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GENOME ANNOUNCEMENT

Complete Genome Sequence of Ikoma Lyssavirus

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Lyssaviruses (family *Rhabdoviridae*) constitute one of the most important groups of viral zoonoses globally. All lyssaviruses cause the disease rabies, an acute progressive encephalitis for which, once symptoms occur, there is no effective cure. Currently available vaccines are highly protective against the predominantly circulating lyssavirus species. Using next-generation sequencing technologies, we have obtained the whole-genome sequence for a novel lyssavirus, Ikoma lyssavirus (IKOV), isolated from an African civet in Tanzania displaying clinical signs of rabies. Genetically, this virus is the most divergent within the genus *Lyssavirus*. Characterization of the genome will help to improve our understanding of lyssavirus diversity and enable investigation into vaccine-induced immunity and protection.

The lyssavirus genome consists of a single-stranded, negative-sense RNA, approximately 12 kb long. The RNA serves as a template for the production of five proteins; nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-dependent RNA polymerase protein (L). The International Committee on Taxonomy of Viruses (ICTV) has classified 12 lyssavirus species, with a further two awaiting classification; Bokeloh bat lyssavirus (BBLV) and Ikoma lyssavirus (IKOV) (1, 2, 4).

IKOV was isolated from the brain of a rabid African civet (*Civettictis civetta*), killed following an unprovoked attack on a child in Tanzania in 2009 (4). Initial phylogenetic analysis of a partial N sequence confirmed that this virus was most closely related to members of the genus *Lyssavirus*, albeit highly divergent. Whole-genome sequencing has now been undertaken to further characterize the isolate. RNA was prepared for next-generation sequencing from brain tissue stored in RNAlater (Ambion). Briefly, TRIzol-extracted viral RNA was depleted of host genomic DNA using RNase-free DNase (Qiagen, United Kingdom), and host rRNA was depleted using Terminator 5′-phosphate-dependent exonuclease (Epicentre Biotechnologies). The RNA was fragmented, and two random-primed cDNA libraries were made and run concurrently using the Roche 454 GS FLX system. The sequencing data were assembled in the GS *de novo* assembly software (Roche). Four viral contigs were identified and aligned with the genomic terminus sequences using Seqman (DNAStar), and the resulting consensus sequence was used in GS Reference Mapper (Roche) to obtain further sequence reads from the original raw data. The total number of assembled viral reads was 2,429, equating to 0.64% of the total reads. Despite the low proportion of viral sequence detected within the total data set, adequate coverage of the entire genome (with the exception of the 3′ untranslated region [UTR]) was obtained. The genomic termini were obtained using methods described previously (3). Briefly, genomic RNA was subjected to ligation by T4 RNA ligase (Promega, Madison, WI) using the manufacturer's instructions. The ligated RNA was subjected to nested PCR using primers located in the 5′ end of the L gene and 3′ end of the N gene. Amplicons were cloned using standard techniques and sequenced. Further Sanger sequencing of noncoding regions and the G gene confirmed the 454 sequence data.

The IKOV genome is 11,902 nucleotides long with 61% to 62.1% sequence identity compared to the other lyssavirus whole-genome sequences. Open reading frame lengths were similar to previously deduced lengths from other lyssavirus species: 3′ UTR, 70 nt; N gene, 1,353 nt; N P region, 66 nt; P gene, 870 nt; P-M region, 74 nt; M gene, 609 nt; M-G region, 209 nt; G gene, 1575 nt; G-L region, 569 nt; L gene, 6381 nt; and 5′ UTR, 126 nt. Analysis of glycoprotein antigenic sites revealed little conservation, suggesting that the currently available vaccine would confer limited or no cross protection. Interestingly, the L-protein has a deletion at residue 2, resulting in the protein being 2,127 residues rather than 2,128 as all other lyssavirus species. The initial and terminal 9 nt are complementary and identical to those in all other lyssaviruses. These data will contribute to our understanding of lyssavirus diversity and evolution and further our knowledge of vaccine-induced immunity and protection.

Nucleotide sequence accession number. The complete genomic sequence of IKOV has been deposited in GenBank under accession number JX193798.

ACKNOWLEDGMENTS

This work was supported by grants from The Department for Environ-

Received 26 June 2012 **Accepted** 27 June 2012

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doi:10.1128/JVI.01628-12

ment, Food and Rural Affairs (SE0424 and SE0427) and by the EU FP7–funded Research Infrastructure Grant European Virus Archive (no. 19 228292).

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