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GENOME SEQUENCES

Coding-Complete Genome Sequences of Emerging Rabbit Hemorrhagic Disease Virus Type 2 Isolates Detected in 2020 in the United States

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ABSTRACT Five rabbit hemorrhagic disease virus type 2 (RHDV2) coding-complete genome sequences were obtained from the livers of domestic and wild rabbits during the 2020 outbreak in the United States. These represent the first available RHDV2 sequences from the United States.

Rabbit hemorrhagic disease (RHD) is a highly contagious disease affecting rabbits. Rabbit hemorrhagic disease virus (RHDV), the causative agent of RHD, is a calicivirus of the genus *Lagovirus* that targets the liver and results in high mortality. RHDV consists of a 7.4-kb positive-sense single-stranded RNA genome encoding two open reading frames (ORF). ORF1 encodes a single polyprotein which is cleaved into nonstructural proteins and the major capsid protein, VP60, while ORF2 encodes a minor structural protein, VP10 (1).

RHDV2, the only RHDV capable of causing disease in domestic and wild lagomorphs, was first detected in France in 2010 (2). The initial detection of RHDV2 in North America was in Quebec, Canada, in 2016. Sporadic, localized outbreaks of RHDV2 in the United States were detected in Ohio in 2018, Washington State in 2019 (domestic pets and farmed and feral animals), and New York in 2020. In the southwestern United States, the virus was first detected in March 2020 in New Mexico and has subsequently spread through Arizona, Texas, Colorado, Nevada, California, and, most recently, Utah. This was the first time that the disease had been detected in wild rabbits in the United States (3, 4).

Total genomic DNA/RNA was extracted directly from liver homogenates of dead rabbits from New Mexico, Arizona, Texas, and New York (Table 1), submitted to the Foreign Animal Disease Diagnostic Laboratory (FADDL) at the Plum Island Animal Disease Center in New York, and sequenced as described elsewhere (5). In brief, nucleic acid extraction was done using the MagMaxPath DNA/RNA kit (Applied Biosystems). First-strand synthesis was performed using SuperScript III reverse transcriptase (Invitrogen) with random primers, followed by second-strand synthesis with Sequenase v2 (Affymetrix) and amplification with *Taq* polymerase (Clontech). Genome sequencing was done on an Illumina MiSeq system using the Nextera XT library preparation kit and the 500-cycle v2 sequencing kit (Illumina).

A custom reference-guided assembly pipeline (Python v2.7.8) was used to produce the consensus sequences from reads passing the default Illumina quality trimming parameter, and the resulting FASTQ files were assembled using a previously resolved RHDV2 genome provided by the Canadian Food Inspection Agency as the reference scaffold. The custom pipeline used Burrows-Wheeler Aligner v0.7.12 (6) for initial reference-guided assembly and SAMtools and BCFtools v1.1 for processing and consensus assembly.

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TABLE 1 Data for the sequences in this report

Isolate	Location	Date of collection[a]	Infected species	Total no. of RHD reads	Total no. of all reads	Avg coverage (fold)	GC content (%)	GenBank accession no.	SRA accession no.	Genome length (nt)[b]
RHDV2/Apr2020/TX1	Levelland, TX	April 2	Domestic rabbit (*Oryctolagus cuniculus*)	12,794	1,411,158	348.6	50.9	MT506233.1	SAMN15946739	7,369
RHDV2/Mar2020/NM1	Candy Kitchen, NM	March 23	Domestic rabbit (*Oryctolagus cuniculus*)	198,523	1,425,743	3,260	50.7	MT506234.1	SAMN15946740	7,369
RHDV2/Mar2020/NY2	New York City, NY	March 2	Domestic rabbit (*Oryctolagus cuniculus*)	162,005	226,226	2,790.7	50.9	MT506235.1	SAMN15946741	7,369
RHDV2/Mar2020/NY1	New York City, NY	March 2	Domestic rabbit (*Oryctolagus cuniculus*)	1,360,442	1,504,926	7,442.77	50.8	MT506236.1	SAMN15946742	7,369
RHDV2/Apr2020/AZ1	Cochise County, AZ	April 1	Cottontail rabbit (*Sylvilagus* sp.), not otherwise specified	486,749	1,236,554	6,434.6	50.9	MT506237.1	SAMN15946743	7,369

[a] All in 2020.
[b] nt, nucleotides.

The five sequences displayed the same genomic length and organization and similar GC content (Table 1), sharing >99.46% identity. The closest BLASTn match for the sequences was a genome from Spain (GenBank accession number KP129398.1) with up to 93.05% identity for the cases from New Mexico, Arizona, and Texas. The New York sequences shared up to 93.15% identity with a genome from Spain (GenBank accession number KM878681.1). A phylogenetic tree (not shown) showed that the genomes from New Mexico, Arizona, and Texas formed a single genetic cluster distinct from the New York viral sequences, potentially indicating distinct incursion events of the virus into the United States.

RHDV2 is a high-consequence animal pathogen in the United States, where transboundary movements of domestic and wild rabbits present a risk of transmission. These sequences will allow for rapid characterization of RHDV2 and quick phylogenetic analysis, facilitating future characterization for vaccine and diagnostic development.

Data availability. The RHDV2 genome sequences in this report have been deposited in GenBank under the accession numbers MT506233.1, MT506234.1, MT506235.1, MT506236.1, and MT506237.1. The raw data are available in the Sequence Read Archive (SRA) under the accession numbers SAMN15946739, SAMN15946740, SAMN15946741, SAMN15946742, and SAMN15946743.

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