Complete Genome Sequences of Three African Foot-and-Mouth Disease Viruses from Clinical Samples Isolated in 2009 and 2010

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The complete genome sequences of three foot-and-mouth disease viruses (one virus of each serotype SAT1, SAT2 and O) were directly sequenced from RNA extracted from clinical bovine samples, demonstrating the feasibility of full-genome sequencing from strong positive samples taken from symptomatic animals.

In Africa, foot-and-mouth disease (FMD) is a primary livestock disease of economic importance, affecting wild and domestic cloven-hoofed animals (1). The etiologic agent, FMD virus (FMDV) (Picornaviridae, Aphthovirus), exists in seven distinct serotypes (Euro-Asian serotypes O, A, C, and Asia 1, and South African territories [SAT] 1 to 3), with multiple subtypes (2).

To evaluate the feasibility of complete genome sequencing from clinical samples using unbiased RNA sequencing (RNA-seq) methods, three FMDV strongly positive epithelial samples from symptomatic cattle were selected using real-time reverse transcription-PCR (RT-PCR) (3). From these samples, originating from Zambia and Namibia, three FMDV isolates (SAT2/ZAM18/2009, O/ZAM14/2010, and SAT1/NAM01/2010) were obtained and characterized (using virus isolation, antigen enzyme-linked immunosorbent assay [ELISA], and partial sequencing). The original clinical samples were homogenized in phosphate-buffered saline (10% [wt/vol]), pretreated by 0.45 M phosphate-buffered saline (PBS) containing 0.45 M poly(C) tract and a 110-nt gap centered around contig position 6,000) were closed using PCR amplification and Sanger sequencing, with average coverages of 2,151× and 732×, respectively. These FMDV genomes contain a single open reading frame (ORF) of 7,008 (SAT2/ZAM18/2009) and 6,999 nucleotides (nt) (O/ZAM14/2010) encoding a polypeptide precursor protein, and they share high nucleotide homology with AF540910, HM191257, and AY593842 using GATU (8).

The complete genome sequences of SAT2/ZAM18/2009 and O/ZAM14/2010 were obtained, with average coverages of 2,151× and 732×, respectively. These FMDV genomes contain a single open reading frame (ORF) of 7,008 (SAT2/ZAM18/2009) and 6,999 nucleotides (nt) (O/ZAM14/2010) encoding a polypeptide precursor protein, and they share high nucleotide homology with AF540910 and HM191257, respectively. The contig representing SAT1/NAM01/2010 contains a single 7,020-nt ORF (polypeptide precursor protein) and shares a high nucleotide homology with AY593842. As only a limited number of FMDV reads were available for the latter sample, two gaps (a 67-nt gap around the poly(C) tract and a 110-nt gap centered around contig position 6000) were closed using PCR amplification and Sanger sequencing, while the average coverage was <10×.

These data demonstrate the feasibility of direct sequencing of complete FMDV coding sequences from samples from symptomatic animals (real-time RT-PCR threshold cycle [Ct] range of 14.63 to 16.18 for the samples used in this study) using an unbiased cDNA sequencing approach. However, targeted approaches using FMDV-specific cDNA synthesis primers (9) or PCR amplification may result in a better sensitivity for whole-genome sequencing.

Nucleotide sequence accession numbers. The complete coding sequences for SAT2/ZAM18/2009, O/ZAM14/2010, and SAT1/NAM01/2010 were assigned DDBJ/EMBL/GenBank accession numbers KU821590 to KU821592.

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