Near-Complete Genome Sequences of Measles Virus Strains from 10 Years of Uganda Country-wide Surveillance

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ABSTRACT
Measles remains a global health challenge despite the availability of a safe and effective vaccine. Sporadic outbreaks of measles virus infections continue in Uganda. We report eight near-complete genome sequences of measles virus strains from Uganda cases from 2011 to 2020, providing useful data for assessing vaccine escape and local/regional transmission.

Measles virus (MV) is classified in the *Measles morbillivirus* species (genus *Morbillivirus*, family *Paramyxoviridae*) and is encoded by a negative-sense single-stranded RNA linear genome of about 16,000 nucleotides (nt). Measles is a very transmissible viral infection and, despite an effective vaccine, MV continues to infect and sicken individuals throughout the world. Disruptions of routine vaccination by the coronavirus disease 2019 (COVID-19) epidemic are resulting in further increases in global measles cases (1). The currently used MV vaccines are based on a genotype A MV isolate from 1954 (2). Continued MV evolution may erode the efficacy of the vaccines currently in use, requiring adjustments in vaccines or vaccination schedules for protection against currently circulating MV strains. Accordingly, full genome sequences of MV strains isolated from contemporary measles cases from all parts of the world are essential for keeping ahead of this pathogen. We have developed a simple amplicon-based sequencing method suitable for the MinION sequencing platform to generate genome sequences of MV strains, which is especially functional in resource-poor settings. Here, we report eight near-complete genome sequences from Uganda cases from 2011 to 2020, addressing the lack of MV full genomes from Uganda and from East Africa.

MV IgM enzyme-linked immunosorbent assay (ELISA)-positive cases (3) were identified by Expanded Programme on Immunization (EPI) MV surveillance activity in Uganda (4), and throat swab samples from IgM-positive cases were cultured on Vero/hSLAM cells (5) for 1 or 2 passages of 5 days. The MV-positive cultures were sequenced using MV-specific genotype B3 amplicon primers that amplify the full-length MV genome (primer sequences are available at https://github.com/mlcotten13/Measles_primers). Briefly, total viral RNA was extracted from cell culture material using QIAamp viral RNA minikits following the manufacturer’s instructions. MV RNA was reverse transcribed using amplicon-specific primers and SuperScript III reverse transcriptase (Invitrogen), followed by PCR amplification using Phusion high-fidelity DNA polymerase (Thermo Fisher Scientific). PCR amplicon products were subjected to MinION sequencing library preparation using the SQK-LSK109 barcoding kit (Oxford Nanopore Technologies) according to the manufacturer’s instructions, followed by sequencing on MinION SpotON R9.4.1 flow cells. The resulting fast5 files were base called and demultiplexed using Oxford Nanopore Technologies Guppy software v.5.0.11 + 2b6dbff (6). Demultiplexed reads for each sample were used for reference-based assembly using minimap2.1 (7) with MVs/California.USA/34.19/[B3] (GenBank accession

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TABLE 1  Sampling details, assembly statistics, and accession numbers for the strains in this study

<table>
<thead>
<tr>
<th>Sample identifier</th>
<th>Strain</th>
<th>BioProject accession no.</th>
<th>SRA accession no.</th>
<th>GenBank accession no.</th>
<th>Location</th>
<th>Collection date (yr-mo-day)</th>
<th>No. of raw reads®</th>
<th>No. of MV-specific reads</th>
<th>Genome length (nt)</th>
<th>GC content (%)</th>
<th>No. of nucleotide differences from reference®</th>
<th>Identity to reference (%)</th>
<th>No. of nucleotide differences from earliest Uganda strain®</th>
<th>Identity to earliest Uganda strain (%)®</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEMVI_11_2</td>
<td>MVi/Kyenjojo.UGA/24.13 [B3]</td>
<td>PRJNA843031</td>
<td>SRX15483626</td>
<td>ON642794</td>
<td>Kyenjojo</td>
<td>2013-6-11</td>
<td>183,718</td>
<td>170,266</td>
<td>15,657</td>
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<td>349</td>
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<tr>
<td>GEMVI_11_3</td>
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<td>PRJNA843031</td>
<td>SRX15483627</td>
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<td>Otuke</td>
<td>2019-3-28</td>
<td>283,310</td>
<td>256,334</td>
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<td>174</td>
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<td>GEMVI_11_4</td>
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<td>ON642796</td>
<td>Kile</td>
<td>2020-1-30</td>
<td>426,125</td>
<td>393,265</td>
<td>15,709</td>
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<td>ON642799</td>
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<td>Homa</td>
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</tbody>
</table>

® Read lengths of 2,000 to 2,500 nt.
® Number of nucleotide differences from the genome of MVs/California.USA/34.19/[B3] (GenBank accession number MT789820).
® Percentage of nucleotide differences from the genome of MVs/California.USA/34.19/[B3] (GenBank accession number MT789820).
® Number of nucleotide differences from the earliest Uganda MV strain (sample identifier GEMVI_12_9) near-complete genome (GenBank accession number ON642797).
® Percentage of nucleotide differences from the earliest Uganda MV strain (sample identifier GEMVI_12_9) near-complete genome (GenBank accession number ON642797).
number MT789820) as the reference sequence. Consensus genome sequences and final sequence checks were determined using Geneious Prime v.2022.1.1 (8). All tools were run with default parameters unless otherwise specified. Differences or ambiguities in the genome sequences were resolved manually by counting coverage with quality-controlled read data. Details for the samples are found in Table 1, including case dates and locations, sequencing metrics, and GenBank and SRA accession numbers for the sequence data.

The eight near-complete MV genome sequences reported here were obtained from Uganda measles cases identified in 2011 to 2020 from locations across the country. Despite small numbers, these eight sequences help address the lack of MV genome sequences from Uganda and from East Africa, where outbreaks of measles occur regularly. The sequences were all classified as genotype B3, the genotype most frequently reported from Africa (9, 10). The MV sequencing primers and method for the MinION platform reported here provide a useful tool for monitoring MV evolution and transmission.

The study was approved by the Uganda Ministry of Health (reference number 105/197/01), the Uganda Virus Research Institute Research and Ethics Committee (reference number GC/127/19/12/740), and the Uganda National Council for Science and Technology (reference number HS2741).

Data availability. The genome sequences described here have been deposited in GenBank with the accession numbers ON642794 to ON642801. The unassembled read data are available in the SRA with the accession numbers SRX15483626 to SRX15483633 under BioProject accession number PRJNA843031 (Table 1). Primer sequences are available at https://github.com/mlcotten13/Measles_primers.

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REFERENCES