STUDY PROTOCOL

Understanding the human, animal and environmental interface of mpox transmission in Nigeria (Mpox One Health Study)

KEY STUDY CONTACTS

(Co)Principal Investigators	Dr Abiodun Egwuenu : Nigeria Centre for Disease Control and Prevention (NCDC) Dr Adeyinka Adedeji : National Veterinary Research Institute (NVRI)
UK-PHRST Lead	Dr Hilary Bower (UK-PHRST/LSHTM): One Health Transmission Study Ms Cristina Leggio, UK-PHRST, UKHSA, Laboratory Prof. Shelley Lees (LSHTM/UKPHRST), Qualitative Research Questions
Co-investigators/Study Coordinators	Mr David Idowu Olatunji: NCDC Dr Cornelius Ohonsi: NCDC Dr Adama Ahmad: National Reference Laboratory (NRL), Nigeria Dr Ogechukwu Maryann: NRL Dr Maurice Nanven: NVRI Nicodemus Mkpuma: NVRI Prof Shelley Lees: LSHTM/UK-PHRST Dr. Natalie Fischer: LSHTM/UK-PHRST Dr. Chioma Achi: LSHTM/UK-PHRST/NCDC Mr Adesuyi Ayodeji Omoare: NCDC Ms Bamidele Oluwafemi (NRL) Mr Abdulakeem Amaoo (CPHL)
Study sponsor	London School of Hygiene and Tropical Medicine
Indemnity	London School of Hygiene and Tropical Medicine
Funder(s)	UK Government Department of Health & Social Care Overseas Development Assistance via UK-PHRST research funds
Other Study Contributors	Dr Emmanuel Feyi Obishakin: previously NVRI, currently Pirbright Institute, UK
Peer Reviewed by:	UK-PHRST Technical Steering Committee

Contents

1. STUDY SUMMARY

Study Title	**Understanding the human, animal, and environmental epidemiology of mpox transmission in Nigeria (Mpox One Health Study)**
Study Design	**1. Human component** Observational cohort study of case-households and non-household close and sexual contacts with immediate and prospective serological sampling **2. Animal component** Cross-sectional study of rodents, domestic and wild animals found within the environs of the case household (immediate compound and close neighbourhood) **3. Environmental sampling component** Surface sampling in selected recently-affected households.
Study Participants and rationale for inclusion	**Human component** **a) Confirmed cases offered participation in the separate but associated Clinical Characterisation Study (see separate protocol)** *Rationale for inclusion:* to investigate risk factors for infection and case experience of the disease b) **Household contacts of confirmed cases** *Rationale for inclusion:* to investigate risk factors for infection; transmission dynamics including evidence of pauci- and asymptomatic infection; incidence and household secondary transmission rates and risks. Uninfected household members (ascertained by serology) will provide the comparison group for risk and exposure analysis **c) Sexual contacts of the same confirmed cases** *Rationale for inclusion*: to investigate sexual contact as a risk factor for infection, prevalence of infection among sexual contacts, role of sexual contact in the transmission chain **d) Non-household close contacts of the same confirmed cases** *Rationale for inclusion:* to investigate risk factors for infection, prevalence of infection among close contacts **Animal component** **Rodents, domestic, and wild animals in environs and neighbourhood of case-households** *Rationale for inclusion*: to explore the prevalence of infection and role of animals in the transmission chain **Environment component** **Surface sampling in selected recently-affected households**

	Rational for inclusion: to inform understanding of the role of environmental contamination in transmission
Planned Sample Size (if applicable)	Sample size depends on the cases recruited to the clinical characterization study but should be as large as possible to allow for valid subgroup analysis. Average household size in Nigeria is 5 people. Based on this we will aim for a minimum of 150 households and maximum of 250 households (approx. 500 – 1000 household participants excluding index cases) over the period of a year which must include the peak season months of July to December. Sample size for close and sexual contact subgroups will be opportunistic but capped at 5 per case for budgetary reasons. All contacts must be first-degree contacts ie. no contacts of contacts. Convenience sampling will be used for the animal study: the aim will be to trap in the vicinity of 30% of case household (75 trapping events) and capture approximately 200 rodents and small mammals. In addition, domestic animals will be sampled.
Planned Study Period	October 2023- November 2024
Research Aim/ Objectives	**Research Aim:** This work aims to understand the roles played by human, animal, and environmental factors in mpox transmission in Nigeria. **Research Objectives:** To elucidate the human, animal and environmental epidemiology of mpox in Nigeria in order to inform and support better epidemic response and control.
Research Questions	**Human component** • What are the risk factors for mpox infection and transmission in case-households and among non-household sexual and close contacts? • What is the incidence and rate of secondary transmission of mpox in case households in Nigeria? • What are the respective roles of human-to-human and animal-to-human contact in mpox infection in Nigeria? • What are the experiences of mpox infection in cases, their households and close contacts? **Animal component** • What is the prevalence of past and present mpox infection in rodents, domesticated and 'bush' animals in the vicinity of case-households?

	• What are the likely animal reservoirs of mpox virus in Nigeria? **Environmental component** • What role does surface contamination play in transmission in case-households? • For how long does the Mpox virus persist in household environments? • Does it evade household cleaning practices? • If the virus persist, is it still infection competent?

2. ROLE OF STUDY SPONSOR

London School of Hygiene & Tropical Medicine is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance and Integrity Office: London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT.
Tel: +44 207 927 2626 Email: RGIO@lshtm.ac.uk

Indemnity: London School of Hygiene & Tropical Medicine holds Public Liability ("negligent harm") insurance policies which apply to this study.

Neither the Sponsor (LSHTM RGIO) or the Funder (NIHR/DHSC) have any functional or decision-making role in the study design, implementation, analysis, interpretation or dissemination of findings.

3. ROLES AND RESPONSIBILITIES OF KEY STUDY PERSONNEL

Nigeria Centre for Disease Control and Prevention (NCDC)

Dr Chinwe Ochu (CO): Principal Investigator for Nigeria Mpox project : oversight and supervision of whole project, including implementation and quality

Dr Abiodun Egwuenu (AE): Joint Study Lead with Dr Adeyinka Adedeji), responsible for protocol and budget development; coordination of implementation including HR organisation; supervision of quality of implementation; analysis and interpretation of results.

Mr David Idowu Olatunji: Co-investigator, responsible for development and implementation of study

Dr Cornelius Ohonsi: Co-investigator, responsible for qualitative input into semi-structured interviews, analysis and interpretation

National Veterinary Research Institute (NVRI)

Dr Adeyinka Adedeji : Joint Study Lead with AE: responsible for protocol and budget development; coordination of implementation of animal and environmental studies including HR organisation; supervision of study quality and implementation; analysis and interpretation of results .

Dr Maurice Nanven: Co-investigator responsible for animal field sampling

Mr. Nicodemus Mkpuma: Co-investigator responsible for laboratory procedure and quality

National Reference Laboratory (NRL)

Ms Adama Ahmad: co-investigator, responsible for laboratory procedures and quality

Ms Ogechukwu Maryann: co-investigator, responsible for laboratory procedures and quality

UK Public Health Rapid Support Team (UK-PHRST)

Prof. Gwenda Hughes: Principal Investigator for Nigeria Mpox project: oversight and supervision of whole project, including implementation and quality

Dr Hilary Bower: Co-investigator, technical advisor for protocol development, implementation planning, analysis and interpretation

Dr Natalie Fischer: Co-investigator, technical advisor for protocol development, implementation planning, analysis and interpretation

Prof Shelley Lees: Co-investigator, technical advisor for social science investigation of human to human and animal to human contacts, and qualitative input into semi-structured interviews, analysis and interpretation

4. BACKGROUND

Mpox (previously known as monkeypox) is a viral zoonotic disease caused by the mpox virus. It is the most important human orthopoxvirus infection, second only to smallpox which was eradicated globally in 1979 (Sklenovska & Van Ranst 2018). It is common in West and Central Africa and has recently spread into different parts of the world (Bunge et al. 2022). Mpox strains were previously named by the region where they occur, namely the Central and West African Clades, but are now designated as clade I and II respectively (Happi et al. 2022). Clade II appears to consist of two subclades, IIa and IIb. On present knowledge, Clade I appear to have a higher case fatality rate (CFR) estimated at 10.6% compared to Clade II which has a CFR of 3.6% (Bunge et al. 2022). Human mpox cases in endemic countries present similar clinical signs to other diseases involving rashes such as chicken pox and measles making differential diagnosis difficult without laboratory confirmation.

Mpox was first discovered in monkeys, but the exact reservoir is still unknown. The virus can infect a broad range of animal hosts (primates and non-primates) such as monkeys, rodents, and domestic animals, but rodents and small mammals are primarily suspected as virus reservoirs. Serologic and molecular studies have detected possible circulation of mpox in populations of rodents such as tree squirrels, Gambian pouched rats, rope squirrels, dormice, and other small mammal species (Parker, 2012; MacNeill, 2022). Additionally, studies have documented over 40 species of animals that have been naturally or experimentally infected with mpox (Parker & Buller 2013). Experimentally, mpox has been shown to infect animals via multiple different inoculation routes, however, the natural route of transmission is not clearly understood and may be species specific (Di Giulio & Eckbury 2004). Transmission may also involve the human-to-animal path as evidenced by a recent study in France where humans transmitted the infection to a dog (Seang et al. 2022), while a recent molecular and serologic study in Nigeria showed evidence of circulation of orthopox virus in rodents trapped in communities/households with confirmed mpox cases (Meseko et al. 2023). However, the study did not confirm presence of mpox virus. Hence, further studies are required to investigate the possible role of rodents in the epidemiology of mpox in Nigeria.

Human cases of mpox were recorded in Nigeria in 1971 and 1978, then almost completely disappeared until 2017 (Foster et al. 2022; Yinka-Ogunleye et al. 2018). Over 120 laboratory-confirmed and probable cases were reported between 2017 and 2018, the majority presenting with classical features of febrile prodrome accompanied by progressive generalised skin rashes (Yinka-Ogunleye et al. 2019). However, there were also reports of cases without the febrile prodrome, those whose symptoms were predominantly genital ulcers, and of mpox associated with HIV-infection. Many aspects of the clinical features and natural history of mpox in Nigeria are yet to be investigated.

The disease has been endemic in Nigeria since its resurgence in 2017 (Happi et al. 2022). But, in 2022, the number of cases reported increased dramatically to over 2000 suspected cases, 700 of which were laboratory confirmed, and 7 deaths (NCDC 2022) (Fig 1), more than the total cases reported in the previous five years. Most of the deaths occurred among patients infected with HIV. There is also a strong suspicion that the number reported represents only a small proportion of the true burden of the disease, due to limited testing capacity, home-management of cases, and incomplete reporting (NCDC 2022). Due to sociocultural and religious factors in Nigeria, it has also been difficult to identify what is driving this outbreak, whether it is linked to increased animal-human transmission or increased human-human transmission, possibly linked to sexual activity, or both.

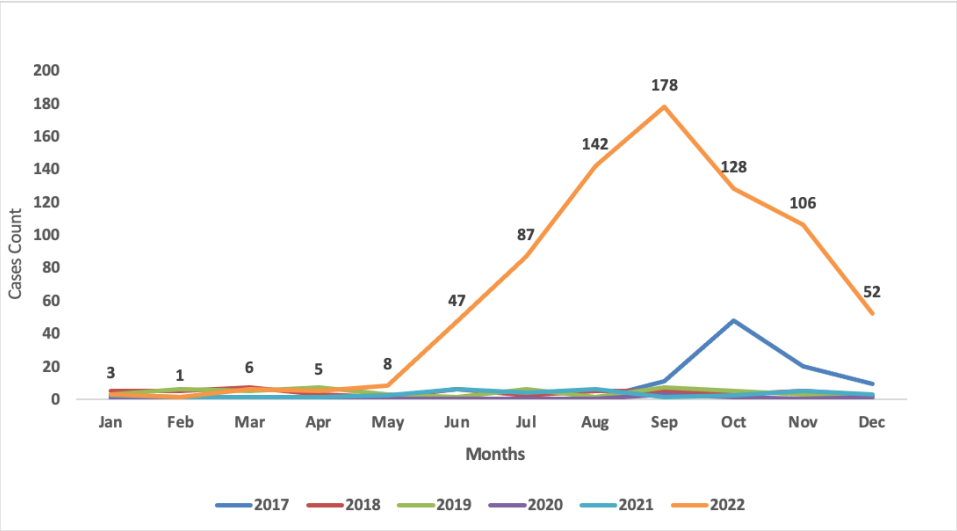

Figure 1: Nigeria confirmed Monkeypox cases by the year of incidence - September 2017 till date

In May 2022, an unprecedented outbreak of mpox across America, Australia and Europe was reported among persons with no travel history to endemic countries (Alder et al. 2022). By 23rd July 2022, the surge in human-to-human cases of mpox led to the declaration of a global public health emergency. From January 2022 to 27 February 2023, 86,173 confirmed cases of mpox and 99 deaths have been reported from 110 countries (WHO 2023). Most confirmed cases have been reported in the WHO region of the Americas (58,578, 67%) which has also accounted for the most deaths (76, 77%). Of the remaining cases, 30% were reported in the European region, 0.3% in the Western Pacific Region, 0.1% in the Eastern Mediterranean Region and 0.05% the South-East Asia Pacific region (WHO 2023). The WHO African region accounted for 2% of the overall cases (1,382) and 17% of deaths (17). Phylogenetic studies identified Clade IIb as the strain responsible for the global outbreak (Gao et al. 2023). Most cases in the global outbreak have occurred in gay, bisexual, and other men who have sex with men (WHO 2023).

While substantial research effort has taken place to understand the global epidemic, the epidemiology, clinical, social, behavioural and ecological characteristics of mpox and mpox transmission in endemic countries such as Nigeria remain under-researched. In August 2022, the Nigeria CDC, the UK-PHRST and partners convened a workshop to define priorities for a research programme on mpox in Nigeria. One research priority identified was a study on *Understanding animal, human and environmental risk factors for mpox transmission through case-households and contacts,* which is described in this protocol.

5. STUDY RATIONALE

Since the beginning of the outbreak in Nigeria, questions have arisen about resurgence of mpox and what may be driving it, particularly the uncertainty around the epidemiology of mpox, the essential parameters of transmission, the risk factors for infection and disease, and the relative importance of different routes of transmission, including animal to human, human to human, and sexual transmission. The limited information available in endemic countries was re-emphasised when Mpox started to spread globally, and particularly when index cases of this wider epidemic were shown to be infected with the variant associated with Nigeria.

This study aims to collect the data necessary to answer some of these questions, using epidemiological and One Health approaches to analyse data across the human/animal interface, focusing on household and close non-household contacts. It aims to gather evidence that can inform prevention and response activities in the present and future outbreaks in Nigeria. The findings, while specific to Nigeria, are likely to be of value to other countries endemic for mpox, and offer insight into the global epidemic.

6. STUDY PARTNERS/TEAM

Main study partners:

Nigeria Centre for Disease Control and Prevention (NCDC)

UK Public Health Rapid Support Team (UK-PHRST)

National Veterinary Research Institute

Other study partners:

AFENET: FELTPs as part of field work teams/supervision; contracting agency

Nigerian Institute of Medical Research: sample collection, laboratory testing, field supervision

State Ministries of Health and Agriculture

7. THEORETICAL FRAMEWORK

Our hypothesis is that by establishing the key epidemiological parameters, risk factors for infection, and relative importance of different routes of transmission for mpox in households and among close contacts, we will be able to assist public health decision makers in Nigeria and elsewhere to improve prevention and response activities.

This study is one of two linked studies. The other is a Clinical Characterisation Study of mpox disease in Nigeria that will be the source of the confirmed cases to be approached for participation in this Mpox One Health Study.

8. RESEARCH QUESTIONS

9.1 Human component

- What are the risk factors for mpox infection and transmission in case-households and among non-household sexual and close contacts?
- What is the incidence and rate of secondary transmission of mpox in case households in Nigeria?
- What are the respective roles of human-to-human and animal-to-human contact in mpox infection in Nigeria?
- What are the knowledge and experiences of mpox infection among cases, their households and close contacts?

9.2 Animal component

- What is the prevalence of mpox infection in rodents, domesticated and 'bush' animals in the vicinity of case-households?
- What are the likely animal reservoirs of mpox virus in Nigeria?

9.3 Environmental component

- What role does surface contamination play in transmission in case-households?
- How long does the Mpox virus persist in household environments
- Does it evade household cleaning practices?

- If the virus persist, is it infection competent?

9. SPECIFIC OBJECTIVES

To establish:

a) The main risk factors for infection, and barriers to preventing infection

b) Key drivers of transmission in case-households and among non-household close contacts

c) The incidence and secondary transmission rates of mpox infection in case households

d) The proportion of infection that is symptomatic, pauci-symptomatic and asymptomatic, and the effect of this on household transmission

e) The prevalence of active and historic infection in pets, domestic animals, rodents and other animals belonging to or in close contact with case-households

f) An indication of the extent and infection-competency of surface contamination in case households

10. EXPECTED OUTCOMES

1. Key risk factors for infection quantified
2. Key drivers of household transmission and transmission among non-household close and sexual contacts established.
3. Main barriers to preventing infection identified
4. Household incidence and secondary transmission rates estimated
5. Proportion of mpox infection that is symptomatic, pauci-, and asymptomatic
6. Rates of active and past infection in pets, domestic animals, rodents in the proximity of households
7. Identification of animal reservoirs of mpox virus in the environment in Nigeria
8. An understanding of surface contamination in case-households

11. METHODOLOGY

12.1 Study Design

This is an observational cohort study with five components:

- A survey and sampling of household contacts of confirmed cases of mpox to ascertain risk factors, exposure, and infection status of each individual at the time of recruitment. Associations will be drawn from comparisons of mpox sero-positive and mpox sero-negative household members.
- A survey and sampling of non-household close and sexual contacts of the index case for the same purposes.
- A follow-up study involving sampling of each enrolled household member at 28 days to capture subsequent development of infection for household parameter calculation.
- Contemporaneous sampling of rodents, domestic and wild/bush animals found in the environs of the household to establish presence of active or past infection.

- Contemporaneous sampling of household surfaces in selected households

12.2 Study Setting

The study settings are dictated by the locations of cases enrolled in the NCDC/UK-PHRST Clinical Characterization Study. This aims to recruit cases in two or more high burden states in Nigeria, with selection of suitable hospitals (2 per state) and primary healthcare centres taking into account factors including mpox case numbers (including historical case numbers), available resources and research capabilities, security issues and accessibility. Where possible, geographically separate states will be selected to increase geographic coverage across Nigeria, but this will be dependent on other factors, particularly accessibility and security issues.

The two studies – Clinical and One Health - will open in parallel, in Lagos state (Lagos University Teaching Hospital; Lagos State University Teaching Hospital), and Rivers state (University of Port Harcourt Teaching Hospital; Rivers State University Teaching Hospital) while Ogun State (Olabisi Onabanjo University Teaching Hospital; Federal Medical Centre Abeokuta) is proposed as a third location for the study.12.3 Study Participants

1. Confirmed cases

Case participants are laboratory-confirmed cases of mpox who have been invited to participate in the Clinical Characterisation Study. All laboratory-confirmed cases approached for the Clinical Characterisation study will be offered the opportunity to take part in the One Health Study with their households regardless of whether they consent to participate in the Clinical Characterisation Study or not,

Rationale for inclusion: to investigate risk factors for infection *and experience of symptomatic cases of mpox. Case participation is also the gateway to identifying households with confirmed exposure to mpox.*

2. Household contacts of same confirmed cases

Household contacts are defined as:

- People who were present in the household for a minimum of 1 month prior to the case's mpox symptom onset and who are present in the household now. A household is defined as people living together in the same residential space and sharing meals.
- People who were present in the household for a minimum of 1 month prior to the cases' mpox symptom onset and died of any cause in this month.
- People who were present in the household for a minimum of 1 month prior to the case's mpox symptom onset but are not present now (proxy reporting*).

Rationale for inclusion: to investigate exposure risk factors; incidence; transmission dynamics including pauci- and asymptomatic infection; and establish household secondary transmission rates and risks. Serologically-confirmed uninfected household members will be the comparison group for risk and exposure analysis. Household members with likely intimate contact (eg. a spouse) will be asked to answer additional questions privately.

*Information on symptoms and exposures for absent or dead household members will be requested from other family members, with the aim of identifying whether there is evidence that either of

these two categories of people had a mpox symptoms, acknowledging the limitation of proxy reporting.

3. Close contacts* of same confirmed cases not living in the same household

Close contacts are defined as people who had bodily contact with the case at least once during the one month prior to the case's symptom onset, or shared enclosed space (e.g. in an occupational setting, extended visits within the period) for at least a day, or shared or handled clothing, bedding or towels used by the case during the same period.

Rationale for inclusion: to investigate risks of infection from non-household casual contact

4. Sexual contacts* of same confirmed cases not living in the same household

Sexual contact is defined as skin-to-skin contact, including sexual intercourse or activities, kissing, cuddling or holding hands.

*Rationale for inclusion***:** to investigate potentially high-risk exposures related to sexual contact and estimate secondary transmission if direction of transmission can be established

*****NOTE: only direct first level contacts will be included, not contacts of contacts, even if first level contacts are found to be antigen or antibody positive.

5. Animals

Small mammals and wild animals found inside the houses, compounds or within perimeter fencing of case households and of households adjacent to case-households, also in nearby farmland and on the perimeter of farms where rural communities are involved. Samples will also be collected from domestic animals in case households.

*Rationale for inclusion***:** to investigate presence of active or past virus in animals as an exposure risk.

12.4 Inclusion and Exclusion Criteria

Confirmed mpox cases and all their household members (defined as people living together within the same residential structure and sharing meals) will be invited to take part regardless of age, sex, pregnancy, smallpox vaccine status, language, or any other variable. Parents will be asked to respond for children under age 12. Questions about sexual activity will only be asked to participants above the legal age of consent in Nigeria, which is 18 years of age. People over 40 years of age will be asked to state whether they were vaccinated for smallpox and, if acceptable, show their vaccination scar to facilitate interpretation of serology. All small mammals, rodents and wild animals found will be included in trapping and all domesticated animals and pets sampled.

There are no exclusion criteria.

12.5 Recruitment methods

Confirmed cases who are approached to offer participation in the Clinical Characterisation Study will also be offered participation in the Mpox One Health Study (see Clinical Characterisation Study recruitment flowchart). In the Clinical Characterisation Study, following case confirmation if the case is not admitted, a study team researcher will accompany the State Disease Surveillance and Notification Officer (SDSNO) to offer participation in that study. At the same time, the participant will be invited to participate in the Mpox One Health Study and asked to provide consent for study researchers to approach members of their household and their other close contacts. Confirmed cases will be offered participation in the Mpox One

Health Study regardless of whether they provide consent to participate in the Clinical Characterisation Study. The approach will be made for all confirmed cases whether they are hospitalised or treated in the community.

Trained study team members will then contact the household and other close contacts to explain the purpose of the study and request participation. After the case, household and/or close contacts agree to participate in the study, written informed consent will be sought.

It will be important that cases, households and close contacts are contacted as soon as possible (ideally within 24 hours) after the case is laboratory confirmed so that baseline sampling can be done rapidly. Where cases are also recruited to the Clinical Characterisation Study, baseline sero-sampling will already have been performed through that study). Registration and sampling of household members and contacts will be done at first contact if agreed. The household and close contact exposure investigation questionnaires will be delivered immediately if all are present, or arranged as soon as possible afterwards (within 48 hours) if people are absent. Questionnaires for hospitalised cases will be performed in hospital by the household study field team or during the household visit should the case already have been discharged at the time. The semi-structured interviews about experiences with mpox disease will take place at the 28-day visit with the case and one contact. At the same visit a short follow-up questionnaire on subsequent exposure and development of mpox symptoms will be administered to contacts. See Mpox One Health Study flowchart which summarises the process.

The aim will be to set a time when all or most household members and contacts are available. External contacts will be interviewed separately away from the case household. This means study teams will need to be available for evenings as well as other times. The intention is to recruit, train and maintain on standby sufficient study teams with catchment areas that allow a team to make rapid contact with a household once identified. The study team will be paid an amount per household, commensurate with the time required to carry out the study protocol.

12.6 Consent

Written consent will be requested from all participants. Parents or guardians of children under the age of 12 years old will give consent for their child. Children aged 12-18 years will be invited to give assent and their parents/guardian will provide consent. All participants will receive a participant information sheet, and be given the opportunity to ask questions before giving consent. Consent forms will be translated into the four main languages spoken in Nigeria: English, Yoruba, Ibo and Pidgin English. Study teams would be recruited based on their ability to speak English and other dialects in that locality. For non-literate participants, **t**he study and the consent form will be explained verbally in the presence of a family member, after which they will be asked to indicate their consent with a fingerprint. For children, study staff obtaining consent will consider the ability of the child to understand the principles of the study and will discuss the study with the child in age appropriate language.

Participants will be informed that their participation is voluntary and that they are free to discontinue their participation at any time. Participation in the study will not affect the participant's ability to receive medical care. Participants will be asked if they are willing to be re-contacted by the study team in the future for one follow-up sample.

12.7 Sample Size

Sample size depends on the cases recruited to the Clinical Characterization Study and the size of their households, but should be as large as possible to allow for valid analysis by age and risk factors, and to increase the possibility of evaluating range of disease severity.

In 2022, 765 confirmed cases were reported across Nigeria. Lagos State had the highest number of cases at 188 over 12 months. The average number of cases from top 10 high-burden states was 40 over 12 months, with 3 to 16 cases reported per month. The average household size in Nigeria is 5 people. The Clinical Characterisation Study has set a recruitment target of 200 participants (range 150-250), considering this to be achievable considering recent data. Based on this, the household study will aim for a minimum over all sites of 150 households and maximum of 250 households of confirmed cases (approx. 900 – 1500 household participants including the index cases) over the period of a year which will include the peak season months of July to December. We will review recruitment at 3 and 6 months and if it becomes apparent that the targeted sample size cannot be reached in these two states, we have contingency plans to extend to a third state.

The generic reproductive rate for mpox is reported to be between 1.10 and 2.40 (Grant et al, 2020), though the rates may be higher or lower in Nigeria**.** Based on this we might expect to identify between 1 and 2 additional cases of secondary infection per household, but this will depend on the extent of symptoms experienced by the case while in the household. Therefore, we will not set a sample size for number of secondary infections.

Sample size for close and intimate contact subgroups will be opportunistic but capped at 5 close contacts per case for budgetary reasons. All contacts must be first-degree contacts ie. no contacts of contacts.

Currently there is no robust estimation of the frequency and magnitude of any risk factor for mpox in the Nigerian context. This is a primary reason for doing the study. We have conducted a sample size calculation to evaluate the degree of precision with which we can detect a significant difference between the importance of different risk factors e.g. different types of human-to-human contact. The following calculation is based on detecting a significantly higher risk for mpox transmission during sexual contact compared to other non-sexual contacts in the household based on various assumptions.

From prior publications, each mpox case is expected to lead to 1 to 2 secondary cases (Grant et al, 2020) and we assumed there is a two-fold higher infection risk during sexual contact compared to non-sexual contact. In our study of 1000 household participants (200 cases x 5 contacts), we estimated 1 sexual contact per household, giving 200 sexual contacts and 800 non-sexual contacts (1:4 ratio) per case. Of these, we estimate that 10% of non-sexual contacts and 20% of sexual contacts of a case will become infected with mpox virus. Using the openepi tool, we calculated the required sample size to detect a two-fold increased risk for sexual contacts with a 95% confidence interval and power of 80% to be between 519-637 participants, which is well within our recruitment target of 1000 household contacts.

However, we acknowledge that if the difference in transmission risk between sexual and non-sexual close contact were smaller, e.g. only 1.5 times higher, our estimates will be less robust. However, given the dearth of evidence available, we think our assumptions are realistic and that the sample size will enable the estimation of risk factors with reasonable precision.

The animal sample will be based on convenience sampling. The aim will be to trap in the vicinity of 30% of recruited case households (75 trapping events) to capture approximately 200 rodents and small mammals. In addition, domestic animals kept in these households will be sampled.

12. IMPLEMENTATION

13.1 Study components

1. **Cases:** demographic, exposure, risk factor, and past and present symptom information will be collected using an individually-applied questionnaire. Exposure questions will be

designed to allow each factor to be reported individually or combined into wider categories according to an scale of risk which will be created from existing literature. Cases above the age of 18 years old with likely sexual contacts (e.g. spouses) will be asked an additional set of questions privately.

The questionnaire will be followed by a semi-structured interview on day 28 to gather qualitative information about their experience of having mpox and their perceptions of the illness and how it transmits.

Cases will also be asked to give a follow-up sample on day 28 and answer a short questionnaire on subsequent exposure to mpox cases and development of symptoms since the initial visit.

2. **Case households:**

a. Case-household member data collection: demographic, exposure, risk factor, and past and present symptom information will be collected using an individually-applied questionnaire with each household member and children at the initial visit. Exposure questions will be designed to allow each factor to be reported individually or combined into wider categories according to an scale of risk which will be created from existing literature. Household members above the age of 18 years old with likely sexual contacts (e.g. spouses) will be asked an additional set of questions in private.

The questionnaire will be followed by a semi-structured household interview on day 28 with 1-2 household member or close contact to gather qualitative information about their experience of having mpox in the household and their perceptions of the illness and associated stigma, as well as a short questionnaire on subsequent exposure to mpox cases and development of symptoms since the initial visit.

The interview will be used to try and construct a matrix of exposure to the case that, once laboratory results are available, may assist in recreating the chain of transmission. Symptom recall of individuals will be checked during this activity, and the family will also be asked to help complete exposure questionnaires on absent members.

b. Case household member baseline sample: all mpox-symptom-free household members will be asked to give a blood sample to ascertain their current antibody status (previously infected (IgG), recently infected (IgM), uninfected). Household members discovered to have active symptoms will be referred immediately to State services for diagnostic testing. This study will not take diagnostic samples.

c. Case household follow-up sample: all household members will be asked to give a second blood sample for serology on day 28 to identify any person who has become infected asymptomatically or pauci-symptomatically during this period. At this visit, household members will also be asked about any mpox-like current symptoms. If active cases are found, they will be referred for diagnostic testing.

2. Non-household contacts

The same questionnaire regarding exposures and risk factors will be administered to the non-household close contacts. Only contacts above the age of 18 years old will be asked about their sexual exposure. Participants will be asked to provide a blood sample at the same time. Follow up

sampling at 28 days will also be requested, together with a short questionnaire about whether they have had subsequent contact with someone other than the case who has gone on to test positive for mpox.

3 3. Animal sampling

Animals living in and around the households of each case-household will be investigated. Sampling locations will depend on the locations of each positive human infection. Traps will be placed inside and around residential buildings. Due to the significant rodent activity, traps will also be place in marketplaces and garbage dumps if they are close to selected households. Trapping of rodents will also take place in agriculture or farming fields with passive rodent-human interactions if the households are in rural communities. For access, cooperation, and the safety of the traps and catch, advocacy will be carried out in the communities where these trappings will be undertaken.

13.2 Sample collection

Human participants

Ten (10) and five (5) mls of blood will be collected using aseptic technique from adults and children contacts respectively**.** Samples will be collected on days 1 and 28 of visiting the household**.** During field work samples will stored at 2- 8^0C using ice packs and vaccine boxes to maintain temperature and transported at the end of the day either to field storage or the testing facility according to the Laboratory Sample Transport SOP. Samples will be tested for recent (IgM antibodies) or past mpox infection (IgG antibodies) using an ELISA serology assay. Contacts with evidence of mpox-like symptoms will be referred to the State team for diagnostic investigation. The study team will take blood samples for serology only and will not carry out any diagnostics sampling.

Animal sampling

Households pets, rodents/small mammals, domestic animals in the enlisted households will be sampled. Rodent traps such as Sherman (alive), snap trap (dead), tomahawk (larger rodents) will be deployed in the evenings (between 5-6pm) around the homes and along drainages. Traps will be retrieved between 6-7am (before heavy human traffic) the following morning.

Mobile laboratories will be setup at a secluded location nearby the households. Such location will be well separated from human activities. At the mobile laboratory site, traps will be handled. Live captive animals (excluding pets and domestic animals) will be euthanized using gaseous anaesthetic agents such as Isoflurane (small rodents) and Ketamine (large rodents). Blood, serum, skin, liver, spleen, lungs, heart samples will be collected from the euthanised animals. Rodent tissue samples will be collected and frozen on site before transportation. Serum samples from rodents will be separated on site before being frozen and shipped in liquid Nitrogen dewars to NVRI, Vom. Animal carcasses will be incinerated/burnt and buried. Pets and domestic animals will be sampled by blood, swab (oral and nasal) skin scab samples collection only after proper restraint.

The animal sampling team will ensure case household neighbours and communities are informed and full sensitised to their work before any activity starts to ensure community collaboration and team safety.

Environmental sampling

Where a case has been hospitalised, environmental sampling will be carried out if their household is visited within 5 days of admission. With specific consent, sampling will be performed in all households where the case has remained at home.

Households of cases that have presented soonest will be prioritised to increase the chance of capturing contamination. Environmental sampling will be carried out by the same team that carries out the animal sampling and, as above, will ensure their activities are well understood by households and neighbours. Electrostatic dust cloths or sterile nylon flocked swabs, moistened with viral transport medium, will be used to swab hard surfaces (e.g. floor, bed, control panels, door handles, chairs, light switches, toilet seat, sinks) and all hard surfaces that are frequently touched by individuals in the households). The hard surfaces will be swabbed twice i.e. Day 1 and day 4. Household members will also be asked about their cleaning practices. Team members collecting environmental samples will use appropriate PPE. Swabs will be transported in viral transport medium under cold chain to the laboratory for analysis and where possible cultured to identify whether any virus found is infection competent.

13.3 Data collection

Data will be collected from both the Clinical Characterization Study and the One Health Study using the same specially designed RedCap e-CIF installed on smartphones/tablets provided to research assistants who will all be trained on study data collection procedures, under the supervision of the national and State teams. Detailed sociodemographic information and clinical characteristics will be collected from cases in the Clinical Characterization Study. Exposure history and risk factor information will be obtained from the cases and contacts in the One Health Study. Field study team members will be trained on the etiquette of contacting respondents, consenting and enrolling them in the study, and on good practice in quantitative and qualitative data collection. Android phones and paper-copies of the data collection tools/forms will be available as back-up.

Households, individual household members and non-household contacts will be assigned a unique study ID number to enable data linking. These numbers will be linked to the Clinical Characterisation Study case ID. Household member questionnaires will be completed by study team members using electronic data collection devices and a new module developed in RedCap specifically for the research. Household interview notes will be captured by contemporaneous study team notes which will written up electronically, imported into Nvivo and coded according to a framework analysis approach. Sampling results will be linked to questionnaire and interview data using unique ID.

Animal sampling results will be collated and linked to households by the unique ID. Excel will be employed to record data from the animal captures. Morphological indices from animals will be recorded using a template used in similar projects (Species, breed, length, tail length, ear length etc).

13.4 Analysis plan

- Describe the distribution of potential risk factors and exposures for mpox infection in case-households contacts and among non-household close and sexual contacts;
- Estimate risk associations for infection by age and sex, and other exposure variables with sufficient numbers
- Estimate incidence, secondary and household transmission rates of mpox infection among household contacts
- Estimate the proportion of mpox infection that is asymptomatic or pauci-symptomatic.
- Describe the incidence of mpox infection (including asymptomatic infection) in non-household close and sexual contacts, the distribution of exposure risk factors, and the risk associations with exposure variables.
- Describe knowledge, perception and experiences of mpox infection as well as everyday human to human and animal to human contacts

- Describe the prevalence of active and past mpox infection in animals in proximity to case households.
- Describe the presence of virus in the environment, including whether it is infection-competent.

NOTE: Mpox-negative household members and non-household contacts will be the comparison group for risk factors and exposures within each group.

13.5 Community engagement

As this study is linked to the Clinical Characterisation Study, it is expected that that study will provide community-level information to explain the nature of the clinical study, raise awareness of mpox and encourage suspect cases to present to the facilities involved. The household contact study team will contribute to this engagement to explain the intentions and benefits of the follow-up of households and non-household contacts. Engagement will need to be done at a population level in the selected areas (the hospital facility and its community catchment area), as prior to recruitment of cases specific local communities will not be known. Delivery methods will vary depending on the context. For example in urban areas, it may focus on public and social media channels, while in more rural areas, local leaders and networks will be used. In both contexts, local health and community workers will be engaged and informed.

The clinical and household field study leads will also ensure that State Epidemiologists and Veterinarians, and their teams, are well informed before any recruitment starts, and are engaged to encourage the reporting of suspect cases in the community.

Household level engagement will necessarily be more individual and discreet but again guided by the context. Cases will be asked to first contact their households to ensure they agree to a visit by the study team and ascertain any conditions or requests about that visit. Study teams will ask households if they wish the team to engage with their neighbours to explain the study, or at minimum ensure householders have enough information to feel comfortable in explaining their role in the study themselves if they prefer. In more rural areas, it is likely that the study team will need to engage with community leaders but this will be discussed with the case and their household before arrival on site to ensure approaches are in line with community norms and acceptable to the household.

After the study is complete, aggregated, anonymised findings and recommendations arising will be shared with participant households, contacts, community leaders and public health authorities in the targeted areas through documents pitched for understanding at each level (see section 16: Dissemination of findings).

13.6 Laboratory

Human sampling

This study requires blood draw for the serological analysis. No sampling or swabbing will be done for acute diagnostics – persons with mpox-like symptoms must be referred to the State's diagnostic service.

The study team visiting households will be constituted to ensure that at least 1 of the team is a trained and qualified phlebotomist. Only qualified study team members will be permitted to take blood samples for the study. Standard PPE for blood-draw from healthy individuals will be worn (gloves, mask and laboratory coat) and universal precautions will be applied to sample storage and disposal of waste products from the blood draw (needles, syringe, gloves etc) as per the field study sample-taking SOP provided by the Lab team.

Samples will be stored in vaccine carriers with ice packs during the period of the field work and transferred to cold chain at the end of the day as specified in the SOP. Samples will remain here until transfer to the study laboratories is arranged, as per the sample transport SOP provided by the lab team. Any breaches in sample cold chain will be recorded with the samples to inform subsequent analysis and interpretation. Study consent will include consent to retain any residual sample for further research/testing if needed.

Animal sampling

A mobile laboratory will be set up near the case household. Traps will be handled and animal samples will be collected in this mobile laboratory. Live captive animals will be sampled after being euthanized using gaseous anaesthetic agents such as Isoflurane (small rodents) and Ketamine (large rodents). Blood, serum, skin, liver, spleen, lungs, heart samples will be collected and frozen in the mobile site before transportation. Serum samples will be separated on site before being frozen and shipped in liquid nitrogen. All samples will be shipped to NVRI, Vom. Animal carcasses will be incinerated/burnt and buried immediately after sampling. Domestic animals will be sampled by blood collection only.

Animals samples will be analysed using both antigen and serological tests (PCR and ELISA) to enable identification of both present and past infection. Sequencing will be carried out for PCR positive animal samples where feasible SOPs will be established for all processes from the mobile laboratory activities and waste disposal to community information regarding the mobile laboratory and all its processes.

13.7 Human resources

1. **Household and close contact sample and data collection teams** (Study Field Teams)

Households will be visited by a study team of 3 trained people: all will be trained to administer the questionnaires and carry out the semi-structured interviews. One of the three will be a trained/qualified phlebotomist responsible for taking and managing the blood samples. To ensure timely contact with households and close contacts, there will be four sample and data collection teams based in each of the two targeted states. These teams will also administer questionnaires and deliver semi-structured interviews of hospitalised cases in hospital, where appropriate; they may also take blood samples where cases have not been recruited to the Clinical Characterisation Study.

These teams will not be full-time study employees but will be contracted to respond to a request to visit a household within 24 hours and paid a set fee per household which will take into account their travel time and costs, the possibility that they may need to return for absent household members, time required to electronically write up qualitative notes, the time spent on ensuring samples are correctly transported, and their subsistence during the visit time (including accommodation if the location requires it).

Staff for these teams will be recruited from the educated local population who are available for work but currently unemployed, and based on their location in relation to the clinical study site facility. The aim is to have a number of study teams on standby across the likely case-catchment area around the facility, to enable each teams to reach households in their area within 24 hours of the case presenting. The number of teams will depend on the area to be covered, but as they will not be full time employees, it will be possible to have maintain coverage across the catchment cost-effectively. These field staff will receive full field study training and although not full time staff will be included in the study support network so that they will also gain experience in research work.

District Surveillance and Notification Officers/Health Educators (1 per LGA in the 2 targeted states ~ 50): will be engaged to work with the Field Study Teams when there is a case in their respective LGA. These MoH staff also form the backbone of the surveillance system in each LGA and support outbreak response including contact tracing and monitoring. As such they will ensure the study is informed as soon as possible of any new cases in the recruited households.

2. **Household and close contact study support staff**

Study supervisors (2): The NCDC State Liaison Officer will supervise the study field teams in their state. Among their responsibilities will be: management of the field study teams ensuring timely quality work; receiving and checking the collected data, ensuring sample storage and transport runs efficiently; communication with the Study Leads, resolution of implementation problems, liaison with the State Epidemiologist and state surveillance teams etc. They will also be responsible for logistics and monitoring wellbeing of the field teams.

State Epidemiologist (2): will support the study through facilitating the activities.

3. **Animal study field teams**

Study Supervisor(1): A part time study supervisor will be required for the animal sampling component of this study. He/she will be responsible for planning and implementing of animal sampling in all selected sampling sites, recruiting of all the ad-hoc staff need for the animal sampling. He will also liaise with the human health component of the study.

Field sampling and data collection teams (3 teams of 5 people): Part-time staff responsible for field animal sampling and data collection and safe transportation of all animal samples collected to the laboratory (NVRI).

Laboratory staff: (2): Part-time and based at the NVRI, he/she will be responsible for storage and analysis of animal sampling collected within the stipulated turn around time.

4. **Study Oversight staff**

The Study Co-ordinator is a full time position responsible for the implementation of the human study in all states. The NVRI Study Lead is a full-time position responsible for the implementation of the animal studies in all states. These staff members lead the oversight of the study in all states, ensure liaison with State Epidemiologists/State Veterinarians and other senior State Level MoH staff. They will ensure the engagement/ collaboration of the State level surveillance/veterinary teams. The study leads line-manage the State-level study supervisors and are ultimately responsible for the effective and efficient implementation of this research study.

13.8 Training

Human study team: 1 week minimum for all field and supervisory staff covering:

- Understanding the study design, aims, objectives, and implementation
- Questionnaire application and non-directive interviewing for household and close contacts
- Semi structured interviews and sexual network interviews
- Intimate contact interviewing
- Human Sample taking – review and technique check for qualified phlebotomists
- Ethical practice training

Animal study team: Refresher training will be conducted by the study team leads from NVRI to all study personnel before commencement.

13.9 Risks to the Project and Project Personnel

a) **Personnel Security:** threats to personnel in the field including kidnapping. *Mitigation: All interstate transportations will be by air travel where possible. Security escorts will be employed if necessary. As far as possible, study team members will be recruited to work in their home State and area.*

b) **Risk of infection/zoonotic transmission:** Rodents/small mammals are reservoirs of several zoonotic pathogens. Staff involved in sample-taking from humans or animals may be exposed to other infections as well as mpox. *Mitigation: staff involved in the sample collection will wear appropriate PPE and be (re)trained on infection, prevention and control to reduce risk of infectio*n.

c) **Political instability in the country** delays study implementation due to travel restrictions or risks. *Mitigation: risk is difficult to mitigate, other than to extend study duration to reach reasonable sample size. If international staff are prevented from visiting, any needed support will continue remotely.*

d) **Conflicting priorities or work burden** reduce NCDC and State staff capacity to implement study in a timely manner. *Mitigation: It is difficult to mitigate this - study leads are required to lead, supervise and monitor the implementation of the study to ensure results. Without a minimum of NCDC staff assigned duties in the field there is risk of the study not producing the required data.*

e) **Geographic overstretch.** *Mitigation: we have limited the studies to small number of high burden states, and are not attempting to recruit all cases across the whole country.*

f) **Late start meaning 2023 high season is missed**. *Mitigation: project to run for 12 months to ensure inclusion of high season, unless maximum number of households are attained before 12 months.*

13. HUMAN SUBJECT PROTECTION

14.1 Ethical Review

The project will be submitted for the approval of the Nigerian National Health Research Ethics Committee, the National Veterinary Research Institute Research Ethics Committee, and London School of Hygiene & Tropical Medicine Research Ethics Committee.

14.2 Risk to human participants

- There will be minimal risk to the participants. Interviews may be time-consuming and some participants may experience some stress from answering questions particularly of a personal nature. Refreshments will be provided during the interview and staff will be trained to create a pleasant atmosphere, and respond to concerns. Questions about sexual relationships will be carried out in private and answers will be provided by the participant directly into the tablet questionnaire without verbalization of the question or the answer.

- There is risk of minor injury from blood draw which will be mitigated by using appropriately trained staff.

- The visit of the study team may expose the household to curiosity from their neighbours: this will be mitigated by keeping the study team to a minimum number of people (2-3) and ensuring that the household is assisted to explain the visit if they wish to.

- The visit of the study team may cause the case's illness to be known in the community and the case and/or their household to be stigmatised in the community. Mitigation: we will ensure the study team visit is as low key as possible, that household confidentiality is strictly maintained by the study team, and that the household is able to explain the disease to their neighbours/ community if needed.

14.3 Risk to animal participants

- Trapped rodents can die of heat stress if not removed from the Sherman traps before sunrise. Dead rodents can quickly decompose. Mitigation: Traps will be removed and the captive animals should be evacuated and euthanized early.

- Risk of harm to domestic animals through sampling: mitigation is to ensure the study team are well trained in this form of sampling.

14. DATA PROTECTION

15.1 Patient confidentiality

Staff will receive training on ensuring patient confidentiality. All data collected on study participants will be secured (locked if paper or password-protected/encrypted if electronic). Only named members of the study team will have access to the raw data including the personal identifiers, for the purpose of entering the information into an electronic database, checking for errors, and producing the anonymised version. Access to the anonymised dataset will be restricted to individuals who are actively involved in the study, and subsequently bone fide researchers with approval from the study Data Governance Committee.

15.2 Data Linkage

Data from this study will be linked with that of the Clinical Characterisation study as recruitment is based on cases recruited into that study. Participant identifiers will be used for these linkages.

15.3 Data ownership

All data generated will be owned by NCDC and NVRI. Data will be stored in the NCDC repository with legitimate requests for access assessed by a Data Management Committee of the principal investigators from NCDC, NVRI and UK-PHRST.

The committee will facilitate and prioritise urgent investigations (from any sector, including public health, academic and commercial) with a high probability of impact in a given outbreak. Where appropriate, priority will be given to scientific work that can be completed within Nigeria, but data and samples may be analysed in other countries in collaboration with Nigerian researchers.

15.4 Data Management and Storage

Data generated will be stored at the NCDC. Data collected by study field staff on mobile devices will be sent automatically to a central server at NCDC. Access to the data will be given to key study personnel like the principal investigators and data managers from NCDC and UKPHRST. A data dictionary will be developed for the study data base containing descriptions of all the data elements, their eligible values and labels and categories to guide analysis and data sharing with the public. Data submitted to the central server will be downloaded for cleaning and then will be exported to an appropriate software for analysis. A dashboard excel template will be used at NCDC for real time data monitoring and validation. This electronic method of

data collection will minimize risk of data loss, optimize efficiency and data quality. Data will be retained for 10 years following project completion.

15.5 Data Quality

Data collected will be monitored through internal quality control and quality assurance mechanisms to ensure conformance to the key elements of data quality; i.e., validity, reliability, precision, completeness, timeliness and integrity. The data will be subjected to initial data logical and consistency checks (skip patterns, range values) as it is collected on the device and additional checks will be conducted when the data is downloaded and merged. RedCap will be configured to capture date time and location stamps at start and end of each enrolled case data entry session to provide insight into the time spent on each session for future planning. Complete audit trail will entail activity/timestamp, user identification, hardware identification, transmission and backup status. Further daily data quality checks of data submitted to the central database will be conducted. Data quality issues arising from submitted data will be detected through eyeballing, running of analysis scripts and automated dashboard functions. All data quality issues and responses will be documented using a designed Error Log Template.

15.6 Authorship provisions

Authorship will consist of all study group members. This study will recognise investigators contributing to research efforts, often in difficult circumstances. They will be given full recognition for their efforts and results from the study will be shared with them.

15. DISSEMINATION OF STUDY RESULTS

Results will be published in peer reviewed articles and webinar/ seminars as approved by the study partners.

Full report of findings and recommendations will be shared with health authorities in the study States and with other States that are experiencing the mpox epidemic. Findings will also be used to inform new guidelines created and published by Nigeria CDC.

Lay versions of aggregated, anonymised results and recommendations will be created to inform participant households and communities of the findings of the study and encourage use of the information to reduce risk of contracting mpox, ensuring a feedback mechanism to affected communities and participants. Nigeria CDC will also disseminate the findings through its social and public media channels.

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APPENDICES

1. Flowcharts for Clinical Characterisation and One Health Studies
2. Consent form
3. Participant information leaflet
4. Questionnaire
5. Interview guide

6. Laboratory SOP for sampling
7. Study schedule: Gantt chart