Acta Scientiae Veterinariae, 2023. 51: 1910.

RESEARCH ARTICLE
Pub. 1910

ISSN 1679-9216

Phylogenetic Classification of Feline Immunodeficiency Virus

Diezza Biondo[1,2], Diéssy Kipper[1,2], Jessica Gomes Maciel[1,2], Weslei de Oliveira Santana[1,2], André Felipe Streck[1,2] & Vagner Ricardo Lunge[1,2]

ABSTRACT

***Background*:** The feline immunodeficiency virus (FIV) is responsible for a retroviral disease that affects domestic and wild cats worldwide, causing Feline Acquired Immunodeficiency Syndrome (FAIDS). FIV is a lentivirus from the family *Retroviridae* and its genome has 3 main structural genes: *gag*, *pol* and *env*. Phylogenetic studies have classified FIV into 7 subtypes according to the diversity among strains from the World, mainly in the *env* gene. Epidemiological analyses have demonstrated the high predominance of FIV-A and FIV-B. This *in silico* study aimed to perform a phylogenetic analysis to study FIV diversity worldwide.

***Materials, Methods & Results*:** A total of 60 whole genome sequences (WGS) and 122 FIV *env* gene sequences were included in 2 datasets, which were aligned using MAFFT version 7. Recombination among genomes and/or *env* genes was analyzed with RDP5 software. Phylogenetic analyses with both datasets were performed, after removing the recombinant sequences, by the W-IQ-TREE and constructed and edited by the FigTree. A total of 12 recombination events involving 19 WGS were detected. In addition, 27 recombination events involving 49 sequences were observed in the *env* gene. A high rate of recombinants was observed inter-subtypes (A/B and B/D) and intra-subtypes (A/A). All recombinants were removed from the subsequent phylogenetic analyses. Phylogenies demonstrated 6 distinct main clades, 5 from domestic cats (A, B, C, E, U) and 1 from wild cat sequences (W) in the WGS, as well as in the specific env gene analyses. Most clustered with subtype B sequences. In the WGS analysis, clade B had a prevalence of 65.9% Brazilian sequences (27/41) and 2.4% Japanese sequences (1/41). In the *env* gene analyses, clade B showed a prevalence of 43.8% of Brazilian sequences (32/73) and 20.5% of USA sequences (15/73). The results of both analyses also confirm the FIV-wide geographical distribution around the world. In the phylogenetic analyses carried out with WGS, sequences from China (1/41; 2.4%), Colombia (1/41; 2.4%) and the USA (1/41; 2.4%) were identified in clade A; sequence from Canada in clade C (1/41; 2.4%); sequence from Botswana belonged to clade E (1/41; 2.4%); sequences from Brazil clustered into clade U (2/41; 5% - data not yet published); and sequences belonging to the clade W were from Canada (1/41; 2.4%) and the USA (5/41; 12.3%). Specific *env* gene phylogenetic analyses showed sequences from Colombia (1/73; 1.4%), France (2/73; 2.7%), the Netherlands (3/73; 4.1%), Switzerland (2/73; 2.7%), USA (6/73; 8.3%), belonging to clade A; sequence from Canada belonging to clade C (1/73; 1.4%); sequences from Brazil belonging to clade U (2/73; 5% - data not yet published); and sequences belonging to clade W from the USA (6/73; 8.3%).

***Discussion*:** The results presented here demonstrate that FIV has a rapid viral evolution due to recombination and mutation events, more specifically in the env gene, which is highly variable. Currently, this retrovirus is classified into 7 subtypes (A, B, C, D, E, F and U-NZenv) according to their high genomic diversity. It also highlighted the importance of *in silico* sequence and phylogeny studies to demonstrate evolutionary processes. This was the first study to address the WGS FIV diversity with a phylogenetic approach.

Keywords: FIV, *in silico*, phylogeny, subtypes, recombination.

DOI: 10.22456/1679-9216.129530

Received: 10 January 2023 Accepted: 14 April 2023 Published: 18 May 2023

[1]Programa de Pós-Graduação em Biotecnologia (PPGBIO) & [2]Laboratório de Diagnóstico em Medicina Veterinária (LDMV), Universidade de Caxias do Sul (UCS), Caxias do Sul, RS, Brazil. CORRESPONDENCE: D. Kipper [diessykipper@hotmail.com]. LDMV - UCS. CEP 95070-560 Caxias do Sul, RS, Brazil.

INTRODUCTION

Feline Immunodeficiency Virus (FIV) is from the family *Retroviridae* [25]. It causes domestic and wild feline progress infections to Feline Acquired Immune Deficiency Syndrome (FAIDS) [17]. The FIV genome comprises 3 main structural genes: *gag*, *pol* and *env*. The *gag* gene encodes internal structural proteins (matrix; capsid and nucleocapsid). The *pol* gene codifies the viral polymerase (reverse transcriptase; protease; integrase; dUTPase). The *env* gene encodes surface and transmembrane glycoproteins. The FIV genome has also other accessory and regulatory genes. The *env* gene is the most variable and the main determinant of antigenic diversity [15].

FIV evolution occurs due to recombination and mutation events [9]. Currently, it is classified into 7 subtypes (A, B, C, D, E, F and U-NZenv) according to genomic diversity [4,26,40]. Epidemiological studies have demonstrated the predominance of FIV-A and FIV-B worldwide. FIV-C was described in North America, Asia and Oceania, while FIV-D was detected only in Asian countries. FIV-E seems to occur in Central / South America and Asia, FIV-F in North America, Europe and Oceania, and FIV U-NZenv in New Zealand [7,8,10,35]. This study aimed to evaluate FIV diversity based on *in silico* analyses of WGS and *env* gene sequences through a phylogenetic approach.

MATERIALS AND METHODS

Dataset

A total of 60 whole genome sequences (WGS) and 62 *env* gene sequences FIV were included in the dataset. All WGS and complete *env* genes were downloaded from the GenBank - National Center for Biotechnology Information (NCBI) [Tables 1 and 2]. The terms "complete genome", "genome", "envelope", "complete cds" and "feline immunodeficiency virus" were used in the search strategy, and only sequences with 3 main information (host, country, and year of isolation) were included in each dataset. Clonal sequences were not included. From the 60 WGS, the whole *env* region was extracted using Geneious version 2021.2.21, and added to the *env* dataset, totaling 122 *env* gene sequences. WGS and *env* gene alignments were generated using MAFFT version 7 [14].

Table 1. WGS dataset of 60 complete FIV genome sequences downloaded from Genbank.

NCBI	Strain/Isolate	Country	Year	Subtype by NCBI	Clade	Organism	Host
MF352016	CHN17	China	NI	A	A	Feline immunodeficiency virus	NI
MN630242	GF6	Colombia	2018	A	A	Feline immunodeficiency virus	*Felis catus*
M25381	Petaluma	USA	NI	A	A	Feline immunodeficiency virus	NI
MF370550	Pequeno_2013	Brazil	2013	B	B	Feline immunodeficiency virus	*Felis catus*
MW142019	1D	Brazil	2015	B	B	Feline immunodeficiency virus	*Felis catus*
MW142020	2B	Brazil	2012	B	B	Feline immunodeficiency virus	*Felis catus*
MW142021	3A	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142022	3B	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142023	4A	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142024	4B	Brazil	2009	B	B	Feline immunodeficiency virus	*Felis catus*
MW142025	5	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142026	6	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142027	9	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142029	12	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142030	13	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142031	14	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142033	17	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142034	18	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142035	19	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142036	20	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142037	21	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142038	22	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142040	25	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142041	27	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142042	28	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142043	29	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*

(continues...)

(...continuation)

MW142046	33A	Brazil	2017	B	B	Feline immunodeficiency virus	*Felis catus*
MW142048	34	Brazil	2002	B	B	Feline immunodeficiency virus	*Felis catus*
MW815635	26	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW815636	30	Brazil	2012	B	B	Feline immunodeficiency virus	*Felis catus*
M59418	NI	Japan	NI	B	B	Feline immunodeficiency virus	NI
AF474246	BM3070	Canada	NI	C	C	Feline immunodeficiency virus	NI
EU117992	1027	Botswana	NI	E	E	Feline immunodeficiency virus	*Panthera leo*
MW142028*	11	Brazil	2007	B	R	Feline immunodeficiency virus	*Felis catus*
MW142032*	16	Brazil	2007	B	R	Feline immunodeficiency virus	*Felis catus*
MW142039*	24	Brazil	2011	B	R	Feline immunodeficiency virus	*Felis catus*
MW142047*	33B	Brazil	2017	B	R	Feline immunodeficiency virus	*Felis catus*
MW815633*	10	Brazil	2008	B	R	Feline immunodeficiency virus	*Felis catus*
MW815634*	15	Brazil	2006	B	R	Feline immunodeficiency virus	*Felis catus*
AY713445*	pOma3	NI	NI	NI	R	Feline immunodeficiency virus	*Otocolobus manual*
X57002*	Z1	Switzerland	NI	A	R	Feline immunodeficiency virus	NI
M36968*	San Diego	USA	NI	A	R	Feline immunodeficiency virus	NI
EU117991*	27B	USA	NI	B	R	Feline immunodeficiency virus	*Panthera leo*
U11820*	USIL2489_7B	USA	NI	B	R	Feline immunodeficiency virus	*Felis domesticus*
EF455607*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455608*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455609*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455610*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455611*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455612*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455613*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455614*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
MW142044	32A	Brazil	2014	U	U	Feline immunodeficiency virus	*Felis catus*
MW142045	32B	Brazil	2014	U	U	Feline immunodeficiency virus	*Felis catus*
DQ192583	PLV-1695	Canada	1995	NI	W	Feline immunodeficiency virus	*Puma concolor*
EF455603	NI	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*
EF455604	NI	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*
EF455605	NI	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*
EF455606	NI	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*
EF455615	CoLV	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*

NI = data not informed; *R = recombinant.

Table 2. *env* gene dataset of the 122 complete FIV sequences

NCBI	Strain/Isolate	Country	Year	Subtype by NCBI	Clade	Organism	Host
MN630242	GF6	Colombia	2018	A	A	Feline immunodeficiency virus	*Felis catus*
L06312	Wo	France	1988	A	A	Feline immunodeficiency virus	NI
AF298778	Wo	France	1988	NI	A	Feline immunodeficiency virus	*Felis catus*
X60725	FIV-UT113	Netherlands	NI	A	A	Feline immunodeficiency virus	NI
M73964	19k32	Netherlands	NI	NI	A	Feline immunodeficiency virus	NI
M73965	19k1	Netherlands	NI	NI	A	Feline immunodeficiency virus	NI
L00607	Dixon	NI	NI	A	A	Feline immunodeficiency virus	NI
X57001	Z2	Switzerland	NI	A	A	Feline immunodeficiency virus	NI
X57002	Z1	Switzerland	NI	A	A	Feline immunodeficiency virus	NI
M36968	San Diego	USA	NI	A	A	Feline immunodeficiency virus	NI
GQ420651	V1CSF	USA	1990	NI	A	Feline immunodeficiency virus	*Felis catus*
KP264269	P7B_C50_2010.87	USA	2010	NI	A	Feline immunodeficiency virus	*Felis catus*
KP264287	P5AC5_2010.36	USA	2010	NI	A	Feline immunodeficiency virus	*Felis catus*
KP264361	M8CC2_2011.06	USA	2010	NI	A	Feline immunodeficiency virus	*Felis catus*
KP264376	M50AC198_2010.09	USA	2010	NI	A	Feline immunodeficiency virus	*Felis catus*
MF370550	Pequeno_2013	Brazil	2013	B	B	Feline immunodeficiency virus	*Felis catus*
MW142019	1D	Brazil	2015	B	B	Feline immunodeficiency virus	*Felis catus*
MW142020	2B	Brazil	2012	B	B	Feline immunodeficiency virus	*Felis catus*
MW142021	3A	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142022	3B	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*

(continues...)

(...continuation)

MW142023	4A	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142024	4B	Brazil	2009	B	B	Feline immunodeficiency virus	*Felis catus*
MW142025	5	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142026	6	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142027	9	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142028	11	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142029	12	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142030	13	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142031	14	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142032	16	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142033	17	Brazil	2007	B	B	Feline immunodeficiency virus	*Felis catus*
MW142034	18	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142035	19	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142036	20	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142037	21	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142038	22	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142039	24	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142040	25	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142041	27	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142042	28	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142043	29	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW142046	33A	Brazil	2017	B	B	Feline immunodeficiency virus	*Felis catus*
MW142048	34	Brazil	2002	B	B	Feline immunodeficiency virus	*Felis catus*
MW815633	10	Brazil	2008	B	B	Feline immunodeficiency virus	*Felis catus*
MW815634	15	Brazil	2006	B	B	Feline immunodeficiency virus	*Felis catus*
MW815635	26	Brazil	2011	B	B	Feline immunodeficiency virus	*Felis catus*
MW815636	30	Brazil	2012	B	B	Feline immunodeficiency virus	*Felis catus*
U11820	USIL2489_7B	USA	NI	B	B	Feline immunodeficiency virus	*Felis domesticus*
KP264262	P9AC19_2010.36	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264320	P21BC76_2010.87	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264331	P17AC99_2010.36	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264356	P11BC35_2010.87	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264372	M5AC13_2010.09	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264382	M49AC85_2010.09	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264400	M44AC91_2010.09	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264415	M3AC168_2010.09	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264495	M28AC70_2010.09	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264501	M26AC109_2010.09	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264506	M25AC98_2010.09	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264527	M15AC42_2010.09	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264560	M11AC238_2010.09	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
KP264562	M10AC285_2010.09	USA	2010	NI	B	Feline immunodeficiency virus	*Felis catus*
AF474246	BM3070	Canada	NI	C	C	Feline immunodeficiency virus	NI
KP264341	P14AC25_2010.36	USA	2010	NI	NC	Feline immunodeficiency virus	*Felis catus*
KP264436	M33AC146_2010.09	USA	2010	NI	NC	Feline immunodeficiency virus	*Felis catus*
KP330209*	FDSydneyC1	Australia	2009	NI	R	Feline immunodeficiency virus	*Felis catus*
EU117992*	1027	Botswana	NI	E	R	Feline immunodeficiency virus	*Panthera leo*
MW142047*	33B	Brazil	2017	B	R	Feline immunodeficiency virus	*Felis catus*
DQ192583*	PLV-1695	Canada	1995	NI	R	Feline immunodeficiency virus	*Puma concolor*
MF352016*	CHN17	China	NI	A	R	Feline immunodeficiency virus	NI
X69496*	UK8	England	NI	A	R	Feline immunodeficiency virus	NI
D37813*	Sendai 1	Japan	NI	A	R	Feline immunodeficiency virus	NI
D37816*	Aomori 1	Japan	NI	B	R	Feline immunodeficiency virus	NI
D37812*	Yokohama	Japan	NI	B	R	Feline immunodeficiency virus	NI
D37814*	Sendai 2	Japan	NI	B	R	Feline immunodeficiency virus	NI
D37817*	Aomori 2	Japan	NI	B	R	Feline immunodeficiency virus	*Felis catus*
M59418*	NI	Japan	NI	B	R	Feline immunodeficiency virus	NI

(cotinues...)

(...continuation)

D37811*	Shizuoka	Japan	NI	D	R	Feline immunodeficiency virus	NI
D37815*	Fukuoka	Japan	NI	D	R	Feline immunodeficiency virus	NI
AF298779*	delta20NaiaWo	NI	NI	NI	R	Feline immunodeficiency virus	*Felis catus*
AY713445*	pOma3	NI	NI	NI	R	Feline immunodeficiency virus	*Otocolobus manual*
L00608*	Dixon	No	NI	A	R	Feline immunodeficiency virus	NI
X69494*	UK2	Scotland	NI	A	R	Feline immunodeficiency virus	NI
M25381*	Petaluma	USA	NI	A	R	Feline immunodeficiency virus	NI
AY621093*	FC1	USA	NI	B	R	Feline immunodeficiency virus	NI
EU117991*	27B	USA	NI	B	R	Feline immunodeficiency virus	*Panthera leo*
EF455607*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455608*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455609*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455610*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455611*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455612*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
EF455613*	NI	USA	NI	NI	R	Feline immunodeficiency virus	*Puma concolor*
KP264267*	P8AC1_2010.36	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264279*	P6AC68_2010.36	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264295*	P4AC34_2010.36	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264297*	P4AC31_2010.36	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264315*	P22CC73_2011.37	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264323*	P2AC12_2010.36	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264324*	P18BC13_2010.87	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264350*	P13AC71_2010.36	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264360*	P10AC51_2010.36	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264389*	M47AC135_2010.09	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264398*	M46AC73_2010.09	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264413*	M41AC119_2010.09	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264439*	M32AC159_2010.09	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264453*	M31AC253_2010.09	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264478*	M30AC39_2010.09	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264480*	M2CC1_2011.06	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264484*	M29CC1_2011.06	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264508*	M20AC284_2010.09	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264513*	M1AC2_2010.09	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264519*	M16AC130_2010.09	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
KP264536*	M14AC254_2010.09	USA	2010	NI	R	Feline immunodeficiency virus	*Felis catus*
MW142044	32A	Brazil	2014	U	U	Feline immunodeficiency virus	*Felis catus*
MW142045	32B	Brazil	2014	U	U	Feline immunodeficiency virus	*Felis catus*
EF455603	NI	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*
EF455604	NI	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*
EF455605	NI	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*
EF455606	NI	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*
EF455614	NI	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*
EF455615	CoLV	USA	NI	NI	W	Feline immunodeficiency virus	*Puma concolor*

NI = data not informed; *R = recombinat; NC = no clade.

Recombinant sequence analysis

To verify the existence of possible recombinant genomes and/or a more specific recombinant event in the *env* gene, both alignments were analyzed with the RDP5 software [20], which applies different methods of recombination and analysis in a set of data. In this research, data were analyzed using the following recombination methods: RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan and 3Seq [41]. The beginning and end breakpoints of the potential recombinant sequences were defined by the RDP5 software. Recombinant events were considered significant when $P \leq 0.01$ was observed for the same event using 4 or more algorithms [20].

Phylogenetic analysis

Phylogenetic analyses with both datasets were performed after recombinant events sequence removal. The phylogenetic relationships were inferred using the maximum likelihood (ML) method with 1000 replicates of the ultrafast bootstrap approximation and the optimal nucleotide substitution model selected using ModelFinder implemented in the W-IQ-TREE web server [37]. The FigTree program was used to construct and edit the phylogenetic trees (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS

A total of 12 WGS recombination events were detected by RDP5 (Table 3). Among these events, 19 sequences were involved and were removed from the phylogenetic analyses. Most of the events in WGS (75%) occurred between wild-type host sequences in similar regions of the genome, end of pol, ORF1, ORF2 and *env* gene (Figure 1). Furthermore, it was possible to verify recombination between subtype A (M36968 and X57002), between subtype B (MW142032, MW142028, MW142047, MW815634, MW815633) and between wild-type host and subtype B (EU117991, EF455614, MW142039, AY713445, EF455609, U11820), sequences. The EF455610 wild-type host strain was present in 5 recombination events, it appeared with the highest frequency (Table 3).

A total of 27 *env* gene recombination events were detected by RDP5 (Table 4). Among the *env* gene events, 49 sequences were involved. D37815 subtype D sequence was recombinant and had the major parent D37811 subtype D and minor parent D37817 subtype B, all sequences from Japan. Sequence D37814 subtype B (from Japan) was recombinant and had the major parent KP264478 (from USA) and minor parent D37811 subtype D (from Japan). Sequence D37816 subtype B was recombinant and had the major parent D37817 subtype B and minor parent D37815 subtype D, all from Japan. Consequently, these events show the occurrence of recombination between subtypes B and D in Japan. Besides, sequence EU117992 subtype E was a minor parent of the recombinant sequences AY13445 (wild-type host) and EU117991 subtype B (Figure 2). Recombination events between wild-host sequences (EF455610, EF455609, EF455607, EF455611, EF455608, EF455612, EF455613) were also found. The L00608 subtype A strain was present in 5 recombination events, it appeared with the highest frequency (Table 4).

Of the initial 60 WGSs, only 41 remained in the study because the recombinant sequences were removed. All 41 WGSs sequences were comparatively analyzed and they could be classified into 6 well-separated clades in the phylogenetic tree. Four clades were composed by WGS from the corresponding subtypes: FIV-A (n = 3), FIV-B (n = 28), FIV-C (n = 1) and FIV-E (n = 1). The remaining genomes clustered into 2 clades: U (n = 2), and W (wild-type host; n = 6) [Figure 3]. Clade A was composed of genomes from China (1/41; 2.4%), Colombia (1/41; 2.4%) and the USA (1/41; 2.4%); clade B was composed of 28 sequences from Brazil (27/41; 65.9%) and Japan (1/41; 2.4%); clade C was composed by 1 genome from Canada (1/41; 2.4%); clade E was composed by 1 genome from Botswana (1/41; 2.4%). Clade U was composed of 2 genomes from Brazil (2/41; 5%); and clade W was composed of 6 genomes from Canada (1/41; 2.4%) and the USA (5/41; 12.3%).

Of the initial 122 *env* gene sequences, only 73 remained because the recombinant sequences were removed from the study. All 73 *env* gene sequences were comparatively analyzed and they could be classified into 5 well-separated clades in the phylogenetic tree. Three clades were composed of *env* gene sequences from the corresponding subtypes: FIV-A (n = 15), FIV-B (n = 47) and FIV-C (n = 1). The remaining *env* gene sequences clustered into 2 clades: U (n = 2), and W (wild-type host; n = 6) [Figure 4]. Clade A was composed of *env* sequences from Colombia (1/73; 1.4%), France (2/73; 2.7%), Netherlands (3/73; 4.1%), Switzerland (2/73; 2.7%), USA (6/73; 8.3%); clade B was composed by *env* gene sequences from Brazil (32/73; 43.8%) and USA (15/73; 20.5%); clade C was composed by 1 *env* sequence from Canada (1/73; 1.4%); clade U was composed by 2 sequences from Brazil (2/73; 2.7%); and 6 *env* sequences were clustered in clade W from the USA (6/73; 8.3%). Two sequences (2/73; 2.7%) from the USA (KP264341 and KP264436) did not cluster into clades.

In the phylogenies (WGS and *env* gene) it was possible to verify the genetic evolutionary difference between the wild-type and domestic cats by the formation of different clades, and the formation of clusters for almost all the FIV subtypes already described (A, B, C, E, U, W).

Table 3. Results of the recombinant WGS FIV sequences, subsequently removed from the phylogenetic study.

WGS									
Event	Recombinat	Major parent	Minor parent	Detection methods						
				R	G	B	M	C	S	T
1	EF455611_USA	EF455610_USA	EF455614_USA	+	+	+	+	+	+	+
2	EF455610_USA	EF455609_USA	EF455608_USA	+	+	+	+	+	+	+
3	EF455610_USA	EF455611_USA	EF455608_USA	+	+	+	+	+	+	-
4	EF455611_USA	EF455612_USA	EF455614_USA	+	+	+	+	+	+	+
5	EF455610_USA	EF455612_USA	EF455607_USA	+	+	+	+	+	+	-
6	EF455613_USA	Unknown	EF455608_USA	+	+	+	+	+	+	+
7	M36968_subtypeA_USA	X57002_subtypeA_Switzerland	Unknown	+	-	+	-	+	-	+
8	MW142032_subtypeB_Brazil	MW142028_subtypeB_Brazil	MW142047_subtypeB_Brazil	-	+	+	+	+	+	-
9	EU117991_subtypeB_USA	EF455614_USA	MW142039_subtypeB_Brazil	+	-	-	+	+	+	+
10	MW815634_subtypeB_Brazil	MW815633_subtypeB_Brazil	Unknown	+	-	+	+	+	+	-
11	AY713445	EF455609_USA	U11820_subtypeB_USA	+	-	-	+	+	+	-
12	EF455610_USA	EF455614_USA	EF455612_USA	+	-	-	+	+	-	+

R = RDP; G = Genconv; B = BootScan; M = MaxChi; C = Chimaera; S = SiScan; T = 3Seq.

Table 4. Results of the recombinant complete env gene FIV sequences, subsequently removed from the phylogenetic study.

env gene									
Event	Recombinat	Major parent	Minor parent	Detection methods						
				R	G	B	M	C	S	T
1	KP264324_USA	L00608_subtypeA	KP264360_USA	+	+	+	+	+	+	+
2	KP330209_Australia	D37813_subtypeA_Japan	KP264279_USA	+	+	+	+	+	+	+
3	D37815_subtypeD_Japan	D37811_subtypeD_Japan	D37817_subtypeB_Japan	–	+	+	+	+	+	+
4	D37812_subtypeB_Japan	D37814_subtypeB_Japan	M25381_subtypeA_USA	+	+	+	+	+	+	+
5	D37814_subtypeB_Japan	KP264478_USA	D37811_subtypeD_Japan	+	+	+	+	+	+	+
6	KP264297_USA	L00608_subtypeA	KP264439_USA	+	+	+	+	+	+	+
7	KP264267_USA	L00608_subtypeA	KP264536_USA	+	+	+	+	+	+	+
8	D37816_subtypeB_Japan	D37817_subtypeB_Japan	D37815_subtypeD_Japan	+	+	+	+	+	+	+
9	EF455610_USA	EF455609_USA	EF455607_USA	+	+	+	+	+	+	+
10	KP264480_USA	L00608_subtypeA	M59418_subtypeB_Japan	+	+	+	+	+	+	+
11	KP264508_USA	X69494_subtypeA_Scotland	KP264478_USA	–	+	+	+	+	+	+
12	EF455610_USA	EF455611_USA	EF455608_USA	+	+	+	+	+	+	+
13	KP264453_USA	KP264508_USA	MW142047_subtypeB_Brazil	+	+	+	+	+	+	+
14	AY621093_subtypeB_USA	Unknown	KP264315_USA	+	+	+	+	–	+	+
15	KP264453_USA	KP3302209_Australia	KP264398_USA	–	+	+	+	+	+	+
16	KP264389_USA	KP264295_USA	KP264513_USA	+	+	+	+	+	+	+
17	AY713445	EF455607_USA	EU117992_subtypeE_Botsuana	+	+	–	+	+	+	+
18	EF455609_USA	EF455612_USA	EF455613_USA	+	+	+	+	+	+	+
19	EU117991_subtypeB_USA	DQ192583_Canada	EU117992_subtypeE_Botsuana	–	+	+	+	+	+	+
20	KP264413_USA	KP3302209_Australia	KP264398_USA	–	+	+	+	+	+	+
21	X69496_subtypeA_England	AF298779	Unknown	–	+	+	+	+	+	+
22	KP264453_USA	MF352016_China	KP264360_USA	+	+	+	–	–	+	+
23	KP264413_USA	D37813_subtypeA_Japan	KP264360_USA	+	+	+	+	+	+	+
24	KP264519_USA	KP264323_USA	Unknown	–	+	+	–	+	+	+
25	KP264350_USA	KP264519_USA	L00608_subtypeA	+	–	+	+	+	+	+
26	KP264448_USA	KP264323_USA	Unknown	+	–	–	+	+	+	+
27	KP264360_USA	KP264315_USA	Unknown	+	+	+	+	+	–	–

R = RDP; G = Genconv; B = BootScan; M = MaxChi; C = Chimaera; S = SiScan; T = 3Seq.

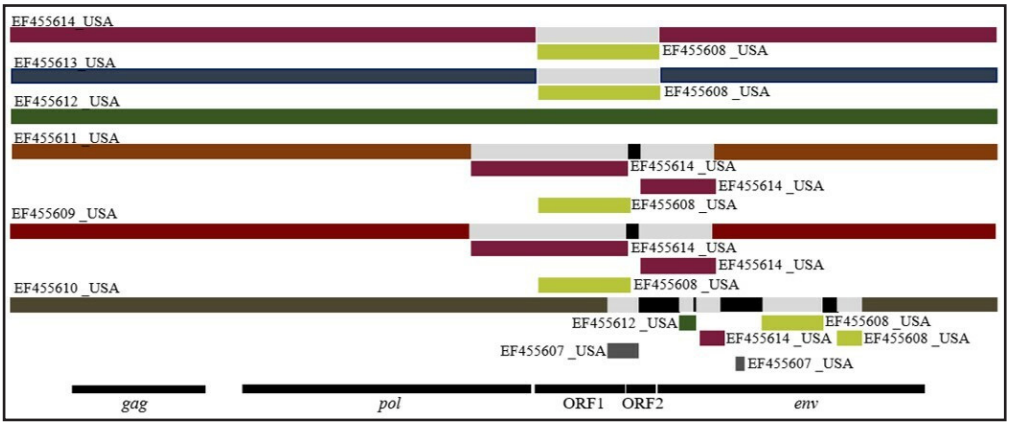

Figure 1. Shows the main recombination site in wild-host sequences of the WGS dataset. The *gag*, *pol* and *env* genes; ORF1 and ORF2 are identified at the bottom of the figure. Different colors (colored by software RDP5) denote recombinant segments in sequences and show their non-recombinant segments.

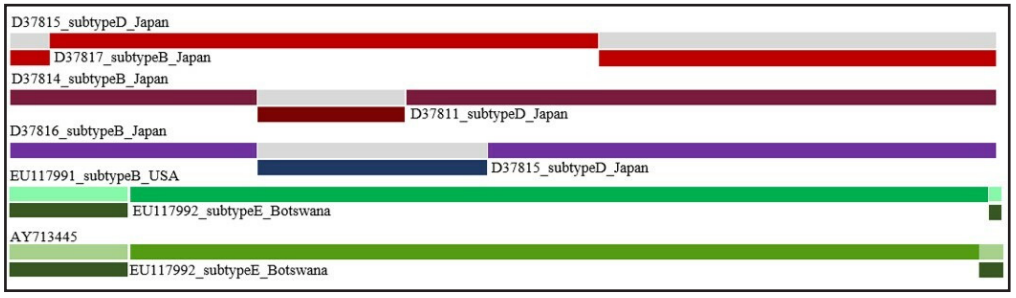

Figure 2. Shows the main recombinant sites between subtypes B, D and E of the *env* dataset. Different colors (colored by software RDP5) denote recombinant segments in sequences and show their non-recombinant segments.

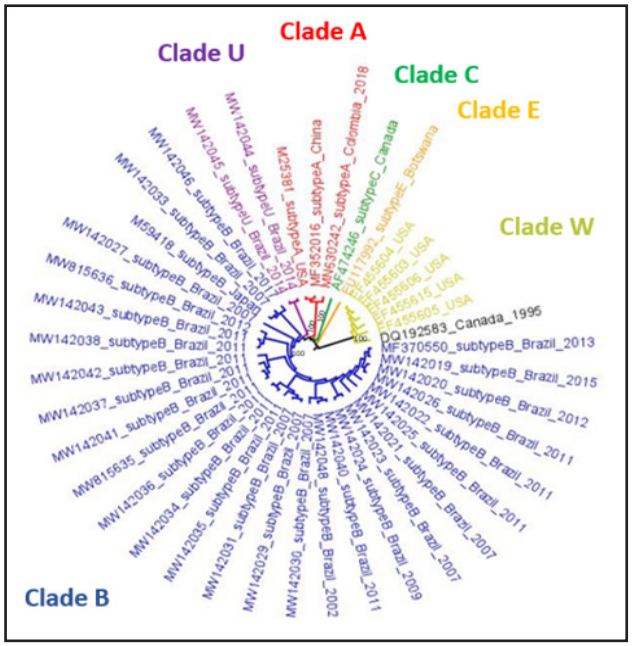

Figure 3. Phylogenetic tree based on 41 WGS. Red labels are Clade A (subtype A); blue labels are Clade B (subtype B); green labels are Clade C (subtype C); orange labels are Clade E (subtype E); purple labels are Clade U (subtype U); and yellow labels are Clade W (wild-host). The evolutionary history was inferred by maximum likelihood, with 1000 bootstrap replicates. The main bootstraps are present in the nodes.

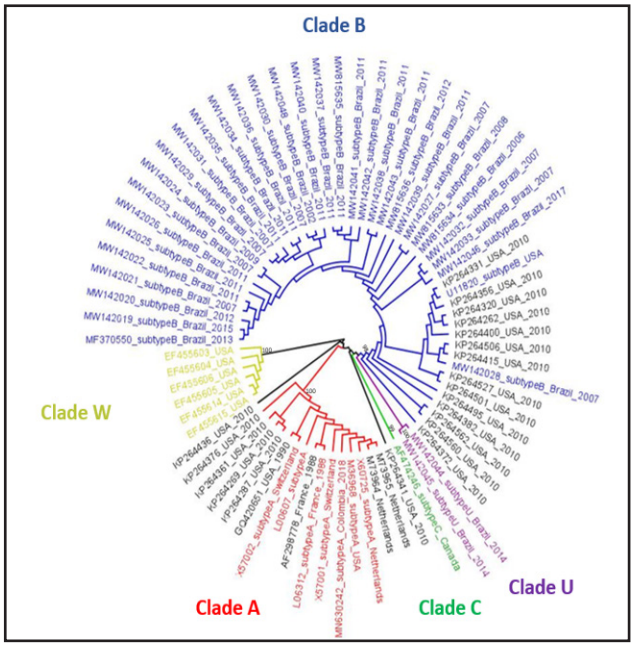

Figure 4. Phylogenetic tree based on 73 env gene sequences. Red labels are Clade A (subtype A); blue labels are Clade B (subtype B); green labels are Clade C (subtype C); purple labels are Clade U (subtype U); and yellow labels are Clade W (wild-host). The evolutionary history was inferred by maximum likelihood, with 1000 bootstrap replicates. The main bootstraps are present in the nodes.

DISCUSSION

This study evaluated the FIV diversity based on in silico analyses of WGS and *env* gene sequences through a phylogenetic approach. The WGS FIV recombinants analysis showed intra-subtype recombination between subtype A (M36968 and X57002) and between subtype B (MW142032, MW142028, MW142047, MW815634, MW815633). There are few reports describing these events, which makes it impossible to know the degree of viral diversity of this type of recombination [30]. A previous study highlighted the difficulty of detecting intrasubtype recombination given the high similarity of the sequences, making this type of event go unnoticed [9]. Noteworthy, this kind of recombination had already been reported in other lentiviruses (HIV) [16,18]. On opposition, similar intersubtype recombination events observed here had already been reported between the most frequent FIV subtypes (A and B) [24].

Recombination events were more frequently observed in an additional analysis of the *env* genes. Interestingly, recombination among sequences of isolates from subtypes B (D37814 and D37817) and D (D37815 and D37811) from Japan were identified, reinforcing data presented by a previous report [1]. Recombination between lentivirus genomes was also demonstrated in HIV-1 and HIV-2, which circulated simultaneously in different geographic regions [27,29]. The analysis of 2 FIV isolates from Japan also suggests that limited similarities in some hot-spot recombination regions can occur in cats coinfected with more than 1 subtype [13].

Recombination events in the whole-genomes and *env* gene sequences obtained in wild cats (EF455610, EF455609, EF455607, EF455611, EF455608, EF455612 and EF455613) were also observed. It was already reported that 3 lineages were derived from recombination events (EF455611, EF455609/EF455610 and EF455613/EF455614) [3]. This previous study highlighted that the ancestral sequences EF455607/EF455608 were involved in all recombination events that resulted in EF455611, EF455609/EF455610 and EF455613/EF455614 strains, making them important precursors of the current circulating FIVs from this subtype. Importantly, FIV sequence EF455610 showed the highest number of recombination events (5/12) in the analysis performed with WGS. In the analysis of the *env* gene sequences, 1 isolate from subtype A (L00608) was more frequently (5/27) demonstrated in the recombination events. These isolates are probably derived from lineages in felines living in mid-distance geographic regions (Greater Yellowstone Ecosystem and Wyoming), which indicates that individuals were infected by ancestral viruses in close contact in the recent past [3].

It was possible to observe the classification of almost all FIV subtypes in clades, both in the phylogeny of the WGS and in the *env* gene. There were 6 distinct clades: A, B, C, E, U and W. In addition, most sequences isolates were from subtypes A and B. This result confirms other studies demonstrating subtypes A and B as the most common and prevalent worldwide [10,30]. Previous reports from northern and western Europe (France, the Netherlands and Switzerland) demonstrated the dominance of subtype A in this continent [28,32]. In addition, studies carried out in Canada and the west coast of the USA also report the prevalence of isolates from clade A [26,38]. Importantly, it was not possible to observe the clade D here because all previous sequence classified as D were removed due to recombination events. It was also not possible to observe clade formation for subtype F because there are no WGS or complete *env* gene sequences available, only partial sequences that were not included in this study. Thus, sequencing FIV complete genomes is important for a more robust classification. Many studies are still carried out with only partial sequences of the selected region of the genome [6,11,23,28].

Brazil had an expressive number of sequences grouped in clade B both in the WGS analysis and in the *env* gene (n = 27 and n = 32, respectively). The predominance of subtype B was already demonstrated in studies carried out in different geographic regions: southeast [5,21,35], midwest [19], south [4] and northeast [36]. Only 1 study reports the occurrence of subtype A circulating in the northeast of Brazil [22]. The USA was the second country to have a high frequency of FIV sequences from clade B in the analysis of the *env* gene (n = 15). Other studies carried out in the country previously showed the circulation of this subtype on the East Coast [38], in the states of Texas [39], Tennessee and Illinois [2]. The sequences grouped in the clade U, from Brazil, have not yet had their data published in an article so there could be some explanation for the possibility of the existence of the subtype in circulation in the country.

Other studies are focusing in the analysis of other FIV genomic regions for phylogenetic classifications: *pol* [33,34], *vif* [17], and *gag* [17]. However, the *env* gene codes for the most exposed protein and is under pressure from immune response, generating positive exhaustion mutation selection and high diversity to classify this virus and compare with vaccine strains [12,23,28]. This gene also has high variable sequences in the corresponding region of loops in the *Env* protein [28,31]. Thus, nucleotide sequences from the V3-V5 region of the *env* gene have been widely used in the genetic subtyping of FIV strains, as well as in molecular epidemiological studies [30].

CONCLUSION

FIV recombination events (inter and intrasubtype) were observed in the whole-genomes and *env* gene sequences evaluated, reinforcing their importance in FIV evolution. The phylogenetic analysis demonstrated 6 different clades (A, B, C, E, U, W). These results highlight the importance of in silico evaluation and phylogenetic studies to elucidate FIV evolutionary processes as well as circulating lineages worldwide.

MANUFACTURER

[1]Software Geneious version 2021.2.2. Boston, MA, USA.

***Funding*.** This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES), process number: 88887.571689/2020-00.

***Acknowledgments*.** We thank to the University of Caxias do Sul, which provided technical support for this article, and the laboratories that shared the data through the NCBI/GenBank, on which this research is based.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 **Bachmann M.H., Mathiason-Dubard C., Learn G.H., Rodrigo A.G., Sodora D.L., Mazzetti P., Hoover E.A. & Mullins J.I. 1997.** Genetic diversity of feline immunodeficiency virus: dual infection, recombination, and distinct evolutionary rates among envelope sequence clades. *Journal of Virology*. 71(6): 4241-4253. DOI: 10.1128/JVI.71.6.4241-4253.1997.

2 **Bęczkowski P.M., Hughes J., Biek R., Litster A., Willett B.J. & Hosie M.J. 2014.** Feline immunodeficiency virus (FIV) *env* recombinants are common in natural infections. *Retrovirology*. 11: 80. DOI: 10.1186/s12977-014-0080-1.

3 **Bruen T.C. & Poss M. 2007.** Recombination in feline immunodeficiency virus genomes from naturally infected cougars. *Virology*. 364(2): 362-370. DOI: 10.1016/j.virol.2007.03.023.

4 **Cano-Ortiz L., Junqueira D.M., Comerlato J., Costa C.S., Zani A., Duda N.B., Tochetto C., Santos R.N., Costa F.V.A., Roehe P.M. & Franco A.C. 2017.** Phylodynamics of the Brazilian feline immunodeficiency virus. *Infection, Genetics and Evolution. Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*. 55: 166-171. DOI: 10.1016/j.meegid.2017.09.011.

5 **Caxito F.A., Coelho F.M., Oliveira M.E. & Resende M. 2006.** Feline immunodeficiency virus subtype B in domestic cats in Minas Gerais, Brazil. *Veterinary Research Communications*. 30(8): 953-956. DOI: 10.1007/s11259-006-3363-8.

6 **Duarte A. & Tavares L. 2006.** Phylogenetic analysis of Portuguese Feline Immunodeficiency Virus sequences reveals high genetic diversity. *Veterinary Microbiology*. 114(1-2): 25-33. DOI: 10.1016/j.vetmic.2005.11.056.

7 **Elder J.H., Lin Y.-C., Fink E. & Grant C.K. 2010.** Feline immunodeficiency virus (FIV) as a model for study of lentivirus infections: parallels with HIV. *Current HIV Research*. 8(1): 73-80. DOI: 10.2174/157016210790416389.

8 **Hartmann K. 2012.** Clinical aspects of feline retroviruses: a review. *Viruses*. 4(11): 2684-2710. DOI: 10.3390/v4112684.

9 **Hayward J.J. & Rodrigo A.G. 2008.** Recombination in feline immunodeficiency virus from feral and companion domestic cats. *Virology Journal*. 5: 76. DOI: 10.1186/1743-422X-5-76.

10 **Hosie M.J., Addie D., Belák S., Boucraut-Baralon C., Egberink H., Frymus T., Gruffydd-Jones T., Hartmann K., Lloret A., Lutz H., Marsilio F., Pennisi M.G., Radford A.D., Thiry E., Truyen U. & Horzinek M.C. 2009.** Feline immunodeficiency. ABCD guidelines on prevention and management. *Journal of Feline Medicine and Surgery*. 11(7): 575-584. DOI: 10.1016/j.jfms.2009.05.006.

11 **Huguet M., Novo S.G. & Bratanich A. 2019.** Detection of feline immunodeficiency virus subtypes A and B circulating in the city of Buenos Aires. *Archives of Virology*. 164(11): 2769-2774. DOI: 10.1007/s00705-019-04363-1.

12 **Iwata D. & Holloway S.A. 2008.** Molecular subtyping of feline immunodeficiency virus from cats in Melbourne. *Australian Veterinary Journal*. 86(10): 385-389. DOI: 10.1111/j.1751-0813.2008.00336.x.

13 **Kakinuma S., Motokawa K., Hohdatsu T., Yamamoto J.K., 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(6): 3639-3646. DOI: 10.1128/JVI.69.6.3639-3646.1995.

14 **Katoh K. & Standley D.M. 2013.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*. 30(4): 772-780. DOI: 10.1093/molbev/mst010.

15 **Kenyon J.C. & Lever A.M.L. 2011.** The molecular biology of feline immunodeficiency virus (FIV). *Viruses*. 3(11): 2192-2213. DOI: 10.3390/v3112192.

16 **Kiwelu I.E., Novitsky V., Margolin L., Baca J., Manongi R., Sam N., Shao J., McLane M.F., Kapiga S.H. & Essex M. 2013.** Frequent intra-subtype recombination among HIV-1 circulating in Tanzania. *PloS One*. 8(8): e71131. DOI: 10.1371/journal.pone.0071131.

17 **Koç B.T. & Oğuzoğlu T.Ç. 2020.** A phylogenetic study of Feline Immunodeficiency Virus (FIV) among domestic cats in Turkey. *Comparative Immunology, Microbiology and Infectious Diseases*. 73: 101544. DOI: 10.1016/j.cimid.2020.101544.

18 **Lau K.A. & Wong J.J.L. 2013.** Current Trends of HIV Recombination Worldwide. *Infectious Disease Reports*. 5(Suppl 1): e4. DOI: 10.4081/idr.2013.s1.e4.

19 **Marçola T.G., Gomes C.P.C., Silva P.A., Fernandes G.R., Paludo G.R. & Pereira R.W. 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(3): 546-550. DOI: 10.1007/s11262-013-0877-3.

20 **Martin D.P., Varsani A., Roumagnac P., Botha G., Maslamoney S., Schwab T., Kelz Z., Kumar V. & Murrell B. 2021.** RDP5: a computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. *Virus Evolution*. 7(1): veaa087. DOI: 10.1093/ve/veaa087.

21 **Martins A.N., Medeiros S.O., Simonetti J.P., Schatzmayr H.G., Tanuri A. & Brindeiro R.M. 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-naïve cats in Rio de Janeiro, Brazil. *Journal of Virology*. 82(16): 7863-7874. DOI: 10.1128/JVI.00310-08.

22 **Martins N.D.S., Rodrigues A.P.S., Luz L.A., Reis L.L., Oliveira R.M., Oliveira R.A., Abreu-Silva A.L., Reis J.K.P. & Melo F.A. 2018.** Feline immudeficiency virus subtypes B and A in cats from São Luis, Maranhão, Brazil. *Archives of Virology*. 163(2): 549-554. DOI: 10.1007/s00705-017-3636-2.

23 **Muz D., Can H., Karakavuk M., Döşkaya M., Özdemir H.G., Değirmenci Döşkaya A., Atalay Şahar E., Pektaş B., Karakuş M., Töz S., Özbel Y., Gürüz A.Y. & Muz M.N. 2021.** The molecular and serological investigation of Feline immunodeficiency virus and Feline leukemia virus in stray cats of Western Turkey. *Comparative Immunology, Microbiology and Infectious Diseases*. 78: 101688. DOI: 10.1016/j.cimid.2021.101688.

24 **Pecon-Slattery J., Troyer J.L., Johnson W.E. & O'Brien S.J. 2008.** Evolution of feline immunodeficiency virus in Felidae: implications for human health and wildlife ecology. *Veterinary Immunology and Immunopathology*. 123(1-2): 32-44. DOI: 10.1016/j.vetimm.2008.01.010.

25 **Pedersen N.C., Ho E.W., Brown M.L. & Yamamoto J.K. 1987.** Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science (New York, N.Y.)*. 235(4790): 790-793. DOI: 10.1126/science.3643650.

26 **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(Pt 7): 1843-1852. DOI: 10.1099/vir.0.19743-0.

27 **Robertson D.L., Hahn B.H. & Sharp P.M. 1995.** Recombination in AIDS viruses. *Journal of Molecular Evolution*. 40(3): 249-259. DOI: 10.1007/BF00163230.

28 **Roukaerts I.D.M., Theuns S., Taffin E.R.L., Daminet S. & Nauwynck H.J. 2015.** Phylogenetic analysis of feline immunodeficiency virus strains from naturally infected cats in Belgium and The Netherlands. *Virus Research*. 196: 30-36. DOI: 10.1016/j.virusres.2014.10.023.

29 **Sabino E.C., Shpaer E.G., Morgado M.G., Korber B.T., Diaz R.S., Bongertz V., Cavalcante S., Galvão-Castro B., Mullins J.I. & Mayer A. 1994.** Identification of human immunodeficiency virus type 1 envelope genes recombinant between subtypes B and F in two epidemiologically linked individuals from Brazil. *Journal of Virology*. 68(10): 6340-6346. DOI: 10.1128/JVI.68.10.6340-6346.1994.

30 **Samman A., McMonagle E.L., Logan N., Willett B.J., Biek R. & Hosie M.J. 2011.** Phylogenetic characterisation of naturally occurring feline immunodeficiency virus in the United Kingdom. *Veterinary Microbiology*. 150(3-4): 239-247. DOI: 10.1016/j.vetmic.2011.01.027.

31 Seibert S.A., Howell C.Y., Hughes M.K. & Hughes A.L. 1995. Natural selection on the *gag*, *pol*, and *env* genes of human immunodeficiency virus 1 (HIV-1). *Molecular Biology and Evolution*. 12(5): 803-813. DOI: 10.1093/oxfordjournals.molbev.a040257.

32 Steinrigl A., Ertl R., Langbein I. & Klein D. 2010. Phylogenetic analysis suggests independent introduction of feline immunodeficiency virus clades A and B to Central Europe and identifies diverse variants of clade B. *Veterinary Immunology and Immunopathology.* 134(1-2): 82-89. DOI: 10.1016/j.vetimm.2009.10.013.

33 Szilasi A., Dénes L., Krikó E., Heenemann K., Ertl R., Mándoki M., Vahlenkamp T.W. & Balka G. 2019. Prevalence of feline immunodeficiency virus and feline leukaemia virus in domestic cats in Hungary. *JFMS Open Reports*. 5(2): 2055116919892094. DOI: 10.1556/004.2020.00056.

34 Szilasi A., Dénes L., Krikó E., Murray C., Mándoki M. & Balka G. 2021. Prevalence of feline leukaemia virus and feline immunodeficiency virus in domestic cats in Ireland. *Acta Veterinaria Hungarica*. 68(4): 413-420. DOI: 10.1556/004.2020.00056. PMID: 33459612.

35 Teixeira B.M., Logan N., Samman A., Miyashiro S.I., Brandão P.E., Willett B.J., Hosie M.J. & Hagiwara M.K. 2011. Isolation and partial characterization of Brazilian samples of feline immunodeficiency virus. *Virus Research*. 160(1–2): 59-65. DOI: 10.1016/j.virusres.2011.05.007.

36 Teixeira B.M., Taniwaki S.A., Menezes P.M.M., Rodrigues A.K.P.P., Mouta A.N., Arcebispo T.L.M., Braz G.F., da Cruz J.C.M., Brandão P.E., Heinemann M.B., Silva M.X. & Hosie M.J. 2019. Feline immunodeficiency virus in Northern Ceará, Brazil. *JFMS Open Reports*. 5(2): 2055116919859112. DOI: 10.1177/2055116919859112.

37 Trifinopoulos J., Nguyen L.-T., von Haeseler A. & Minh B.Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*. 44(W1): W232-5. DOI: 10.1093/nar/gkw256.

38 Weaver E.A. 2010. A detailed phylogenetic analysis of FIV in the United States. *PloS One*. 5(8): e12004. DOI: 10.1371/journal.pone.0012004.

39 Weaver E.A., Collisson E.W., 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(4): 2158-2163. DOI: 10.1128/jvi.78.4.2158-2163.2004.

40 Westman M.E., Coggins S.J., van Dorsselaer M., Norris J.M., Squires R.A., Thompson M. & Malik R. 2022. Feline immunodeficiency virus (FIV) infection in domestic pet cats in Australia and New Zealand: Guidelines for diagnosis, prevention and management. *Australian Veterinary Journal*. 100(8): 345-359. DOI: 10.1111/avj.13166.

41 Yang H., Peng Q., Lang Y., Du S., Cao S., Wu R., Zhao Q., Huang X., Wen Y., Lin J., Zhao S. & Yan Q. 2022. Phylogeny, Evolution, and Transmission Dynamics of Canine and Feline Coronaviruses: A Retro-Prospective Study. *Frontiers in Microbiology*. 13: 850516. DOI: 10.3389/fmicb.2022.850516.