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Short communication

Genetic and phylogenetic analyses of capsid protein gene in feline calicivirus isolates from Rio Grande do Sul in southern Brazil

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ABSTRACT

Feline calicivirus (FCV) is an important pathogen that affects domestic cats, inducing acute oral and upper respiratory tract clinical signs. The aim of this study was to analyze the variability of the capsid protein in different FCV isolates from southern Brazil. The sequencing analyses of thirteen Brazilian FCV samples, phylogenetic analyses and assessments of ten previously published sequences were conducted by examining the open reading frame 2 (ORF2, regions B–F). Comparisons of the predicted amino acid sequences of the ORF2 in Brazilian FCV isolates with those of the FCV-F9 strain indicated that the main differences are located within the regions C and hypervariable E (HVR_E). Epitopes that were mapped to the regions D, 5′HVR_E and conserved E also presented with some variability when compared to the strain F9. This is the first study describing sequence analyses and the phylogenetic relationships among FCV isolates from Brazil. The results presented here may expand upon current knowledge regarding aspects of FCV biology, epidemiology and genetic diversity and provide insights into improving the efficacies of current FCV vaccines.

The feline calicivirus (FCV) is a highly infectious pathogen that affects cats worldwide (Radford et al., 2007). This virus is associated with a variety of clinical presentations, including respiratory disease, acute and chronic stomatitis, acute arthritis, limping syndrome and hemorrhagic-like fever, also known as FCV-associated virulent systemic disease (FCV-VSD) (Pedersen et al., 2000; Radford et al., 2007). FCV is a member of the genus *Vesivirus* in the family *Caliciviridae*. The FCV genome consists of a positive-sense, single-stranded RNA molecule of approximately 7.7 kb that contains three open reading frames (ORF) (Radford et al., 2007). ORF 1 encodes the non-structural proteins, ORF 2 encodes the major capsid protein, VP1 (viral protein) and ORF 3 encodes a minor structural protein, VP2 (Neill, 1990; Neill et al., 1991; Sosnovtsev and Green, 2000).

ORF 2 contains conserved and variable sequences and is divided into six regions (A–F) (Neil, 1992; Seal et al., 1993). In contrast to regions C and E, the regions B, D and F are relatively conserved among the FCV isolates (Carter et al., 1992). Region E is further divided into two hypervariable regions (5′ and 3′ hypervariable or 5′HVR_E and 3′HVR_E, respectively) that are separated by a conserved central sequence, named conserved region E (conE) (Seal et al., 1993; Seal, 1994). The variable region E contains the major B-cell epitopes, making this region a target for virus-neutralizing antibodies (Tohya et al., 1997; Radford et al., 1999; Geissler et al., 2002).

The basis of FCV antigenicity resides in the capsid protein, which is important for receptor binding, internalization and uncoating (Geissler et al., 2002). Additionally, the RNA genome of FCV has elevated mutation rates that contribute to its molecular diversity, and it is therefore referred to as a quasispecies (Kreutz et al., 1998; Radford et al., 1998). Vaccinations for FCV were initiated in the 1970s, and the majority of vaccines available worldwide are based on the broadly cross-reactive FCV-F9 strain, although other FCV strains are commercially used as well, such as F7 and 255 (Radford et al., 2006). Despite the use of vaccines, FCV remains widespread in the domestic cat population (Radford et al., 2007).

In Brazil, little is known about FCV in the domestic cat population. After its first isolation (Weiblen et al., 1988), the pathogenicity was analyzed (Pereira et al., 1994), and some serological studies were also conducted in non-domestic (Filoni et al., 2006) and

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Table 1
Brazilian isolates and previously published strains of feline calicivirus (FCV).

FCV isolate/strain (# animal)	Year/country	Clinical origin	Age/gender	Vaccination status	Samples (swab)	GenBank access number
SV520/09#2	2009/Brazil[a]	Stomatitis in the faucets	12 years/male	No	Oral	HQ260661
SV147/09	2009/Brazil	Acute respiratory disease	4 months/female	Yes	Nasal	HQ260673
SV45/09	2009/Brazil	Without	1 year/female	Yes	Nasal	HQ260672
SV306/08	2008/Brazil	Oral disease, ulcers in both lips	1 year/male	Yes	Oral	HQ260671
SV57/08	2008/Brazil	Without	adult/female	No	Conjunctival and nasal	HQ260670
SV56/08	2008/Brazil	Without	3 months/female	Ni[b]	Nasal	HQ260669
SV55/08	2008/Brazil	Without	1.5 years/male	No	Conjunctival	HQ260668
SV38/08	2008/Brazil	Acute respiratory signs and with 7 months old had pneumonia	8 years/female	No	Conjunctival	HQ260667
SV142/07#07	2007/Brazil	Acute respiratory disease	2 months/female	No	Conjunctival and nasal	HQ260666
SV127/07	2007/Brazil	Without	adult/female	Ni	Nasal	HQ260665
SV368/06#11	2006/Brazil	Acute respiratory disease	2 months/female	No	Conjunctival and nasal	HQ260664
SV160/02	2002/Brazil	Ni	Ni	Ni	Conjunctival and nasal	HQ260662
SV1425/93	1993/Brazil	Ni	Ni	Ni	Ni	HQ260663
F9	1958/USA	Acute respiratory and oral disease				M86379
F4	1971/Japan	Acute respiratory signs				D90357
CFI/68	1960/USA	Acute respiratory signs and stiffness				U13992
255	1970/USA	Pneumonia and oral lesions				U07130
LLK	1983/Canada	Lameness				U07131
NADC	1993/USA	Acute respiratory signs				L09718
Urbana	1960/USA	Acute respiratory signs				L40021
KCD	1957/New Zealand	Acute respiratory signs				L09719
2280	1982/Canada	Limping syndrome				X99445
F65	1990/UK	Lameness and oral lesion				AF109465

[a] Isolate obtained from Porto Alegre, all the others were isolated from Santa Maria/Rio Grande do Sul/Brazil.
[b] Not informed.

domestic felines (Johann et al., 2009). A study performed by our group between the years of 2006–2009, reported the isolation of FCV and the feline herpesvirus type-1 in domestic cats from southern Brazil (author's data). The aim of the present study was to investigate the variability in the capsid proteins of different Brazilian FCV isolates and compare them to the reference FCV-F9 strain and other published FCV sequences. This is the first report of sequence and phylogenetic analyses of FCV Brazilian isolates.

The origin, clinical history and GenBank access number of the thirteen FCV Brazilian isolates and the ten previously published sequences that were analyzed in this study are listed in the Table 1. The CRFK (Crandell-Reese feline kidney) cells were used for virus isolation and amplification. The RNA extraction was performed using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) and RNA samples were reverse transcribed (Super-ScriptTM III RT–Invitrogen). The ORF2 (regions B–F) of the FCV isolates was PCR amplified. The primers used for the PCR were designed based on the ORF2 sequence of the complete genome of the FCV-F9 strain and included FCV_Capfor 5′-TTCGGCCGTTTGTCTTCC-3′ [position 6401–6419 (region B of ORF2)] and FCV_Caprev 5′-TTGTGAATTAAAGACATCAATAGACCT-3′ [position 7080–7053 (region F of ORF2)], resulting in a 679 bp product (Fig. 1). The commercial live attenuated vaccine, Felocell CVR-C (Pfizer Animal Health, USA), was used to optimize the PCR reaction and as a positive control in all reactions. The negative control consisted of mock-infected CRFK cells. The PCR products were purified using the PureLink PCR kit (Invitrogen, Carlsbad, CA, USA)

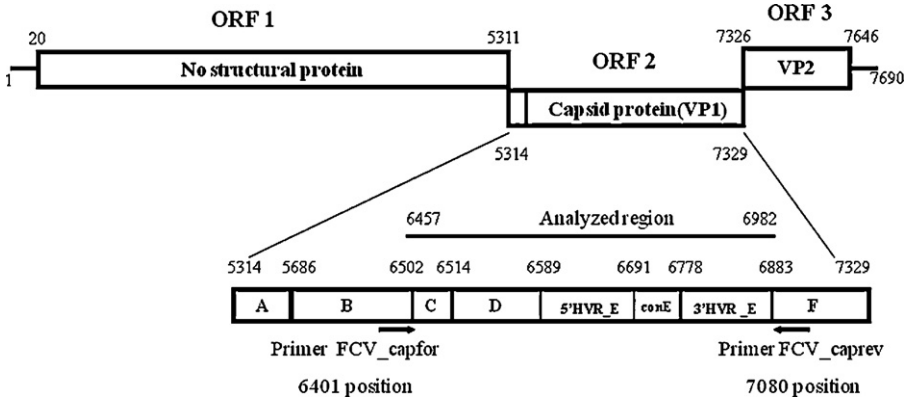

Fig. 1. The genome map of the feline calicivirus (FCV), showing the sequence amplified by the primers set and the region of open reading frame 2 (ORF2) analyzed in this study.