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BRIEF REPORT

TRANSFUSION

Absence of detectable monkeypox virus DNA in 11,000 English blood donations during the 2022 outbreak

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Abstract

Background: A large, worldwide outbreak of mpox (formerly referred to as monkeypox) involving mainly men who have sex with men commenced in May 2022. We evaluated the frequency of positivity for the causative agent, monkeypox virus (MPXV), in blood donations collected in August 2022, during the outbreak period in Southern England.

Methods/Materials: The sensitivity and specificity of an MPXV-specific PCR and a generic non-variola orthopoxvirus (NVO) PCR were evaluated using samples from mpox cases and synthetic DNA standards. Residual minipools from nucleic acid testing were obtained from 10,896 blood donors in Southern England, with 21% from London.

Results: MPXV and NVO PCRs were both capable of detection of single copies of target sequence with calculated limits of detection $(LOD)_{90}$ s of 2.3 and 2.1 DNA copies and analytical sample sensitivities of 46 and 42 MPXV DNA copies/ml, respectively. 454 minipools produced from 10,896 unique donors were assayed for MPXV DNA by both methods. No positive minipools were detected by either PCR.

Conclusions: Although blood donors are unrepresentative of the UK population in terms of MPXV infection risk, the uniformly negative MPXV DNA testing results provide reassurance that MPXV viraemia and potential transmission risk were rare or absent in donors during the outbreak period. Minipools from blood donors allow rapid implementation of large-scale population-based screening for emerging pathogens and represent an important resource for pandemic preparedness.

Chanice Knight and Julien Andreani contributed equally to this work and share first authorship.

1 | INTRODUCTION

Monkeypox virus (MPXV) is a large DNA virus infecting rodents in sub-Saharan Africa and is classified as a member of the genus *Orthopoxvirus* in the family *Poxviridae*. Human infections from zoonotic sources have been frequently described in Central and West Africa, but MPXV and the associated disease, mpox (formerly referred to as monkeypox[1]), have spread rapidly through Europe and the United States in 2022, mainly but not exclusively, involving men who have sex with men (MSM).[2] Infections are manifested by prodromal fever and other systemic symptoms, followed by a characteristic centrifugal rash.[3]

The first mpox cases in the United Kingdom were reported on the 13th May in MSM with no links to Africa or other imported cases.[4] Infection frequencies increased exponentially in subsequent weeks, peaking in July with 40–50 new diagnoses reported daily and a cumulative total of 3485 confirmed diagnoses reported to the UK Health Security Agency (UKHSA) by 26/09/2022.[2] Similar outbreaks have been recorded in Spain, Germany, and a range of other European countries[5] and the United States, over this period, in each country, mainly affecting MSM with shared risk factors for HIV-1 infections and other sexually transmitted infections (STIs).

The occurrence of a large-scale outbreak of mpox in the United Kingdom with frequent atypical or only mildly symptomatic clinical presentations has a potential impact on transfusion safety,[6,7] especially given the occurrence of often prolonged MPXV DNA detection during symptomatic stages of infection,[8] and the frequent (7%–67%) detection of DNA in plasma of mpox cases from different countries (reviewed in Ref. 9). To evaluate potential transfusion transmission risk, we screened plasma from donors collected at the end of August using polymerase chain reaction (PCR) methods specific for the MPXV tumor necrosis factor receptor (TNFR) gene[10] and a second generic non-variola orthopoxvirus (NVO) PCR that detects the majority of orthopoxviruses.[11] Although specific detection of MPXV is preferable as donors may be infected with other poxviruses through vaccinia-based vaccines,[12] some recent outbreak strains possess large deletions of the TNFR gene and are falsely negative in the specific PCR.[13]

2 | MATERIALS AND METHODS

A total of 10,896 donation samples collected between the 19th and 24th August, 2022 were analyzed in the study. These were combined to 454 minipools by NHBT for routine HIV and hepatitis B, C and E virus nucleic acid testing (NAT), each comprising 24 blood or platelet donation samples. Extraction of donor demographic data was performed by NHS Blood and Transplant (Table S1; Suppl. Data).

A pre-quantified MPXV DNA standard was provided by the National Institute of Standards and Technology (NIST), United States. Serial dilutions were made in the supplied dilution buffer and subsequently in RNA buffer solution (1 mM sodium citrate, pH 6.4 supplemented with 50 ng/µl of herring sperm DNA and 0.14 units/µl RNAsin (Thermofisher) for larger scale limiting dilution assays). Pre-extracted samples from mpox cases were kindly provided by the Institute of Virology-Charité, Berlin, for an inter-laboratory study and proficiency test.

An MVA strain of vaccinia virus[14] was used to validate target specificities of the MPXV and NVO PCRs. Serial dilutions of DNA extracted from virus stock with a mid-range infectivity of 1.3×10^8 plaque-forming units (PFUs) / ml were made in RNA dilution buffer.

2.1 | Nucleic acid extraction

Two hundred microliters of plasma pools were extracted using the Quick-DNA/RNA 96 Viral Kit (Zymo Research) following the manufacturer's instructions. Extracted DNA was eluted into 20 µl of DNase/RNase-free water and; 5 µl of extracted DNA was used for PCR.

2.2 | Real-time PCR

Real-time PCR (RT-PCR) for MPXV DNA used previously described primers, probes, and protocols (Suppl. Methods).

3 | RESULTS

3.1 | MPXV PCR assay sensitivity and specificity

The sensitivity and specificity of the MPXV and NVO RT-PCRs were determined through testing of qualitative and quantitative controls. Five microliters of extracted DNA from a panel of samples from five virologically confirmed mpox cases and an uninfected patient control that had been pre-tested in the Tib Molbiol Orthopoxvirus PCR were re-assayed by both PCRs (Table 1A). Both assays reproduced the testing results of the reference laboratory, although Ct values in the MPXV PCR were consistently 3–4 (MPXV) or 1–2 (NVO) cycles higher than those obtained by the reference lab despite testing equal volumes of extracted DNA (5 µl). All samples were reactive, but with higher Ct values on triplicate re-testing

TABLE 1 Validation of MPXV DNA PCR sensitivity and specificity.

(A) Clinical sample panel				
	Ct values			
Sample	**Reference**	**MPXV PCR**	**NVO PCR**	**MPXV PCR @1/24[a]**
1	15.5	19.7	17.6	25.3
2	21.9	25.0	24.0	31.7
3	23.2	28.7	28.6	34.6
4	25.9	30.8	28,1	36.1
5	31.9	37.4	34.7	40.1[b]
6	Neg.	>40	>40	

(B) Dilution series of NIST control				
	MPXV PCR		**NVO PCR**	
Copies/reaction	**Detection**	**Mean Ct**	**Detection**	**Mean Ct**
550,000	3/3	19.09	3/3	21.30
55,000	3/3	23.18	3/3	23.32
5500	3/3	27.36	3/3	28.35
550	3/3	30.52	3/3	31.40
55	3/3	35.81	3/3	34.77
5.5	3/3	39.54	3/3	38.47
2.2	24/31	39.39	19/30	39.76
0.88	17/29	39.26	18/30	40.24
0.55	0/3	-	0/3	-
0.055	0/3	-	0/3	-

(C) Dilution series of MVA control				
	MPXV PCR		**NVO PCR**	
Pfu/reaction[c]	**Detection**	**Mean Ct**	**Detection**	**Mean Ct**
2,600,000	0/3	-	3/3	17.26
260,000	0/3	-	3/3	20.81
26,000	0/3	-	3/3	24.14
2.600	0/3	-	3/3	27.09
260	0/3	-	3/3	31.32
26	0/3	-	3/3	34.19
2.6	0/3	-	3/3	37.14
0.26	0/3	-	3/3	40.30
0.026	0/3	-	0/3	-
0.0026	0/3	-	0/3	-
0.00026	0/3	-	0/3	-
0.000026	0/3	-	0/3	-

[a]Mean values of three replicates shown.
[b]1 from 3 replicates positive.
[c]Based on midpoint of infectivity range on original stock determined by supplier.

in the MPXV PCR after 1/24 dilution in DNA extracted from negative plasma pools (with one positive from 3 replicates from sample 5).

PCR sensitivity was evaluated using a dilution series of the pre-quantified MPXV DNA control distributed by NIST (Figure 1). Modification to the amplification

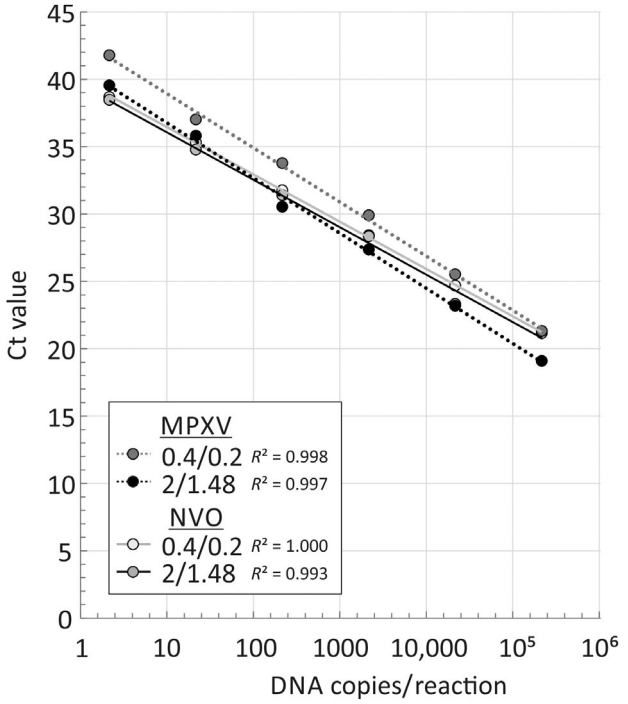

FIGURE 1 Detection of MPXV DNA by MPXV-specific and orthopoxvirus generic real-time PCRs. Detection of a dilution series of the pre-quantified NIST MPXV control DNA by RT-PCR using MPXV-specific and orthopox generic (NVO) primers. Datapoints represent the mean of three replicates; lines of best fit were calculated by linear regression of log-transformed viral load values; and correlation coefficients (R^2) are shown in figure key. The analysis compares amplification achieved by PCRs using different primer (0.4 and 2.0 μM and probe (0.2 and 1.48 μM) concentrations in each PCR (see key).

protocols by increasing primer concentrations to 2 μM in the MPXV PCR led to a consistent reduction of around 2 Ct cycles using different plasmid control dilutions (Figure 1; Table S2—Suppl. Data).

Endpoint analytical sensitivity of both assays was determined through testing of multiple replicates with 0.8 and 2.2 copies of MPXV DNA using optimized conditions. The observed 17/29 positivity in the MPXV PCR on testing replicates containing 0.88 copies equated to a target frequency of 0.88 using standard Poisson formulae (Suppl. Methods), equivalent to single copy sensitivity (Table 1B) with a mean Ct value of positive replicates of 39.3. The calculated LD_{90} of 2.3 DNA copies of MXPV would correspond to an overall analytical sensitivity of 46 DNA copies/ml using the extraction method with a sample representation of 50 μl. Detection of 18/30 replicates of 0.88 copies in the NVO PCR similarly predicted an LD_{90} of 2.1 DNA copies and sensitivity of 42 copies/ml.

A vaccinia virus vaccine construct was assayed by MPXV and NVO PCRs. All tested amounts of MVA (which contains a deletion in the TNFR gene) were negative in the MPXV PCR but were positive in the NVO assay down to a virus amount of 0.26 PFUs and negative at 0.026 PFUs (Table 1C).

Assay sensitivity was unaffected by other nucleic acid or other components co-extracted from experimental samples (Figure S1; Suppl. Data). Spiking 18 nominal copies of MPXV DNA into 5 μl DNA extracted from six EDTA plasma pools and testing in replicate demonstrated mean Ct values comparable to the unspiked control in both PCR assays.

3.2 | PCR-based screening of donation samples

EDTA-anticoagulated plasma samples from a total of 10,896 donations were obtained from donors on 19th–24th August, 2022, representing 1 week's donations from the South of England (one-third of all NHSBT donations), with 21% of the sample set derived from donations collected in London. Donors were approximately equally split between males and females (53%/47%) with a median age of 47 (Table S1; Suppl. Data).

All 454 plasma pools were assayed in MPXV and NVO PCRs, using one-quarter of DNA extracted from 200 μl volumes (sample representation of 50 μL). All samples were negative in both PCRs, with undetectable amplification of target (Ct values >45). With a pool size of 24, and a per sample representation of 2.1 μl, negative results correspond to MPXV viral loads of less than 480 DNA copies/ml of each minipool component.

4 | DISCUSSION

Mpox outbreaks have become prevalent in the United Kingdom, elsewhere in Europe, the United States, Canada, and many other countries worldwide.[15,16] Reported case numbers peaked worldwide in July and August coinciding with the collection period for the samples analyzed in the study with approximately 150 new diagnoses per week in England. However, reporting data for confirmed diagnoses is unlikely to reflect the full extent of MPXV spread, given the ongoing limited availability of diagnostic testing and the asymptomatic or mild nature of many MPXV infections.[17]

The Joint Professional Advisory Committee of the UK Blood Services, responsible for donor selection guidelines, has updated its guidance for donor selection, and these have been introduced to reduce MPXV transmission risk. These exclude donors who have been diagnosed with MPXV infections within 28 days or close contacts of

individuals who have been diagnosed with MPXV infection within the last 21 days.[18] A substantial overlap of risk factors for MPXV infections with those for HIV further contributes to exclusion of at-risk donors. Although MSM represent the main demographic group affected by mpox, those infected in the current UK outbreak[2] also show high rates of HIV-1 co-infection (26%), typically disclose large number of previous sexual partners, frequent sexually transmitted infections in the previous year (53%) and a high rate of HIV pre-exposure prophylaxis use (77%). These are all elements that would lead to donor deferral by NHSBT irrespective of pre-donation reporting of mpox contacts or symptoms. Nevertheless, a small number of donors may continue to donate despite pre-donation questions on risk that should lead to deferral[19]; nondisclosure of infection risk is similarly apparent from studies that document high frequencies of PrEP use in U.S. and UK donors.[20,21]

Although the experimental findings in the current study are clear, it is evident that the absence of detectable PCR positivity in the minipools screened cannot be directly equated to an absence of MPXV infection in the donor population. There is, for example, little information on the duration of DNA detection and its relationship to MPXV infection and the extent to which it occurs pre-disease onset or persists beyond resolution of symptoms when blood donation is more likely. Although MPXV DNA is frequently detected in the plasma of mpox cases,[8,9,22] one current model proposes that MPXV, and associated potential infectivity, may reside more in the cellular (monocyte) fraction of blood.[23] However, the operational conclusion that can be drawn from the data is that detectable MPXV DNA in plasma at a level of >485 DNA copies/ml was rare during a period of extensive MPXV circulation in 2022. Although based on a relatively small sample, findings indicate limited or absent contamination of the blood supply by MPXV over this period, consistent with the lack of reported mpox-associated adverse events reported to date to the UK Serious Hazards of Transfusion haemovigilance scheme. The findings support a low transmission risk of MPXV by substances of human origin reported by ECDC.[24,25]

The study further highlights the potential value of blood donation monitoring to evaluate ongoing threats to blood safety. The use of surplus minipools and the unlinked, anonymised design of the study enable screening to be applied extremely rapidly as a monitoring tool for the spread of an emerging, potentially blood-borne pathogen. Indeed, routine storage of surplus minipools or other donation samples will enable retrospective longitudinal studies of pathogen emergence and provide an important resource in ongoing strategies for pandemic preparedness. Based on the currently used pool size of 24, it was relatively straightforward to test a whole week of donations from one of the two blood processing centers in England in real time.

Although negative results provide reassurance for blood safety, timely detection of MPXV DNA in one or more minipools allows much earlier intervention than possible through conventional haemovigilance. As discussed previously,[7] the existence of an early warning system, uncoupled by design to individual donors or recipients may provide a framework to rapidly evaluate other potential infectious risks to blood safety.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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