AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements™

VIRUSES

Genome Sequences of Seven Foot-and-Mouth Disease Virus Isolates Collected from Serial Samples from One Persistently Infected Carrier Cow in Vietnam

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ABSTRACT Several foot-and-mouth disease virus (FMDV) carrier cattle were identified in Vietnam by the recovery of infectious virus from oropharyngeal fluid. This report contains the first near-complete genome sequences of seven viruses from sequential samples from one carrier animal collected over the course of 1 year. The characterization of within-host viral evolution has implications for FMDV control strategies.

Received 10 July 2017 **Accepted** 12 July 2017 **Published** 24 August 2017

Citation Pauszek SJ, Bertram MR, Vu LT, Hartwig EJ, Smoliga GR, Brito B, Stenfeldt C, VanderWaal K, Fish IH, Hung VV, Phuong NT, Hoang BH, Rodriguez LL, Dung DH, Arzt J. 2017. Genome sequences of seven foot-and-mouth disease virus isolates collected from serial samples from one persistently infected carrier cow in Vietnam. Genome Announc 5:e00849-17. https://doi.org/10.1128/genomeA.00849-17.

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Foot-and-mouth disease virus (FMDV; *Aphthovirus*, *Picornaviridae*) causes one of the most important transboundary livestock diseases. Acute FMD is characterized by the formation of characteristic vesicles on the feet and in the oral cavity (1–3). Following acute infection, a large proportion of FMDV-infected ruminants become persistently infected carriers, defined by the detection of FMDV in oropharyngeal fluid (OPF) beyond 28 days postinfection (4, 5). Transmission from carrier cattle to naive cattle has not been convincingly demonstrated (6–9). However, depopulation, quarantine, and trade restrictions are imposed subsequent to FMD outbreaks, due largely to the perceived risk of transmission from carrier animals (10–12).

In a recent study in Vietnam, FMDV O/ME-SA/PanAsia was the lineage most frequently recovered from persistently infected animals, and it was the most common lineage circulating in the region during the study (13, 14). Analysis of the VP1 sequences of serial isolates from persistently infected animals demonstrated genetic divergence between viruses isolated from individual animals over the course of 1 year (13, 14). However, the VP1 region covers only about 7.5% of the FMDV genome (15), and analysis of the whole genome is required for a more detailed elucidation of evolutionary changes that occur during persistent infection.

The viruses described herein, O/VIT/366/2012_pro, O/VIT/383/2012_pro, O/VIT/407/2012_pro, O/VIT/414/2012_pro, O/VIT/428/2013_pro, O/VIT/431/2013_pro, and O/VIT/433/2013_pro, were isolated from OPF samples collected from one FMDV carrier cow (*Bos indicus*) in Long An Province, Vietnam, between 7 June 2012 and 17 June 2013. All isolates belong to the O/ME-SA/PanAsia lineage. There was no clinical or molecular evidence of incursion of novel FMDV in the herd during the study period, and the most recent outbreak in the herd was reported to have occurred in 2010. Virus isolation was

achieved from all samples by a single passage in LFBK-$\alpha_V\beta_6$ cell culture (16), as previously described (17). Viral RNA was extracted, and the partial 5′ untranslated region (UTR), complete open reading frame (ORF), and partial 3′ UTR were covered with three overlapping reverse transcription-PCR (RT-PCR) amplicons and were Sanger sequenced, as previously described (18). Chromatogram analysis and consensus sequence identification were performed with Sequencher version 5.4.6, as previously described (19). These sequences code for the complete 6,999-nucleotide (nt) ORF, 115 to 116 nt in the 5′ UTR, and 27 to 45 nt in the 3′ UTR. Among the seven sequences, the pairwise nucleotide and amino acid differences within the ORF region ranged from 26 to 147 nt, and 2 to 19 amino acids (aa), respectively. There was one insertion in the 5′ UTR of the isolate O/VIT/428/2013_pro which was not observed in temporally subsequent isolates. Across these samples, 25 sites exhibit nonsynonymous substitutions. These observations suggest a dynamically evolving viral population in carrier animals.

To our knowledge, this is the first report of near-complete sequences of FMDVs isolated from sequential samples obtained from one persistently infected individual under natural conditions. The characterization of these viruses provides insights into within-host evolution of FMDV during persistent infection and has implications for FMD control in areas that are endemic for the disease. Further characterization of FMDV evolution within carriers will help clarify the role of persistently infected animals in FMD epidemiology and this potential source of outbreaks and emergence of novel viral strains.

Accession number(s). The genome nucleotide sequences of O/VIT/366/2012_pro, O/VIT/383/2012_pro, O/VIT/407/2012_pro, O/VIT/414/2012_pro, O/VIT/428/2013_pro, O/VIT/431/2013_pro, and O/VIT/433/2013_pro described herein have been deposited in GenBank under the accession numbers MF143572 to MF143578, respectively. The versions described in this paper are the first versions, MF143572.1 to MF143578.1.

ACKNOWLEDGMENTS

This research was funded in part by ARS-CRIS project 1940-32000-061-00D. Additional funding was provided by the Cooperative Biological Engagement Program of the U.S. Department of Defense, Defense Threat Reduction Agency.

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