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Phylogenetic analysis of feline immunodeficiency virus strains from naturally infected cats in Belgium and The Netherlands

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ABSTRACT

Feline immunodeficiency virus (FIV) is a major pathogen in feline populations worldwide, with seroprevalences up to 26%. Virus strains circulating in domestic cats are subdivided into different phylogenetic clades (A–E), based on the genetic diversity of the V3-V4 region of the *env* gene. In this report, a phylogenetic analysis of the V3-V4 *env* region, and a variable region in the *gag* gene was made for 36 FIV strains isolated in Belgium and The Netherlands. All newly generated *gag* sequences clustered together with previously known clade A FIV viruses, confirming the dominance of clade A viruses in Northern Europe. The same was true for the obtained *env* sequences, with only one sample of an unknown *env* subtype. Overall, the genetic diversity of FIV strains sequenced in this report was low. This indicates a relatively recent introduction of FIV in Belgium and The Netherlands. However, the sample with an unknown *env* subtype indicates that new introductions of FIV from unknown origin do occur and this will likely increase genetic variability in time.

1. Introduction

Feline immunodeficiency virus (FIV) is a common pathogen in cat populations worldwide. The virus is mainly spread by biting, especially during fights between tomcats (Natoli et al., 2005; Yamamoto et al., 1989). It causes a dysfunction of the immune system, in part by directly targeting cells of the immune system, leading to a persistent infection (Tompkins and Tompkins, 2008). After a long asymptomatic phase, infection with FIV can cause an AIDS-like disease, characterized by a depletion of $CD4^+$ T-cells, increase in plasma viral load and severe immune suppression. Clinical signs are usually not directly caused by the FIV infection, but are the result of secondary infections or immune hyper-stimulation.

FIV is divided into five different clades (A–E), which vary in prevalence by geographic location (Yamamoto et al., 2007). These clades are based on phylogenetic analyses of variable regions 3–5 in the *env* gene. The intra-clade diversity in these regions ranges from 2.5 to 15% at the nucleotide level. A new clade arises when a sequence shows more than 20% genetic distance from other clades (Kakinuma et al., 1995; Sodora et al., 1994). While clade A strains are found worldwide, other clades indicate a separate evolution of these viruses in geographical isolated locations. For instance, subtype E has only been found in South-America and subtype D in Japan (Kakinuma et al., 1995; Pecoraro et al., 1996). Several reports have been made of *env* sequences clustering into new FIV clades, these were designated "F" (Duarte and Tavares, 2006; Hayward et al., 2007; Weaver, 2010) or "New Zealand-Unknown" (NZ-U) (Hayward et al., 2007). A cat can be infected by multiple FIV strains. In places where multiple subtypes circulate, this can give rise to recombinant viruses. Recombination can occur either between gene regions, giving rise to, for example, A/C subtypes or within single gene regions, forming for instance A/B envelope chimaeras (Hayward and Rodrigo, 2008).

Routine vaccination with the dual clade (A and D) vaccine (Fel-O-Vax, Boehringer Ingelheim) is not recommended in Europe. Safety concerns have been raised since there are several reports of vaccine-mediated enhancement of disease (Lecollinet and Richardson, 2008). This enhancement might be related to humoral factors (Siebelink et al., 1995) and/or by inducing memory cells that are rapidly activated upon FIV infection. These activated lymphocytes are an ideal target for FIV (de Parseval et al., 2004; Gramaglia et al., 1998; Joshi et al., 2005; Richardson et al., 2002). Furthermore, it is important to mention that cross-protection against European isolates is under discussion (Dunham et al., 2006).

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Clades A and B are reported to circulate in Western Europe (Samman et al., 2011; Steinrigl et al., 2010; Yamamoto et al., 2007), but data from Belgium are lacking. Serological surveys in Belgium have shown a FIV prevalence of 11% in urban stray cats (Dorny et al., 2002) and 14% in healthy cats of 6 years and older (Paepe et al., 2013). However, virus was not isolated and phylogenetic analyses were not performed. In the present study, *env* and *gag* sequences derived from 36 naturally infected cats living in Flanders and The Netherlands were phylogenetically analyzed.

2. Materials and methods

2.1. Samples

Samples were obtained from naturally infected cats living in Belgium ($n = 26$) and The Netherlands ($n = 10$). Cats were presented to the Clinic for Small Companion Animals of Ghent University. All cats were privately owned and owners provided a signed consent. Blood samples were taken by jugular venipuncture. Genomic DNA was isolated from 50 μl heparin-treated blood with the Quick-gDNA™ Blood Microprep kit (Zymo Research).

2.2. PCR and sequencing

Sequences for variable regions in *gag* and *env* (V3-V4) were amplified, using primers FIV-960F and FIV-1709R for *gag*, and primers FIV-7224F and FIV-8000R for *env* (Iwata and Holloway, 2008; Steinrigl and Klein, 2003). All primer sequences can be found in Table 1. PCR was performed using Herculase II Fusion DNA polymerase (Aligent Technologies) in a 50 μl reaction mix containing 0.25 μM of the forward and reverse primer, 0.5 μl template DNA, 1 mM dNTPs, 0.5 μl polymerase and 2.5 mM $MgCl_2$. The PCR product was visualized on a 1% agarose gel, excised and purified with the NucleoSpin® Gel and PCR Clean-up kit (Machery-Nagel GmbH & Co. KG, Düren). Sequencing was performed by GATC Biotech (Constance, Germany) with both forward and reverse primers on an ABI 3730XL Sanger sequencing platform. When sequencing failed, the fragment was cloned into a commercial vector (pCR®-Blunt) with the Zero Blunt PCR cloning

Table 1
Location and 5′–3′ sequence of primers used for sequencing the variable *gag* fragment and the V3-V4 region of the *env* gene (Iwata and Holloway, 2008; Steinrigl and Klein, 2003).

Gene	Location	Forward primer	Reverse primer
gag	960–1709	TCA GAT GGG ATT AGA CAC	CTT CTG CCA AGA GTT GC
env	7224–8000	GTA CAG ACC CAT TAC AAA	CTG CCA CTG GGT TAT ACC

Table 2
Sample identification, origin, age and subtype assignment. All samples were named following Bachmann (Bachmann et al., 1997), the first two letters indicate the home country, followed by two letters indicating the province, and the next four letters are based on the cat's name and town. *Gag* and *env* genbank accession numbers are indicated for samples that generated new unique FIV sequences.

Identification	Location	Age	Sex	Clade *gag*	Clade *env*	Accession number	
						gag	*env*
BEWVdooo	Oostkamp	8	M	A	A	KM880118	KM880108
BEWVkhde	Deerlijk	6	M	A	A	KM880119	
BEWVflpo	Poelkapelle	5	M	A	A		
BEWVprpi	Pittem	8	M	A	A		
BEWVslin	Ingelmunster	12	M	A	A		
BEWVvisa	Sint-Andries	8	M	A	A		
BEOVbaoo	Oostakker	4	M	A	A		KM880105
BEOVboze	Zele	12	M	A	A		
BEOVmame	Melsele	3	M	A	A		
BEOVmido	Drongen	10	M	A	A		
BEOVvias	Assenede	2	M	A	A		KM880106
BEOVzwni	Ninove	4	M	A	A		
BEANchgr	Grobbendonk	8	M	A	A		
BEANcuhe	Heist-op-den-berg	6	M	A	A		
BEANslbe	Bevel	11	M	A	A	KM880116	
BEANzwbo	Boom	2	M	A	A		
BEVBcahe	Hever	3	F	A	A		
BEVBfika	Kampenhout	7	F	A	A		
BEVBozke	Kessel-lo	15	M	A	A		KM880107
BEVBshvi	Vilvoorde	8	M	A	A	KM880121	KM880109
BEVBziel	Elewijt	15	M	A	A		
BEANfrme	Meulebeke	3	M	A	A		
BEHEramo	Moeskroen	10	M	A	A	KM880120	
BELIflzo	Zonhoven	12	M	A	A		
BELIomst	Sint-Truiden	4	M	A	A		
BELIpopa	Paal	12	M	A	A		
NLFLsnal	Almere	8	M	A	A		KM880113
NLFRfrgr	Grou	8	M	A	A		KM880114
NLNBbrhe	Helmond	8	M	A	A		
NLNBtahe	Helmond	13	F	A	A		
NLNHpial	Alkmaar	8	M	A	A		KM880115
NLNHzwud	Uden	8	M	A	A		
NLUTkuvi	Vinkeveen	8	M	A	Unknown	KM880117	KM880110
NLUTpimi	Mijdrecht	8	M	A	A		KM880112
NLZEkowa	Walcheren	8	F	A	A		KM880111
NLZHrole	Leiden	8	M	A	A		