




# Novel Recombinant Foot-and-Mouth Disease Virus Circulating in Vietnam

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**ABSTRACT** We report the genome sequences of 12 recombinant foot-and-mouth disease virus isolates from Vietnam. The recombinant strain has a capsid region from an A/Sea-97 strain and a nonstructural segment from an O/ME-SA/PanAsia strain. The isolates were obtained from two outbreak samples collected in June 2017 and 10 subclinical samples collected between 2017 and 2019.

Foot-and-mouth disease (FMD), caused by FMD virus (FMDV; *Aphthovirus*, *Picornaviridae*), is an important infectious disease of livestock. Acutely infected animals develop characteristic vesicles on their feet and mouth (1). The seven distinct FMDV serotypes (A, Asia 1, C, O, SAT 1, SAT 2, SAT 3) are divided into topotypes, lineages, and sublineages based on VP1 sequence similarity (2). FMDV diversity is driven in part by a high mutation rate. Additionally, recombination is increasingly recognized as an important and common factor in FMDV evolution (3–5).

The viruses described herein were obtained from vesicular epithelium (clinical;  $n = 2$ ) or oropharyngeal fluid (subclinical;  $n = 10$ ) collected from cattle and buffalo in three provinces in Vietnam during 2017 to 2019 (Table 1). FMDV was confirmed by virus isolation (VI) on LFBK $\alpha$ v $\beta$ 6 cells, followed by detection of viral RNA in VI supernatant by real-time reverse transcription PCR (qRT-PCR) (6, 7). VI supernatant RNA was subjected to viral deep sequencing as previously described (8, 9). Briefly, RNA was extracted using the MagMAX total RNA isolation kit, and host DNA was depleted using the DNA-free DNase kit (Ambion). The RNA underwent first-strand synthesis using the Superscript II first-strand synthesis system (Invitrogen), coupled with random primers and two FMDV-specific primers (10). Double-stranded cDNA was generated using the NEBNext Ultra nondirectional RNA second-strand synthesis module and sequenced as previously described (8) using the Nextera XT kit on a NextSeq 500 platform with paired-end reads (Table 1). All analyses were performed in CLC Genomics Workbench v11.0. The paired reads were quality trimmed using default parameters and then mapped to previously published A/Sea-97 (GenBank accession no. [KJ933864](https://doi.org/10.1128/MRA.01263-20)) and O/ME-SA/PanAsia ([KR265075](https://doi.org/10.1128/MRA.01263-20)) sequences, representative of strains circulating in the region. Mapping to the A reference genome yielded high coverage in the capsid region but low coverage in the nonstructural regions, while mapping to the O reference yielded the opposite coverage. A consensus sequence was extracted from each mapping using default parameters (Table 1) and aligned for each sample to account for the different areas of low coverage in each mapping. Consensus sequences extracted from the alignment were annotated based on comparison with the references, and

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**TABLE 1** Sampling locations and dates, sequencing metrics, and accession numbers for sequences in this report

| Sequence                  | Province | Species | Date collected | Genome length (nt) | Total no. of reads generated | Avg read length (nt) | No. of mapped reads | Avg coverage (no. of reads) | GC content (%) | GenBank accession no. | SRA accession no. |
|---------------------------|----------|---------|----------------|--------------------|------------------------------|----------------------|---------------------|-----------------------------|----------------|-----------------------|-------------------|
| A/VIT/17-11648/2017       | Dak Lak  | Cattle  | Jun 2017       | 8,165              | 2,496,838                    | 74.1                 | 2,035,029           | 17,981.0                    | 53.7           | MT340197              | SAMN14596608      |
| A/VIT/17-13002/2017       | Dak Lak  | Cattle  | Jun 2017       | 8,165              | 3,902,130                    | 74.5                 | 2,911,130           | 25,956.0                    | 53.6           | MT340198              | SAMN14596609      |
| A/VIT/DL-P087-1/2017_pro  | Dak Lak  | Cattle  | Sep 2017       | 8,166              | 4,380,148                    | 74.5                 | 1,705,913           | 15,203.3                    | 53.8           | MT340199              | SAMN14596610      |
| A/VIT/DL-P-106-1/2018_pro | Dak Lak  | Cattle  | Jun 2018       | 8,165              | 1,096,324                    | 146.5                | 772,946             | 13,513.3                    | 53.6           | MT340209              | SAMN14596611      |
| A/VIT/DL-P-186-1/2018_pro | Dak Lak  | Cattle  | Jul 2018       | 8,165              | 584,244                      | 147.5                | 439,255             | 7,723.2                     | 53.7           | MT340211              | SAMN14596612      |
| A/VIT/DL-P-96-2/2018_pro  | Dak Lak  | Cattle  | Aug 2018       | 8,165              | 1,164,424                    | 144.1                | 1,020,807           | 17,451.2                    | 53.7           | MT340207              | SAMN14596613      |
| A/VIT/DL-P-193-4/2018_pro | Dak Lak  | Cattle  | Sep 2018       | 8,175              | 3,214,770                    | 144.3                | 2,582,692           | 43,707.9                    | 53.3           | MT340218              | SAMN14596614      |
| A/VIT/DL-P-73-4/2018_pro  | Dak Lak  | Cattle  | Oct 2018       | 8,184              | 3,172,284                    | 143.1                | 2,355,116           | 39,642.1                    | 53.4           | MT340220              | SAMN14596615      |
| A/VIT/DL-P-492-1/2019_pro | Dak Lak  | Cattle  | Jan 2019       | 8,180              | 2,692,232                    | 142.1                | 2,324,200           | 38,716.4                    | 53.5           | MT340222              | SAMN14596616      |
| A/VIT/DL-P-605-2/2019_pro | Dak Lak  | Cattle  | Jun 2019       | 8,189              | 2,607,374                    | 144.7                | 2,089,976           | 35,489.6                    | 53.8           | MT340223              | SAMN14596617      |
| A/VIT/BK-P181-3/2018_pro  | Bac Kan  | Buffalo | Aug 2018       | 8,189              | 3,366,252                    | 143.8                | 2,875,136           | 48,769.8                    | 53.3           | MT340224              | SAMN14596618      |
| A/VIT/LA-P12-10/2018_pro  | Long An  | Cattle  | Mar 2018       | 8,163              | 2,012,536                    | 145.3                | 1,661,319           | 28,044.1                    | 53.5           | MT340225              | SAMN14596619      |

the poly(C) tract in the 5' untranslated region (UTR) was standardized to 12 nucleotides (nt) (11).

The 8,163- to 8,189-nt genome sequences encode a 6,999-nt open reading frame (ORF) flanked by a 1,068- to 1,094-nt 5' UTR and a 92- to 93-nt 3' UTR, excluding the poly(A) tail. The pairwise identity among these sequences was 97.9%. A BLASTn search showed that the sequences were 92% similar to that of A/VIT/42/2013 (GenBank accession no. [KY322680](#)). Similarity improved to 97% when searching the capsid (P1) region only, whereas the nonstructural region (2B through 3D) was 97% similar to that of O/VIT/106131/2013 (GenBank accession no. [MF947143](#)), an O/ME-SA/PanAsia isolate. Recombination analysis in RDP4 (12) confirmed recombination in these sequences, with the beginning breakpoint at position 1688 in the alignment (99% confidence interval [CI], 1653 to 1825) and the ending breakpoint at position 4123 (99% CI, 4047 to 4155).

These recombinant genome sequences highlight the need for continued surveillance combined with full-genome sequencing to identify emerging novel FMDV strains in regions of endemicity. Because routine sequencing of FMDV typically includes only the VP1 coding segment, related viruses likely have been reported as simply A/SEA-97 without awareness of the chimeric nature of the viruses.

**Data availability.** The genome nucleotide sequences have been deposited in GenBank under accession no. [MT340197](#) through [MT340225](#). The raw sequence data are available in the NCBI Sequence Read Archive under BioProject accession no. [PRJNA625284](#).

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