Complete Genomic Sequences of Rabbit Hemorrhagic Disease Virus G1 Strains Isolated in the European Rabbit Original Range

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The complete genomic sequences of rabbit hemorrhagic disease virus (RHDV) strains isolated in 1995 (CB137) and 2006 (CB194) from wild European rabbits from Portugal are described. The strains were isolated in the original range of the European rabbit and assigned to genogroup 1 (G1), which is known to have persisted only in the Iberian Peninsula. ORF2 of isolate CB137 might encode a shorter minor structural protein, VP10.

Rabbit hemorrhagic disease virus (RHDV) belongs to the genus *Lagovirus* of the family *Caliciviridae* and causes high mortality rates in both domestic and wild adult European rabbits. The European rabbit originated in the Iberian Peninsula, where two subspecies, *Oryctolagus cuniculus algirus* and *O. cuniculus cuniculus*, coexist, the former being restricted to the southwest region of the Peninsula and the latter being distributed worldwide, mostly through human-mediated dispersal (6). Both subspecies are fatally susceptible to RHDV (10) despite their known genetic differences (6). The evolution of RHDV seems to follow a chronological rather than geographical pattern (8), and six genogroups (G1 to G6) have been defined. However, while in other European countries genogroups have been successively replaced, in the Iberian Peninsula only G1 strains have been isolated, even among contemporary strains (4, 10).

The genome of RHDV consists of a positive-sense single-stranded RNA molecule of 7,437 nucleotides (nt) organized into two slightly overlapping open reading frames (ORF): ORF1, from nucleotide 10 to 7044, and ORF2, from nucleotide 7025 to 7378 (9). ORF1 encodes a large polyprotein which by cleavage produces a major structural protein (VP60), the capsid protein, and several nonstructural proteins, including a helicase, a protease, and an RNA-dependent RNA polymerase, and ORF2 encodes a minor structural protein (VP10).

The complete genome was amplified and sequenced using RNA extracted with an RNeasy minikit (Qiagen) from liver samples of dead European rabbits collected in Portugal within the area of distribution of *O. cuniculus algirus*. Upon necropsy, the animals presented histopathological lesions compatible with rabbit hemorrhagic disease (reviewed in reference 3). cDNA was synthesized using oligo(dT) as primers and SuperScript III reverse transcriptase (Invitrogen). Full-genome amplification was achieved with a combination of eight pairs of primers designed based on publicly available sequences. After purification, PCR products were sequenced on an ABI Prism 310 genetic analyzer (PE Applied Biosystems).

At the nucleotide level, the strains have the highest homology (94 to 97%) to strains that had been assigned to genogroup G1 and the lowest (88 to 92%) to strains of genogroup G6 (RHDVa). Identity with the nonpathogenic RCV-like strains MRCV and RCV-A1 (1) ranges between 78 to 80%. For CB137, a nucleotide substitution at position 7026 (ACG for ATG) disrupts the initiation codon of ORF2. The closest in-frame ATG is located at position 7037 and might lead to the production of a shorter VP10 protein. This feature has never been reported for other pathogenic RHDV strains but was observed in MRCV (5). No evidence of recombination was found for these strains, although it has been documented for RHDV (2, 7).

Nucleotide sequence accession numbers. The full-genome sequences of CB194 and CB137 are available in GenBank under accession numbers [JX886001](#) and [JX886002](#).

ACKNOWLEDGMENTS

The Portuguese Foundation for Science and Technology (FCT) supported the doctoral fellowship of Ana M. Lopes (SFRH/BD/78738/2011) and the postdoctoral fellowships of Joana Abrantes (SFRH/BPD/73512/2010) and Pedro J. Esteves (SFRH/BPD/27021/2006). The FCT Project PTDC/CVT/108490/2008 supported this work.

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Received 1 October 2012 Accepted 2 October 2012

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[doi:10.1128/JVI.02683-12](https://doi.org/10.1128/JVI.02683-12)