Supplementary data for paper:

Monkeypox virus isolation from longitudinal samples in 11 hospitalised patients

1. Authorship

ISARIC4C's Clinical Characterisation Protocol (UK) is a generic protocol for the rapid, coordinated clinical research-based investigation of exposure to pathogens, chemicals, toxins, or potentially harmful energy sources of public health concern. Ethical permission was granted by the South Central—Oxford C Research Ethics Committee in England (Ref 13/SC/0149), the Scotland A Research Ethics Committee (Ref 20/SS/0028), and the WHO Ethics Review Committee (RPC571 and RPC572, 25 April 2013) approved. The protocol is ISRCTN registered (https://doi.org/10.1186/ISRCTN66726260). Further NHS REC permission for the study to be carried out at UKHSA (Ref 22/EE/0258)

ISARIC4C Investigators Clinical Characterisation Protocol UK - Mpox activation

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2. Supplementary data

Supplementary Methods

Mpox diagnosis was on clinical grounds, confirmed by samples taken at presentation (day 0) using a pan-*Orthopoxvirus* real-time polymerase chain reaction (PCR) and an MPXV generic (G2R_G) real-time PCR based on a published assay (1). Once diagnosed, patients were monitored with a pan-*Orthopoxvirus* PCR only.

169 samples in total were included comprising; 131 samples, in which PCR detected *Orthopoxvirus* DNA.

Samples from the same participants (33 throat swabs, lesion swabs, urine, CSF, serum and plasma samples) in which *Orthopoxvirus* DNA was not detected were included as negative controls.

Five additional samples (serum, urine and lesion swabs) that were not previously tested were included. The baseline MPXV DNA Ct value from the clinical diagnosis at day 0 of the study was used for further analysis.

Each sample was inoculated into African green monkey kidney (Vero E6) cells, termed passage 1 (P1). Briefly, 100 μl of sample was diluted with 0.4 ml of Minimum Essential Medium-Glutamax, 5% fetal bovine serum, 4x antimycotic/antibiotic (complete medium), added to T25 cm^2 tissue culture flasks and incubated at 37^{o}C with no CO_2 for 1 hour, followed by the addition of 4.5 mls of complete medium, additionally containing 1x Non-Essential Amino Acids and 25mM HEPES. P1 cultures were incubated for seven days and monitored daily for cytopathic effects (CPE). P1 cultures were sampled on days 0 and 7, and lysed cells from day 7 frozen for further use. P2 cultures were established using 0.5 ml clarified supernatant from P1 day 7 cultures if infectious virus was not recovered. However, as P2 did not show differing results from P1, the authors elected to perform P1 only for subsequent work after preliminary publication (2). Previous work has shown that it is sufficient to perform up to two sub-cultures (P1/P2) to recover infectious MPXV from environmental samples with high PCR Ct values (≤31.6) (3).

Samples from day 0 and day 7 underwent virus inactivation and nucleic acid (NA) extraction using the QIAamp Viral RNA kit (Qiagen), according to manufacturer's instructions. The G2R_G MPXV-specific real-time PCR, multiplexed with MS2 bacteriophage as an internal extraction control, was performed on all samples using the TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems), according to manufacturer's instructions and analysed on an Applied Biosystems 7500 Fast PCR machine.

All work involving clinical samples and recovery of infectious virus was performed in a Containment Level 3 laboratory within a Class III Microbiological Safety Cabinet (UKHSA, Porton Down).

Supplementary Results

Table S1 demonstrates the duration of MPXV DNA detection by PCR and MPXV isolation by cell culture from onset of symptoms, separated by sample type. A cut off Ct <38 was used for sample inclusion.

Table S1. Duration of MPXV detection by PCR and MPXV isolation by sample type

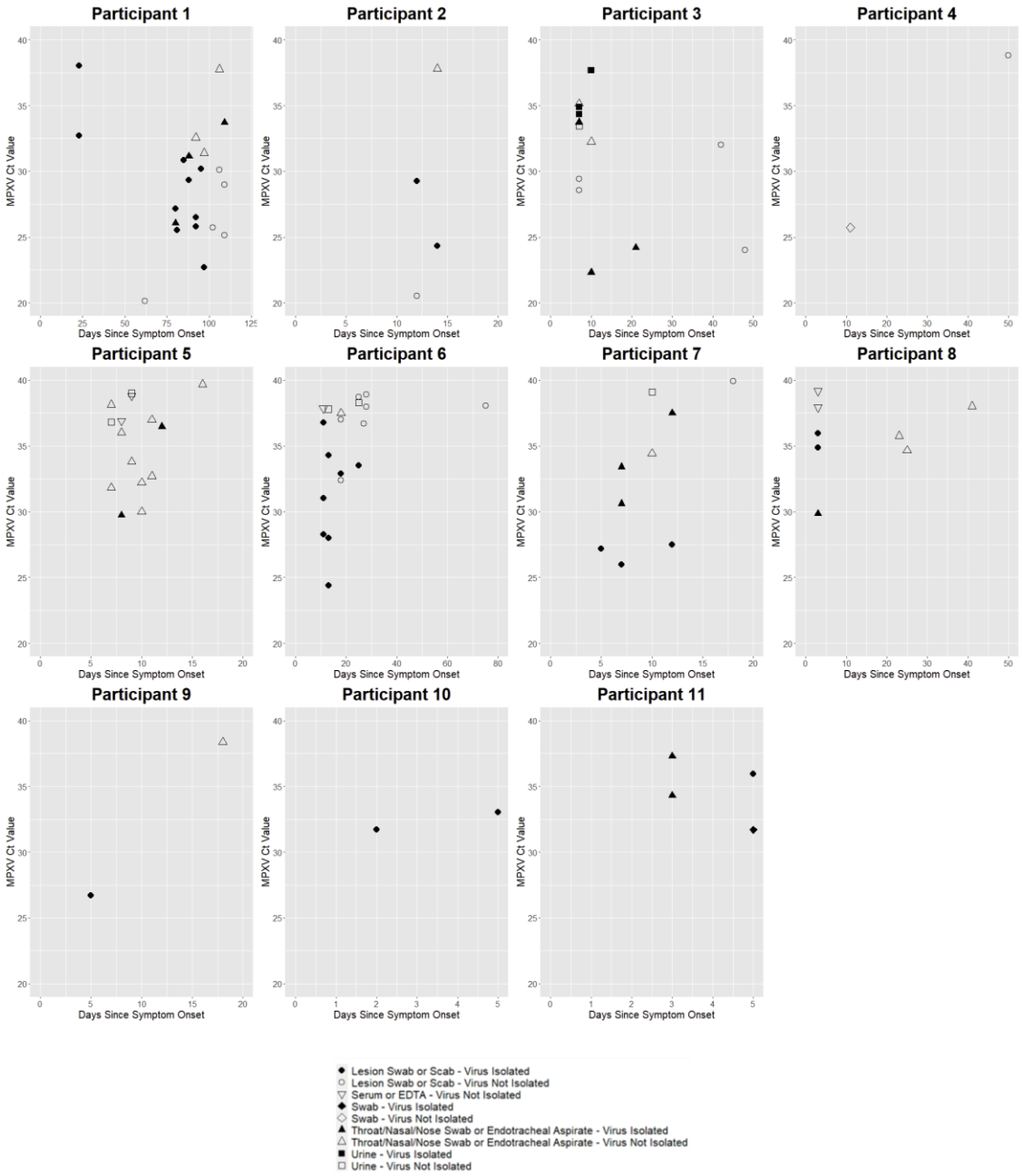
Sample type	Number of samples where MPXV was detected by PCR	Median duration of detection of MPXV by PCR from symptom onset, in days (range)	Number of samples from which MPXV was isolated	Median duration in of MPXV isolation from symptom, in days (range)
Lesion swab or scab	42	18.0 (2 – 109)	29	13.0 (2 – 97)
Throat/nasal/nose swab or endotracheal aspirate	30	10.5 (3 – 109)	10	9.0 (3 – 109)
serum or EDTA	3	8.0 (3 – 11)	0	-
Urine	6	7.0 (7 -13)	3	7.0 (7 – 10)
Swab - unknown source	3	3.0 (3 – 11)	2	3.0*

* These two samples were collected on the same number of days since symptom onset and from the same participant, therefore this value is not a median.

Table S2. Summary of participant characteristics

Patient	Immune status	Recorded sex	Age group (years)	Location of lesions	Duration of lesions	Vaccinated	Antiviral
1	Immunosuppressed – HIV $CD4^+$ T-lymphocyte count 57 cells/mm^3 and HIV RNA viral load of 52 800 copies/mL at presentation	Male	40-50	Limbs and oral cavity	Lesions resolved between 120 and 155 days after onset during outpatient follow up	No	Tecovirimat and cidofovir
2	Immunocompetent	Male	30-40	Face, trunk, limbs, palms, and penile shaft	16 days	No	No
3	Immunocompetent but neonate	Female	0-10	Palms, soles, face and trunk	23 days	No	Tecovirimat and cidofovir
4	Immunocompetent	Male	30-40	Widespread including eye	65 days	No	Tecovirimat and 1% trifluridine topical eye drops
5	Immunocompetent	Male	30-40	Face, trunk, limbs, palms, soles, and scrotum	11 days	No	Brincidofovir
6	Immunocompetent	Male	40-50	Face, scalp, trunk, limbs, penile shaft, palms, and soles	13 days	No	No
7	Immunocompetent	Male	30-40	Face, scalp, trunk, limbs, palms, glans penis, and scrotum	14 days	No	Brincidofovir
8	Immunocompetent	Female	30-40	Face, trunk, hands (including nail bed), and labia majora	13 days	MVA 6 days post exposure, 12 days pre-illness	Brincidofovir
9	Immunocompetent	Female	30-40	Vulva, limbs, hands, torso	20 days	No	Tecovirimat and cidofovir
10	Immunocompetent	Female	30-40	Face, trunk, arms, and hands	5 days	No	Tecovirimat
11	Immunocompetent	Female	0-10	Face, trunk, arms, and legs	16 days	No	None

Figures S1. Cycle threshold value of the MPXV PCR against the number of days since symptom onset.



Supplementary figure S1: On the y-axis, a decrease in Ct value corresponds to an increase in MPXV DNA in the sample. The days since symptom onset is plotted on the x-axis – different axes limits are used for each participant. Different symbols are used to denote each sample type. Circles are used to denote a lesion swab or a scab; a triangle used to denote respiratory samples (throat, nasal or nose swab and endotracheal aspirate); an inverted triangle to denote blood samples (serum or EDTA); a square to denote urine samples, and a diamond to denote a swab. The symbol is filled if MPXV was able to be isolated at day 7 of culture, and not filled if virus could not be isolated.

Sequencing

For MPXV genome sequencing, primary clinical samples were subjected to DNase treatment prior to extraction then directly sequenced using the Illumina DNA prep kit in a 2x150 bp sequencing run on a NextSeq1000 to a depth of ~20M reads per sample. Sequence reads were aligned to NC_063383 using BWA and consensus sequences were generated using an in house programme QuasiBAM. Consensus sequences were aligned using mafft and manually corrected around indels. Single nucleotide changes were visualized using snipit.

Regarding the participant with prolonged MPXV detection, whole genome sequencing was carried out for three samples from the participant with sufficiently low Ct value at various time points during their illness. There were at least 6 weeks between samples and samples were compared between initial and re-presentation. The consensus sequences generated from each sample were compared to identify any single nucleotide differences. The level of diversity within the samples was as expected for an infection of this duration (<5 SNPS). Whilst we cannot rule out reinfection from the same source, given the diversity observed in UK cases at the time, reinfection is unlikely in this case. Sequence data is available on GenBank (accession numbers available on request).

References for supplementary data

1. Yu Li, Hui Zhao, Kimberly Wilkins, Christine Hughes, Inger K. Damon. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. Journal of Virological Methods. 2010 Oct; 169(1):223-227.
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3. Atkinson B, Burton C, Pottage T, Thompson K, Ngabo D, Crook A, et al. Infection-competent monkeypox virus contamination identified in domestic settings following an imported case of monkeypox into the UK. Environ Microbiol. 2022 Oct 20;24(10):4561–9.

3. Funding

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