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GENOME SEQUENCES

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 LFBKαvβ6 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) and O/ME-SA/PanAsia (KR265075) 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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REFERENCES

1. Arzt J, Juleff N, Zhang Z, Rodriguez LL. 2011. The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. Transbound Emerg Dis 58:291–304. https://doi.org/10.1111/j.1865-1682.2011.01204.x.
2. Knowles NJ, Samuel AR. 2003. Molecular epidemiology of foot-and-mouth disease virus. Virus Res 91:65–80. https://doi.org/10.1016/S0168-1702(02)00260-5.
3. Jamal SM, Nazem Shirazi MH, Ozyoruk F, Parlak U, Normann P, Belsham GJ. 2020. Evidence for multiple recombination events within foot-and-mouth disease viruses circulating in West Eurasia. Transbound Emerg Dis 67:979–993. https://doi.org/10.1111/tbed.13433.
4. Bachanek-Bankowska K, Di Nardo A, Wadsworth J, Mioulet V, Pezzoni G, Grazioli S, Brocchi E, Kafle SC, Hettiarachchi R, Kumarawadu PL, Eldaghayes IM, Dayhum AS, Meenowa D, Sghaier S, Madani H, Abouchoaib N, Hoang BH, Vu PP, Dukpa K, Gurung RB, Tenzin S, Wernery U, Panthumart A, Seeyo KB, Linchongsubongkoch W, Relmy A, Bakkali-Kassimi L, Scherbakov A, King DP, Knowles NJ. 2018. Reconstructing the evolutionary history of pandemic foot-and-mouth disease viruses: the impact of recombination within the emerging O/ME-SA/Ind-2001 lineage. Sci Rep 8:14693. https://doi.org/10.1038/s41598-018-32693-8.
5. Brito B, Pauszek SJ, Hartwig EJ, Smoliga GR, Vu LT, Dong PV, Stenfeldt C, Rodriguez LL, King DP, Knowles NJ, Bachanek-Bankowska K, Long NT, Dung DH, Arzt J. 2018. A traditional evolutionary history of foot-and-mouth disease viruses in Southeast Asia challenged by analyses of non-structural protein coding sequences. Sci Rep 8:6472. https://doi.org/10.1038/s41598-018-24870-6.
6. Stenfeldt C, Pacheco JM, Smoliga GR, Bishop E, Pauszek SJ, Hartwig EJ, Rodriguez LL, Arzt J. 2016. Detection of foot-and-mouth disease virus RNA and capsid protein in lymphoid tissues of convalescent pigs does not indicate existence of a carrier state. Transbound Emerg Dis 63:152–164. https://doi.org/10.1111/tbed.12235.
7. Pacheco JM, Arzt J, Rodriguez LL. 2010. Early events in the pathogenesis of foot-and-mouth disease in cattle after controlled aerosol exposure. Vet J 183:46–53. https://doi.org/10.1016/j.tvjl.2008.08.023.
8. Palinski RM, Bertram MR, Vu LT, Pauszek SJ, Hartwig EJ, Smoliga GR, Stenfeldt C, Fish IH, Hoang BH, Phuong NT, Hung VV, Vu PP, Dung NK, Dong PV, Tien NN, Tho ND, Dung DH, Arzt J. 2019. First genome sequence of foot-and-mouth disease virus serotype O sublineage Ind2001e from southern Vietnam. Microbiol Resour Announc 8:e01424-18. https://doi.org/10.1128/MRA.01424-18.
9. Bertram MR, Palinski RM, Ranjan R, Biswal JK, Pauszek SJ, Hartwig EJ, Smoliga GR, Fish IH, Vierra D, Subramaniam S, Mohapatra JK, Das B, Pattnaik B, Arzt J. 2019. Genome sequences of 18 foot-and-mouth disease virus outbreak strains of serotype O sublineage Ind2001d from India, 2013 to 2014. Microbiol Resour Announc 8:e00776-19. https://doi.org/10.1128/MRA.00776-19.
10. Xu L, Hurtle W, Rowland JM, Casteran KA, Bucko SM, Grau FR, Valdazo-González B, Knowles NJ, King DP, Beckham TR, McIntosh MT. 2013. Development of a universal RT-PCR for amplifying and sequencing the leader and capsid-coding region of foot-and-mouth disease virus. J Virol Methods 189:70–76. https://doi.org/10.1016/j.jviromet.2013.01.009.
11. Carrillo C, Tulman ER, Delhon G, Lu Z, Carreno A, Vagnozzi A, Kutish GF, Rock DL. 2005. Comparative genomics of foot-and-mouth disease virus. J Virol 79:6487–6504. https://doi.org/10.1128/JVI.79.10.6487-6504.2005.
12. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B. 2015. RDP4: detection and analysis of recombination patterns in virus genomes. Virus Evol 1:vev003. https://doi.org/10.1093/ve/vev003.