Shotgun metagenomic sequencing of the first case of monkeypox virus in Brazil, 2022

Ingra Morales Claro1,2, Camila Malta Romano1, Darlan da Silva Candido1,2,3, Evelyn Lepka de Lima4, José Angelo Lauletta Lindoso1,4,5, Mariana Severo Ramundo1, Filipe Romero Rebelo Moreira2,4, Luiz Alberto Costa Barra1, Luciana Marques Sansão Borges4, Lucas Alberto Medeiros4, Marcia Y. S. Tomishige4, Tomas Moutinho2,1, Anderson José Dias da Silva4, Camila Cristina Martini Rodrigues4, Luiz Cesar Fernandes de Azevedo4, Lucy Santos Vilas-Boas4, Camila Alves Maia da Silva1, Thaís Moura Coletti1, Erika R. Manuli1, Aine O’Toole7, Joshua Quick8, Nicholas Loman8, Andrew Rambaut7, Nuno R. Faria1,2,3, Claudia Figueiredo-Mello4, Ester Cerdeira Sabino1

ABSTRACT

Monkeypox virus (MPXV), a zoonotic virus endemic to the African continent, has been reported in 33 non-endemic countries since May 2022. We report an almost complete genome of the first confirmed case of MPXV in Brazil. Shotgun metagenomic sequencing was completed in 18 hours, from DNA extraction to consensus sequence generation.

KEYWORDS: Viral metagenomics. Monkeypox virus. West African clade. Genomic surveillance. Brazil.

INTRODUCTION

Monkeypox virus (MPXV) is a double-stranded DNA zoonotic virus with a 197-kb genome, member of the Orthopoxvirus (OPV) genus and Poxviridae family, which also includes smallpox virus that causes smallpox. MPXV was first identified in a 9-month-old boy in 1970 in the Democratic Republic of Congo. Since then, several outbreaks of monkeypox have been reported in the African continent, where 1,408 suspected cases and 66 deaths were reported in 2022 alone1,2. MPXV is currently classified into two lineages, the West and Central African clades, although a novel classification into clades 1, 2 and 3 has recently been proposed3. The MPXV Clade 3 includes most human outbreaks from 2017, 2018 and 2022, and can be further divided into lineages A, A.1, A.1.1, and B.13. In early May 2022, cases of MPXV were detected in the UK and Portugal, most of them with no known travel history to endemic countries. As of June 9, 2022, 1,240 cases have been confirmed in 33 countries on all continents4, most of them in European countries and the United States5. Available epidemiological and contact tracing data revealed that most cases are associated with men who have sex with men (MSM)6. The first two MPXV cases from South America were reported from Argentina on May 27, 20226. Both reported cases traveled to Spain 2 and 9 days prior to case notification, and the two MPXV partial genomes (942 bp) were made available as the first monkeypox sequences from Latin America. As of June, 8, 2022, Brazil had 8 suspected monkeypox infections...
in the States of Santa Catarina, Ceará, Mato Grosso do Sul, Rio Grande do Sul, Rondônia and São Paulo.7

Here we report an almost complete genome of the first confirmed case of monkeypox detected in Brazil. A skin swab of the lesions (vesicle and crust) was collected on June 7, 2022, at the Emílio Ribas Institute of Infectious Diseases from a 41-year-old male patient with a recent travel history to Portugal and Spain, and clinical manifestation suggestive of monkeypox signs and symptoms.

Ethical aspects

The authors applied the free and informed consent form that was signed by the patient, authorizing the collection of the biological sample and its use in the sequencing of the complete genome of the virus. In addition, the patient also consented to the sequencing results being released to the scientific community.

MATERIALS AND METHODS

Viral DNA was isolated from 200 μL of the recovered material using the QIAamp Viral DNA Mini Kit (Cat No. 51304, Qiagen, Germany) according to the manufacturer’s instructions and eluting in 60 μL of elution buffer. DNA was quantified using fluorimetry with the Qubit dsDNA High Sensitivity Assay (Cat No. Q32854, Life Technologies, Waltham, USA) on the Qubit 3.0 instrument (Life Technologies, USA). Shotgun metagenomics was performed using 10 ng of the extracted DNA and the Rapid PCR Barcoding kit (SQK-RPB004) - Oxford Nanopore Technologies (ONT, UK), adapted from the Rapid SMART-9N protocol8. PCR products were then purified using a 1:1 ratio of AMPure XP beads (Cat No. A63881, Beckman Coulter, UK) and quantified.

MinION libraries were prepared using an input of 50 ng per sample, pooled in an equimolar way followed by the rapid adapter ligation. The final libraries were loaded onto FLO-MIN106 flow cells on the MinION device (ONT, UK) and sequenced using MinKNOW 1.15.1 with the standard 48-hour run script. FASTQ files were demultiplexed and trimmed using Guppy V5.0.16 1 (ONT, UK), and the barcoded FASTQ files were aligned and mapped to the reference genome (GenBank accession No. MN648051) using minimap2 version 2.28.09 and converted to a sorted BAM file using SAMtools10. NanoStat version 1.1.2 11 was used to compute the number of raw reads and minimum contig length to cover 50 percent of the genome (N50) of the aligned reads. Table 1.19.05.2827 was used for genome visualization and to compute the number of mapped reads, percentage of genome coverage, and coverage depth12.

Variants were detected with medaka_variants and the consensus sequence was built using medaka_consensus (ONT, UK). Genome regions with <20x coverage were masked.

The dataset was aligned to the reference genome using MAFFT version 7-45313. Maximum likelihood analyses of almost complete and complete genome sequences were conducted using IQTree214 under a Jukes Cantor nucleotide substitution model.

RESULTS

Our sequencing run generated a total of 954,284 reads, with 784,000 reads with quality score > 8. The average depth was 277.7x, covering 100% of the viral genome with at least 1 read and an N50 of 4,493. Complete MPXV genome sequence has been submitted to NCBI GenBank accession number ON751962, and raw data can also be found in our project’s dedicated GitHub repository15.

To contextualize the novel monkeypox genome, we downloaded 102 whole-genome sequences from NCBI GenBank (Supplementary Table S1). Of these, 81 were genomes from the ongoing MPXV B.1 multi-country outbreak.

The complete genome sequences revealed that this first case of MPXV in Brazil clustered within the newly proposed B.1 lineage, and was closely related to sequences from Portugal, Germany, USA and Spain (Figure 1). We identified 3 unique SNPs compared to the updated US-CDC genome (Accession No. ON563414.3): GA->AA (10118; non-synonymous), TC->TT (15004; synonymous) and GA->AA (169928; non-synonymous). The G->A and C->T mutations such as those observed in the new Brazilian MPXV genome, may be an effect of APOBEC3 deaminase editing, as previously suggested16.

We then evaluated whether the recommended diagnostic RT-qPCR primers were suitable for the identification of the newly sequenced virus genome2. Investigating putative mismatches in the binding sites of primers and probes for both the generic MPXV and the specific B.1 MPXV detection sets2,16, we found two mutations in the binding regions for forward (T2676C) and reverse (G2608A) primers of the generic set. These mutations are located six and eight nucleotides away from the 3’OH end of the forward and reverse primers, respectively, and are unlikely to affect the sensitivity of this primer set, indicating that existing primers are capable of detecting infections from the current outbreak.

DISCUSSION

Here we describe the genome of the first monkeypox
CONCLUSION

The almost complete genome sequencing of this first Brazilian case of MPXV was crucial to confirm the diagnosis by means of a fast and reliable sequencing technique, to ensure the molecular diagnosis of future cases by PCR and characterize the newly proposed B.1 lineage.

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AUTHORS’ CONTRIBUTIONS

Conceptualization: IMC, CMR, DSC, MSR, FRRM, NRF, and ECS; data curation: IMC, CRM, DSC, FRRM, AOT, NL, AR, NRF, CFM, and ECS; formal analysis: IMC, CMR, DSC, FRRM, AOT, NL, AR, and NRF; funding acquisition: AR, NL, NRF, and ECS; investigation: IMC, CMR, DSC, ELL, JALL, MSR, FRRM, LACB, LMSB, LAM, MYST, TM, AJDS, CCMR, LCFA, LSVB, CAMS, TMC, ERM, AOT, NL, AR, NRF, and ECS; methodology: IMC, CMR, DSC, MSR, FRRM, TM, LSVB, CAMS, TMC, ERM, AOT, NL, AR, and NRF; resources: AR, NL, NRF, CFM, and ECS; validation: IMC, JQ, NL, AR, and NRF; writing (original draft): IMC, CMR, DSC, MSR, NRF; writing (review and editing): IMC, CMR, DSC, JALL, MSR, FRRM, NL, AR, NRF, CFM, and ECS.

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