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Epidemiological Investigation and Genetic Evolution Analysis of Feline Calicivirus during 2025–2026 in Shandong Province

Yuqing Wang
Qingdao Agricultural University
Ang Tian
Shandong Academy of Agricultural Sciences
Kefeng Wei
Shandong Yiyuan Pharmaceutical Co., Ltd
Shuo Yang
Shandong Academy of Agricultural Sciences
Lei Zhao
Shandong Agricultural Seed Industry and Technology Co., Ltd
Lihong Qi
Shandong Academy of Agricultural Sciences
Xue Lin
Xinyi Animal Hospital
Wangwang Sun
Shandong Academy of Agricultural Sciences
Fengzhou Ma
Shandong Yiyuan Pharmaceutical Co., Ltd
Lichun Fang
Shandong Academy of Agricultural Sciences
Lin Zhang
Qingdao Agricultural University
Jiaqiang Wu
wujiaqiang2000@sina.com

Qingdao Agricultural University

Short Report

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Abstract

Feline calicivirus (FCV) is a major viral pathogen associated with feline respiratory diseases. To characterize the epidemiological and evolutionary features of FCV in Shandong Province, China, 656 clinical feline specimens were collected during 2025–2026. RT-PCR was used to screen for common feline viral pathogens, and FCV-positive samples were subjected to virus isolation, purification and whole-genome sequencing. The overall FCV-positive rate was 1.67% (11/656). Co-infections predominated among FCV-positive specimens, with feline parvovirus (FPV) as the most frequent co-infecting agent. Eleven local FCV strains were isolated; genogroup analysis showed that both GI (six strains) and GII (five strains) were circulating. Four high-confidence recombination events were identified. Notably, strain F1481 (genogroup GI) originated from inter-genogroup recombination between a GII-type isolate (F1506) and a GI-type isolate (F1498), providing bioinformatic evidence for natural inter-genogroup recombination between GI and GII FCV strains. These findings indicate that recombination and antigenic drift are important evolutionary forces shaping local FCV populations and provide empirical evidence for regional disease control and the development of geographically matched vaccine candidates.

Introduction

Feline calicivirus (FCV), a member of the genus Vesivirus within the family Caliciviridae, is one of the most prevalent pathogens of feline upper respiratory tract disease (URTD) (Wei et al. 2024). Two major pathotypes are recognized according to clinical manifestations: classical respiratory FCV (FCV-URTD), characterized by fever, oral ulceration, respiratory signs and gingivostomatitis, and virulent systemic-disease-associated FCV (FCV-VSD), which can cause multi-organ damage with mortality rates exceeding 50% (Caringella et al. 2019; Guo et al. 2018). Since the first report of FCV-VSD in the United States in 1998 (Pedersen et al. 2000), such highly virulent strains have attracted widespread concern.

FCV is a non-enveloped positive-sense RNA virus with a genome of approximately 7.7 kb comprising three open reading frames (ORF1–ORF3) (Carter et al. 1992). ORF1 encodes a polyprotein precursor processed into six non-structural proteins, ORF2 encodes the major capsid protein VP1, and ORF3 encodes the minor capsid protein VP2. VP1 comprises six functional regions (A–F); domains D and E harbour major linear B-cell epitopes and are key targets of neutralizing antibodies (Geissler et al. 2002; Seal et al. 1993), making ORF2 a pivotal marker for FCV genotyping (Prikhodko et al. 2014). As an RNA virus lacking proofreading activity, FCV readily accumulates mutations and undergoes recombination, driving antigenic drift and the emergence of novel variants. Notably, the first naturally occurring inter-genogroup GI/GII recombinant FCV was isolated from domestic cats in China, exhibiting enhanced in-vitro replication and poor cross-neutralization by GI-based vaccines (Tan et al. 2025).

Molecular epidemiological investigations in Hangzhou, Jiangsu, Guangzhou and Beijing have shown endemic circulation of both GI and GII genogroups, with GII predominating in East Asian countries (Kim et al. 2024; Mao et al. 2022). However, systematic whole-genome-based surveillance remains limited in

Shandong Province, particularly in Jinan. In the present study, clinical feline specimens collected during 2025–2026 were screened for common viral pathogens, and FCV isolates were characterized by whole-genome sequencing, phylogenetic, antigenic-variation and recombination analyses to delineate the epidemic status and evolutionary trends of locally circulating FCV.

Materials and Methods

Sample collection and PCR screening

Oropharyngeal and rectal swabs were collected from cats with suspected respiratory-tract infections, or with limb and pulmonary edema, at multiple pet hospitals in Shandong Province during 2025–2026; in total, 656 specimens were recruited. Each swab was transferred into a centrifuge tube pre-filled with 1 mL PBS, vortexed, and preserved at − 80°C. Viral nucleic acids were extracted with the Simply P Viral DNA/RNA Extraction Kit (BioFlux, USA). Target-specific primers based on conserved regions of FCV, FeLV, FCoV, FAstV, FeChV, FPV and FBoV reference sequences were used with TaKaRa Ex Taq DNA polymerase (TaKaRa, Japan). Amplicons were purified with a Gel Extraction Kit (CWBIO, China), cloned into the pMD18-T vector (TaKaRa), and subjected to bidirectional Sanger sequencing (Sangon Biotech); sequences were confirmed by BLAST.

Virus isolation and purification

FCV-positive specimens were filter-sterilized (0.22 μm) and inoculated onto confluent F81 cell monolayers in RPMI-1640 medium containing 2% fetal bovine serum. Cultures were monitored daily for cytopathic effect (CPE); when CPE was observed, viruses were harvested after three freeze–thaw cycles, and specimens without detectable CPE underwent three blind passages. Samples co-positive for FPV were subjected to serial propagation combined with three rounds of plaque purification to eliminate FPV contamination. Multi-step growth kinetics were determined by $TCID_{50}$ assays using the Reed–Muench method.

Bioinformatic analysis

Whole-genome sequences of 41 globally distributed FCV reference strains were retrieved from GenBank. MEGA 11.0.13 was used to calculate nucleotide and amino-acid p-distances and to construct maximum-likelihood phylogenetic trees based on the whole genome, ORF1, ORF2 and ORF3, with 1,000 bootstrap replicates. Putative recombination events were screened using seven algorithms in RDP4; breakpoints supported by at least four algorithms (Bonferroni-corrected $P < 0.01$) were considered high-confidence and validated with SimPlot (200 bp window, 20 bp step).

Results

Epidemiological analysis and isolation identification of FCV

Screening of 656 clinical specimens revealed co-existence of single-pathogen infections and heterogeneous co-infections. The FCV nucleic-acid positive rate was 1.67% (11/656). Co-infections constituted the majority among FCV-positive specimens, with FPV as the dominant co-infecting pathogen (Fig. 1A-B). FCV positive clinical specimens were inoculated onto F81 cells and subjected to three rounds of blind serial passaging. Distinct cytopathic effects (CPE), typified by cell rounding, aggregation and detachment, were observed for 11 isolates; the severity of cytopathic lesions increased alongside incubation duration(Fig. 1C-D). Transmission electron microscopy revealed spherical, non enveloped virions of approximately 30 nm diameter in purified virus preparations, consistent with canonical morphological features of caliciviruses (Fig. 1E). Multi step growth curve assays revealed divergent replication kinetics among the 11 isolates propagated in F81 cells, with pronounced inter strain differences in peak viral titres (Fig. 1F).

Virus genogrouping and phylogeny

Eleven FCV strains were successfully isolated from FCV-positive specimens. Whole-genome-based phylogenetic analysis assigned six isolates (F3, F513, F1481, F1485, F1494 and F1498) to genogroup GI and five isolates (F1493, F1496, F1497, F1504 and F1506) to GII, indicating co-circulation of both genogroups in Jinan (Fig. 2). Except for F3, local isolates clustered apart from vaccine strains F9 and F255 and grouped predominantly with previously reported Chinese domestic strains, reflecting geographically clustered evolution. Pairwise whole-genome nucleotide identities among the 11 isolates ranged from 76.2% to 99.7%; four GII isolates shared > 95% identity, suggesting a local transmission cluster. Identities between local isolates and vaccine strains F9 and F255 were both below 80% (76.2–79.1% and 76.6–79.3%, respectively)(Supplementary Fig. S1).

Recombination and gene mutation

Four high-confidence recombination events were identified (Fig. 3 and Supplementary Fig. S2). Strain F513 contained a recombinant region spanning nt 3853–5224, consistent with a previously identified recombination locus in Qingdao strains. Notably, inter-genogroup recombination was detected: GI strain F1481 originated from recombination between GII isolate F1506 and GI isolate F1498 (Fig. 3). Previous studies have reported multiple amino-acid substitutions in domain E associated with FCV virulence. Alignment of eight virulence-related sites within domain E showed that VSD reference strains harboured 3–7 substitutions relative to vaccine strains, while our local isolates exhibited 1–6 amino-acid replacements, indicating substantial sequence variation (supplementary Table S1).

Discussion

The FCV nucleic-acid positive rate (1.67%) was lower than the national average of 10.86% reported for 2018–2020 (Cao et al. 2023), likely reflecting differences in sampling population (pet-hospital versus household and shelter cats) and sampling period. Most FCV-positive samples were involved in complex co-infections, with FPV predominant. Because FPV preferentially infects dividing lymphoid and myeloid tissues and induces immunosuppression, it increases susceptibility to secondary FCV infection (Balboni et al. 2014; Stuetzer and Hartmann 2014); routine pathogen detection is therefore recommended for clinically suspected cases.

Co-circulation of GI and GII strains in relatively balanced proportions was confirmed. The distribution of isolates across multiple subclades suggests multiple independent cross-regional introductions, and none of the GII isolates clustered with European or American strains, consistent with geographical clustering in FCV molecular evolution (Sun et al. 2017). The low whole-genome identity (< 80%) between local isolates and vaccine strains F9 and F255 raises the risk of immune escape by circulating field strains (Wang et al. 2021), while the accumulation of 3–7 amino-acid substitutions in the VP1 5’-HVR across all isolates identifies this region as the major driver of FCV antigenic drift, potentially compromising the cross-protection afforded by existing vaccines (Brunet et al. 2019). Because only in-silico prediction was performed here, definitive conclusions on the pathogenicity of these field isolates require in-vivo challenge studies.

Recombination is a key evolutionary driver for FCV. Four high-confidence events were detected, including a recombinant region in F513 consistent with a locus reported in Qingdao (Liu et al. 2023). Notably, the identification of inter-genogroup recombination generating GI strain F1481 from GII F1506 and GI F1498, together with the co-circulation of both genogroups, provides favourable conditions for cross-genogroup recombination (Tan et al. 2025). Continuous whole-genome surveillance is required to capture emerging inter-genogroup recombinant variants within local cat populations.

In conclusion, this study provides the first systematic molecular epidemiological investigation of FCV in Shandong Province. The FCV-positive rate was 1.67% (11/656), co-infections with FPV were common, and eleven strains (six GI and five GII) were isolated, indicating co-circulation of both genogroups. Whole-genome identity with vaccine strains was below 80%, the VP1 5’-HVR harboured 3–7 amino-acid substitutions, and four recombination events, including one inter-genogroup GI/GII recombinant, were identified. These findings indicate that recombination and antigenic drift are important evolutionary forces shaping local FCV populations. Future serological neutralization and animal-challenge studies are warranted to assess vaccine cross-protection and support regionally matched vaccine development.

Declarations

Conflict of Interest Statement

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Yuqing Wang: data curation, investigation, software, visualization, formal analysis; Ang Tian: conceptualization, methodology, writing—original draft, writing—review and editing; Kefeng Wei: resources; Shuo Yang: investigation, resources; Lei Zhao: resources; Lihong Qi: supervision, resources; Xue Lin: resources; Wangwang Sun: investigation; Fengzhou Ma: resources; Lichun Fang: resources; Lin Zhang: resources, funding acquisition, project administration, supervision; Jiaqiang Wu: funding acquisition, project administration, supervision.

Data Availability

All data relevant to this study are included within the article or provided as supplementary information.

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Figures

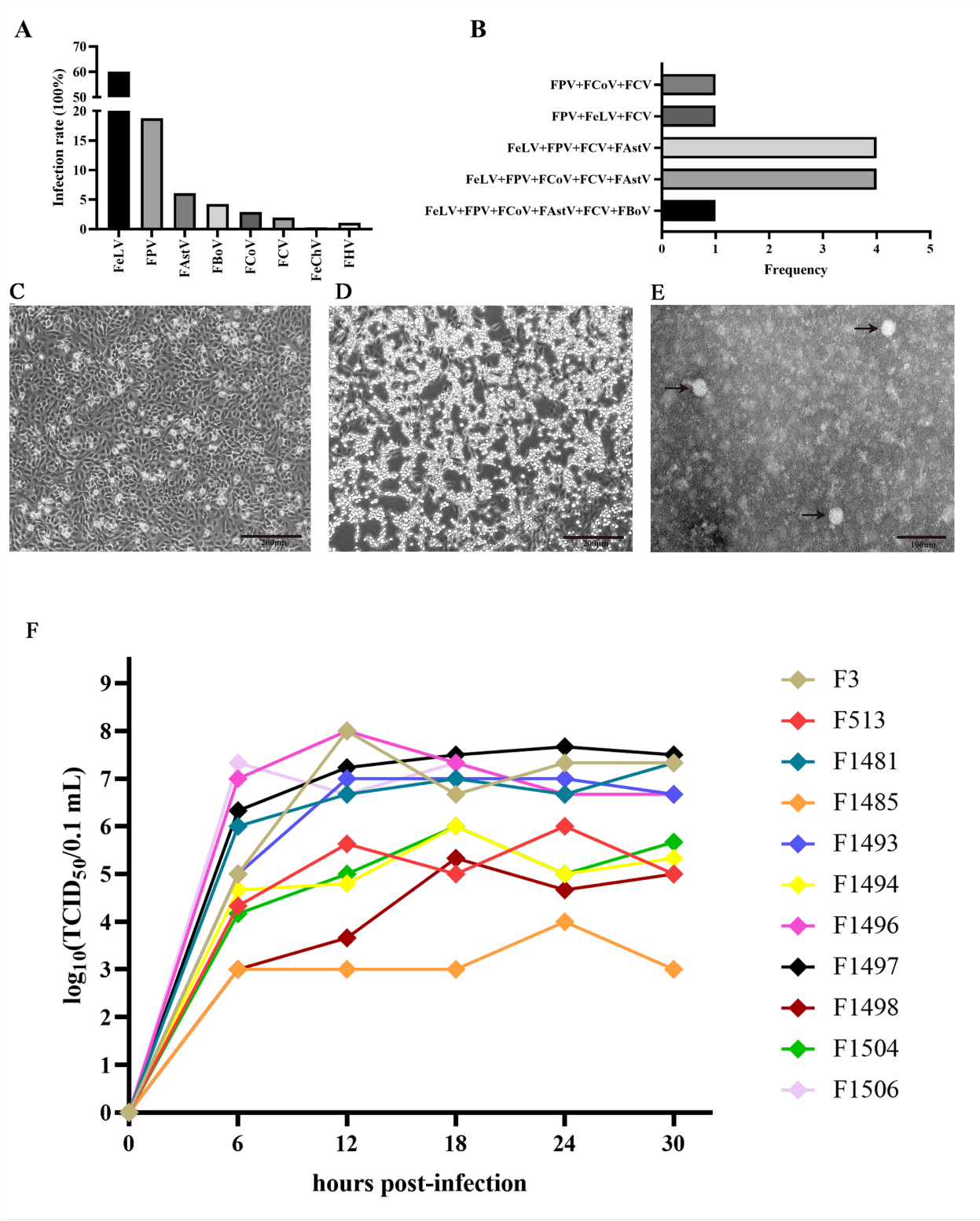


Figure 1

Epidemiological analysis and isolation identification of FCV. (A) Positive detection rates of different feline pathogens; (B) diverse co-infection patterns among FCV-positive clinical samples.(C) Uninoculated F81 control cells (100×), scale bar = 200 μm. (D) Infected cells displaying CPE characterized by cell rounding, aggregation and detachment at 12 h post-inoculation (100×), scale bar = 200 μm. (E) Electron micrograph of purified isolates showing spherical non-enveloped viral particles ~30 nm in diameter

(100,000×), scale bar = 100 nm. (F) Growth-curve assays demonstrating divergent replication profiles of the 11 isolates in F81 cells. Most strains reached peak viral titres at 12–24 h post-infection, with substantial variability in peak $\log_{10}$ ($TCID_{50}$/0.1 mL) values across isolates.

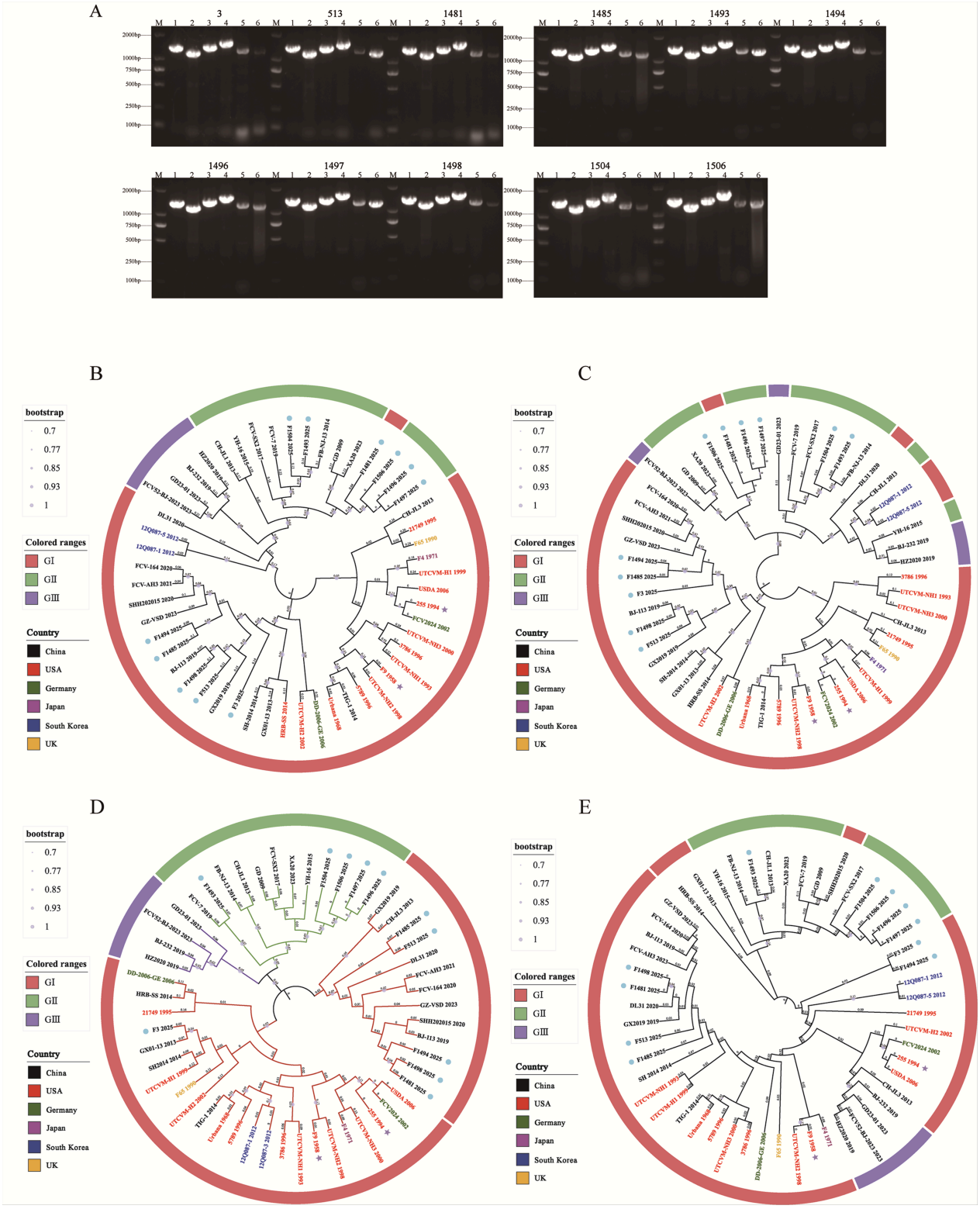


Figure 2

FCV genetic evolutionary analysis. (A) Agarose-gel electrophoresis of segmented PCR amplicons for FCV full-length genome. (B-E) Maximum-likelihood phylogenetic trees inferred from whole-genome, ORF1, ORF2 and ORF3 sequences. Red branches represent GI; green branches represent GII; purple branches represent GIII; blue circles mark isolates obtained in this study, purple stars denote vaccine strains; sequence-label colours correspond to strain geographic origins.

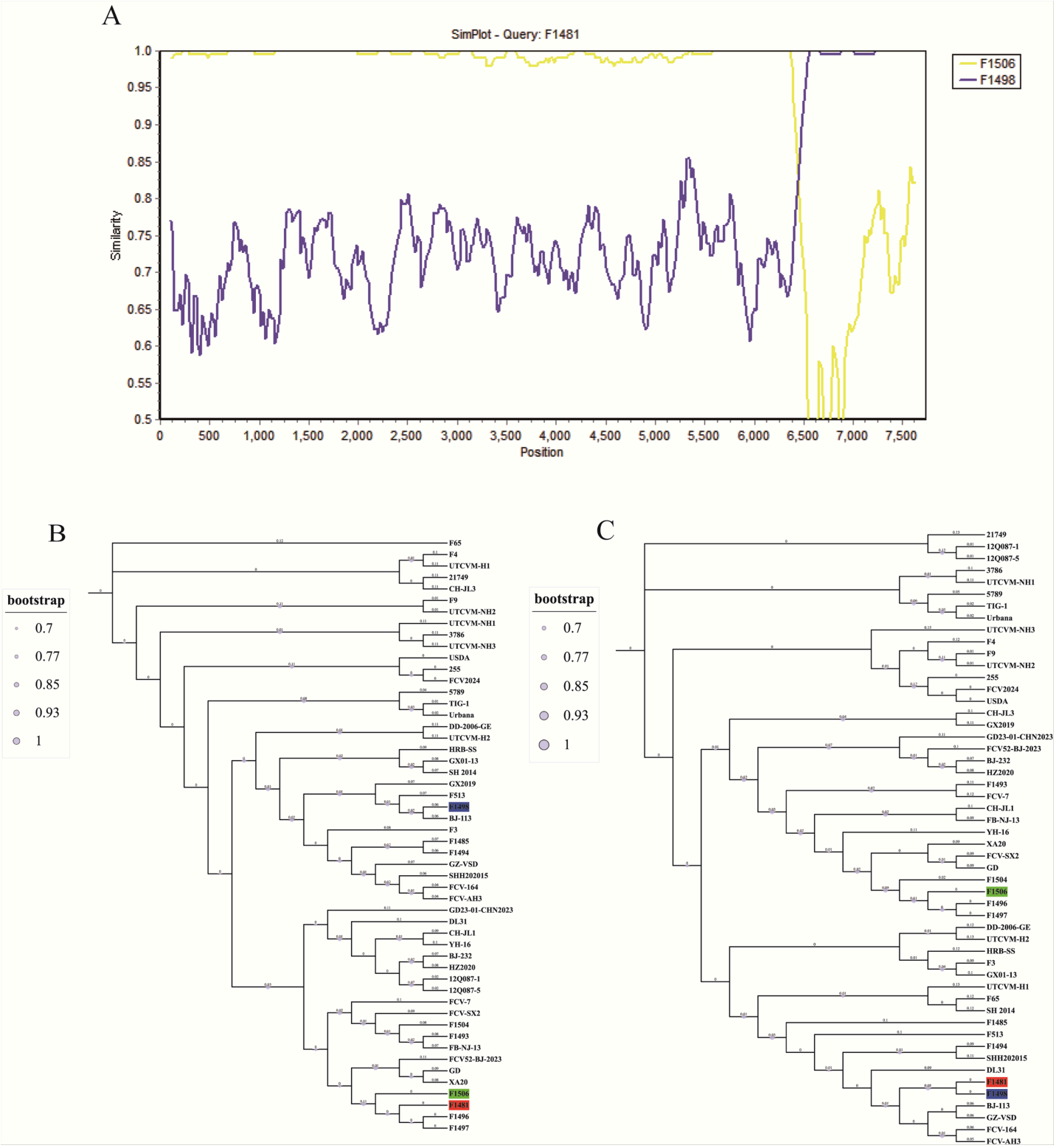


Figure 3

Recombination analysis of FCV isolate F1481 from Jinan. (A) SimPlot v3.5.1 similarity plot showing pairwise sequence similarity between F1481 and its putative parental strains; (B–C) phylogenetic trees built for genomic fragments split at predicted recombination breakpoints, whose discordant topologies validate the recombination signal identified by RDP4 v4.1.

Supplementary Files

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