Monkeypox virus isolation from longitudinal samples in 11 hospitalised patients.

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Monkeypox virus (MPXV) causes mpox, a disease largely confined to West and Central Africa until 2022, when the global spread of MPXV clade IIb was declared a public health emergency of international concern (PHEIC). Another PHEIC was declared in 2024 following the spread of MPXV clade I in Africa. Human transmission occurs mainly through contact with infectious lesions (1). While diagnostic swabs' PCR cycle threshold (Ct) is assumed to correlate with infectiousness, the evidence is weak (2,3). Prolonged MPXV shedding is widely reported, but the duration of infectiousness remains unclear (4–5).

We used the ISARIC WHO Clinical Characterisation Protocol UK to enrol patients admitted to UK hospitals for severe mpox. Seven patients were enrolled from 2018 to 2021 and four in 2022, with a median age of 35 years (range <1-48 years), seven male and four female. One patient was living with HIV. 169 samples were collected,

including lesion swabs, throat swabs, scabs, cerebrospinal fluid, endotracheal aspirates, plasma, and urine. The samples were taken from 13 days before symptom onset to 109 days after onset (supplementary Figure S1, patient 1).

The samples were inoculated into African Green Monkey Kidney (Vero E6) cells, incubated, and monitored for cytopathic effects (CPE). MPXV PCR of culture supernatant on days 0 and 7 confirmed CPE observations if there was a decrease in Ct. All MPXV detected were clade II. 99 samples were positive for MPXV by PCR on day 0 (Ct range 20.1-39.9), and virus was isolated from 47 samples (47.5%). The median Ct value for which MPXV could be isolated was 31.01 (range 22.3 – 38.0).

The longest duration of culture positivity was 109 days, observed in a throat swab with an MPXV Ct of 33.7 from a man with poorly controlled HIV (CD4+ count of 57 cells/mm3 and an HIV viral load of 52,800 copies/mL). He presented with an ulcerating limb rash and a necrotic tongue lesion, was treated with tecovirimat, and was discharged, only to return two weeks later with worsening symptoms (6). Sequencing confirmed a prolonged infection rather than reinfection (see supplementary material). Since his disease was influenced by immune response and antiviral treatment, his data was included in Figure 1A but removed from 1B.

Our data showed MPXV isolation from lesion swabs, throat swabs, tracheal aspirates, scabs, and urine samples from day 2 to 109 days after illness onset. MPXV was isolated at higher Ct values (Ct=37) than previously reported, adding uncertainty to the assumption that patients with high Ct values on PCR are not infectious (2,3).

The duration of infectiousness for MPXV is still unclear, particularly in hospitalised patients (7). A Canadian cohort study found viral DNA detectable for 3-4 weeks,

though this was not linked to infectiousness (8). A Spanish study of outpatients with mpox, including culture data, found the median time to viral clearance to be 25 days, likely due to milder disease (9). Similarly, a 2023 review showed culture data varied by site, with testing ceasing after 19 days (10). The median time of isolation in our study, 12.0 days, fits within these thresholds but was far exceeded by the immunocompromised patient at 109 days.

A limitation of our study is that patients were sampled for clinical diagnosis with inconsistent sampling frequency. Additional freeze-thaw cycles may have impacted culture success. Despite this, we observed the longest virus culture period from a throat sample at over three months post-diagnosis. This study only included MPXV clade II samples, while clade I, which may be associated with a higher case fatality rate, may have different characteristics.

Immunocompromised patients may remain contagious with MPXV for extended periods, particularly through respiratory and oral routes, especially when oropharyngeal lesions are present. Infection prevention guidance should recommend close virological follow-up for these patients who may need extended isolation. High Ct values from oropharyngeal PCRs may not reliably indicate the end of infectiousness.

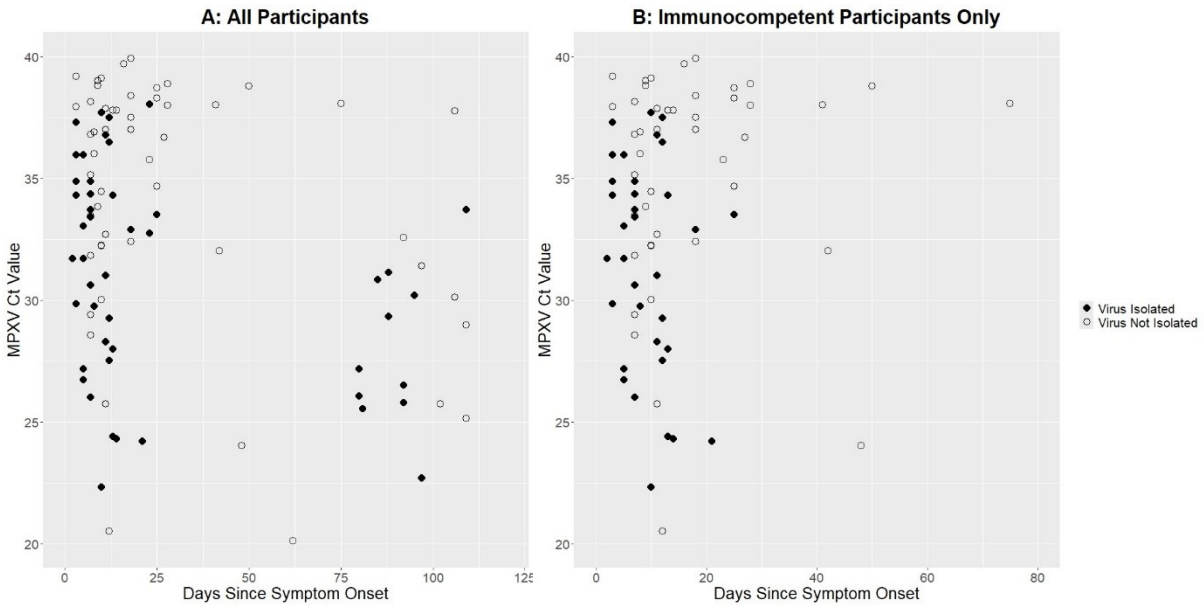

Figure 1. Participants' MPXV PCR Ct value against the number of days since symptom onset. Panel A displays all participants, panel B displays only immunocompetent participants (excluding participant 1). The x-axis limit changes in each figure depending on the maximum number of days since symptom onset. On the y-axis, a decrease in Ct value corresponds to an increase in MPXV DNA in the sample. The symbol used to denote each value is solid black if the virus was able to be isolated at day 7 of culture and hollow if the virus could not be isolated. Graphs were produced using the ggplot2 package (version 3.4.4) in R (version 4.3.2).

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