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Persistence of Monkeypox Virus DNA in Clinical Specimens

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Title page

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Running title: persistence of monkeypox virus

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Dear Editor,

Recently, Eva et al [1] reported the monkeypox outbreak in Madrid of Spain which indicated accurate clinical and virological aspects of the disease outside endemic areas are needed, particularly for early diagnosis in clinic. In early May 2022, the re-emerging outbreak of a resting zoonotic virus, monkeypox virus (MPXV) (the genus *Orthopoxvirus*, the family *Poxviridae*) which was originally reported in humans in central Africa in 1970, attracts great concerns of global public authorities. As of August 29, 2022, MPXV has expanded to 99 countries, and over 48,000 confirmed cases recorded according to the latest CDC data. Clinically, monkeypox cases (MPX) are manifested mainly with skin/mucosal lesions at variable sites including genital, oropharyngeal or perianal areas. Men who have sex with men (MSM) are the mostly affected population. The confirmation of MPXV infection relies on detection of virus DNA in various body fluids. Recent studies reported the shedding of MPXV DNA in various samples [2-3]. However, the information of how frequently the MPXV DNA can be detected in variable clinical samples and how long it remains detectable are still very limited. To understand the dynamics of the early stages of MPXV infection is essential for clinical diagnostic testing and prevention interventions, in that, available observations evidence is only based on case reports.

In this study, we collected the laboratory detection data of MPX cases from the archived reports which included clear and consistent methods, and predict the duration of detecting MPXV DNA in various body fluids through mathematics model analysis. A total of 62 archived MPX cases (49 males, 13 females; median age 31y, range 0.4–50y) from 22 observation studies were included, of which 51 were non-HIV infected MPX cases (38 males, 13 females; median age 32y, range 0.4–49y), and remained 11 were HIV-infected MPX cases (11 males; median age 33y, range 26-50y) (Appendix Table 1). The main clinical manifestations of 62 MPX cases were rash (n=44, 70.97%), fever (n=28, 45.16%),

lymphadenopathy (n=22, 35.48%), myalgia (n=11, 17.74%), headache (n=9, 14.52%), fatigue (n=4, 6.45%) and chills (n=4, 6.45%). Among the non-HIV infected MPX cases, skin rash were the most frequent clinical symptoms (n=34, 66.67%) (6-17 days, median 13d) followed by fever (n=23, 45.10%) (2-5d, median 3d), lymphadenopathy (n=19, 37.25%), myalgia (n=9, 17.65%), headache (n=9, 17.65%), chills (n=4, 7.84%) and fatigue (n=3, 5.88%). However, within the 11 HIV-infected MPX cases, the proportion of MPX with the symptoms of skin rash reach to 90.91%, no case with headache or chills (Appendix Table 1, Appendix Table 2).

We collected the molecular detection data of MPXV by PCR in 62 MPX patients. The parametric Weibull regression models (AFT) was employed to estimate the time until the loss of MPXV DNA detection in each body fluid and reported findings in medians and 95th percentiles using R software version 3.6.1 with flexsurv, survival, and survminer packages. Additional Lnorm and gamma models were used as to evaluate the sensitivity and stability of Weibull regression models. The time until loss of MPXV DNA detection in variable clinical samples was defined as the number of days after the first negative PCR result after the onset of symptoms. For the cases with intermittent results of MPXV detection, we used the date of the first negative result after the final recorded positive PCR results.

A total of 269 specimens of 62 MPX cases were included in this modeling analysis, including 23 urine samples (8.55%), 19 rash or skin lesions samples (7.06%), 17 nasopharyngeal swabs samples (6.32%),17 rectal swabs samples (6.32%), 16 semen samples (5.95%), 15 blood samples (5.58%), 14 fecal samples (5.20%) and 14 saliva samples (5.20%). The results of Weibull models showed that the median time of MPXV DNA persistence ranged from 5.7d to 13.5d in the nasopharynx swabs (8.6d, 95% CI 6.94-10.4d), feces (9.4d, 95% CI 7.15-12.4d), semen (11.4d, 95% CI 8.58-14.9d), urine (13.5d, 95% CI 10.3-17.5d), rash or skin lesions (5.7d, 95% CI 3.86-8.1d), saliva (8.9d, 95% CI 7.14-11.2d),

blood (10.6d, 95% CI 6.37-16.9d), and rectal swabs (8.3d, 95% CI 6.58-10.7d) samples, while 11.3d (95% CI 9.88-13.1d) when using all samples data (Figure 1, Appendix Figure 1). The additional comparisons of sensitivity and stability among Weibull, Lnorm, and gamma models showed no differences among them ($p<0.05$) (Appendix Table 3; Appendix Figure 2, 3).

Pettke et al. reported positive PCR results in semen samples from patients who recovered from MPX for 54 d at maximum [3]. Nörz et al. found the prolonged presence of MPXV DNA in rash or skin lesions and blood samples [4]. In this study, we estimated the time for MPX cases to clear MPXV DNA in the acute phase of infection through an AFT-based modeling study. We found that the median time for semen samples from cases was 11.4d (95% CI 8.58–14.9d) and the 95th percentile was 24.7d (95% CI 17.8–35.5d). Therefore, detection of MPX DNA for cases in semen samples at the 54th day after illness onset should be rare, beyond the 95th percentile limit. Similarly, the detection of MPXV DNA in rash or skin lesions or blood samples from Nörz et al. were also close to the 95th percentile limit as we estimated (14.7d, 95% CI 12.0-18.2d; 41.5d, 95% CI 23.2-84.0d).

As shown in the table, among all the fitted median times, the time in urine (13.5d) was the longest, followed by semen (11.4d) and blood (10.6d) (Table). We found that there was no difference in the median time fitted by the three models (Table; Appendix Table 2). In addition, the estimated time until the loss of DNA detection in various clinical samples was consistent with previous findings in case reports [5-6].

Our study has limitations. First, the infectivity of the specimens was not estimated. We focused on estimating the duration of MPXV DNA in various body fluids among MPX cases but did not imply the existence of infectious virus particles. Second, the date of first samplings may be deviated from the date of symptoms onset. We defined the time until loss of MPXV DNA detection in each specimen as the

number of days between the day after illness onset and the day of the first negative PCR result, which means that the median and 95th percentile we estimated were shorter than expected because of the uncertainty of incubation time. Third, time estimated in this study may not be generalizable to all infections with MPXV, e.g. asymptomatic cases.

Our results provide a reference for the appropriate time for MPXV detection in clinic, which should be considered for clinical diagnostic recommendations, as well as control and prevention of MPXV onward transmission.

Authors' contributions

Study design: ZW Li, GX Zhu and JF Sun ; Data analysis: ZW Li, XX Li, YL Chen and QQ Ruan; Administrative, technical, or material support: ZW Li, XX Li, YL Chen, QQ Ruan, XR Huang, HM Chen, XM Hu, GX Zhu and JF Sun; Critical revision of manuscript: ZW Li,QQ Ruan, GX Zhu and JF Sun. All authors reviewed and approved the final manuscript.

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Availability of data and materials

The code used for the model is available from the corresponding author

Conflict of interest

All the authors declare that they have no conflicts of interest.

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Legends

Figure 1. Time until clearance of monkeypox DNA in nasopharyngeal swabs(A), feces(B), semen(C), urine(D), rash or skin lesions(E), saliva(F), blood(G),and rectal swabs(H) samples among MPX patients, as estimated with the use of Weibull regression. The medians and 95th percentiles of the time until the loss of detection are indicated; bars and shading indicate 95% CIs.

Appendix material legends

Appendix Table 1.Clinical symptoms of 62 MPX cases

Appendix Table 2. Persistence of MPXV DNA in body fluids.

Appendix Table 3. Laboratory detection of MPXV DNA in 22 literatures.

Appendix Figure 1. Time until clearance of monkeypox DNA in any clinical specimens of nasopharyngeal swabs, feces, semen, urine, rash or skin lesions, saliva, blood, or rectal swabs samples among patients with monkeypox virus infection, as estimated with the use of Weibull, Lnorm, and gamma regression. (A) Weibull regression for monkeypox cases; (B) Lnorm regression for monkeypox cases; (C) gamma regression for monkeypox cases. The medians and 95th percentiles of the time until the loss of detection are indicated; bars and shading indicate 95% CIs.

Appendix Figure 2. Time until the clearance of MPXV DNA in nasopharyngeal swabs, feces, semen, urine, rash or skin lesions, saliva, blood, and rectal swabs, by using an Lnorm regression model. The exhibitions are the time until the loss of monkeypox DNA detection after the onset of symptoms in nasopharyngeal swabs (A), feces (B), semen (C), urine (D), rash or skin lesions (E), saliva (F), blood (G), and rectal swabs (H) from 62 MPX cases. The medians and 95th percentiles of the time until the loss of detection is shown in each panel with 95% confidence intervals (blue shading).

Appendix Figure 3. Time until the clearance of MPXV DNA in nasopharyngeal swabs, feces, semen, urine, rash or skin lesions, saliva, blood, and rectal swabs by using a Gamma regression model. The

exhibitions are the time until the loss of MPXV DNA detection after the onset of symptoms in nasopharyngeal swabs (A), feces (B), semen (C), urine (D), rash or skin lesions (E), saliva (F), blood (G), and rectal swabs (H) from 62 cases. The medians and 95th percentiles of the time until the loss of detection is shown in each panel with 95% confidence intervals (blue shading).