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

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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 (Peczoraro 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 95°C 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).
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 TA1, according to the manufacturer’s instructions. Three clones of each strain were sequenced using the dideoxynucleotide chain terminator method and an automated DNA sequencers2 with M13 primers using a Big Dye Terminator Sequencing Kit for MegaBace3.

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), ITTOO2PIU (M2) (Y13867), USgaB01 (AY220071), TX77 (AY139108), TX78 (AY39107) and TX84 (AY139108); subtype C: T11 (AB027298), TI2 (AB027299) and TI4 (AB027301); subtype D: Fukuoka (D37822) and Shizuko (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 sequências 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

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of Msc. Betânia Paiva Drumond. We thank Dr. Stephen P. Dunham for providing FIV-positive control.
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

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