Monkeypox virus neutralising antibodies detected against Clade Ib and Clade IIb in healthy individuals following vaccination with MVA-BN

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ABSTRACT

We show that a two-dose regimen of the vaccine modified Vaccinia Ankara-Bavarian Nordic (MVA-BN) can generate neutralising antibodies against monkeypox virus (MPXV) Clade Ib and IIb, with higher responses observed to the latter. This result provides evidence that vaccination can induce cross-neutralising antibodies against currently circulating MPXV clades.

RESEARCH LETTER

Mpox is a zoonotic viral disease caused by the monkeypox virus (MPXV), which is divided into Clades I (originally Central African) and II, with Clade II further subdivided into subclades IIa and IIb *(1,2).* In 2023 a new subclade of Clade I, termed Clade Ib, emerged in the Eastern Democratic Republic of the Congo (DRC). Since the first human case discovered in August 1970 in DRC, mpox has been reported in 11 African countries, with a small number recorded outside of Africa. However, in 2022 a global outbreak occurred in nonendemic areas caused by the Clade IIb strain *(1).* More recently, the emergence of Clade Ib, which became a Public Health Emergency of International Concern (PHEIC) in August 2024 and is associated with more severe disease and death, particularly in children continues to be a public health threat *(3).* The WHO recommends those at high-risk of contracting mpox, especially during an outbreak receive vaccination *(2)* with the modified Vaccinia Ankara-Bavarian Nordic (MVA-BN) smallpox vaccine, which is a live attenuated vaccine used worldwide, including in the UK, Europe, and the USA *(1).*

Evidence demonstrates that vaccination with MVA-BN can generate low levels of neutralising antibodies against Clade IIb and Clade Ia *(4,5).* In the UK, a single dose of MVA-BN gives a short-term protection of 78% against mpox predominantly in the MSM (men who have sex with men) community *(6).* However, whether vaccination can also induce

neutralising antibodies to Clade Ib has not been addressed. Using a small cohort of healthy healthcare workers (n=25) vaccinated with MVA-BN due to occupational exposure to mpox, this study measured neutralising antibodies against Clades Ib and IIb using a plaque neutralisation assay (PRNT). Our results demonstrate that MVA-BN vaccination can elicit antibodies that can neutralise Clade Ib, albeit at low levels, and provides comparative data against Clade IIb for which the vaccine is known to be effective *(6).*

The importance of complement in relation to neutralisation levels has been reported for MPXV *(7)* in addition to other viruses *(8).* To assess the contribution of complement in our cohort, serum samples were exposed to different conditions (heat-inactivation (HI), HI supplemented with guinea pig serum as a complement source, and non-HI). Here we found, as previously reported *(7),* complement is required for neutralisation of MPXV *in vitro* (Figure 1A). Additionally, no significant difference in MPXV neutralisation was detected (p=0.0625, Wilcoxon signed-rank test) between HI serum in the presence of a complement source and non-HI. Based on these data we used non-HI serum for the remainder of the experiments.

Neutralisation against MPXV Clade Ib and IIb was measured in non-HI serum samples from twenty-five vaccine recipients. The vaccine group which is comprised of healthcare workers with high occupational exposure to MPXV had three individuals with comorbidities consisting of multiple sclerosis, psoriasis, and asthma (Table 1). The median $PRNT_{50}$ value for Clade Ib was 25.9 (IQR = 10.05 – 49.7) compared to Clade IIb which had a median of 44.8 (IQR = 19.55 – 89.4). Comparisons across these samples demonstrated that two doses of MVA-BN generate greater neutralisation against MPXV Clade IIb than Ib, a difference which was found to be statistically significant (p-value=0.0028, Wilcoxon signed-rank test) (Figure 1B). Although this difference in neutralizing antibody titers is small, its biological relevance remains uncertain. The protective threshold for MPXV neutralizing

antibodies is still not well defined and prospective cohort studies are required to define antibody-specific correlates of protection.

Our results show low levels of MPXV neutralisation; consistent with previous studies *(4,5,9)*. We also found that neutralisation of Clade Ib was lower than Clade IIb. Although the present study is limited by the relatively low sample size it demonstrates neutralisation of MPXV Clade Ib in vaccine recipients without prior disease and includes a comparison with Clade IIb neutralisation . Moreover, given that the study cohort includes healthcare workers at highest risk of exposure evidence of vaccine-associated neutralisation is relevant for policies regarding future vaccine rollouts.

MPXV neutralisation is known to require complement *(7)*. Low levels of neutralisation were observed with the addition of guinea pig serum to virus without the addition of serum and when pooled human plasma was added to virus (data not shown) highlighting the non-specific effect that foreign complement sources can have on MPXV neutralisation. An effect which has also been observed with guinea pig serum alone exhibiting neutralisation activity against mumps virus comparable to purified antibodies alone *(10)*. Therefore, like other studies *(9)* an approach to use non-heat-inactivated serum to measure MPXV neutralisation was undertaken.

The low levels of neutralisation identified in our study, particularly for Clade Ib, may suggest that at least moderate protection against disease from MPXV Clade Ib can be expected following vaccination with MVA-BN. However, the durability of these responses and whether a third ‘booster’ dose may be required to increase immunity to mpox Clade Ib infections *(4,5,9)* requires further investigation.

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The views expressed are those of the author(s) and not necessarily those of the DECIPHER consortium, The Pandemic Institute or ISARIC4C Investigators.

CONFLICTS OF INTEREST

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Table 1. Demographics of the study cohort

	n	Age*	Ethnicity (%)	Sex (%)	Comorbidities
MVA-BN vaccine recipients	25	39 (30 – 45)	White (80%) Asian (12%) Latin (8%)	Male (36%) Female (64%)	Multiple Sclerosis (n=1), Psoriasis (n=1), Asthma (n=1)

*Represents the interquartile range.

FIGURE LEGEND:

Figure 1. PRNT50 titres for participants vaccinated with two doses of MVA BN vaccine to assess neutralising antibody responses to MPXV Clade Ib and Clade IIb. Each datapoint represents the geometric mean titre of two experimental replicates. Black line represents the median. $PRNT_{50}$ values were determined using Probit regression. A) Assesses the contribution of complement on neutralisation. Y-axis represents log-$PRNT_{50}$ values. X-axis illustrates the different conditions tested: HI=heat-inactivation; GPS=guinea pig serum. P-value determined using Mann Whitney U test. B) $PRNT_{50}$ values for Clade Ib and IIb. P-value determined using Wilcoxon matched pairs signed rank test.

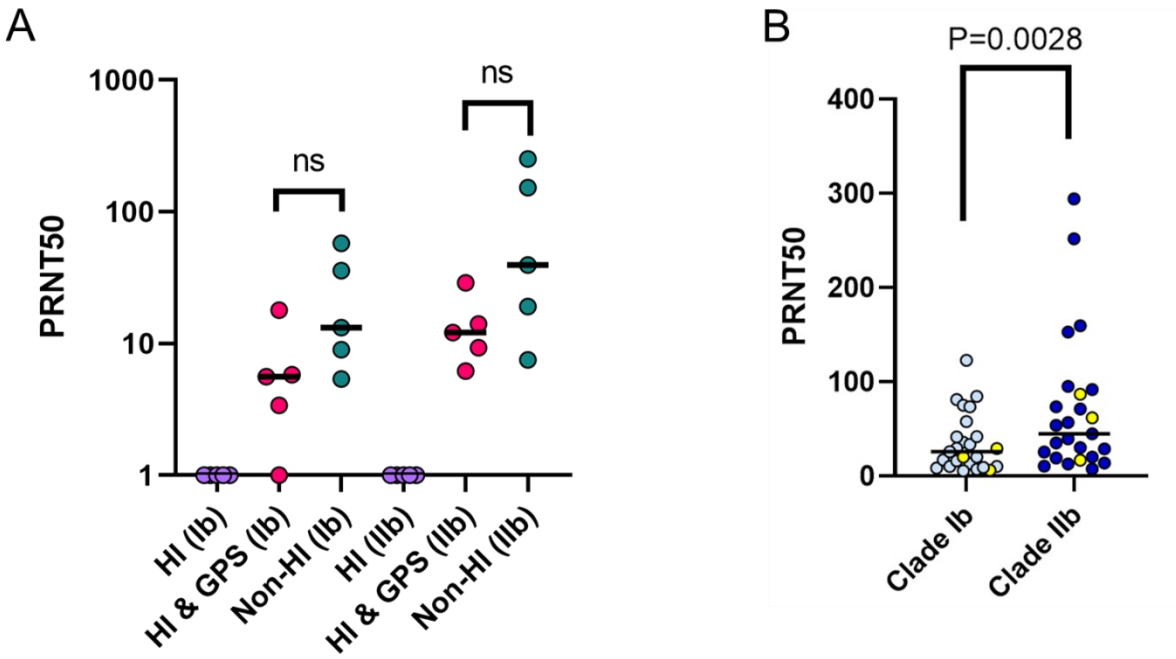

Figure 1. $PRNT_{50}$ titres for participants vaccinated with two doses of MVA BN vaccine to assess neutralising antibody responses to MPXV Clade Ib and Clade IIb. PRNT50 values of less than 4 were given a value of 0. (A) Effect of complement on neutralising antibody titres (B) Comparison of antibody titres against MPXV Clade Ib and Clade IIb.

APPENDIX: Monkeypox virus neutralising antibodies detected against Clade Ib and Clade IIb in healthy individuals following vaccination with MVA-BN

Methods

Study design and participants

Blood samples were obtained from twenty-five healthcare workers with occupational exposure to mpox who received two doses of the Modified Vaccinia Ankara (MVA) Bavarian Nordic (MVA-BN) vaccine via subcutaneous or intradermal routes (Appendix Table 1). Vaccine recipients were recruited into the Acute Virus Immunity Study (AVIS; REC 16/NW/0170) at the University of Liverpool. All participants provided written informed consent before sample collection.

Viruses and Cells

The Clade IIb mpox virus, 2022, Slovenia ex Gran Canaria was obtained from the European Virus Archive Global. MPXV Clade Ib, 2024, London ex East Africa (October 2024) was isolated from a clinical sample collected under the ISARIC Clinical Characterisation Protocol-UK (approved in England by Oxford Research Ethics Committee, ref 13/SC/0149). We passaged virus stocks in Vero E6 cells (African green monkey kidney, C1008 Public Health England PHE – UK Health Security Agency (UKHSA)) maintained in Dulbecco's Modified Eagle Medium (DMEM) with 2% heat-inactivated fetal bovine serum and 0.05mg/ml gentamicin and harvested 72 h post inoculation.

Virus Sequencing

DNA was extracted from MPXV stocks using Zymo DNA/RNA viral extraction kit. The NextGen PCR MPXV Sequencing Library Prep Kit (MBS) was used in conjunction with Oxford Nanopore Rapid Sequencing DNA kit V14 - barcoding (SQK-RBK114.24) to amplify viral DNA *(1).* Mpox PCR amplicons were sequenced on an Oxford Nanopore P2 R10.4 flow

cell following manufacturer's instructions. Low quality reads (average q-score <9) were discarded during base calling. Reads passing initial QC were trimmed with porechop_abi to remove barcodes. Reads were mapped to MPOX clade 1 and clade 2 reference genomes using minimap2 v2.26 with map-ont presets *(2)*. Consensus sequences were created from individual bam files using SAMtools v1.18 consensus using the Bayesian model *(3)*. Sequences were mapped to both clades to confirm that the choice of reference sequence did not affect clade assignment during construction of the tree.

Consensus sequences were combined with MPOX Clade 1 and 2 reference sequences NC003310 and NC063383 and 100 previously published MPOX sequences with known clade assignments. Previously published GenBank sequences and clade assignments were retrieved from the Nextstrain MPOX instance (https://github.com/nextstrain/mpox) and filtered to retain sequences with >95% coverage relative to the reference *(4)*. 25 sequences were randomly selected for each of the major subclades (1a, 1b, 2a, 2b) for inclusion in the tree. Sequences were aligned with MAFFT v7.525 using automatic parameter determination *(5)*. An approximate maximum-likelihood neighbour joining tree was constructed with FastTree v2.1.11 under a Jukes-Cantor GTR model of nucleotide evolution, with the tree visualised in R using ggtree *(6,7)* (Appendix Figure 1).

Plaque Reduction Neutralisation Titres (PRNT)

PRNTs were performed using Vero E6 cells. Sera were diluted 1:4 in DMEM (2% FBS; 0.05 mg/mL gentamicin) followed by serial twofold dilutions. MPXV at 700 PFU/mL was added to an equal volume of diluted sera and incubated at 37°C for 1 h. In some experiments, 10% guinea pig serum (a source of complement), was added to the starting serum dilution (final concentration 5% when mixed with MPXV). The virus-serum dilution was inoculated onto Vero E6 cells in duplicate. Cells were incubated for 72 h at 37°C and 5% CO2 before being fixed with 10% formalin and stained with crystal violet solution. The 50%

plaque reduction titre ($PRNT_{50}$) was determined via probit regression analysis using a script adapted from Bewley et al. 2021 *(8).* In brief plaque counts were entered into Microsoft Excel, under four column headers, labelled as conc, success, failure and VOC (Virus Only Control). In the 'conc' column, the reciprocal of the dilutions used for the sample being analysed was added (4, 8, 16, 32 etc.). In the 'failure' column, the average plaque counts from two wells containing virus and serum was input alongside the corresponding serum dilution. In the 'VOC' column, the average plaque count from the virus control was input. To calculate the 'success' rate, the plaque count (failure) was subtracted from the VOC. The worksheet was saved as a .csv file with the sample ID. The software package 'R' was used with the script available from Bewley et al. 2021. A graph was generated within R which calculated the median plaque reduction neutralising dose ($PRNT_{50}$) with confidence intervals. When a 50% reduction in number of plaques was not seen at 1:4 dilution a value of 0 was assigned to that sample.

Statistical Analysis

Statistical analysis was done using GraphPad Prism version 10 (GraphPad Software). The non-parametric Mann-Whitney U-test and Wilcoxon matched-pairs signed-rank tests were used to compare differences across the samples. P values less than 0.05 were considered significant.

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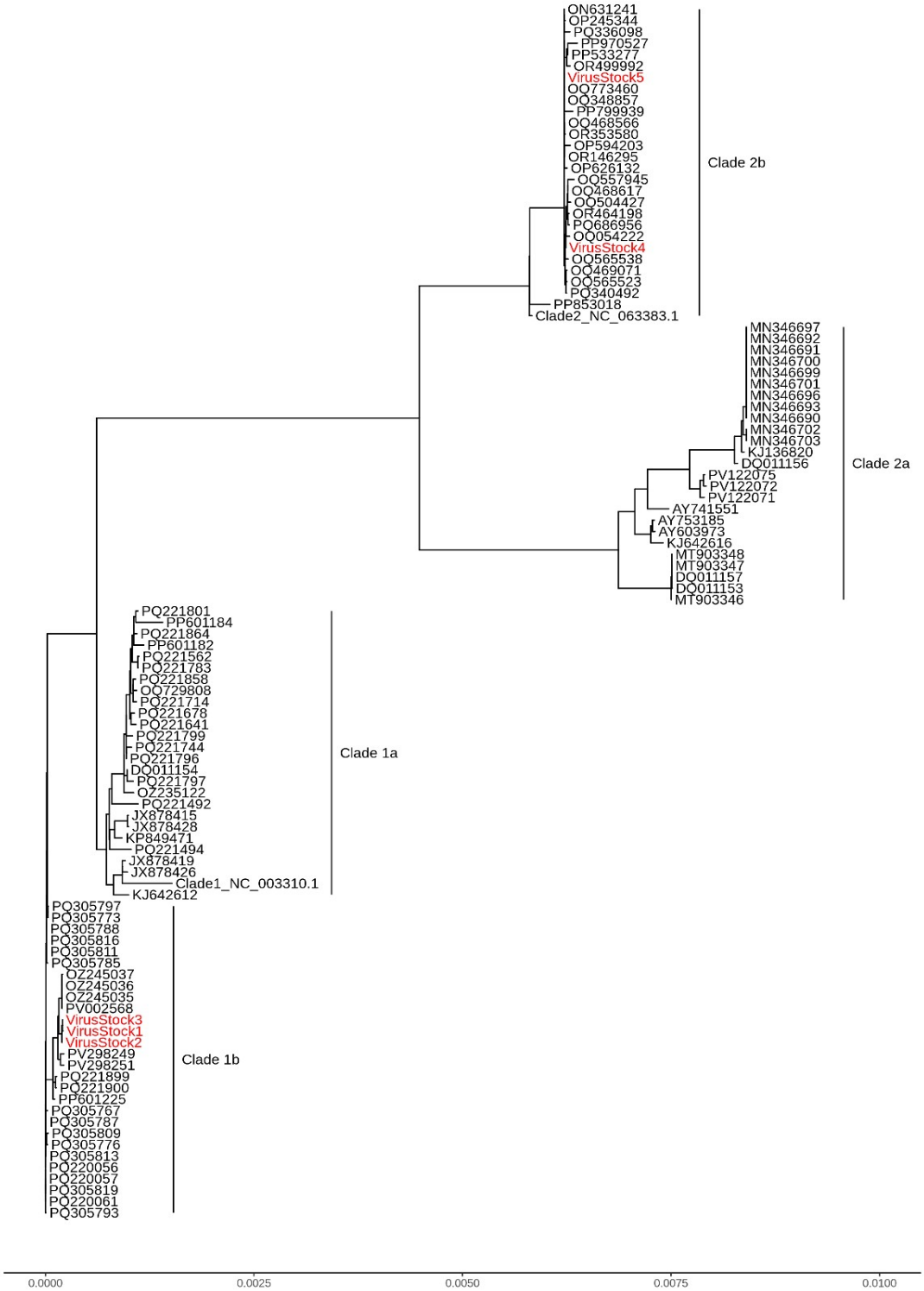

Appendix Figure 1: Phylogenetic tree of MPXV Clades. Highlighted in red are virus stock sequences mapped to the corresponding clades.