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VIRUSES

Sequence of Reston Virus Isolate AZ-1435, an Ebolavirus Isolate Obtained during the 1989–1990 Reston Virus Epizootic in the United States

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ABSTRACT Reston virus (RESTV) was discovered in 1989–1990 during three connected epizootics of highly lethal viral hemorrhagic fever among captive macaques in primate housing facilities in the United States and Philippines. Currently, only one RESTV isolate from that outbreak (named Pennsylvania) has been sequenced. Here, we report the sequence of a second isolate, Reston virus/M.fascicularis-tc/USA/1990/Philippines89-AZ1435.

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Reston virus (RESTV; *Filoviridae*: *Ebolavirus*: *Reston ebolavirus* [1]) was discovered in 1989–1990, during a highly lethal viral hemorrhagic fever epizootic among crab-eating macaques (*Macaca fascicularis*) in primate-holding facilities in Reston, Virginia (1989 and 1990), Philadelphia, Pennsylvania (1989), and Alice, Texas (1989), USA. The affected animals were traced to a single primate export facility in the Philippines (2–5). RESTV reemerged in crab-eating macaques imported from the same facility in 1992 in Siena, Italy (6, 7), and in 1996 in Alice, Texas, USA (8–11), and was found in sick domestic pigs (*Sus scrofa*) and unspecified nonhuman primates in the Philippines in 2008 (12) and 2015 (13), respectively.

RESTV isolate sequence data are sparse (14), and only a single complete (15) genome (Reston virus/M.fascicularis-tc/USA/1989/Philippines89-Pennsylvania; RefSeq no. NC_004161 [16]) is available from the 1989–1990 epizootic. We sequenced RESTV AZ-1435, an isolate obtained from the spleen of a crab-eating macaque with signs of respiratory infection sampled during the 1990 Reston, Virginia, USA, episode of the 1989–1990 outbreak (17, 18). RESTV AZ-1435 was isolated by inoculating MA-104 cells with 10% spleen homogenate, followed by passage of MA-104 cell supernatant onto Vero E6 cells (17). We further passaged this sample by infecting Vero E6 cells and harvesting supernatant.

The virus was inactivated with Trizol LS (Thermo Fisher Scientific), and RNA was extracted as described previously (19). rRNA removal (low-input Ribo-Zero, Illumina) was performed prior to cDNA synthesis. cDNA libraries for sequencing were prepared using the Ovation RNA-Seq version 2 system (NuGEN Technologies) and the Ultralow DR (directional read) multiplex system (NuGEN Technologies) following the manufacturers' recommendations. After single-primer isothermal amplification (SPIA, NuGEN Technologies), cDNA was purified using Agencourt RNAClean XP beads (Beckman Coulter, Inc. Life Sciences), sheared, and sequenced via paired-end (2 × 100 bp) in rapid chemistry mode on a HiSeq 2500 sequencing system (Illumina). SMARTer rapid ampli-

fication of cDNA ends (RACE) 5′/3′ kit (Clontech Laboratories, Inc.) was used to amplify the 5′ end of RNA. The 3′ end was amplified by adding adapter oligonucleotides with T4 RNA ligase I (New England BioLabs) and reverse transcriptase (RT)-PCR. RT-PCR products were cloned into pGEM T-easy vectors (Promega) for sequencing.

Trimmomatic (20), was used to trim reads for quality and length prior to assembly. Read quality was assessed pre- and post-trimming using open source FastQC version 0.11.5 software (Babraham Bioinformatics, http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Reads were mapped to the RESTV reference genome (RefSeq no. NC_004161) using the Burrows–Wheeler aligner MEM (BWA-MEM) algorithm (21). Variants were identified using genome analysis toolkit (GATK) best practices (22, 23) and were manually inspected with Integrative Genomics Viewer (IGV) (24, 25). A final sequence was identified by comparison to NC_004161.

The RESTV AZ-1435 genome differs from the Pennsylvania genome at few locations, supporting previous investigations indicating that the 1989–1990 U.S. episodes were directly connected. Our data support the hypothesis that both RESTV isolates share an ancestor virus that was exported to all affected U.S. locations.

Accession number(s). The GenBank accession number of RESTV AZ-1435, now designated Reston virus/M.fascicularis-tc/USA/1989/Philippines89-AZ1435 based on current filovirus nomenclature standards (26), is KY008770.

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