Viral dynamics in patients with monkeypox infection: a prospective cohort study in Spain

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Abstract

Background

Monkeypox DNA has been detected in skin lesions, saliva, oropharynx, urine, semen, and stool of patients infected during the 2022 clade IIb outbreak; however, the viral dynamics within these compartments remain unknown. We aimed to characterise the viral load kinetics over time in various parts of the body.

Methods

This was an observational, prospective, multicentre study including outpatients diagnosed with monkeypox in hospitals and sexual health clinics in Spain between June 28, 2022 and Sept 22, 2022. Men and women aged over 18 years were eligible if they reported having symptom onset within the previous 10 days of presentation, and were ineligible if disease was severe enough to be admitted to hospital. Samples were collected from five body locations (skin lesions, oropharynx, rectum, semen or vagina, and a dried blood spot) at six time points up to 57 days after the screening visit. Samples were analysed by quantitative PCR and a subset by cell culture. The primary endpoint was time from symptom onset to viral DNA clearance.

Findings

Overall, 1,663 samples were collected from 77 study participants. 75 (97%) participants were male, the median age was 35·0 years, and 39 (51%) were people living with HIV. The median time to viral clearance was 25 days in the skin lesions, 16 days in the oropharynx, 16 days in the rectum, 13 days in semen, and 1 day in blood. The time to viral clearance for 90% of cases was 41 days in skin lesions, and 39 days in semen. The median viral load in skin lesions was 7.3 $\log_{10}$ copies/mL at baseline, compared to 4.6, 5.0, 3.5, and 4.0 $\log_{10}$ copies/mL in oropharynx, rectal, semen, and blood specimens, respectively. Replication-competent viruses were isolated in samples with high DNA levels (>6.5 $\log_{10}$ copies/mL).

Interpretation

In immunocompetent patients with mild monkeypox disease, PCR data alone would suggest a contact isolation period of three to six weeks but, based on detection of replication-competent virus, this time could be reduced. Based on findings from this cohort of patients, semen testing after recovery and prolonged use of condom may not be necessary.

Funding

The study was funded by emergency response funds of the University Hospital Germans Trias i Pujol and the YoMeCorono crowdfunding campaign.

Research in context

Evidence before this study

We searched Pubmed for articles on monkeypox viral dynamics published from inception up to October 10, 2022. We used the terms "Monkeypox" AND ("viral detection", "viral dynamics", "viral load", "viral shedding"). Only 8 papers reported quantitative measurements of viral DNA. Five studies had small sample sizes, inconsistent sampling, and short follow-up periods. One study from France, conducted during the 2022 clade IIb outbreak, included samples from 50 and 24 patients at enrolment and after 14 days, respectively. The authors observed a reduction in the proportion of positive PCR results on day 14 after diagnosis. However, the study collected specimens at only two time-points, had no samples collected beyond day 14 of illness, and lacked viral culture data. A UK study on eight hospitalized patients reported prolonged viral shedding in a range of samples, including blood, urine, lesions, and the respiratory tract but was limited by the small sample size, included mostly patients with severe disease and did not include viral culture data. A study from the Democratic Republic of Congo, of clade I virus, examined an undetermined number of patients using PCR and found higher viral loads in skin lesions than in other body sites, and that viral DNA was detectable in blood and pharyngeal samples prior to rash appearance. Several small studies have cultured monkeypox virus from lesion swabs, anal, urethral or seminal samples, but all were small in size and had tested only a few timepoints. Overall, there is a significant gap in knowledge on the dynamics of monkeypox viral clearance in the outbreak being reported in 2022.

Added value of this study

In this prospective analysis of 77 monkeypox patients, each had 26 specimens collected over time, resulting in 1663 PCR results, which provides a substantial amount of data for describing viral kinetics. We systematically collected specimens from five body locations at six different times and analysed them using PCR quantification of viral DNA and viral culture for a comprehensive assessment of viral dynamics. Our results indicate that swabs of skin lesions have higher viral loads and longer time to clearance than other locations, including oropharynx, rectum, semen, and blood. We found that viral DNA remains detectable in skin lesions for a median of 25 days, and most patients no longer have detectable viral DNA after 41 days. Most positive samples on viral culture were collected before day 15 of illness. Although qPCR can detect DNA for up to six weeks, the lack of culture viability could indicate a shorter infectious period than that indicated by PCR alone. Importantly, only 3 (1%) of 219 semen samples had a viral load above the level at which culture was likely to be positive. These data suggest prolonged transmission in semen is unlikely.

Implications of all the available evidence

Based on PCR results, immunocompetent patients with mild monkeypox disease might require an isolation period of 3 to 6 weeks. However, if our findings regarding the shorter duration of replication-competent virus detected on viral cultures are confirmed, the contact isolation period could be reduced. Although sufficient data is not yet available to be completely conclusive, our data suggest testing semen after recovery or prolonged use of the condom may not be needed.

Further studies in patients with more severe disease or marked immunosuppression are required to understand viral dynamics in these patient groups.

Introduction

Monkeypox, a zoonotic illness caused by the monkeypox virus, has affected rural communities in West and Central Africa since 1970.[1] In July 2022, the World Health Organization (WHO) declared monkeypox as a public health emergency of international concern on account of an unprecedented global spread of the disease outside previously endemic countries in Africa. The multi-country outbreak has involved extensive human-to-human transmission in Europe and North America.[2]

The transmission of monkeypox virus between humans has historically been thought to occur primarily through respiratory droplets.[3] However, during the 2022 clade IIb outbreak, direct contact with infectious material from skin lesions, lesions on mucous membranes, and body fluids occurring during sexual and/or close intimate contact is believed to constitute the primary mode of transmission.[4–8] However, the frequency and duration with which viral DNA and viable virus is found in each body location and fluids and the relative contribution each makes to transmission remain unclear.

Viral DNA has been detected in skin lesions, saliva, oropharynx, urine, semen, and stool of patients infected during the 2022 outbreak[4,9] A study from France observed that samples taken 14 days after diagnosis from 24 patients had a highly reduced proportion of positive PCR results as compared to baseline samples.[10] However, the study collected specimens at only two time points, had no samples collected beyond day 14 of illness, and lacked viral culture data. Another study on eight patients hospitalized with monkeypox in the UK reported prolonged viral shedding (22-39 days) in a range of samples but was limited by the small sample size.[11] Finally, a study from the Democratic Republic of Congo, of patients with clade I virus, demonstrated higher viral loads in skin lesions than in other body sites, and also indicated the virus DNA may be detectable in blood and pharyngeal samples before the appearance of the rash.[12]

In the absence of high-quality empirical evidence regarding the period of infectiousness, current infection prevention and control measures consist of respiratory isolation of patients with monkeypox virus infection until all lesion scabs have fallen off and the skin underneath has re-epithelialized, which may take up to three weeks. Also, in the absence of empirical data on persistence in semen over time, WHO has made a recommendation on condom use during any sexual activity for 12 weeks after recovery.[13] There is an urgent need to better understand how the virus is transmitted and when it is cleared from each body compartment to inform isolation requirements and precautions after recovery. Therefore, we aimed to characterize the viral load kinetics over time in various parts of the body, combining both PCR and viral culture data.

Methods

Study design and setting

This was an observational, prospective, multicentre cohort study of patients diagnosed with monkeypox in Spain between Jun 28, 2022, and Sep 22, 2022. Participating centres included two tertiary hospitals and two sexual health clinics in Madrid and Barcelona.

Consecutive patients seen in the outpatient medical department were invited to participate in the study if they were women and men aged 18 years or older who presented with signs of monkeypox infection, and who reported having symptom onset within the previous 10 days. Participants were ineligible if they had severe disease (defined as requiring admission to hospital). Participants without a confirmed diagnosis of monkeypox after initial molecular testing were subsequently withdrawn from the study. The study protocol was approved by the Ethics Committee of the Hospital Germans Trias i Pujol and written informed consent was obtained from all participants before enrolment.

Study procedures

Patients who consented to participate were interviewed for demographic, epidemiological, and clinical characteristics at baseline (day 0). Data collected included information on the number and location of monkeypox lesions, the presence of systemic symptoms, lymphadenopathies, and proctitis. Physical examination and diagnostic testing (by quantitative PCR [qPCR]) for monkeypox were performed on day 0. Patients underwent Sexually Transmitted Infections (STIs) screening (including HIV, *Chlamydia trachomatis*, *Neisseria gonorrhoea*, *Treponema pallidum,* and *Herpes Simplex Virus* tests). A symptom diary card was provided, and participants were asked to report the list of symptoms experienced over time, along with their onset and resolution dates. On day 29, the research team performed a telephonic interview to assess the clinical evolution of symptoms and lesions.

As part of the follow-up process, participants were provided with six packs of five sample self-collection devices, one for each day the participant was required to self-collect samples. Participants were asked to collect samples from their skin lesions (vesicle fluid or dry scraping of scabs or scars), oropharynx (swab), and blood (dried blood spot) on days 1, 8, 15, 22, 29, and 57 after the screening visit, and samples from their rectum (swab), semen (collection container), and vagina (swab) on days 1, 15, 29, and 57. The swabs (Item Number: 310202) and viral transport medium (Item Number: 304305KF) were purchased from Deltalab (Barcelona, Spain). The details regarding training and instructions given to patients for self-collection of samples are

provided in the Appendix (page 3), and the accuracy of self-sampling using these methods is reported elsewhere.[14] The swabs were immediately placed in 3 mL of viral transport medium. For the dried blood spot, participants were asked to completely fill a circle with a diameter of 1·2 mm, which equates to an estimated blood volume of 50 µL.[15]

A courier service was used to transport self-collected samples from participants' homes to the microbiology laboratory at the University Hospital Germans Trias i Pujol (Badalona, Spain) under optimal conditions for sample stability. During transport, samples were kept at 4ºC; time from sample collection to laboratory receipt was not allowed to exceed 24 hours; once received, samples were processed immediately or stored at -80ºC until processing. All samples were analysed for the detection of monkeypox virus DNA by quantitative polymerase chain reaction (qPCR). Detailed protocols on nucleic acid extraction and qPCR are included in the Appendix, page 4. Copy number per mL was determined using a linear dilution series of a quantified standard monkeypox virus DNA standard (AMPLIRUN® Monkeypox virus DNA control, Vircell Spain SLU, Santa Fe, Granada, Spain). Three replicates were included for each concentration. Viral loads from body fluids (i.e., blood and semen) were expressed in DNA copies per mL, whereas the amount of virus in swab-collected samples (i.e., skin lesions, oropharynx, and rectum) were expressed as copies per mL of viral transport medium.

MDF and MAM conducted cell culture using a subgroup of specimens. Criteria for specimen selection and cell culture protocols are included in the Appendix, page 4. Upon observation of the cytopathic effect and at the end of the cell culture incubation period, culture supernatants were collected from each well, and RT-PCR was performed, which was considered positive if it was at least 3 cycle thresholds (Ct) lower than the original sample. All cell culture-related procedures were performed at a biosafety level 3 facility.

Study Variables and Outcomes

The primary outcome was the median time to viral clearance for each body region, defined as the interval between the onset of symptoms and the date when samples tested negative on PCR. For each sample, we converted the study day into the number of days from symptom onset. As a post-hoc analysis, we assessed the time to viral clearance for 90% and 95% of patients.

Prespecified secondary outcomes were viral load at each body location at each timepoint, presence of replication-competent viruses based on viral culture, and association between clinical features and the time to viral clearance. Other secondary outcomes included: the association between clinical features and demographic and epidemiological factors; behavioural factors associated with monkeypox acquisition, and barriers and facilitators to health services;

monkeypox-specific humoral and cellular responses in a subset of individuals and associations with time to viral clearance or reinfection; and intrahost viral evolution in distinct locations during infection evolution. These four prespecified secondary outcomes will be reported elsewhere as they were related to ancillary substudies. As an exploratory analysis, we assessed the effect of age and HIV infection status on the predicted time to viral clearance in all compartments but did not incorporate this in our modelling approach.

Statistical analysis

The demographic and clinical characteristics of study participants were described using the median and interquartile range (IQR, defined by the 25th and 75th percentiles) or the number and percentage over available data. We used a linear mixed effect models to describe the log viral load of individuals with monkeypox and infer the time to viral clearance and describe the viral load over time (Appendix, page 4). We assumed all bodily fluid compartments (skin lesions, rectum, oropharynx, semen, and blood) to be independent and fitted them separately. Values lower than the limit of detection of the assay were considered right censored. A limit of detection of 4·04 $\log_{10}$ copies/mL was used in the blood compartment and of 2·9 $\log_{10}$ copies/mL for all other samples. Model parameters were estimated using the stochastic approximation expectation maximisation algorithm implemented in Monolix 2019 R2.[17] We used the Conditional Sampling use for Stepwise Approach on Correlation tests (COSSAC) to identify potential effects on both parameters.[18] The probability of detectable virus in each compartment was calculated by simulations, sampling 500 population parameters in their asymptotic estimation distribution accounting for parameter uncertainty. Then, for each set of population parameters, we sampled 300 individual parameters (leading to a total 150,000 individual parameters per compartment), and calculated the time to viral load clearance in each individual. We then derived the proportion of detectable virus in each dataset at each time, and obtained the mean and the 95% confidence interval from the distribution observed in the simulated datasets. As an exploratory analysis, we have assessed at the impact of age and HIV infection status on the predicted time to viral clearance in all compartment but did not incorporate this in our modelling approach. No imputation methods were used for missing values. For all hypothesis tests, the significance threshold was set at a two-sided alpha value of 0·05.

Role of the funding source

The study was funded by emergency response funds of the University Hospital Germans Trias i Pujol and the YoMeCorono crowdfunding campaign. The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The

corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The analysis included 1,663 clinical samples collected at six time points from 77 confirmed monkeypox cases enrolled between Jun 28, 2022, and Sep 22, 2022 in Spain (Figure 1). Two participants were recruited more than ten days after symptom onset (day 11 and 14) and were included in the group of 77 analysed people. The demographic and clinical characteristics of the study participants are summarized in Table 1 and Table S1 (Appendix, page 6). Seventy-five (97%) participants were male, the median age was 35·0 years (IQR 29·0 – 46·0), and 39 (51%) were people living with HIV. Among participants living with HIV, 38 (97%) were taking antiretroviral medication, 34 (87%) had undetectable viral loads, 9 (23%) had CD4 counts below 500 cells/μL, two (5%) having CD4 counts below 300 cells/μL, and none had CD4 counts below 100 cells/μL. Seventy-three (95%) of 77 patients presented with systemic symptoms (described in Table S1, Appendix, page 6), 69 (90%) with a rash on the anogenital and/or perioral region, and 54 (70%) with a rash on a distant site from the point of inoculation. 46 (59.7%) participants presented with ulcerated skin lesions. The systemic illness lasted for a median of 5·0 days (IQR 3·0 - 8·0), whereas local and distant site rashes lasted for a median of 21 days (13·0 – 26·0) and 12·0 days (8·8 – 18·3), respectively. The median duration of lymphadenopathy was 11·0 days (7·8 – 19·0) and the median duration of proctitis was 12·0 days (7·0 – 16·0; Figure S1, Appendix, page 10). Only one patient required hospitalization (due to a pulmonary venous thromboembolism), and no patients received systemic antiviral therapy.

Among the 77 participants, we collected a total of 367 swabs of skin lesions, 425 oropharyngeal samples, 258 rectal samples, 391 blood samples, 219 semen samples, and 3 vaginal specimens (collected from the same patient at three time points). The median time from symptoms onset to first study PCR was 7 days (IQR 5-8). Within the first 10 days following the onset of symptoms, 75 (100%) of 75 swab samples of skin lesion tested positive by PCR, 57 (76%) of 75 oropharyngeal samples, 51 (77%) of 66 rectal samples, 33 (67%) of 49 semen samples, and 17 (24%) of 72 blood samples. In samples collected more than 25 days after symptom onset, 36 (26%) of 138 skin lesion samples were positive by PCR, 21 (11%) of 187 oropharyngeal samples, 5 (4%) of 113 rectal samples, 4 (4%) of 98 semen samples, and 5 (3%) of 168 blood samples (Figure S2, Appendix, page 11). One woman enrolled in the study tested positive in her vaginal sample on study day 1. Intermittent shedding (i.e., negative PCR results in a specific body location that became positive at a later time point) was observed in all sample types (Table S2, Appendix, page 8).

The model of viral clearance replicated the observed data across all sample types (Figure 2 and Figure S3, Appendix, page 12). We found that skin lesions had the longest median time to viral clearance, of 25 days (95% CI 23 – 28), from symptom onset. The corresponding value for the other body locations were as follows: 16 days (13 – 19) for oropharyngeal samples, 16 days (13 – 23) for rectal samples, 13 days (9 – 18) for semen, and 1 day (0 – 5) for blood. According to the model, 90% of individuals would have undetectable viral DNA in skin lesions 41 days (95% CI 34 – 47) after symptom onset. The corresponding estimates for other samples was 34 days (27 – 42) in oropharyngeal samples, 27 days (21 – 38) in rectal samples, 39 days (27 – 56) in semen specimens, and 13 (6 – 23) days in the blood (Figure 2). Time to viral clearance for 95% of patients was 47 days (38–56) for skin lesions, 42 days (32–53) for oropharyngeal samples, 31 days (23–42) for rectal samples, 53 days (34–84) for semen samples, and 20 days (10–39) for blood samples. Our exploratory analysis showed that age was not associated with time to viral clearance, and that time to viral clearance in skin, throat, rectal, or blood samples did not differ, but the time to viral clearance in semen appeared to be possibly faster in individuals living with HIV (Figures S4, Appendix, page 13). Individual viral load profiles for each sample type, correlations between compartments, and diagnostic plots for each compartment are shown in appendix 2 (Figures S5-7, Appendix, pages 14–16).

Median viral load at baseline was highest in swabs of skin lesions (7·3 $\log_{10}$ copies per mL [IQR 6·5 – 8·2]), followed by rectal (5·0 $\log_{10}$ copies per mL [2·9 – 7·5]) and oropharyngeal samples (4·6 $\log_{10}$ copies/mL [2·9 – 5·8]). Semen had median viral loads of 3·5 $\log_{10}$ copies per mL (2·9 – 4·7) and blood had 4·0 $\log_{10}$ copies per mL (4·0 – 4·0) $\log_{10}$ viral copies per mL. The viral load decreased markedly in all sample types over time (Figure 3A,B). Four (5%) participants shed viral DNA more than 57 days from symptom onset: 3 (5%) from skin lesions (with viral loads of 3·2 $\log_{10}$ copies per mL, 3·2 $\log_{10}$ copies per mL and 4·0 $\log_{10}$ viral copies per mL), and 1 (2%) from the rectum and semen (both samples with 4 $\log_{10}$ viral copies per mL).

Viral culture was performed on 174 samples in total (65 skin lesion samples, 52 oropharynx samples, 34 rectum samples, and 23 semen samples). The result was positive, as detected by cytopathic effect, in 33 of 47 samples with viral loads of 6.5 $\log_{10}$ copies per mL or higher (approximately a Ct value of 26). The 14 negative samples included eight lesional swabs, four oropharyngeal swabs, one rectal swab and one semen sample (Figure 3C). None of the 127 samples with viral loads below 6.5 $\log_{10}$ copies per mL were positive. Overall, three (1%) of 219 semen samples had a viral load of 6·5 log10 copies per mL or higher, and out of two tested only one was positive on viral culture.

The presence of monkeypox virus was confirmed by real-time qPCR on DNA purified from cell growth medium collected after five days in all the samples that showed a cytopathic effect. Only one sample collected after day 15 from symptom onset tested positive on viral culture (Figure 3C). Based on the viral load culture results (i.e., threshold of 6.5 $\log_{10}$ copies per mL to determine viability) we conducted an exploratory analysis to calculate the time until the viral load was lower than 6.5 $\log_{10}$ copies per mL. For 90% of individuals to achieve a viral load below this threshold the corresponding values for each body location were 14 days (95% CI 11 – 17) for skin lesion samples, 5 days (0 – 10) for oropharyngeal samples, 10 days (8 – 14) for rectal samples, 2 days (0 – 11) for semen, and 0 days (0 – 0) for blood (Table S3, Appendix, page 9).

Discussion

In this prospective study, we have characterized the time to viral clearance of outpatients without substantial immunosuppression, diagnosed with mild monkeypox disease, in a real-world community setting utilising longitudinal data. Our findings indicate that viral DNA detected by qPCR remains present in swab samples of skin lesions for a median period of 25 days, and that most patients would no longer have detectable viral DNA after 41 days. Furthermore, culture viability data suggests that the infectious period may be shorter than the one established by PCR data alone. However, both DNA positivity and culture have limitations as PCR cannot distinguish viable and non-viable virus and culture may have inadequate sensitivity.[19]

We found that viral DNA was detectable by qPCR in skin lesions for a median time of 25 days since symptom onset and in other body locations or fluids for slightly shorter periods between 1 to 16 days. According to our model, it would take until day 41 for 90% of individuals to have undetectable viral DNA levels in swabs of skin lesions. Taken alongside data from previous studies,[6,9-11,20,21] we conclude that whilst monkeypox DNA is nearly always found in skin lesions in the early illness, it is detected less frequently (30-70%) and at lower viral loads in other samples. During the course of the disease, lesions on the skin had viral DNA levels that were at least 2 orders of magnitude higher than other samples, and remained above the threshold for a positive viral culture for more than one week from symptom onset. We did not find convincing evidence that HIV status or age impacted on viral kinetics.

A key question that remained unanswered during the first months of the 2022 global outbreak was whether monkeypox virus could be transmitted through semen after recovery, as previously observed in other zoonotic viruses.[22] Small studies of patients with monkeypox had found a reportedly high prevalence of PCR-positivity in their semen specimens but the data on the time to viral clearance or the presence of viable viruses in these samples was scarce.[5,9,10,23]

We found that the median time to viral clearance from the semen was 13 days since symptom onset, the time for 90% of individuals to have undetectable viral DNA levels was 39 days, and viral loads in semen were generally low throughout the course of infection, with only 1% of samples having a viral load of 6.5 $\log_{10}$ copies/mL or higher (above the limit for successful viral culture). Moreover, only a single sample, collected early in the illness, was positive on culture. Based on these findings the overall risk of transmission through seminal fluid is likely to be low and the time to clearance of replication-competent virus appears shorter than the duration of viral detection by qPCR. However, for many viruses, shedding in semen can be intermittent and extended follow-up will be of value to establish when monkeypox DNA is permanently undetectable in semen.

We found that samples from oropharynx and rectum contained replication-competent viruses and could therefore be sources of infection. However, exposure to oropharyngeal secretions may be associated with a lower risk of infection than exposure to skin lesions, as viral DNA loads were lower and detection of replication-competent virus was less frequent from oropharyngeal samples in our study. The median time of viral shedding from blood was only 1 day from symptom onset and viral loads in blood were generally low. This finding is much shorter than as reported in a previous study that included individuals who required hospital admission, often with more severe disease, than typically described for most community cases in the 2022 outbreak.11 The short duration seen in our study probably reflects the relatively mild clinical course of our patient cohort, in which only one patient required hospitalisation. Of note, the viral dynamics could have differed in the very first days of infection, which could have implications for viable transmission routes, even though we recruited patients within 10 days of symptom onset.[12]

A potential limitation of our study is that the self-sampling strategy, whilst facilitating the study implementation and reducing loss to follow-up, might have resulted in samples of lower accuracy than those collected by healthcare professionals. However, self-collected samples have been demonstrated to be highly accurate for a variety of pathogens, and we have previously demonstrated that lesion and oropharyngeal swabs collected by patients with monkeypox are comparable to those collected by healthcare professionals.[14] Second, we used dried blood samples, which may not be as accurate as samples collected by venipuncture. Third, we did not include saliva samples, which have been suggested to have a potential role in disease transmission.[9,24] Additionally, there is currently no international standardized methodology for analyzing monkeypox virus viability in cell culture. The lack of standardization may hinder comparisons of our analysis with other studies evaluating viral viability, even though we successfully isolated replication-competent viral particles from all body locations. Finally, our

study cohort consisted solely of outpatients, almost all of whom were young men. Viral dynamics may differ, in particular in severely unwell patients who may shed virus for longer, but our results should be applicable to the vast majority of patients who are managed outside of the hospital.

In summary, our study provides a comprehensive perspective of the dynamics of monkeypox viral clearance within the first two months following symptom appearance in a cohort of patients with relatively mild disease, managed as outpatients and without significant immunocompromise. For this group of patients, our findings may help inform isolation and post-recovery precautions. Current recommendations, for all patients regardless of immune status or disease course, consist of respiratory isolation for up to 3 weeks and condom use during any sexual activity for 12 weeks after recovery. If isolation decisions are made on PCR results alone, our data would suggest a requirement for contact isolation lasting from 3 to 6 weeks in immunocompetent patients with mild monkeypox disease. However, integrating our data on the shorter duration of replication-competent virus detected on viral cultures, suggests the time to ending isolation could be reduced. In addition, if further research confirms our findings, semen testing after recovery and prolonged use of condoms may not be necessary for this group of patients. Further studies are needed to better inform decisions on infection prevention and control, particularly in individuals with more marked immunosuppression and with asymptomatic infection.

Contributors

OM, MM, CS, MU and EJT-V conceived and designed the study. CS, MU, EJT-V, AMe, AA, AH-R, CC, VD, ARi, PC, XO, JMC, MV-M, MDF, MAM, MA-D, EG-C, APL, ARa, VB, acquired the data. CS, MU, EJT-V, DO, AMA, JG, MM and OM analysed and interpreted the data. CS, MU, DO, AMA, JG, MM and OM did the statistical analysis. CS, MU, EJT-V, MM, and OM drafted the manuscript. CS, MU, EJT-V, AMe, AA, AH-R, CC, VD, DO, AMa, ARi, PC, XO, JMC, MV-M, MDF, MAM, MA-D, EG-C, APL, ARa, VB, CG-C, RP, NP, M-RSF, JMB-S, MF, PLO-R, BC, VG-P, JC, JG, P-JC, IB, MM and OM reviewed and approved the manuscript; vouched for the accuracy and completeness of the data and for the adherence of the study to the protocol; and were responsible for the final decision to submit for publication. CS, MU, EJT-V, MM, and OM had full access and verified all the data in the study.

Declaration of interests

We declare no competing interests.

Data sharing

De-identified participant data collected for the study, including individual participant data and a data dictionary defining each field in the set, will be made available from the corresponding author on reasonable request.

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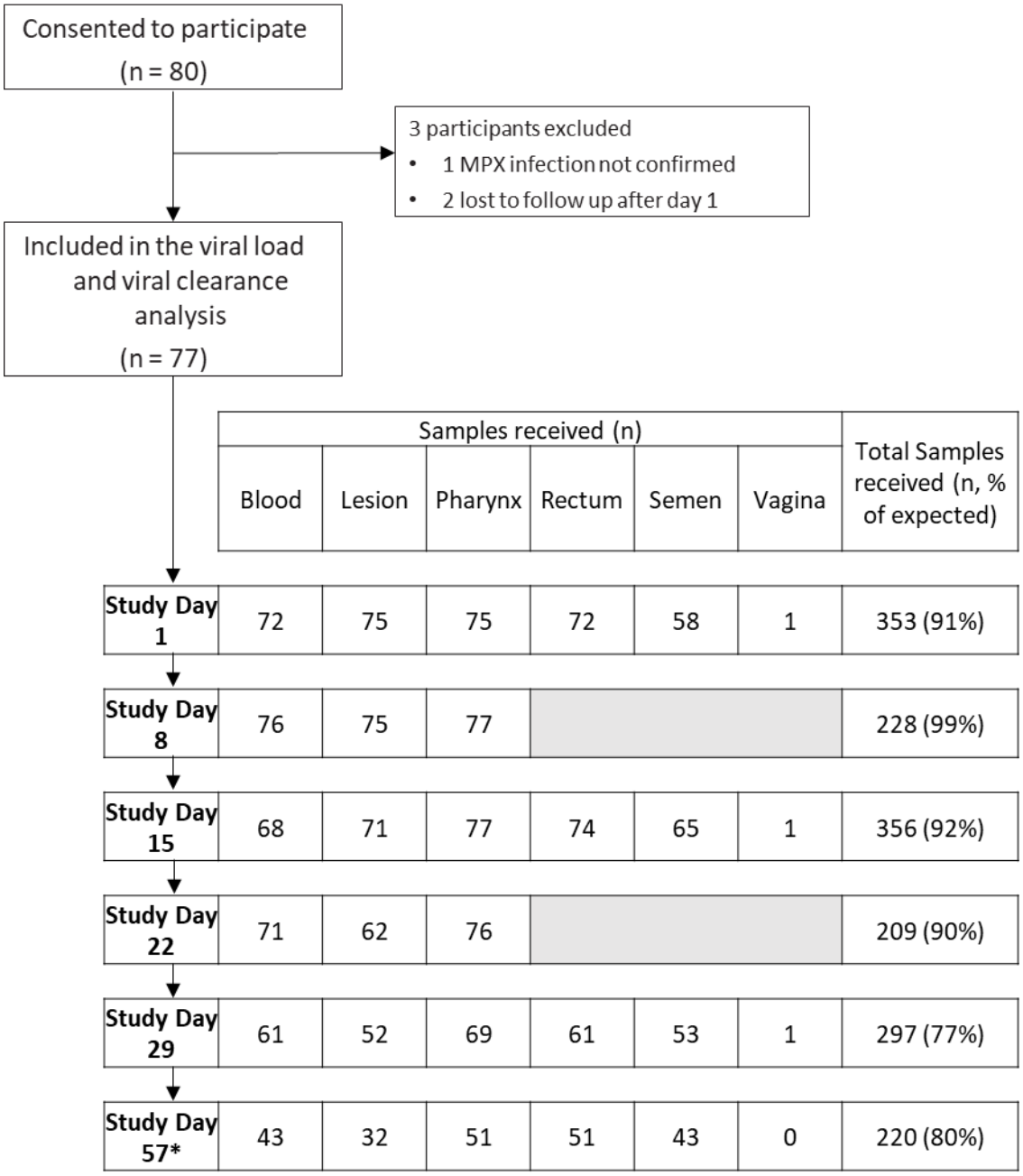
Figure 1. Trial flowchart

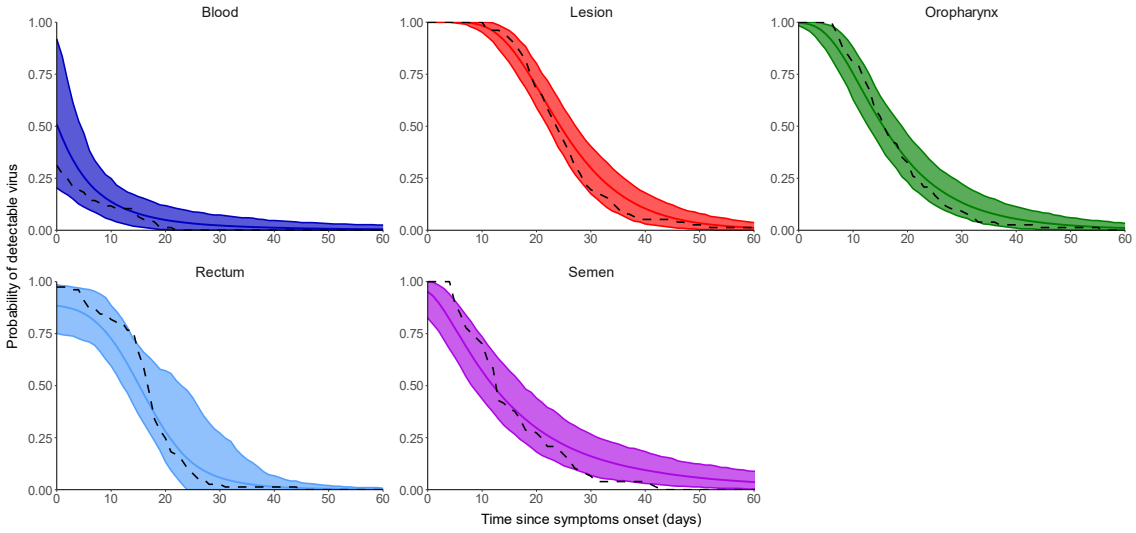


	Samples received (n)						Total Samples received (n, % of expected)
	Blood	Lesion	Pharynx	Rectum	Semen	Vagina	
Study Day 1	72	75	75	72	58	1	353 (91%)
Study Day 8	76	75	77				228 (99%)
Study Day 15	68	71	77	74	65	1	356 (92%)
Study Day 22	71	62	76				209 (90%)
Study Day 29	61	52	69	61	53	1	297 (77%)
Study Day 57*	43	32	51	51	43	0	220 (80%)

Figure 1. Trial flowchart. A total of 80 individuals consented to participate in the study; three had exclusion criteria; the remaining 77 were included in the study. On each study day 1, 8, 15, 22, 29, and 57, each participant provided a set of samples. The number of specimens received at each time point is indicated by body region. On study days 8 and 22, rectum and semen/vaginal samples were not required. *At the time of data lock, 55 participants had reached study day 57. Thus, the denominator used to calculate the number of expected samples at this timepoint was 55 participants. For the rest of timepoints, the number of samples expected was based on the 77 participants that had reached study day 29.

Figure 2. Time to viral clearance in all patients (A) and in 50%, 90%, and 95% of patients (B).

A



B

Location	Time to clearance in 50% of patients Days (95% CI)	Time to clearance in 90% of patients Days (95% CI)	Time to clearance in 95% of patients Days (95% CI)
Blood	1 (0 – 5)	13 (6 – 23)	20 (10 – 39)
Semen	13 (9 – 18)	39 (27 – 56)	53 (34 – 84)
Rectum	16 (13 – 23)	27 (21 – 38)	31 (23 – 42)
Oropharynx	16 (13 – 19)	34 (27 – 42)	42 (32 – 53)
Skin lesion	25 (23 – 28)	41 (34 – 47)	47 (38 – 56)

Figure 2. Time to viral clearance in all patients (A) and in 50%, 90%, and 95% of patients (B). In A, the simulated means and 95% CIs of time to viral clearance are shown as a solid lines and shaded area, respectively. The dashed line represents the cumulative incidence calculated on the observed data. Model fits for each sample type are shown the appendix (p 12).

Figure 3. Median viral load (A), individual participant viral load (B), and viral culture (C) in lesions, oropharyngeal, rectal, and semen samples by time from symptom onset

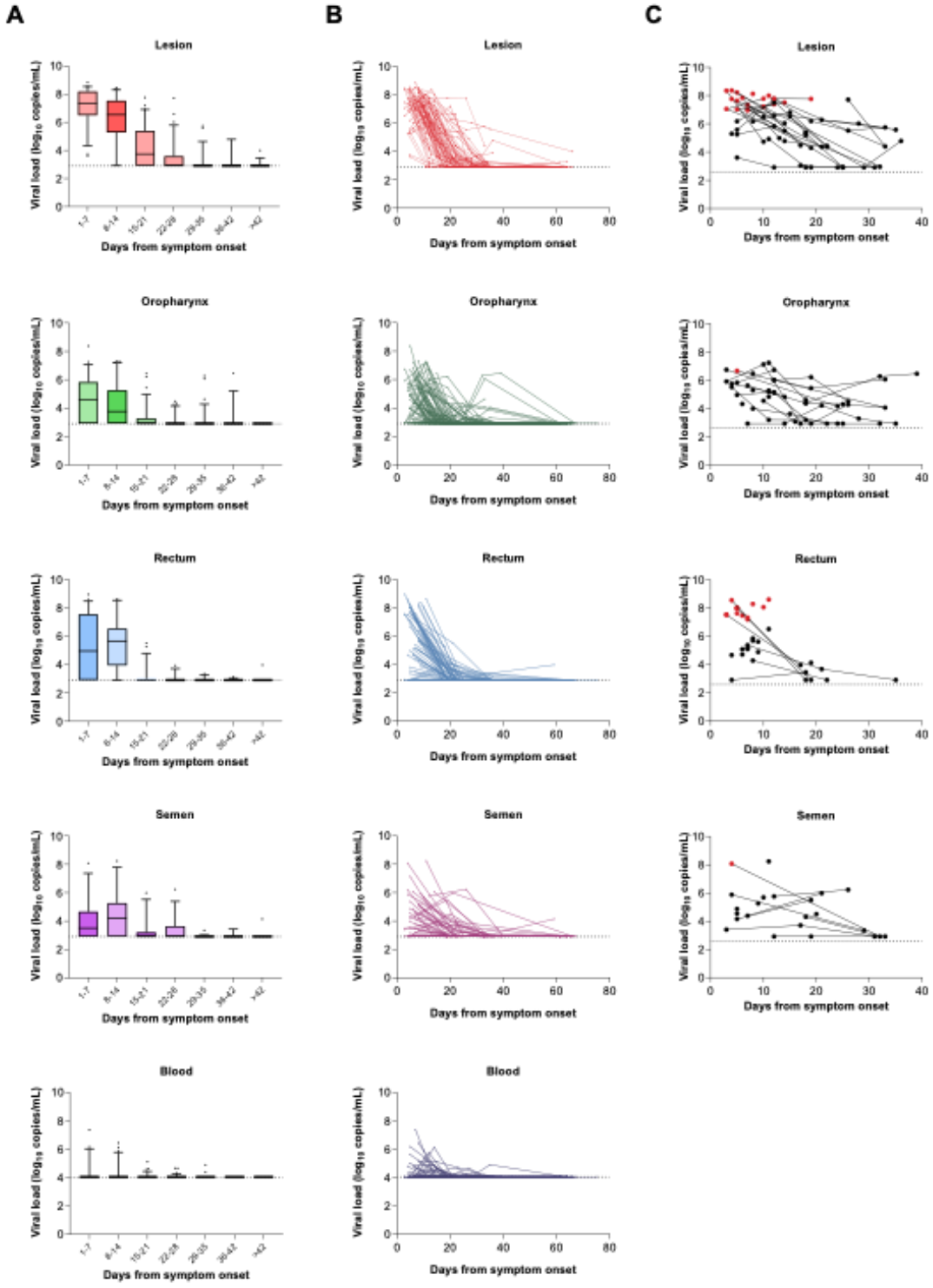


Figure 3. Median viral load (A), individual participant viral load (B), and viral culture (C) in lesions, oropharyngeal, rectal, and semen samples by time from symptom onset. Viral load measures are expressed as log10 copies per mL (mL of viral transport media for lesion, pharynx,

and rectum, and mL of blood and semen). The limit of detection was 4·0 log10 copies per mL for the blood samples and 2·9 log10 copies per mL for the rest of the samples, both of which are indicated with dashed lines. For each patient, the sample collection day was adjusted for the date of onset of symptoms. In A, box plots represent median, IQR, and minimum and maximum viral load. In C, samples in red had replication-competent viruses detected by culture.

Table 1. Baseline and Clinical Characteristics

BASELINE CHARACTERISTICS	
N	77
Gender (Male), n (%)	75 (97)
Age (years), median (IQR)	35·0 [29·0, 46·0]
Origin, n (%)	
South/Latin America	31 (40)
Spain	36 (47)
People living with HIV[1], n (%)	39 (51)
Concomitant *N. gonorrhoea* Infection	9 (12)
Concomitant *T. pallidum* infection	4 (5)
Concomitant *C. trachomatis* infection	3 (4)
CLINICAL CHARACTERISTICS	
Recent smallpox vaccination, n (%)	2 (3)
Incubation period	6·0 [4·0, 8·0]
Time from symptoms onset to first study PCR, in days (median [IQR])	7·0 [5·0, 8·0]
Systemic illness, n (%)	73 (95)
Lymphadenopathies, n (%)	64 (83)
Proctitis, n (%)	24 (31)
Anogenital and/or perioral skin rash, n (%)	69 (90)
Number of localized lesions[2], n (%)	
0	8 (11)
1	17 (22)
2 to 5	32 (42)
>5	19 (25)
Lesion location, n (%)	
Perioral	13 (17)
Perianal	21 (27)
Genital	36 (47)
Oral mucosa	5 (7)
Other locations	6 (8)
Skin rash at a distant site from the inoculation point, n (%)	54 (70)
Number of distant lesions[2], n (%)	
0	17 (23)
1-4	20 (27)
5-20	30 (41)
>20	6 (8)
Lesion location, n (%)	
Trunk, armpit, neck	46 (60)
Upper extremities	47 (61)
Lower extremities	36 (47)
Face	31 (40)
Complications (recorded at d29), n (%) (N=64)	29 (38)
Required hospitalization	1 (1)

[1]Nine (12%) participants were people living with HIV with a CD4 cell count below 500 cells/µL with two having a CD4 cell count below 300 cells/µL. None had a CD4 cell count below 100 cells/µL. [2] Highest count of lesions during the follow-up period.