THE LANCET Infectious Diseases

Supplementary webappendix

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

Supplement to: Yinka-Ogunleye A, Aruna O, Dalhat M, et al, for the CDC Monkeypox Outbreak Team. Outbreak of human monkeypox in Nigeria in 2017–18: a clinical and epidemiological report. *Lancet Infect Dis* 2019; published online July 5. http://dx.doi.org/10.1016/ S1473-3099(19)30294-4.

APPENDIX 1: Monkeypox case investigation form

(This form was modified in the course of the outbreak**)**

Nigeria Monkeypox Case Investigation form

Epid number**:**
Date of investigation: ____/___/___

Case reported by *name* _____

*Title*_____

*Phone No*_____

Section 2: Patient identity

1. Last Name_____ First Name _____

2. For children, father's name _____

3. Date of birth ___/___/___

4. Age (years) _____

5. Gender □ M □ F

6. Village/settlement/street of residence during the last 6 months_____

7. State _____ LGA _____ WARD_____

8. Nationality _____ Ethnicity / tribe_____

9. Occupation of the patient_____

_____

Section 2: Patient status

10. Status of the patient: □ Alive □ Dead

11. If dead, date of death ___/___/___ Place of death: _____

12. Place of the funeral, name village: _____ LGA_____ state _____

13. Is a smallpox vaccination scar present? □ Yes □ No □ Do not know

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Section 3: Clinical history/Presentation

14. Date of onset of symptoms**:** __/___/___

15. Name of the village where the patient got ill _____ *Country*_____

16. a. Did the patient travel anytime in the three weeks before becoming ill: □ Yes □ No □ DNK

    b. If Yes, indicate the places __ (1)_____(2)_____

17. a. Did the patient travel during illness : □ Yes □ No □ DNK

    b. If Yes, indicate the places __(1)_____(2)_____

18. Does the patient have a cutaneous eruption**?** □ Yes □ No □ DNK.

19. If yes, date of onset for the rash: ___/___/___

20. Did the patient have fever**?** □ Yes □ No □ DNK. If yes, date of onset for the fever: ___/___/___

21. If there is active disease,

    a. Are the lesions in the same state of development on the body? □ Yes □ No □ Do not know

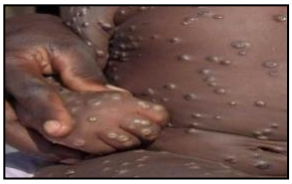
    b. Are all of the lesions the same size and state of development? □ Yes □ No □ Do not know

    c. Are the lesions deep and profound? □ Yes □ No □ Do not know

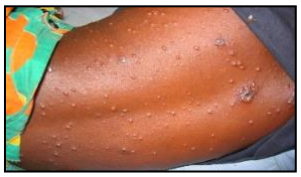
22. Localisation of the lesions. □ Face □ Legs □ Soles of the feet □ Palms of the hands

    □ Thorax □ Arms □ Genitals □ All over the body

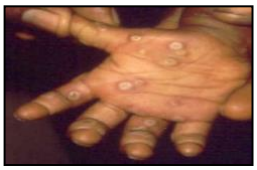
23. Do the lesions resemble (for each photo):



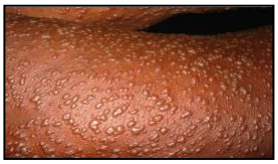
a. □ Yes □ No



b. □ Yes □ No



c. □ Yes □ No



d. □ Yes □ No

24. Does or did the patient have any of the following symptoms (check all that apply)

Vomiting/nausea	□ Yes	□ No	□ DNK	Headache	□ Yes	□ No	□ DNK
Cough	□ Yes	□ No	□ DNK	Lesions that itch	□ Yes	□ No	□ DNK
Lymphadenopathy, inguinal	□ Yes	□ No	□ DNK	Muscle pain (myalgie)	□ Yes	□ No	□ DNK
Lymphadenopathy, axillary	□ Yes	□ No	□ DNK	Fatigue	□ Yes	□ No	□ DNK
Lymphadenopathy, cervical	□ Yes	□ No	□ DNK	Conjunctivitis	□ Yes	□ No	□ DNK
Chills or sweats	□ Yes	□ No	□ DNK	Sensitivity to light	□ Yes	□ No	□ DNK
Sore throat when swallowing	□ Yes	□ No	□ DNK	Is the patient bedridden?	□ Yes	□ No	□ DNK
Oral ulcers	□ Yes	□ No	□ DNK				

Section 4: Exposures

25. During the three weeks preceding the onset of symptoms, did the patient have contact with one or more persons who had with similar symptoms? □ Yes □ No □ Do not know

If Yes, respond to the following questions concerning these additional ill people (indicate all of the ill people). There is additional space for multiple contacts at the end of this form.

26. Last name _____ First name _____

27. Relationship with the patient _____

28. First date of contact with the ill person ___/___/___

29. Did the patient touch a domestic or wild animal during the three weeks preceding symptom onset?

□ Yes □ No □ Do not know

30. If Yes, what kind of animal_____

31. Date of contact ___/___/___

32. Type of contact (*check all that apply*)

□ Rodents alive in the house □ Dead animal found in the forest

□ Alive animal living in the forest □ Animal bought for meat

Section 5: Laboratory

33. Was a specimen collected? □ Yes □ No 35. If Yes, date ___/___/___

34. Type: □ Crust □ Swab □ Blood

Collect at least two types of specimens from each patient. For each specimen: place a label on this form and a label on the specimen tube. Ensure that the two labels have the same name/number of the

Section 6: Update on the Hospital information

35. Was the patient sent to a hospital? □ Yes □ No

36. Was the patient admitted in the isolation ward? □ Yes □ No

37. If Yes, name of Hospital_____ Hospitalization date ___/___/__

38. Date of discharge___/___/___OR Date of death___/___/__

Section 7: Medical History (Please provide information on the

39. If female, Pregnancy status: □ Pregnant □ Not pregnant

40. HIV status: □ Negative □ Positive □ Unknown

41. Any other known medical condition (Please state)

……

……

Section 8: Additional contacts of the patient

Full Name	Location/Address	Sex	Relationship	Type of contact e.g. touch, breastfeeding, sexual

Appendix 2

MONKEYPOX SPECIMEN PROCESSING:

We cut swab tips obtained from lesions into 2.0mL microcentrifuge tubes and hydrated these with 400uL of phosphate buffered saline (PBS). Hydrated swabs were incubated for 10 minutes at room temperature before a brief 1-minute centrifugation in a swab extraction tube system (Roche Diagnostics, Indianapolis, IN) to collect the eluate for DNA extraction. One crust was placed in a 2.0mL microcentrifuge tube with 300uL of PBS and thoroughly homogenized using a sterile, disposable pestle until cloudy in appearance. Homogenates were incubated for 10 minutes at room temperature prior to DNA extraction.

We votexted serum specimens and used a 100uL aliquot directly for DNA extraction. using the EZ1 Advanced DNA Tissue Kit and EZ1 Advanced instrument (Qiagen N.V.;Germantown, MD) in U.S. CDC laboratories (Atlanta, GA) or manually with the QIAmp DNA Mini Kit (Qiagen N.V.;Germantown, MD) at the NCDC National Reference Laboratory (Gaduwa, Abuja, Nigeria). US-CDC and NCDC laboratories conducted real time PCR for MPX and VZV using a West African monkeypox virus specific (MPXV-WA) real-time PCR assay based on TaqMan® chemistry and technology21. The assay was performed in duplicate at a final volume of 20uL containing 6.5uL of RNase/DNase free water (Clontech, Mountain View, CA), on the 7500 Fast Real-Time PCR Instrument (Life Technologies, Grand Island, NY) [15]. Real-time PCR assay specific for VZV was used for Chikenpox testing[17].

We performed Enzyme-linked immunosorbent assays (ELISAs) of anti-OPXV antibodies as previously described.[16] Sera were tested by IgM ELISA at 1:50 dilution and by IgG ELISA at 1:100 dilution to detect antibodies purified VACV Vaccinia virus. Cutoff values (COVs) were determined based on the mean optical density (OD) plus 3 standard deviations for five negative control sera. Resulting OD minus COV (OD-COV) values determined if a sample was positive (>0.0 OD-COV) or negative (<0.0 OD-COV) for presence of anti-OPXV antibodies.

We utilized a targeted hybridization protocol to enrich MPXV DNA from the clinical samples, with the captured MPXV DNA amplified and sequenced using the Illumina MiSeq benchtop system. Whole genome sequences for seven MPXV positive cases from Rivers State were generated and assembled following published protocols.[18] Given the high number of cases and variable epidemiological linkages identified in Rivers State, these seven outbreak samples (selected based on geographic information and quality of epidemiological data) were selected for haplotype analysis along with four references of WA MPXV