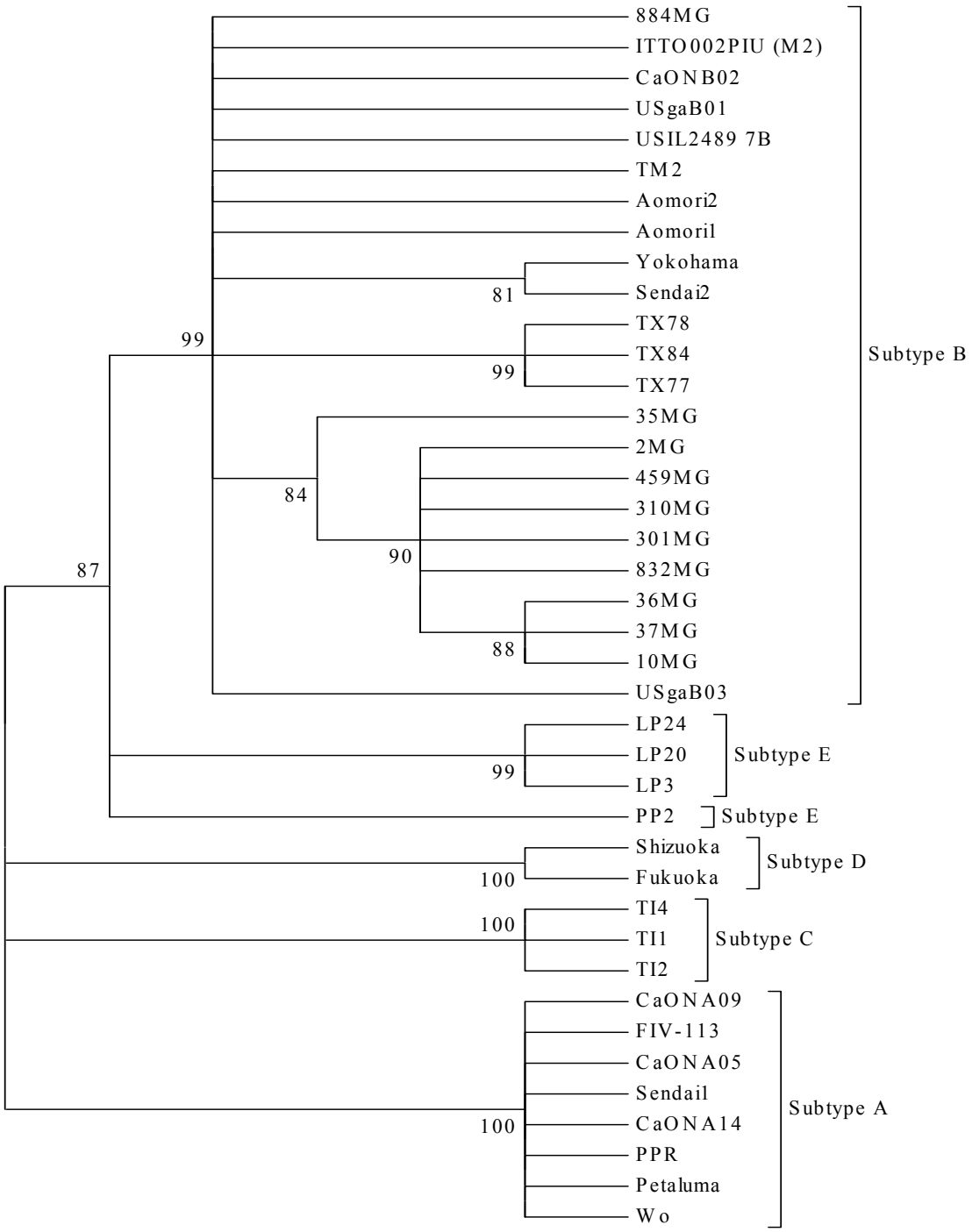


Figure 1. Unrooted condensed phylogenetic tree of 329bp sequences from the p17-p24 region of *gag* gene of feline immunodeficiency virus strains from the state of Minas Gerais, Brazil. The tree was constructed from synonymous substitutions by the neighbor-joining algorithm using Kimura´s two parameter model. Bootstrap values are shown at the branch points.

Table 1. Percent nucleotide similarity among *gag* p17-p24 region of feline immunodeficiency virus strains previously described and FIV Brazilian strains

| Subtype | Strain | Brazilian strain | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2MG | 10MG | 35MG | 36MG | 37MG | 301MG | 310MG | 459MG | 832MG | 884MG |
| A | CaONA09 | 85 | 85 | 84 | 85 | 85 | 85 | 85 | 85 | 84 | 84 |
| | Petaluma | 83 | 83 | 82 | 83 | 83 | 83 | 83 | 83 | 82 | 82 |
| B | TM2 | 97 | 97 | 98 | 97 | 97 | 97 | 97 | 97 | 96 | 96 |
| | Aomori2 | 97 | 96 | 97 | 96 | 96 | 97 | 97 | 97 | 96 | 96 |
| C | TI4 | 84 | 83 | 84 | 83 | 83 | 84 | 84 | 84 | 83 | 85 |
| | TI2 | 84 | 83 | 84 | 83 | 83 | 84 | 84 | 84 | 83 | 85 |
| D | Shizuoka | 87 | 86 | 87 | 86 | 86 | 87 | 87 | 87 | 86 | 87 |
| | Fukuoka | 85 | 85 | 85 | 85 | 85 | 85 | 85 | 85 | 84 | 85 |
| E | LP20 | 92 | 91 | 91 | 91 | 91 | 91 | 92 | 92 | 91 | 90 |
| | LP24 | 93 | 92 | 92 | 92 | 92 | 92 | 93 | 93 | 92 | 91 |

## REFERENCES


BACHMANN, M.H.; SODORA, D.L.; MATTHIASON-DUBARD, M. et al. Genetic diversity of feline immunodeficiency virus: dual infection, recombination and distinct evolutionary rates between envelope sequences clades. *J. Virol*., v.71, p.4241-4253, 1997.,

BENDINELLI, M.; PISTELLO, M.; LOMBARDI, S. et al. Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen. *Clin. Microbiol. Rev*., v.8, p.87-112, 1995.

CAXITO, F.A.; MAGALHÃES-COELHO, F.; PINTO, F.F. et al. Study of feline immunodeficiency virus (FIV) in Minas Gerais by nested PCR-RFLP analysis of the gag gene. In: NATIONAL MEETING OF VIROLOGY, 14., 2003, Florianópolis. A*nais*…, Florianópolis, 2003. p. 209.

DUARTE, A.; MARQUES, M.I.; TAVARES, L. et al. Phylogenetic analysis of five Portuguese strain of FIV. *Arch.Virol*. v.147, p.1061-1070, 2002.

HOHDATSU, T.; MOTOKAWA, K.; USAMI, M. et al. 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. Meth*., v.70, p.107-111, 1998.

INADA, G.; MIYAZAWA, T.; INOSHIMA, Y. et al. Phylogenetic analysis of feline immunodeficiency virus isolated from cats in Taiwan. *Arch. Virol*., v.142, p.1459-1467, 1997.

KAKINUMA, S.; MOTOKAWA, K.; HOHDATSU, T. et al. Nucleotide sequence of feline immunodeficiency virus: Classification of Japanese isolates into two subtypes which are distinct from non-Japanese subtype. *J. Virol*., v.69, p.3639-3646, 1995.

KUMAR, S.; TAMURA, S.; NEI, M. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence aligmment. *Brief Bioinform,* v.5, p.150-163, 2004.

NISHIMURA, Y.; GOTO, Y.; PANG, H. et al. Genetic heterogeneity of *env* gene of feline immunodeficiency virus obtained from multiple districts in Japan. *Virus Res*., v.57, p.101-112, 1998.

OLMSTED, R.A.; HIRSCH, V.M.; PURCELL, R.H. et al. Nucleotide sequence analysis of feline immunodeficiency virus: genome organization and relationship to other lentiviruses. *Proc. Natl. Acad. Sci. USA*, v.86, p.8088-8092, 1989.

PECORARO, M.R.; TOMONAGA, K.; MIYAZAWA, T. et al. Genetic diversity of Argentine isolates of feline immunodeficiency virus. *J. Gen. Virol*., v.77, p.2031-2035, 1996.

PEDERSEN, N.C.; HO, E.W.; BROWN, M.L. et al. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science,* v.235, p.790-793, 1987.

RICHARDS, B.; SKOLETSKY, J.; SHUBER, A.P. et al. Multiplex PCR amplification from the CFTR gene using DNA prepared from buccal brushes/swabs. *Hum. Mol. Gen*., v.2, p.159-163, 1993.

SODORA, D.L.; SHAPER, E.G.; KITCHELL, B.E. et al. Identification of three feline immunodeficiency virus (FIV) *env* gene subtypes and comparison of the FIV and human immunodeficiency virus type evolutionary patterns. *J. Virol*., v.68, p.2230-2238, 1994.

TOTH, T.E.; SMITH, B.; PYLE, H. Simultaneuos separation and purification of mononuclear and polymorphonuclear cells from the peripheral blood of cats. *J. Virol. Methods*, v.36, p.185-196, 1992.