Genomic epidemiology study of monkeypox virus isolates circulating in Barcelona during the 2022 multi-country outbreak

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Summary

Introduction: Monkeypox virus (MPXV) infection was previously considered a rare zoonotic infection, endemic in West and Central Africa. However, since May 2022, the virus has spread globally, resulting in the first multi-country outbreak without known epidemiological links to endemic regions.
Material and method: This study aimed to describe the viral lineages circulating in Barcelona among cases diagnosed from June to August 2022 by whole genome sequencing.
Results: The tiling amplicon sequencing approach used is a simple methodology that enabled us to obtain complete MPXV genomic sequences in a high percentage of cases. We identified several sublineages of the B.1 variant of MPXV, the predominant outbreak variant worldwide, circulating in Barcelona over the study period.
Conclusion: Genomic surveillance by whole genome sequencing is recommended to track the virus's spread and understand its evolution. This study's findings highlight the global spread of MPXV and the importance of genomic surveillance to detect circulating strains. The tiling amplicon sequencing approach used in this study was a simple and effective method for obtaining complete genomic sequences of MPXV.

Key words:
Monkeypox. MPXV. Human-to-human transmission. Genomic surveillance. Tiling amplicon sequencing. Whole genome sequencing.

Estudio de epidemiología genómica de los aislados del virus de la viruela del mono circulantes en Barcelona durante el brote multipaís de 2022

Resumen

Introducción: La enfermedad causada por el virus del monkeypox (MPXV) anteriormente se consideraba una enfermedad zoonótica rara endémica de África occidental y central. Sin embargo, a partir de mayo de 2022, el virus se extendió globalmente, resultando en el primer brote multipaís sin vínculos epidemiológicos conocidos con las regiones endémicas.
Material y método: El objetivo de este estudio fue describir los linajes virales circulantes en Barcelona entre los casos diagnosticados de junio a agosto de 2022 mediante una técnica de secuenciación de genoma completo basada en amplicones.
Resultados: La metodología de secuenciación empleada nos permitió obtener secuencias genómicas completas de MPXV de una manera sencilla en un alto porcentaje de casos. Se identificaron varios sublinajes de la variante predominante del brote en todo el mundo (linaje B.1), circulando en Barcelona durante el periodo de estudio.
Conclusión: Se recomienda la vigilancia genómica mediante secuenciación de genoma completo para rastrear la propagación del virus y comprender su evolución. Los hallazgos de este estudio destacan la propagación mundial de MPXV y la importancia de la vigilancia genómica para detectar cepas circulantes. La metodología de secuenciación basada en amplicones utilizada en este estudio fue simple y efectiva para la obtención de secuencias genómicas completas de MPXV.

Palabras clave:
Viruela del mono. MPXV. Transmisión de humano a humano. Vigilancia genómica. Secuenciación por amplicones. Secuenciación de genoma completo.

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Introduction

Human monkeypox (mpox) is a disease caused by the monkeypox virus (MPXV), which is closely related to the variola virus (smallpox) and vaccinia virus, within the genus *Orthopoxvirus* of the family *Poxviridae*[1]. Monkeypox infections present with localized maculopapular rash lesions followed by the development of lymphadenopathy, fever, malaise, and pain associated with lesions[2]. Until recently, mpox was considered a rare zoonotic disease endemic in West and Central Africa, although being the most prevalent zoonosis infection caused by this genus in humans after the smallpox eradication in 1980[3]. However, since May 2022, mpox cases have been increasingly growing after the detection of an infected UK resident returning from Nigeria[4], resulting in the first multi-country outbreak without known epidemiological links to such endemic regions. Most cases (>86,000 as of April 2023) have occurred in men who have sex with men (MSM) in the urban areas of several European countries and the USA, with Spain being the third country with the highest number of cases[3]. Specifically, a total of 2134 cases were confirmed in Catalonia (from May to November, 2022)[5]. At the end of July 2022, the WHO recommended strengthening the genomic surveillance of circulating monkeypox strains[2]. Germany and Canada are the leading countries on the number of genomic sequences published, while the genetic diversity of MPXV in Spain is markedly underrepresented in public sequence repositories (62 genomic sequences available in GenBank as of April 2023).

Recently, the WHO announced the new neutral nomenclature suggested for MPXV clades according to Happi *et al.*[6]; Clade I represents the previous "Congo Basin (CB)" Clade, while Clades IIa and IIb represent the previous "West African (WA)" Clade. Additionally, Clade IIb contains a group of genomes from recent 2017-2022 mpox outbreaks, which are entitled "hMPXV1"[6]. MPXV clades IIa and IIb have most commonly been reported in outbreaks occurring from western Cameroon to Sierra Leone, and usually have a low case–fatality ratio (<1%). In contrast, viruses from clade I (mostly found in Central Africa) are considered more virulent with a case–fatality ratio >10%[7].

Since 2017-18, infections outside of Africa have been caused by isolates from clade IIb, despite its reported poorer human-to-human transmission[8]. It has also been suggested that the 2017 MPXV epidemic favored the emergence of a single human strain[9]. The surge in the number of cases among MSM and those without a reported travel history during the ongoing multi-country outbreak is highly suggestive of sustained human-to-human transmission. Accordingly, Happi *et al.*[6] suggested to name this subclade "hMPXV1", and its genome diversity be classified in several sub-lineages, such as A, A.1, A.1.1, B.1 (i.e., A.1.1.1), and so on, to support fine-scale real-time genomic surveillance. The major lineage worldwide in the 2022 outbreak has been B.1, with up to 17 sub-lineages from B.1.1 to B.1.17). Additionally, "A" sub-lineages (A.2, A.2.1, A.2.2, A.2.3, and A.3) have been detected worldwide but in a minor proportion of cases[10].

At the beginning of the outbreak, when no MPXV-targeted WGS assays were available, the use of a virome-sequencing protocol allowed us to obtain the first Spanish draft genome sequence in May 2022 (ON622718.1)[11], albeit providing a low yield for MPXV reads. Since then, a handful of sequencing approaches have been developed swiftly (as reviewed by Chen *et al.*[12]). The most relevant being: i) a commercially available metagenomic strategy with enrichment by capture probes (which captures the target sample and therefore has a better yield than the non-targeted metagenomic approach); ii) a tiling PCR approach (which allows a better genome coverage in samples with a high Ct value); and iii) a directed sequencing approach (or super amplicon technology), which allows to perform PCR in a single tube and is less sensitive to mutations.

Here, we used an amplicon-based whole genome sequencing approach to perform a genomic epidemiology study from a subset of mpox cases from the Northern Metropolitan area of Barcelona and Barcelona city (Spain) with the goal to characterize the isolates circulating during the 2022 outbreak..

Materials and method

Ninety-three clinical samples with a Ct≤26 from the LightMix Modular Monkeypox virus Real-Time PCR assay (Roche Diagnostics, Switzerland) were included. In 92/93 cases, samples consisted of a single skin lesion swab preserved on viral transport media, while 1/93 was a rectal exudate preserved on viral transport media as well.

Nucleic acids, extracted with the automated extraction system Microlab STARlet (Hamilton Medical, Switzerland) using the STARMag Universal Cartridge Kit (Seegen, South Korea), were directly used for MPXV PCR amplification. The whole genome of the virus was amplified adapting the amplicon tiling approach described by Tutu van Furth *et al.*[13], consisting of a total of 88 primer sets divided in two pools, for the amplification of 44 fragments with an average size of 2.5 Kb. Briefly, 2.5 µL of nucleic acids were used for each of the two primer pools. Amplification was performed using Q5™ Hot Start High-Fidelity 2x Master Mix (New England Biolabs, USA) with the following cycling conditions: 30s at 98 ºC, and then 35 cycles for 10 s at 98 ºC and 5 min at 65 ºC. The two pools for each individual sample were

combined, purified using Ampure XP Beads (Beckman Coulter, USA), with a final elution of 18 µL, and quantified using Qubit Flex Fluorometer (Life Technologies, USA).

Sequencing libraries were prepared using the Rapid Barcoding Kit 96 (Oxford Nanopore Technologies, UK), with a starting quantity of 100 ng of DNA for each sample. Then, libraries were pooled, loaded onto R9.4.1 flow cells and sequenced in four independent runs for 72 h on a MinION Mk1C device following manufacturer's instructions. A negative control of the whole process was added for each sequencing run.

Raw sequencing data were analysed using the INSaFLU online platform[14] to obtain consensus genomic sequences. Nextclade (v2.5.0) was used to assess consensus quality and assign MPXV lineages.

Results

A total of 93 samples were included in this study, obtained from 92 males and 1 woman, ages ranging from 17 to 60 years old, with sample collection dates between 2nd June 2022 and 8th September 2022. These specimens represented 37.2% of patients who tested positive in this period at Hospital Germans Trias i Pujol (Barcelona, Spain).

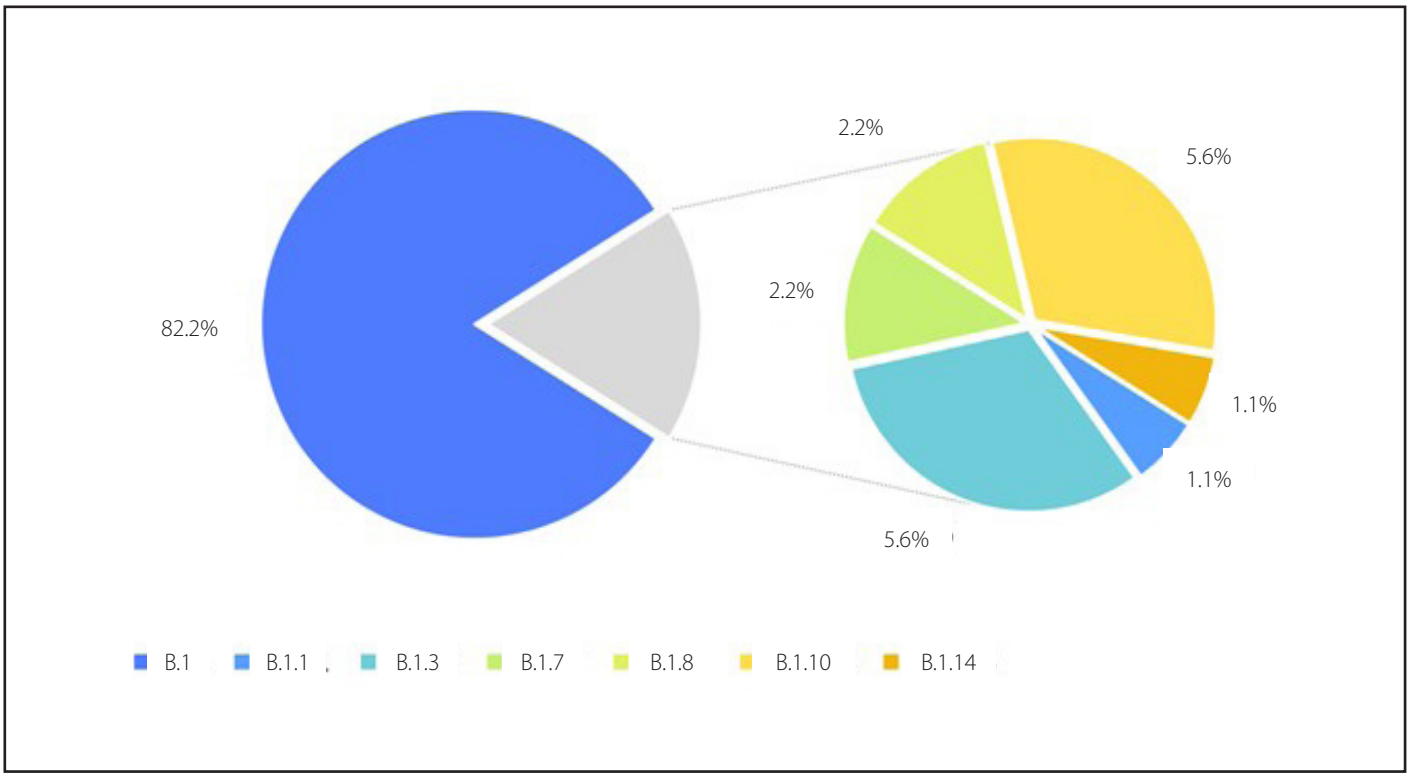
The MPXV genomic sequence was successfully obtained for 90/93 samples, with a mean genome coverage of 97.1% (range, 82.9-99.9%) and a mean sequencing depth of 1043x (IQR, 618.5). The remaining three samples were excluded because their genome coverage was lower.

All the 90 genomic sequences obtained were classified within the major outbreak lineage B.1. More specifically, hMPXV1 lineages identified were B.1 (82.2%), B.1.10 (5.6%), B.1.3 (5.6%), B.1.7 (2.2%), B.1.8 (2.2%), B.1.1 (1.1%) and B.1.14 (1.1%) (Figure 1).

Discussion

This study provides a genomic epidemiology overview from a relatively large subset of samples from mpox cases in the Barcelona greater area during the 2022 international outbreak. The tiling amplicon sequencing approach used enabled us to identify several different lineages, with a similar proportion to that observed in Portugal except for A lineages, which were not detected in this study. Among lineages detected, B.1.10 is the one accounting for most cases in Colombia, and lineages B.1.1, B.1.7 and B.1.14 had not previously been described in Spain. Among the isolates sequenced, we found identical sequences from different individuals, suggesting close epidemiological links through local

Figure 1. Monkeypox virus lineage distribution from the 90 samples analysed from June to August 2022.



community transmission chains. On the other hand, the fact that we have found several different sub-lineages probably suggests that multiple introductions occurred during the outbreak in our area, in agreement with the widespread nature of the outbreak affecting many countries.

Previous studies related to the mpox outbreak suggest that most cases occurred in MSM communities and in an older range age group in comparison with past outbreaks[3]. Our epidemiological data suggests a similar trend, with a broad age range (17 to 60 years old) and nearly all samples being collected from men.

The genetic traits of MPXV have been quite relevant in this outbreak and sequencing is essential for tracking the spread and evolution of viral linages. For starters, MPXV is a double-stranded DNA virus from the *Orthopoxvirus* genus, for which a mutation rate of 1-2 substitutions per genome per year had been described[15]. However at least 46 mutations have been identified when comparing 2022 epidemic outbreak isolates from those previously reported in 2018-19[16]. This large number of mutations might be partially explained by APOBEC3 enzymes, which have been previously described to impact the mutation rate of the virus. APOBEC3 enzymes might induce mutations that do not affect the viability of the virus but produce hypermutate variants with altered characteristics[16]. However, the effect of these mutations is not fully understood. There is ongoing work to better explain whether the observed genomic mutations lead to specific phenotypic changes such as enhanced transmissibility, virulence, immune escape, resistance to antivirals, or reduced impact of countermeasures, therefore further work is needed to understand the appearance of these mutations[2].

This study has some limitations: the lack of in-depth clinical information, that could have been useful to give a more detailed picture of the outbreak in our area, and the fact that we sequenced a subset of all diagnosed cases (44% of the samples with a real-time PCR Ct value <26). However, it must be borne in mind that 40% of all samples received at the laboratory during that period had higher Ct values, which makes whole genome sequencing more difficult.

As mpox cases are still being reported in Spain almost one year later, genomic surveillance may be important to shed light on the origin of possible future outbreaks (imported vs local isolates).

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