THE LANCET
Infectious Diseases

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

This online publication has been corrected. The corrected version first appeared at thelancet.com/infection on June 2, 2022.

Supplement to: Adler H, Gould S, Hine P, et al. Clinical features and management of human monkeypox: a retrospective observational study in the UK. *Lancet Infect Dis* 2022; published online May 24. https://doi.org/10.1016/S1473-3099(22)00228-6.

Supplementary appendix

Clinical features and management of human monkeypox, United Kingdom, 2018-2021: a retrospective observational study

Hugh Adler, PhD[1,2], Susan Gould, MRCP[1,2], Paul Hine, MRCP[1,2], Luke B Snell, MRCP[3], Waison Wong, MRCPCH[4], Catherine F Houlihan, PhD[5,6], Jane C Osborne, PhD[5], Tommy Rampling, DPhil[5,6], Mike BJ Beadsworth, MD[1,2], Christopher JA Duncan, DPHil[7,8], Jake Dunning, PhD[9,10,11], Tom E Fletcher, PhD[1,2], Ewan R Hunter, PhD[7], Michael Jacobs, PhD[6,7], Prof Saye H Khoo, MD[1,12], William Newsholme FRCP[3], David Porter, PhD[4], Robert J Porter, FRCPath[13], Libuše Ratcliffe, MRCP[1], Matthias L Schmid, MD[7], Prof Malcolm G Semple, PhD[14,15], Anne J Tunbridge, FRCP[16], Tom Wingfield, PhD[1,2,17]*, Nicholas M Price, PhD[3]*

On behalf of the NHS England High Consequence Infectious Diseases (Airborne) Network

Affiliations:

1. Tropical and Infectious Disease Unit, Liverpool University Hospitals NHS Foundation Trust, Liverpool, UK
2. Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK
3. Directorate of Infection, Guy's & St Thomas' NHS Foundation Trust, London, UK
4. Department of Paediatric Infectious Diseases, Alder Hey Children's NHS Foundation Trust, Liverpool, UK
5. Rare and Imported Pathogens Laboratory, UK Health Security Agency, Porton Down, Salisbury, UK
6. University College London, London, UK
7. Department of Infection and Tropical Medicine, Newcastle-upon-Tyne Hospitals NHS Foundation Trust, Newcastle, UK
8. Translational and Clinical Research Institute, Immunity and Inflammation Theme, Newcastle University, Newcastle Upon Tyne, UK
9. Department of Infectious Diseases, Royal Free London NHS Foundation Trust, London, UK
10. Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, UK
11. National Infection Service, UK Health Security Agency, UK
12. Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, UK
13. Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
14. Respiratory Unit, Alder Hey Children's Hospital NHS Foundation Trust, Liverpool, UK
15. NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Institute of Infection Veterinary and Ecological Sciences, University of Liverpool, Liverpool, UK
16. Department of Infectious Diseases, Sheffield Teaching Hospitals NHS Foundation Trust, Royal Hallamshire Hospital, Sheffield
17. World Health Organization Collaborating Centre on Tuberculosis and Social Medicine, Department of Global Public Health, Karolinska Institute, Stockholm, Sweden

*These authors contributed equally to the work

Corresponding author:

Dr Nicholas M Price, Directorate of Infection, Guy's & St Thomas' NHS Foundation Trust, Westminster Bridge Road, London, SE1 7EH.

Email: nicholas.price@gstt.nhs.uk

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Supplementary methods

Patient care provision in the High Consequence Infectious Disease (HCID) centres

All patients were cared for in HCID centres by staff wearing appropriate personal protective equipment (PPE) as designated by national consensus guidelines.[1] Briefly, PPE comprised a fluid resistant surgical gown, plastic apron, plastic visor, filtering face-piece 3 respirator, hood, rubber boots and three pairs of latex gloves, with a buddy system for supervision of donning and doffing. HCID centre staff receive regular training in PPE donning and doffing, and additional staff (e.g. interventional radiologists) receive training and buddy doffing on an as-needed basis. Patient care took place in lobbied single rooms with negative pressure ventilation, HEPA filtration of vented air and clearly demarcated zones for donning and doffing PPE. Staff providing direct patient care to people with confirmed monkeypox were offered prophylactic vaccination with modified vaccinia Ankara (MVA, Imvanex®). Physician reviews typically took place twice daily (more or less frequent depending on acuity of illness), with infectious diseases physicians (or paediatricians, in the case of children) available 24 hours a day. Patients typically had routine blood tests performed at admission, in response to clinical deterioration, and every 24—48 hours if receiving antiviral therapy.

Molecular testing for monkeypox virus

Monkeypox viral testing was performed in high containment facilities at the Rare and Imported Pathogens Laboratory. All samples—scabs, skin swabs, blood (EDTA or plasma), upper respiratory tract, and urine—were subjected to the same PCR protocol. Skin and respiratory swabs were transported in viral transport medium, urine in universal containers and scabs in either viral transport medium or universal containers, with no pre-processing for any samples. Pustules were typically unroofed/lanced prior to swabbing, at the discretion of the physician collecting the sample.

Each patient's first skin sample was tested using an in-house developmental pan-orthopoxvirus and pan-parapoxvirus PCR assay adapted from a previously published method,[2] as well as PCR for herpes simplex and varicella zoster viruses (Qiagen GmbH, Hilden, Germany). If a sample tested positive for orthopoxvirus DNA, monkeypox was confirmed using a PCR assay which detected the VETF and Rp018 genes.[3] Finally, a clade-specific PCR assay[4] confirmed that all cases reported here were West African Clade. After the confirmation of monkeypox on an initial skin sample, all subsequent samples from each patient (from any site) were run only on the pan-orthopoxvirus PCR panel. This assay provided results in the form of cycle thresholds (Ct), with lower Ct values corresponding with a higher titre of viral DNA in the sample. The pan-orthopoxvirus PCR assay has undergone internal validation to determine a Ct cut-off of 40 cycles indicating undetectable DNA. Ct values <38 are interpreted as positive. Ct values ≥38 but <40 with acceptable amplification plots are initially interpreted as indeterminate and repeated on clinician request.

Orthopoxvirus serology

Total orthopoxvirus antibodies (IgG+IgM) in serum were assessed using an in-house IFA established at the Bundeswehr Institute of Microbiology in Munich. This assay had previously been employed to confirm cowpox infection in humans and animals.[5] Briefly, chamber slides with African green monkey kidney epithelial cells (MA104; ATCC CRL-2378.1) were infected with Vaccinia Virus Elstree in MEM with 5% FBS. Visible plaques were fixed in methanol/acetone 40 hours post infection. Chambers were blocked with dilution buffer (PBS - 10% goat serum) for 1 hour at 37°C, then two-fold dilutions of human sera (samples and controls) in dilution buffer were prepared and incubated

for 1 hour at 37°C. Samples were washed with PBS-0.25 Tween20. FITC-labelled anti-human IgG was added with Evans Blue as counterstain at 1/20 and 1/50 dilutions, respectively, and incubated for 30 minutes at 37°C. After a washing step with sterile water the chamber slides were air-dried, followed by immunofluorescence microscopy using a Nikon Eclipse 50i instrument with a 40x objective. Human reference sera (https://www.instand-ev.de/) were used as positive controls (cutoff titre >1/80) and dilution buffer as negative control.

Data sharing statement

Participant data, beyond those reported in the published manuscript, will not be made available to others.

Additional members and contributors to the UK High Consequence Infectious Diseases (Airborne) Network

Tropical and Infectious Diseases Unit, Liverpool University Hospitals NHS Foundation Trust, Liverpool, UK

- Mike Abouyannis MRCP DTM&H
- Asma Al-Balushi MRCP
- Stephen Aston MRCP PhD DTM&H
- Robert Ball MRCP DTM&H
- Nicholas J Beeching FRCP MD DTM&H
- Thomas J Blanchard FRCP PhD DTM&H
- Ffion Carlin MRCP DTM&H
- Prof Geraint Davies FRCP PhD DTM&H
- Angela Gillespie RN
- Scott R Hicks MRCP DTM&H
- Marie-Claire Hoyle RN
- Chinenye Ilozue MRCP MSc
- Luke Mair MRCP DTM&H
- Suzanne Marshall RN
- Anne Neary
- Emmanuel Nsutebu MRCP MD DTM&H
- Samantha Parker RN
- Hannah Ryan MRCP DTM&H
- Lance Turtle MRCP PhD DTM&H
- Chris Smith MRCP DTM&H
- Jon van Aartsen MRCP
- Naomi F Walker MRCP PhD DTM&H
- Stephen Woolley MRCP MD DTM&H

Department of Virology, Liverpool University Hospitals NHS Foundation Trust, Liverpool, UK

- Anu Chawla FRCPath
- Ian Hart FRCPath
- Anna Smielewska FRCPath

Department of Radiology, Liverpool University Hospitals NHS Foundation Trust, Liverpool, UK

- Elizabeth Joekes FRCR

Alder Hey Children's NHS Foundation Trust, Liverpool, UK

- Cathryn Benson
- Cheryl Brindley
- Urmi Das
- Chin Kien Eyton-Chong
- Claire Gnanalingham
- Clare Halfhide
- Beatriz Larru
- Sarah Mayell
- Joanna McBride
- Claire Oliver
- Princy Paul
- Andrew Riordan
- Lekha Sridhar
- Megan Storey

Departments of Infectious Diseases[I], Microbiology[M], and Virology[V], Royal Free London NHS Foundation Trust, London, UK

- Audrey Abdul[I]
- Jennifer Abrahamsen[I]
- Breda Athan[I]
- Sanjay Bhagani[I]
- Colin S Brown[I,M]
- Oliver Carpenter[I]
- Ian Cropley[I]
- Kerrie Frost[I]
- Susan Hopkins[I]
- Jessica Joyce[I]
- Lucy Lamb[I]
- Adrian Lyons[M]
- Tabitha Mahungu[I,V]
- Stephen Mepham[I.M]
- Edina Mukwaira[I]
- Alison Rodger[I]
- Caroline Taylor[I]
- Simon Warren[I,M]
- Alan Williams[M]

Department of Clinical Psychology, Royal Free London NHS Foundation Trust, London, UK

- Debbie Levitt

Department of Infection and Tropical Medicine, Newcastle-upon-Tyne Hospitals NHS Foundation Trust, Newcastle, UK

- Denise Allen RN
- Jill Dixon MRCP MSc DTM&H
- Adam Evans FRCPE DTM&H
- Pauline McNicholas RN
- Brendan Payne FRCPath PhD
- D Ashley Price FRCP MD DTM&H
- Uli Schwab FRCP FRCPE MD DTM&H
- Allison Sykes MSc
- Yusri Taha MD
- Margaret Ward RN

Department Paediatric Immunology and Infectious Diseases, Great North Children's Hospital, Newcastle-upon-Tyne

- Marieke Emonts FRCPH MD PhD MSc
- Stephen Owens MRCPCH PhD

Directorate of Infection, Guy's and St. Thomas' NHS Foundation Trust, London, UK

- Alina Botgros
- Sam T Douthwaite
- Anna Goodman
- Akish Luintel
- Eithne MacMahon
- Gaia Nebbia
- Geraldine O'Hara
- Joseph Parsons
- Ashwin Sen
- Daniel Stevenson
- Tadgh Sullivan
- Usman Taj
- Claire van Nipsen tot Pannerden
- Helen Winslow
- Ewa Zatyka

Department of Infection Prevention and Control, Guy's and St. Thomas' NHS Foundation Trust, London, UK

- Ekene Alozie-Otuka
- Csaba Beviz
- Yusupha Ceesay
- Latchmin Gargee
- Morloh Kabia
- Hannah Mitchell
- Shona Perkins
- Mingaile Sasson

- Kamal Sehmbey
- Federico Tabios
- Neil Wigglesworth

Department of Infectious Diseases, Blackpool Teaching Hospitals NHS Foundation Trust, Blackpool, UK

- Peter Flegg

Rare and Imported Pathogens Laboratory, UK Health Security Agency, Porton Down, Salisbury, UK

- Emma J Aarons
- Tim Brooks
- Matthew Dryden
- Jenna Furneaux
- Barry Gibney
- Jennifer Small
- Elizabeth Truelove
- Clare E Warrell

Public Health Wales

- Richard Firth
- Gemma Hobson
- Christopher Johnson

Royal Naval Air Station Culdrose

- Surg Cdr Alison Dewynter
- Lt Cdr Sebastian Nixon
- MA Oliver Spence

Bundeswehr Institute of Microbiology, Munich, Germany

- Joachim J. Bugert MD, PhD

SIGA Technologies Inc, Corvallis, Oregon

- Dennis E Hruby, PhD

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