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Intergenic recombination in feline calicivirus associated with a hemorrhagic-like disease in the Republic of Korea

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Summary. – Feline calicivirus (FCV) is a common cause of upper respiratory tract disease in cats. In this study, the complete genome sequence of FCV 14Q315, which was detected from a dead domestic cat with a hemorrhagic-like disease, was analyzed to identify the genetic characteristics. The FCV 14Q315 genome was 7,684 bp. Phylogenetic analyses based on the ORF1, ORF2, and ORF3 sequences indicated that FCV 14Q315 is more closely related to FCV 15D022 than to other FCV strains. ORF1 of FCV 14Q315 shared high sequence similarity with ORF1 of FCVs 15D022 and UTCVM-H1. We further evaluated genetic recombination in ORF1 of FCV 14Q315 and detected intergenic recombination between p30 and the ORF1/ORF2 junction with high significance. Particularly, the non-recombination region in ORF1 of FCV 14Q315 showed high sequence similarity with FCVs GX2019, CH-JL2, and 15D022. The recombination region in ORF1 of FCV 14Q315 showed the highest similarity with FCV UTCVM-H1, which is associated with a hemorrhagic-like disease. The results suggest that the UTCVM-H1-like FCV was introduced into the Republic of Korea and presumably recombined with Korean FCVs by occasional mixed infections. In addition, the Korean FCV strains were located in several phylogenetic clusters with marked genetic diversity in the ORF2 region. These results imply that Korean FCVs possess high genetic diversity owing to mutations and recombination. Furthermore, it is possible that certain FCVs caused cyclical infections in the Korean cat population based on a phylogenetic analysis of FCVs isolated at different time points.

Keywords: calicivirus; virulent systemic feline calicivirus; recombination; hemorrhagic-like disease

Introduction

Feline calicivirus (FCV) belongs to the genus *Vesivirus* of the family *Caliciviridae.* FCV is prevalent in cat populations and is a common cause of mild upper respiratory tract disease. FCV infection can be accompanied by various symptoms, such as ulceration of the upper respiratory tract, ocular discharge, lethargy, loss of appetite, arthritis, and lameness (Radford *et al*., 2007). However, in the past decade, several virulent FCV mutants that cause a severe and acute virulent systemic disease with persistent high fever, anorexia, depression, necrotic lesions, pancreatitis, and hepatic necrosis as well as high mortality have been identified in many countries (Radford *et al*., 2007; Guo *et al*., 2018; Caringella *et al*., 2019). In particular, a strain associated with a systemic hemorrhagic-like fever and a high rate mortality in cats has been reported (Pedersen *et al*., 2000; Abd-Eldaim *et al*., 2005).

FCV contains a non-enveloped, positive-sense, single-stranded RNA genome of approximately 7.7 kb (Smertina *et al*., 2019). The viral genome contains coding sequences organized in three partially overlapping open reading frames (ORFs). ORF1 encodes non-structural proteins (p5.6, p32, p39, p30, and p13), including RNA-dependent

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Abbreviations: FCV = feline calicivirus; ML = maximum likelihood; nt = nucleotide(s) ORF = open reading frame; RdRp = RNA-dependent RNA polymerase; ROK = Republic of Korea

RNA polymerase (RdRp). ORF2 encodes viral protein 1, the major capsid protein. ORF3 encodes VP2, the minor structural protein (Sosnovtsev *et al*., 2002; Radford *et al*., 2007).

Genetic recombination events have been observed in different viruses of the family *Caliciviridae*, including recombination between different lagoviruses, noroviruses, and feline caliciviruses (Bull *et al*., 2005; Coyne *et al*., 2006; Mahar *et al*., 2013; Lopes *et al*., 2015; Hall *et al*., 2018). In these RNA viruses, recombination events are associated not only with an increase in virulence but also with the expansion of viral host range (Simon-Loriere *et al*., 2011).

In this study, the complete genome sequence of FCVs obtained from a dead domestic cat with a hemorrhagic-like disease was analyzed to identify the genetic characteristics.

Materials and Methods

Samples. Cat 14Q315 died after 1 week of hyperpyrexia (≥40°C) and anorexia. The histological findings in this cat included a marked fibrin thrombus in a lung, marked multiple hemorrhages and multifocal microvascular thrombosis involving the kidney, alymphocytosis in the spleen, and hemorrhage in the neck skin (Supplementary Table 1).

RNA extraction, detection of FCV, and RT-PCR. Viral RNA was extracted from multiple organs, including the brain, kidneys, liver, lymph nodes, spleen, heart, and intestines using the RNAeasy Mini Kit (Qiagen, USA). The presence of FCV genome was tested in multiple organs using the Feline Calicivirus Detection kit (iNtRON Biotechnolgy Inc., Korea) according to the manufacturer's instructions. cDNA was synthesized using the PrimeScrip First-strand cDNA Synthesis Kit (TaKaRa, Kusatsu, Japan), and PCR for the entire genome sequencing was performed using the HotStarTaq Plus Mater Mix Kit (Qiagen) using primer pairs listed in Supplementary Table 2. Amplification was performed at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 45 s and extension at 72°C for 3 min in a Mastercycler (Eppendorf, Hamburg, Germany). PCR products were sequenced using an ABI 3130XL Genetic Analyzer (Applied Biosystems, Waltham, MA, USA) and the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems).

Phylogenetic analysis. Phylogenetic analyses were conducted based on the ORF1, ORF2, and ORF3 sequences of FCV 14Q315 (Supplementary Table 3) using the maximum likelihood (ML) method with 1,000 bootstrap replicates and general time reversible (GTR) model using MEGA 7.0 software.

Recombination event. Recombination in the FCV 14Q315 genome was evaluated using the RDP, GENECONV, Bootscan, MaxChi, Chimaera, Siscan, and 3seq methods implemented in the RDP4.0 with the following parameters: sequences set to linear, Bonferroni correction, highest acceptable *p*-value set to 0.05, and 100 permutations. In addition, this was further confirmed by a SimPlot analysis.

Results

The 14Q315 FCV genome was detected in brain, kidneys, liver, lymph nodes, spleen, heart, and intestines and the complete genome length of FCV 14Q315 obtained from lung tissue sample was 7,684 bp. In the phylogenetic analysis of ORF1, the FCV 14Q315 clustered with FCV 15D022, showing the highest nucleotide sequence similarity of 89.1% (Supplementary Table 4), followed by FCV UTCVM-H1 (88.7%) (Fig. 1a). The complete ORF2 of FCV 14Q315 clustered with the Korean FCV strains, which included FCVs 88 (89.4%), 15D022 (88.9%), and SU (88.0%). However, it showed a low sequence similarity with ORF2 of FCV UTCVM-H1 (76.4%) (Fig. 1b). With regard to ORF3, FCV 14Q315 shared a high sequence similarity with FCV 15D022 (91.6%), but low sequence similarity with FCV UTCVM-H1 (84.0%) (Fig. 1c). Based on the phylogenetic analyses, recombination events were evaluated and a recombination event between ORF1 of FCV 14Q315 and FCV HTCVM-H1 was confirmed (p = 8.88E-16 to 1.50E-62) by RDP, GENECONV, Bootscan, MaxChi, Chimaera, Siscan, and 3seq methods. A weak partial recombination event (p = 1.37E-05 to 3.33E-15) was also predicted in the 5´ region (nt 67–1,395) of FCV 14Q315 ORF1 by RDP, Bootscan, MaxChi, Chimaera, and 3seq (Supplementary Table 5). The recombination at this location was also confirmed by a SimPlot analysis (Fig. 2b). To confirm the history of major recombination events with the FCV UTCVM-H1 strain, ML phylogenetic trees were constructed based on the non-recombination (nt 20–2,365) and recombination (nt 2,366–5,198) regions

Table 1. Nucleotide similarity of non-recombination and recombination regions in ORF1 of FCV 14Q315 strain

Position		Nucleotide similarity (%)				
		15D022	JL4	UTCVM-H1	UTCVM-H2	GX2019
20–2,365 nt	Non-recombination region	85.0	85.0	77.8	78.6	85.6
2,366–5,179 nt	Recombination region	92.5	79.5	97.4	79.5	79.8

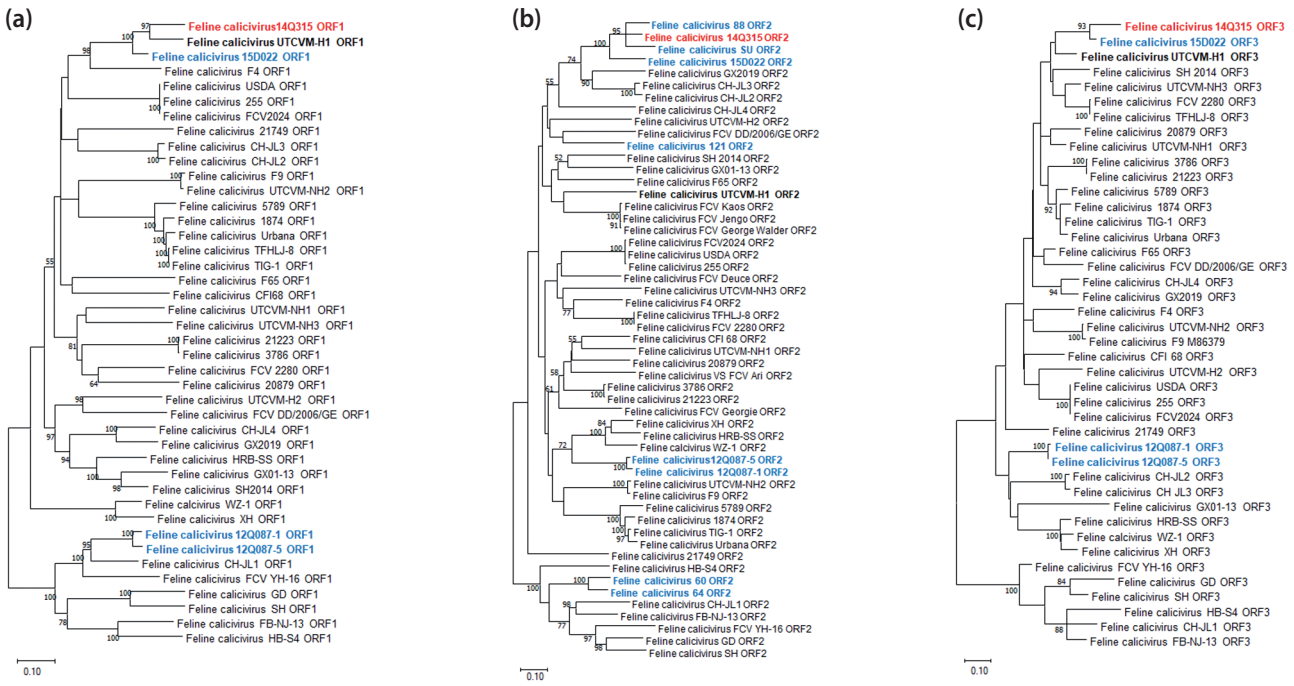

Fig. 1

Phylogenetic trees of feline caliciviruses (FCVs)

Phylogenies were obtained based on complete **(a)** ORF1, **(b)** ORF2, and **(c)** ORF3 nucleotide sequences. The FCV strain discovered in this study is indicated in red, and the other Korean FCV strains are indicated in blue. The phylogenetic trees were generated using the ML method, and branch support was determined by 1,000 bootstrap replicates.

according to the RDP4 analysis. The non-recombination region of FCV 14Q315 clustered with those of FCV GX2019 (85.6%) and CH-JL4 (85%) from China. In this region, the similarity between FCVs 14Q315 and 15D022 was 85.0% (Fig. 2c). However, nucleotide sequence similarity in the non-recombination region between FCV 14Q315 and FCV UTCVM-H1was 77.8% (Table 1). In contrast, FCV 14Q315 shared 97.4% nucleotide sequence similarity with FCV UTCVM-H1 and 92.5% with FCV 15D022 in the region of recombination (Fig. 2d).

In the phylogenetic analysis of ORF2, the Korean FCV strains shared sequence similarities ranging from 73.3 to 97.1%. In particular, ORF2 of FCV 88 and FCV SU clustered with ORF2 of FCV 14Q315 with sequence similarities ≥88% (Fig. 1b). However, the ORF2 of FCV 14Q315 showed a low sequence similarity ranging from 73.7 to 77.1% with the Korean FCV strains (Supplementary Table 4).

Discussion

FCV strains associated with a systemic hemorrhagic-like disease and a high mortality rate have been reported in literature. The clinical signs caused by the FCVs are similar to those observed in the calicivirus-induced rabbit hemorrhagic disease, and the viruses were easily transmitted and rapidly spread to other cats in the facility (Pedersen *et al*., 2000; Abd-Eldaim *et al*., 2005; Abrantes *et al*., 2012). In the present study, the FCV 14Q315 strain with genetic recombination was isolated from a dead domestic cat with a hemorrhagic-like disease. In particular, this recombination event was verified to inherit the RdRp gene of FCV UTCVM-H1 strain isolated from a cat with a hemorrhagic-like disease, which displayed high fever, anorexia, and marked hemorrhage in the lung and kidney, consistent with the symptoms of the cat infected with FCV 14Q315 (Abd-Eldaim *et al*., 2005). In a previous study, a recombinant FCV strain was also identified within an endemic cat colony infected with the two parental strains (Coyne *et al*., 2006). Such recombination events occur frequently in RNA viruses. This can be explained by the widely accepted recombination model of copy-choice recombination, which is mediated by template switching in the course of RNA synthesis during co-infection with two viruses (Coyne *et al*., 2007; Simon-Loriere *et al*., 2011). Therefore, we suggest that the FCVs associated with the hemorrhagic-like disease (UTCVM-H1 FCV) or UTCVM-H1-like FCV, which were not detected in Republic

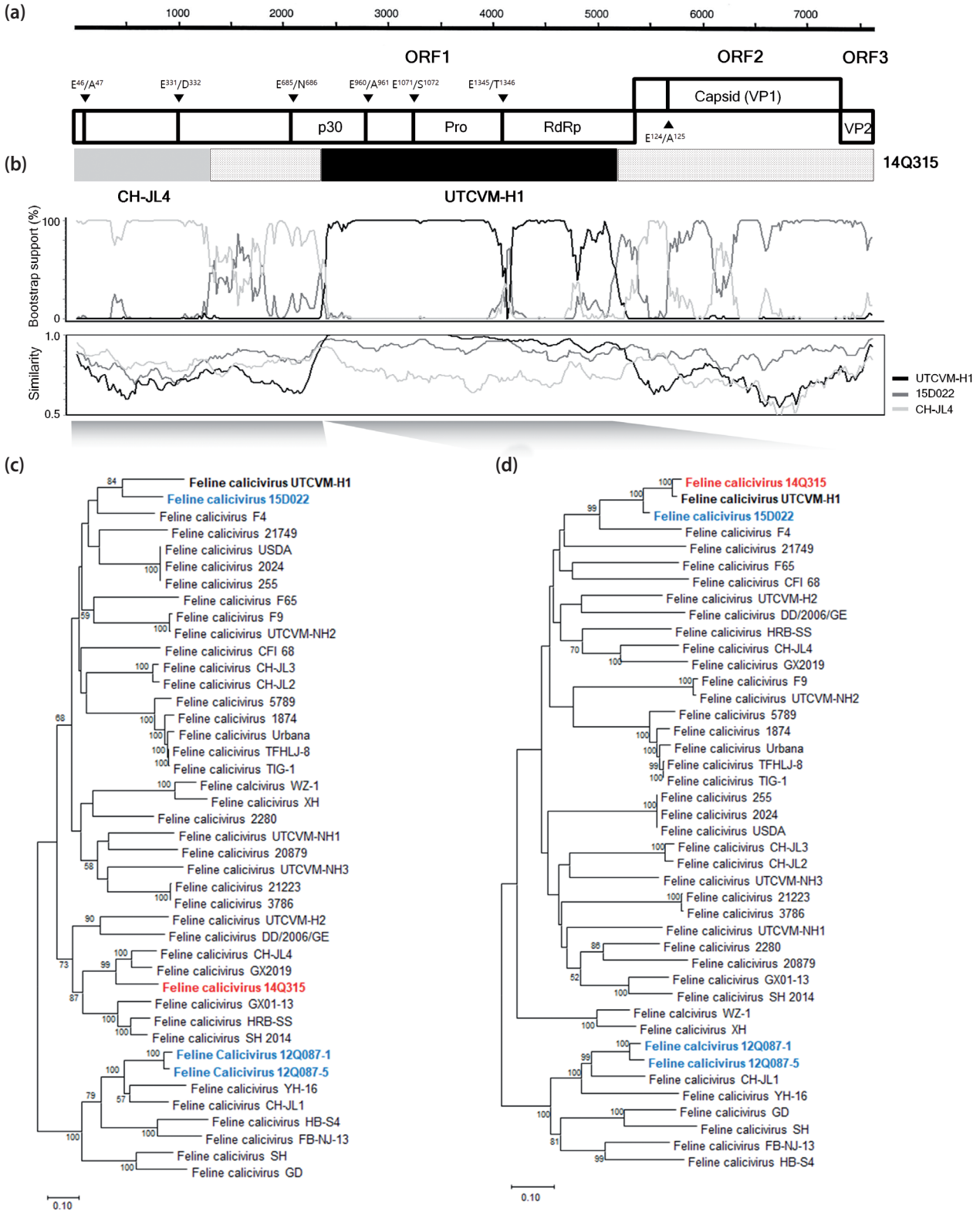

Fig. 2

Genetic recombination in the FCV 14Q315 strain discovered in this study

(a) The schematic genetic map and **(b)** RDP graphs indicate recombination in FCV 14Q315. The phylogenetic trees were constructed using nucleotide sequences of the **(c)** non-recombination and **(d)** recombination regions.

of Korea (ROK) previously, were introduced into ROK and recombined with Korean FCV strains. These recombination events may have occurred by viral replication during a mixed infection in an individual cat; however, no such case has been detected to date.

Based on the phylogenetic analysis of ORF2, the Korean FCV strains shared wide sequence similarity ranges and clustered into several groups. However, the genome sequences of ORF1 and ORF3 of several Korean FCV strains (i.e., FCV 88, SU, 60, 64, and 121) have not been reported and therefore were not included in the comparative analysis. In addition, clinical information on FCV 88 and SU could not be obtained; accordingly, it was not possible to infer the recombination or the relationship between symptoms and recombination. Nevertheless, considering the fact that FCVs reported in ROK were assigned to different clusters in the phylogenetic tree based on ORF2, Korean FCVs are considered to have high genetic diversity owing to genetic recombination and mutation. Furthermore, FCV strains may have caused cyclical reinfections in the cat colony within ROK based on the close phylogenetic relationship between Korean FCVs detected continuously from 2014 to 2017.

Our results indicated that the newly sequenced FCV strain, which caused a hemorrhagic-like disease in domestic cat, exhibits intergenic recombination with UTCVM-H1-like FCV in the non-structural region. In addition, our phylogenetic analysis indicated that the Korean FCV strains possess extensive genetic diversity.

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References

Abd-Eldaim M, Potgieter L, Kennedy M (2005): Genetic analysis of feline calicivirus associated with a hemorrhagic-like disease. J. Vet. Diagn. Invest. 17, 420–429.

Abrantes J, van der Loo W, Le Pendu J, Esteves PJ (2012): Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. Vet. Res. 43(1), 12. https://doi.org/10.1186/1297-9716-43-12

Bull RA, Hansman GS, Clancy LE, Tanaka MM, Rawlinson WD, White PA (2005): Norovirus recombination in ORF1/ORF2 overlap. Emerg. Infect. Dis. 11(7), 1079–1085. https://doi.org/10.3201/eid1107.041273

Caringella F, Elia G, Decaro N, Martella V, Lanave G, Varello K, Catella C, Diakoudi G, Carelli G, Colaianni ML, Bo S, Buonavoglia C (2019): Feline calicivirus infection in cats with virulent systemic disease, Italy. Res. Vet. Sci. 124, 46–51.

Coyne KP, Reed FC, Porter CJ, Dawson S, Gaskell RM, Radford AD (2006): Recombination of Feline calicivirus within an endemically infected cat colony. J. Gen. Virol. 87, 921–926.

Coyne KP, Gaskell RM, Dawson S, Porter CJ, Radford AD (2007): Evolutionary mechanisms of persistence and diversification of a calicivirus within endemically infected natural host populations. J. Virol. 81(4), 1961–1971. https://doi.org/10.1128/JVI.01981-06

Guo H, Miao Q, Zhu J, Yang Z, Liu G (2018): Isolation and molecular characterization of a virulent systemic feline calicivirus isolated in China. Infect. Genet. Evol. 65, 425–429.

Hall RN, Mahar JE, Read AJ, Mourant R, Piper M, Huang N, Strive T (2018): A strain-specific multiplex RT-PCR for Australian rabbit haemorrhagic disease viruses uncovers a new recombinant virus variant in rabbits and hares. Transbound. Emerg. Dis. 65(2), e444–e456. https://doi.org/10.1111/tbed.12779

Lopes AM, Dalton KP, Magalhães MJ, Francisco P, Esteves P, Holmes EC, Abrantes J (2015): Full genomic analysis of new variant rabbit hemorrhagic disease virus revealed multiple recombination events. J. Gen. Virol. 96, 1309–1319.

Mahar JE, Bok K, Green KY, Kirkwood CD (2013): The importance of intergenic recombination in norovirus GII.3 evolution. J. Virol. 87(7), 3687–3698.

Pedersen NC, Elliott JB, Glasgow A, Poland A, Keel K (2000): An isolated epizootic of hemorrhagic-like fever in cats caused by a novel and highly virulent strain of feline calicivirus. Vet. Microbiol. 73, 281–300. https://doi.org/10.1016/S0378-1135(00)00183-8

Radford AD, Coyne KP, Dawson S, Porter CJ, Gaskell RM (2007): Feline calicivirus. Vet. Res. 38(2), 319–335. https://doi.org/10.1051/vetres:2006056

Simon-Loriere E, Holmes EC (2011): Why do RNA viruses recombine? Nat. Rev. Microbiol. 9, 617–626. https://doi.org/10.1038/nrmicro2614

Smertina M, Urakova N, Strive T, Frese M (2019): Calicivirus RNA-dependent RNA polymerase: evolution, sturcutre, protein dynamics, and function. Front. Micorbiol. 10, 1280.

Sosnovtsev SV, Garfield M, Green KY (2002): Processing map and essential cleavage sites of the nonstructural polyprotein encoded by ORF1 of the feline calicivirus genome. J. Virol. 76(14), 7060–7072. https://doi.org/10.1128/JVI.76.14.7060-7072.2002