The Veterinary Journal 220 (2017) 24–27

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Short communication

Genetic and phylogenetic analysis of feline calicivirus isolates in China

Yaxin Sun, Mingliang Deng, Zhong Peng, Ruiming Hu, Huanchun Chen, Bin Wu *

Department of Preventive Veterinary Medicine, State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China

ARTICLE INFO

Article history:
Accepted 19 December 2016

Keywords:
Feline calicivirus
ORF2
Phylogenetic relationship
Genogroup
China

ABSTRACT

The aim of this study was to determine the genetic diversity of Chinese feline calicivirus (FCV) isolates and their phylogenetic relationship with isolates from elsewhere in the world. Phylogenetic analysis was performed based on the partial open reading frame (ORF) 2 sequences (regions B–F) of 21 Chinese FCV isolates and 30 global isolates. The Chinese isolates included 13 isolates from Wuhan, which were isolated in this study, and eight previously published isolates. Sixteen Chinese isolates and two Japanese isolates formed a distinct phylogenetic cluster. Phylogenetic analysis based on the sequences of the complete genome, ORF1, ORF2 and ORF3 of selected isolates supported the above findings. Genogroup analysis revealed that FCV genogroup II is present in China. These findings suggest that Chinese FCV isolates are closely related to Japanese FCV isolates.

Feline calicivirus (FCV) is widely distributed and is associated with respiratory disease, stomatitis, arthritis and haemorrhagic-like fever, also known as FCV-associated virulent systemic disease (Radford et al., 2007). The FCV genome is a 7.7 kb, positive sense, single stranded RNA molecule with three open reading frames (ORF1, ORF2 and ORF3) (Prikhodko et al., 2014). ORF2 encodes a capsid protein and contains both conserved and variable sequences.

Comparative analysis of ORF2 sequences has been used to elucidate phylogenetic relationships among different FCV isolates (Glenn et al., 1999; Prikhodko et al., 2014). FCV isolates appear to belong to a single diverse genetic group (Coyne et al., 2012). Analysis of Japanese isolates has suggested that there are two FCV genogroups and that genogroup II was present only in Japan (Sato et al., 2002). FCV is prevalent in some regions of China (Chen et al., 2013), but little is known about the genetic relationships among FCV strains in China. The aim of this study was to determine the genetic variability and phylogenetic relationships between Chinese FCV isolates and isolates from elsewhere in the world.

Thirteen FCV isolates (defined as Wuhan isolates) were recovered from conjunctival, nasal or oral swabs collected from nine cats treated in an animal hospital in Wuhan, China in 2014 (Table 1; See Appendix: Supplementary Table S1). Feline kidney (F81) cells were used for virus isolation and culture (Henzel et al., 2012). Viral RNA was extracted from the supernatant of the third passage of FCV-infected cell cultures using the EZNA Total RNA Kit (Omega, Bio-Tek). cDNA was synthesised using the PrimeScript reverse transcription (RT) kit with gDNA Eraser (TaKaRa).

A partial ORF2 sequence of 955 base pairs, corresponding to amino acids 277–594 of the FCV capsid protein (FCV-F9 strain, GenBank M86379) was amplified by PCR from each FCV isolate (Henzel et al., 2012). The complete genome of one isolate (WH7-92113) was amplified (see Appendix: Supplementary Table S2). Amplicons were purified using the TIANgel Midi Purification Kit (Tiangen, Biotech) and sequenced. Partial ORF2 sequences (regions B–F) of eight previously published Chinese isolates and 30 isolates from elsewhere in the world were retrieved from GenBank (see Appendix: Supplementary Table S3).

Comparison of partial ORF2 sequences (regions B–F) of Chinese FCV isolates and isolates from elsewhere in the world showed that the nucleotide sequence similarity (BioEdit) was 67.7–98.7% and the deduced amino acid identity (MEGA 5.0) was 74.4–98.3% (See Appendix: Supplementary Table S4). High variability among Chinese FCV isolates and those from elsewhere in the world was observed in the region of linear B-cell epitopes in the ORF2 sequence, as described by Radford et al. (1999). High variability was also observed in Asn-Gly-Thr (amino acid positions 439–441) of the 5′ hypervariable region (HVR) of region E, which has strong immunoreactivity (Seal, 1994) (see Appendix: Supplementary Fig. S1). Similar diversities in these positions were observed when the Chinese isolates were compared with vaccine strains of FCV (F9, F4 and 2024).

On the basis of phylogenetic analysis of partial ORF2 sequences (regions B–F), 16 Chinese isolates and two Japanese isolates formed a distinct cluster (Fig. 1). Phylogenetic analysis of the complete genome, as well as ORF1, ORF2 and ORF3 sequences, of three Chinese

* Corresponding author.
E-mail address: wub@mail.hzau.edu.cn (B. Wu).

http://dx.doi.org/10.1016/j.tvjl.2016.12.014
1090-0233/

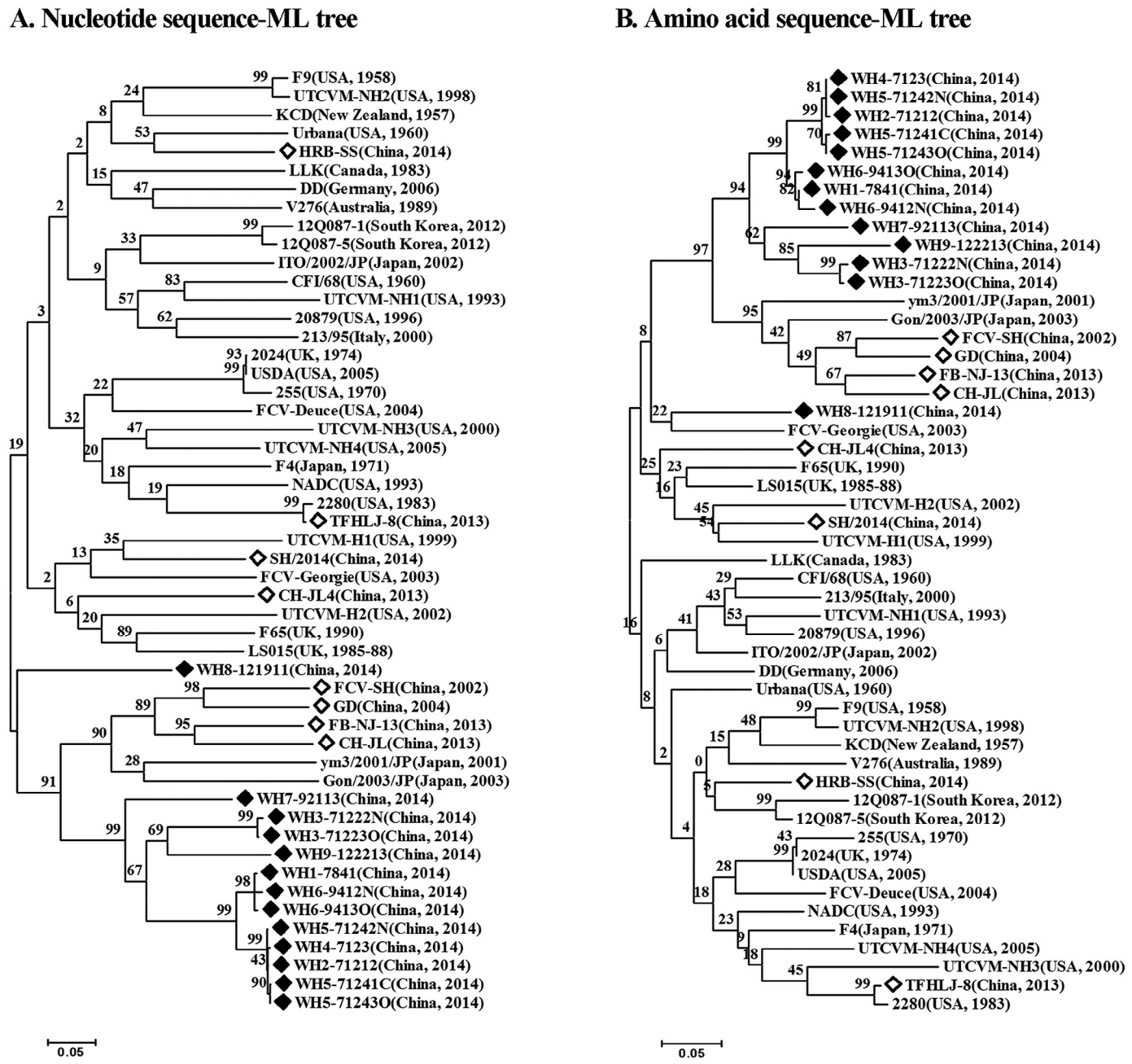

Fig. 1. Phylogenetic trees of feline calicivirus (FCV) isolates based on the nucleotide sequences (A) and amino acid (B) sequences of partial open reading frame 2 (ORF2 regions B–F) of Chinese FCV isolates and global isolates. The phylogenetic trees were constructed using the maximum-likelihood (ML) method with the Kimura 2 parameter (A) and the JTT amino acid substitution model, respectively (B). One thousand bootstrap repetitions were performed for each analysis. Positions that contained gaps and/or missing data were eliminated. ♦ Wuhan FCV isolates. ◊ Previously published Chinese isolates.

isolates (WH7-92113, GD and FB-NJ-13) supported this distinct phylogenetic cluster (see Appendix: Supplementary Fig. S2).

A phylogenetic tree constructed on the basis of ORF2-derived amino acid sequences 370–580 (Sato et al., 2002) demonstrated that most of the Chinese FCV isolates were clustered into genogroup II, which included some Japanese isolates (Fig. 2). Genogroup I and II strains differ in three amino acid residues; at positions 377, 539 and 557, genogroup I strains have Asn, Ala or Pro and Gly residues, respectively, whilst genogroup II strains have Lys, Val and Ser (see Appendix: Supplementary Table S5).

In the present study, most Chinese isolates were more closely related to genogroup II Japanese strains than to genogroup I strains from elsewhere in the world. This could be explained by the geographical proximity of China to Japan. The widespread FCV carrier state may facilitate the spread of feline caliciviruses within the cat population within and between the two countries (Glenn et al., 1999), although it is noted that there is no evidence of widescale national or international dispersal of individual strains in Europe (Hou et al., 2016). Some of the Chinese isolates clustered with genogroup I strains, indicating a relationship with more geographically distant regions.

When the characteristic amino acid residues were compared between these two genogroups, only three amino acid residues (positions 377, 539 and 557) differed between genogroup I and II strains, whilst the amino acid residue at position 566 exhibited variation in an internal genogroup. This is different from previous studies,