



First Complete Genome Sequence of a Hare Calicivirus Strain Isolated from *Lepus europaeus*

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ABSTRACT The first full-genome sequence of a hare calicivirus (HaCV), recently characterized as a novel member of the *Caliciviridae*, is described. This presumed nonpathogenic lagovirus is 7,433 nucleotides long, shows the same genomic organization as that of other lagoviruses, and has the highest nucleotide identity (79%) with pathogenic European brown hare syndrome viruses.

The *Caliciviridae* family comprises five assigned genera, *Norovirus*, *Vesivirus*, *Sapovirus*, *Lagovirus*, and *Nebovirus* (1). The *Lagovirus* genus contains a single species, named *Lagovirus europaeus*, which infects leporids. Its isolates are divided into two genogroups, with GI corresponding to rabbit hemorrhagic disease virus (RHDV)-related viruses, and GII. GII contains the pathogenic European brown hare syndrome virus (EBHSV, GII.1 genotype) and the hare calicivirus (HaCV), an unassigned genotype because still based on a single available capsid protein (VP60) sequence (2). HaCV, first detected in 2014 from healthy hares (*Lepus europaeus*) born and reared in an Italian farm, is presumed to be nonpathogenic (3).

In 2015 in France, HaCV strain E15-431 was detected by reverse transcription-PCR (RT-PCR) using PCR primers located in conserved regions for lagoviruses within the VP60 gene (4), in a hunted wild hare collected as part of the ECALEP European project. This project aimed at understanding the origin of pathogenic lagoviruses (5). Viral RNA was extracted from a piece of duodenum (tissue and content) and was reverse transcribed to cDNA using oligo(dT) and Maxima reverse transcriptase (Thermo Scientific). The genome was PCR amplified with the Expand high-fidelity PCR system (Roche) using combinations of primers designed in conserved regions of the lagovirus genomes or by using a genome-walking strategy. Eight overlapping fragments between 800 and 3,000 bp in length were generated. They covered the entire coding sequence. PCR products were sequenced with an Applied Biosystems 3130 Sanger-based genetic analyzer in both directions, and the consensus sequence was compiled using Vector NTI Advance 11. To confirm the obtained sequence, the amplification process was repeated but this time using primer pairs designed on the consensus. Five overlapping fragments were generated of approximately 2,000 bp, and these were again sequenced in both directions using Sanger technology. The genome extremities were acquired using the rapid amplification of cDNA ends (RACE) method (6). The complete genome sequence was compiled using Vector NTI Advance 11. Pairwise distance analysis was performed using the MEGA7 software (7).

The E15-431 genome sequence is 7,433 nucleotides (nt) in length, excluding the poly(A) tail. BLAST analysis of the entire genome shows that the most closely related viruses are the GII.1 lagoviruses, with 79% identity with the four characterized GII.1 entire genomes (GenBank accession numbers [KC832838](#), [KC832839](#), [Z69620](#), and [MF356366](#)). The nearest GI lagovirus shows 73% identity (GenBank accession number [KX357675](#)). The genomic RNA is organized into two open reading frames (ORFs). ORF1 is 7,008 nt long and encodes a 2,335-amino-acid (aa)-long protein, and ORF2 is 345 nt long and encodes a

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114-aa-long protein. The length of the 5' untranslated region (UTR) is 9 nt, and that of the 3' UTR is 79 nt, excluding the poly(A) tail. The deduced ORF1 protein sequence was compared with a GII.1 polyprotein sequence for which cleavage sites have been experimentally described (8). From this comparison, it was clear that the deduced ORF1 of HaCV had the same order of nonstructural and structural proteins obtained after the proteolytic processing of the polyprotein. The nonstructural proteins p16, p23, helicase, p29, VPg, 3C-like protease, and RNA-dependent RNA polymerase (RdRp) are 139, 224, 349, 275, 115, 143, and 516 aa long, respectively. The major structural protein VP60 is 574 aa long. However, one out of the seven cleavage sites is different, since the E¹²⁵¹-T¹²⁵² site is replaced by E¹²⁴⁵-N¹²⁴⁶, as reported for some GII.1 sequences (9, 10). In addition, the comparison of the E15-431 polyprotein with the four GII.1 ones shows a single amino acid insertion in HaCV p16. ORF2 encodes a protein similar to the GII.1 minor structural protein VP10. Pairwise distance analysis with the available HaCV VP60 sequence (GenBank accession number [KR230102](#)) shows 84.7% nucleotide identity, underlying the high genetic distance between the two HaCV sequences. This study adds a new genomic sequence to the few sequences available on GII lagoviruses and will facilitate research on the phylogenetic relationships of lagoviruses and on the origin of their pathogenicity.

Data availability. The full-genome sequence of HaCV E15-431 has been deposited in GenBank under the accession number [MH204883](#).

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