Project tittle: **Investigating MPXV Viral Clearance in Mpox Cases and Secondary Attack Rate in Contacts** **Principal Investigador (PI):** *Professor Oriol Mitjà, Professor Hypolite Muhindo*	**Date:** *28/05/2025* **Version 1.1**

Note: This amendment (Version 1.1) includes administrative updates only to align dates and language with the ClinicalTrials.gov registration. No methodological changes have been introduced.

List of participant institution

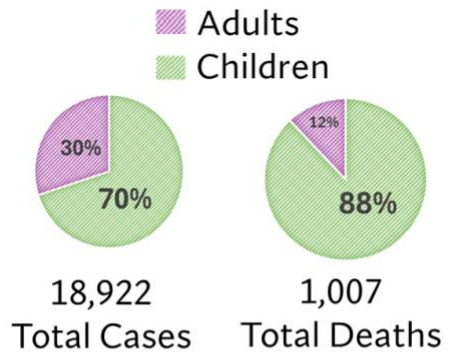
Participant No. *	Participant organisation name	Country
1 (Coordinator)	University of Kinshasa (UNIKIN)	DRC
2	Fundación Lucha contra el Sida, las Enfermedades Infecciosas y la Promoción de la Salud y la Ciencia (FLS)	Spain
3	London School of Hygiene & Tropical Medicine (LSHTM)	United Kingdom

1. BACKGROUND

A mpox outbreak is currently ongoing in the Democratic Republic of Congo (DRC), involving primarily clade 1b, along with cases linked to other clades. Unlike previous outbreaks of clade 1, which were mostly associated with zoonotic transmission, this outbreak has demonstrated sustained human-to-human transmission, including direct and sexual contact in urban centers.

Mpox is a zoonotic viral disease caused by the monkeypox virus (MPXV) that results in an illness and rash similar to that of smallpox. Based on clinical presentation and genomic sequencing results, MPXV isolates were classified into two clades: clade-I, predominant in Central Africa, with high fatality rates (1-12%), (1) (2) and clade-II, predominant in West Africa, with lower fatality rates (0.1%) unless the affected person is immunosuppressed. (3,4)

In recent years, the Democratic Republic of Congo (DRC) has faced substantial outbreaks of clade-I MPXV. (5) Recurrent instances of Mpox outbreaks have affected the DRC, with the most recent occurring between 2023 and 2024. This outbreak has resulted in over 18,000 reported cases and approximately 1,000 fatalities as of May 2024, 88% of deaths occurred in children under 15 years old, and the age group under 5 had a case fatality rate of over 10% (Fig. 1). In the DRC outbreak, MPXV is primarily thought to spread through respiratory droplets through coughs, sneezes or talks that facilitate the transfer of virus to others who are nearby.(6) Previous outbreaks, particularly among children in West and Central Africa, have demonstrated this mode of transmission. (7,8) Clade-I MPXV outbreaks present significant challenges to public health, given this clade' propensity for high transmission rates and severe clinical manifestations.

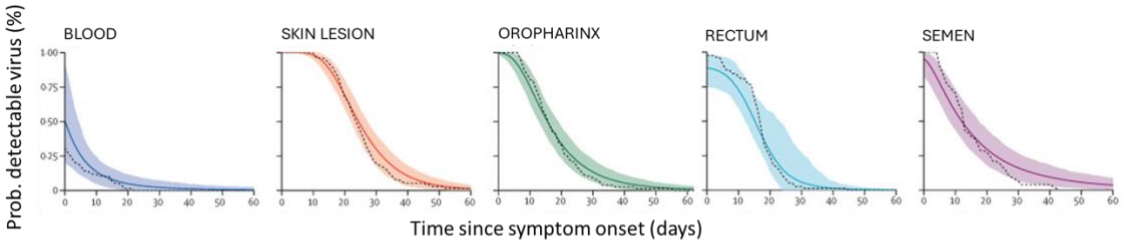


Age group	Case fatality rate
0-11 months	11.1%
12-59 months	10.2%
5-15 years	4.8%

Figure 1.
Democratic Republic of Congo Mpox outbreak statistics as of May 2024.

In 2022, a global outbreak of clade-II MPXV, primarily linked to sexual transmission, affected 116 countries, predominantly high-income nations. During this outbreak, there were more than 90,000 cases, with about 170 deaths occurring in immunosuppressed adults. Our group published several landmark papers. The first provided a clinical description of cases, (9) showing that the clinical presentation was generally mild, with only a small proportion of people needing hospitalization due to local complications. The second focused on the presentation in women, (10) and a third study, demonstrated that people living with advanced HIV (CD4 count less than 200 cells/uL) had severe clinical manifestations and a case fatality rate of 15%. (11)

The global clade-II MPXV outbreak highlighted the potential for transmission through intimate contact and sexual activity. (12) Our group also led key investigations shedding light on viral dynamics and transmission patterns, (13) as well as the accuracy of self-collected swabs for MPXV DNA detection. (14) In a longitudinal cohort study, we demonstrated a rapid decline of the viral load in blood compared to a more prolonged viral shedding from skin lesions. (13) Pharyngeal viral dynamics fell between these two groups. Time to clearance in 50% of patients (95% CI) was 1 (0-5) day in blood, 16 (13-23) days in oropharynx, and 25 (23-28) days in skin lesions (Fig. 2). Overall, our data suggested that, for clade-II MPXV, skin-to-skin contact was the predominant driver of transmission.



Time of clearance (days, 95% CI) in	*50% patients*	*90% patients*	*95% patients*
Blood	1(0-5)	13 (6-23)	20 (10-39)
Semen	13 (9-18)	39 (27-56)	53 (34-84)
Rectum	16 (13-23)	27 (21-38)	31 (23-42)
Oropharynx	16 (13-19)	34 (27-42)	42 (32-53)
Skin lesion	25 (23-28)	41 (34-47)	47 (38-56)

Figure 2.
Viral dynamics and time to clearance in Mpox patients (Suñer et al., 2023)

Firstly, efforts to control clade-I Mpox outbreaks rely heavily on public health interventions, particularly the identification and isolation of cases. However, there are no data regarding the dynamics of viral clearance in clade-I infection to determine the duration of isolation protocols. Understanding the kinetics of viral clearance is crucial for determining the appropriate duration of isolation and guiding clinical management practices. Moreover, understanding viral clearance may pave the way for future research because it will provide information of outcome measurement that can be used in future treatment efficacy RCTs.

Moreover, understanding the Secondary Attack Rate (SAR) holds several important implications for managing and controlling the outbreak. By quantifying the risk of secondary transmission, SAR can guide decisions on the prioritization of contact tracing efforts, deployment of healthcare personnel, and distribution of medical supplies to mitigate further spread of the virus. SAR data can inform the development and adjustment of isolation and quarantine policies. High SAR values suggest a greater likelihood of secondary transmission, prompting stricter isolation measures and longer quarantine periods for contacts of Mpox cases. Conversely, low SAR values may indicate that existing control measures are effective, allowing for more targeted interventions. Importantly, SAR is instrumental in evaluating the effectiveness of vaccine efficacy trials and vaccination campaigns. SAR data can lay the groundwork for future trials on vaccine efficacy and effectiveness and can guide decisions on the deployment and prioritization of vaccines in Mpox-affected communities.

To address these gaps, the ambition of this project is to provide crucial insights into the dynamics of MPXV clearance and person-to-person transmission in the DRC. By focusing on both the infected individuals and their contacts, the project aims to fill critical knowledge gaps that will inform effective public health interventions such as isolation and provide the basis for future trials on vaccine efficacy/effectiveness.

1.1 OBJECTIVES

This proposal outlines two linked epidemiological studies aimed at addressing the public health challenge of Mpox in the DRC provide insights into pathogenicity, clinical information on host susceptibility, and immune responses. The MOVIE study focuses on understanding the kinetics of viral elimination, shedding light on how MPXV interacts with host tissues and immune defences, and informing endpoint selection in therapeutic trials. The TRACE study aims to determine Secondary Attack Rate (SAR) in MPXV outbreaks, assessing host susceptibility within

specific populations, offering vital data to target interventions towards vulnerable groups and informing vaccine efforts by contributing to the assessment of vaccine efficacy endpoints.

OBJECTIVE 1 (MOVIE Study): Understanding MPXV Viral Clearance in Mpox Patients

Rationale: Efforts to control Mpox outbreaks rely heavily on identifying and isolating cases. However, there is a critical gap in our understanding of the dynamics of viral clearance among clade-I MPXV infected individuals of all ages, particularly children and pregnant women. This knowledge gap hampers our ability to determine the optimal duration of isolation protocols and guide clinical management practices effectively.

Objective: The MOVIE study aims to address this gap by describing the time to viral clearance in patients with PCR-confirmed Mpox infection in the DRC. By systematically collecting and analysing data on viral presence across different body compartments over a long period of time, the study seeks to provide essential insights into the kinetics of MPXV elimination and inform evidence-based strategies for isolation and clinical management.

Feasibility and Measurability: The objectives of the MOVIE study are both measurable and verifiable. Time to viral clearance can be quantified through laboratory testing of patient samples, allowing for objective assessment of the effectiveness of interventions and the progression of infection. With access to appropriate resources and collaboration with local health authorities and research institutions, the study's objectives are realistically achievable within the proposed timeline.

OBJECTIVE 2 (TRACE Study): Evaluating Transmission Dynamics of MPXV in Mpox Cases and Contacts

Rationale: In addition to understanding MPXV viral load, there is a pressing need to evaluate transmission dynamics of clade-I MPXV within Mpox-affected communities. Secondary transmission plays a significant role in sustaining outbreaks, yet the factors influencing transmission rates and the risk of infection among contacts of all ages remain poorly understood. This lack of knowledge hampers efforts to make decisions on contact tracing, healthcare personnel deployment, medical supply distribution, contact quarantine policies, and the assessment of effectiveness of vaccine trials or campaigns.

Objective: The TRACE study aims to fill this knowledge gap by evaluating transmission dynamics of MPXV, including Secondary Attack Rate (SAR), in Mpox cases and their contacts. By systematically collecting data on contact exposure and subsequent transmission events, the study seeks to quantify the magnitude of transmission rate, identify risk factors associated with further transmission.

Feasibility and Measurability: Like in MOVIE study, the objectives of the TRACE study are measurable and verifiable. Secondary Attack Rate (SAR) and transmission dynamics can be quantified through epidemiological analysis of contact tracing data and collection and testing of specimens from contacts. With appropriate resources and collaboration with local stakeholders, the study's objectives are realistically achievable within the proposed timeframe.

1.2 INNOVATIVE APPROACH AND AMBITIOUS GOALS

The viral clearance and dynamics of transmission are likely to differ significantly in clade-II sexual transmission compared to clade-I droplet transmission in the current outbreak in DRC (Fig. 3). (3) Sexual transmission of clade-II MPXV involves the virus infecting epithelial cells in the genital area,(15) resulting in low-level viremia and limited presence in respiratory excretions.(13) Prolonged viral clearance in skin lesions serves as a critical marker to determine isolation duration, and the Secondary Attack Rate (SAR) depends on subsequent sexual or intimate encounters. In clade-I respiratory transmission by droplets, the virus initially infects airway epithelial cells, then spreads to lymphatic vessels, spleen, and liver, triggering a significant viremia.(16–18) This dissemination facilitates widespread infection in various tissues, including the skin and distant organs, and the presence of viruses in respiratory excretions facilitates transmission to the next person. Our approach centers on investigating clade-I MPXV transmission in high-risk groups, with a focus on understanding viral replication in the respiratory tract compared to other body compartments. Additionally, we aim to assess the potential for higher Secondary Attack Rate (SAR) based on proximity for respiratory droplet transmission.

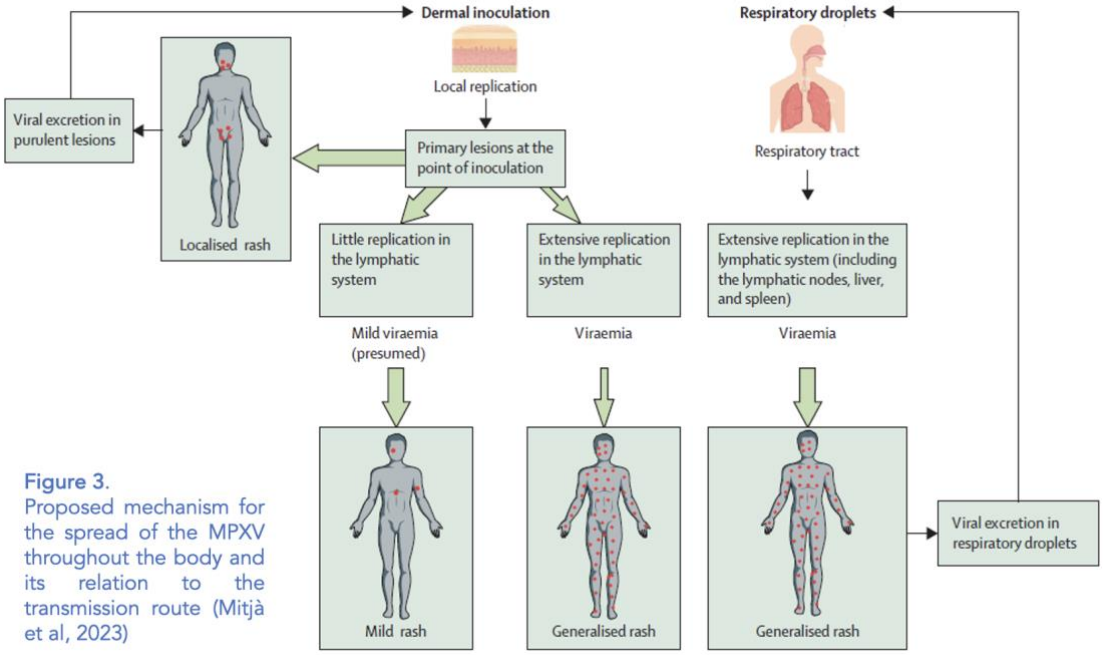


Figure 3.
Proposed mechanism for the spread of the MPXV throughout the body and its relation to the transmission route (Mitjà et al, 2023)

Objective 1: MOVIE Study

The MOVIE Study stands as a groundbreaking initiative in clade-I MPXV research, specifically aiming to explore the complex dynamics of clade-I MPXV viral load. Previous investigations in clade-I endemic regions have predominantly centered on the clinical manifestations of Mpox, owing partly to the limited access to laboratory facilities for conducting extensive virological analyses in Low- and Middle-Income Countries (LMIC) where the outbreaks occurred.(2,19,20) This gap in laboratory-based research has hindered a comprehensive understanding of the disease's pathogenesis in affected individuals.

In contrast, some attention was devoted to studying the clade-II MPXV outbreak in 2022, particularly in high-income countries. At least three studies, characterized by their meticulous examination of viral detection and clearance dynamics, have significantly contributed to our understanding of clade-II MPXV infection transmitted sexually or by close direct contact.(13,21,22) However, it is imperative to recognize that the transmission patterns and viral clearance observed in the clade-II 2022 global outbreak, primarily driven by sexual transmission, may not necessarily mirror those of clade-I MPXV, which, as mentioned above, is predominantly

transmitted through respiratory dropplets. Thus, there remains a critical knowledge gap concerning the transmission dynamics of clade-I MPXV.

The proposed work is ambitious in its scope and potential impact. By elucidating the kinetics of clade-I MPXV elimination in different body compartments, the MOVIE study aims to guide current approaches to Mpox management and prevention with the potential to inform evidence-based strategies for isolation, treatment, and infection control, ultimately reducing morbidity and mortality associated with Mpox outbreaks.

Objective 2: TRACE Study

This study also represents a unique opportunity to determine Secondary Attack Rate (SAR) in clade-I MPXV outbreaks. During the clade-II outbreak in 2022, the SAR could not be clearly defined due to challenges in reporting and identifying sexual contacts. The nature of sexual interactions often led to ambiguity or incomplete reporting, making it difficult to accurately assess transmission rates within this context. Previous experiences with clade-I MPXV outbreaks provided some insights into SAR, particularly in household transmission settings reporting values of up to 9% (23) or in congregate living situations (i.e., prisons, or healthcare facilities).(24,25)

However, the evidence supporting SAR calculations for clade-I MPXV outbreaks is not strong due to methodological limitations in previous studies. SAR investigations were started based on suspected index cases rather than cases confirmed by positive PCR testing. Additionally, contact classification relied on symptoms without confirmation through PCR testing, and therefore missed a fraction of asymptomatic infections. The previously described approach introduces uncertainty into SAR estimations, as suspected cases may not always represent true infections, and symptom-based diagnoses of contacts may lack both sensitivity and specificity. Without confirmation through PCR testing, there is a risk of misclassification and overestimation or underestimation of SAR values.

The TRACE study seeks to address these limitations by employing a rigorous methodology, including systematic PCR testing of all contacts at least at two different time points to confirm infections, and systematic symptom-based assessments, thus providing accurate and reliable data on SAR in clade-I MPXV transmission settings.

2. METHODOLOGY

This project will be delivered over 24 months and will be led by the Fight Infections Foundation (FLS, coordinator) along with the University of Kinshasa (UNIKIN, partner) and London School of Hygiene and Tropical Medicine (LSHTM, partner). Our consortium brings together partners in both Europe and the DRC with expertise in clinical, epidemiological and virological research into Mpox. Collectively, this consortium provides the breadth of experience needed to undertake our two proposed studies and deliver critical information to guide the response to the Mpox epidemic.

Individual work packages are described in detail in Section 3. Below we outline the methodology for the two Scientific Work packages which are designed to provide critical data to inform the Mpox response in DRC.

Ethics Approval

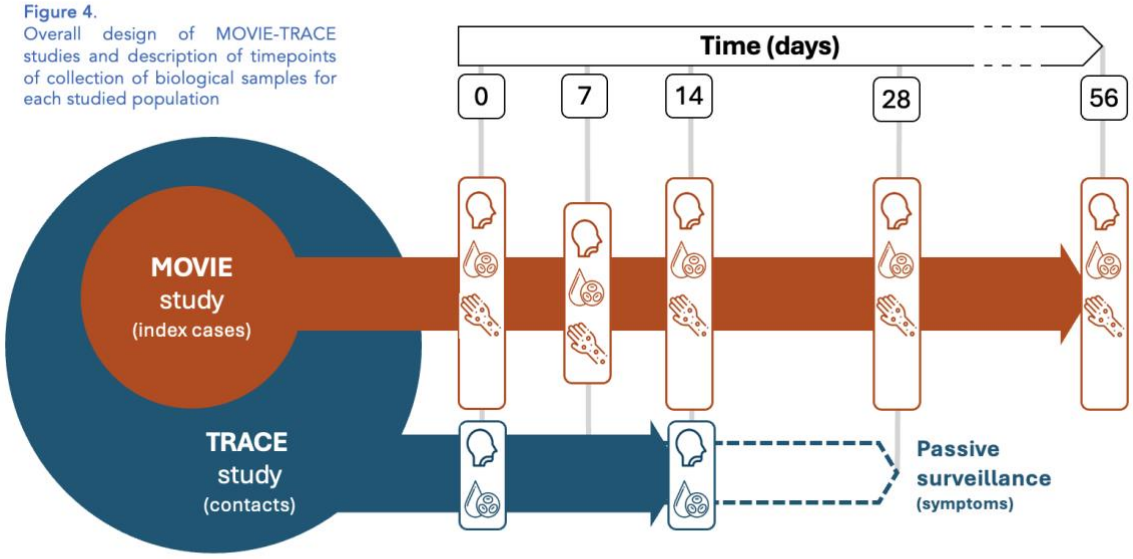
Ethical approval will be obtained from the Observational Ethics Committee of HUGTiP (law 14/2007 on Biomedical Research and Royal Decree 1716/2011 on Biobanks regulation) and from the DRC Ministry of Health National Ethics Committee (Comité National d'Éthique de la Santé, CNES). All participants will be asked to provide written informed consent. Where children are

enrolled, we will obtain assent from the child over 12 years old in addition to consent from a parent or guardian.

Overall Design of the LLEGAT study

The overall project design combines 1) identification and follow-up of Mpox index cases (MOVIE) to understand viral kinetics and duration of infectivity and 2) identification of close contacts of these index cases (TRACE) to understand Secondary Attack Rates (SAR) and determinants of transmission. A high-level scheme for the two studies is shown in Fig. 4.

Figure 4.
Overall design of MOVIE-TRACE studies and description of timepoints of collection of biological samples for each studied population



2.1 MOVIE study of MPXV Viral Kinetics (Work Package 5)

Study sites: The main study site for participant recruitment will be in Èquateur, province. This site is well suited as i) it reports ongoing transmission of Mpox and ii) UNIKIN has been actively involved in research and epidemiological surveillance in the province of Équateur, where it has established a solid presence and collaborative networks in the field of infectious diseases.

Design and population: The proposed design for our study is a prospective observational study, aimed at understanding the dynamics of MPXV infection in both children and adults.

Sample Size: We plan to enrol approximately 50 cases of Mpox to ensure an adequate sample size for meaningful analysis. The study utilizes a convenience sample size, chosen to be within budget while still being sufficiently large for descriptive analyses.

Inclusion and Exclusion criteria: Inclusion criteria for participation in the study include (1) individuals of any gender and age who (2) have been diagnosed with lesions suggestive of Mpox by a trained health worker; (3) have experienced symptom onset within the past 10 days prior to baseline assessment; (4) comply with the study protocol, and (5) be available for follow-up visits. (6) Informed consent will be obtained from all participants, or consent may be provided by a

legally authorized representative if non-adults or in case the individual is unable to provide it themselves, with assent from children of 12 years of age or older. Witnessed consent will be used for those who cannot read/write.
Exclusion criteria include (1) cases of severe Mpox that necessitate hospital admission, (2) individuals with an alternative confirmed diagnosis explaining their illness, (3) prior vaccination against Mpox, (4) individuals over 40 years old who report having received smallpox vaccination during infancy.

Case definitions and variables:

'**Mpox case' case definition**: An Mpox case for MOVIE study will consist of an individual who exhibits symptoms suggestive of Mpox, such as characteristic skin lesions, symptom onset within 10 days prior to baseline sample collection, meets the inclusion criteria for the study and tests positive for MPXV by PCR.

Screening failure: Screening failure occurs when a suspected case, enrolled for participation, tests negative on a PCR test, leading to their exclusion from further procedures or participation.

Severity Score: We will use a modified Mpox-Severity Score (Mpox-SS) to determine severity: The Mpox-SS has six main components: Rash extent and burden of lesions, secondary infections, mucosal areas affected, level of care, analgesia requirement. In addition to that we will consider additional components: Symptom assessment (fever), and internal organ involvement (respiratory, gastrointestinal, neurological symptoms). Each component will be graded from 0 to 4, and the sum of scores will provide a total severity score. The Mpox severity score has been validated in adult individuals only, so this research provides an opportunity to validate the score in pediatric population (https://mpoxseverityscore.com/uploads/MPOX_SSS_V9.pdf).

We will collect the following variables:

Demographic variables: Age; Sex/Gender; Geographic location.

Epidemiological and behavioral variables: Exposure history and contact with known or suspected Mpox cases, personal protective measures and hygiene practices; Occupation; Engagement in sex work; Involvement in sexual activity outside of the partnership; Engaging the services of sex workers in the past 2 weeks.

Clinical variables: Comorbidities (measles, TB, malaria); HIV Status: HIV test results; Previous smallpox vaccination; Date of prodrome symptoms start; Date of vesicular rash start (date of the first vesicle); Approximately maximum number of concurrent skin lesions (e.g., 1, 30, 100); Distribution of lesions (face, scalp, trunk, arms, hands, legs, feet, soles, palms, penis, scrotum, labia majora, groin, buttocks); Distribution (localized to 1 region, 2 regions, more than 2 regions); Distribution in generalized rash (centrifugal, centripetal, even distribution); Development (monomorphic, pleomorphic); Duration of symptoms; Mpox Diagnosis: PCR test results; Mpox-SS components (graded 0 to 4): Rash extent and burden of lesions, secondary infections, mucosal areas affected, level of care required, analgesia requirement, symptom assessment (fever, respiratory, gastrointestinal, neurological symptoms); Treatments received for Mpox.

Photography of lesions

Contact tracing information to identify secondary cases

Laboratory variables: MPXV viral load measurements at skin lesions swabs, blood, oropharyngeal swabs, and urine at different time points; Viral culture: Presence of replication-competent virus; Immunological Responses: MPXV-specific humoral and cellular responses.

Outcome variables:

Primary endpoint: time to viral clearance for each body compartment, which is defined as the interval from the onset of symptoms to the first negative PCR result. This will be measured in skin lesions swabs, blood, oropharyngeal swabs, and first-void urine samples.

Secondary outcomes: (1) Describing the viral load at each body compartment at different time points. (2) Exploring the association between severity of clinical features and the time to viral clearance. Severity of clinical features will be assessed using the mMpox-SS. (4) Investigating behavioural factors associated with Mpox acquisition by means of questionnaires. (5) Explore intrahost viral evolution in distinct compartments during infection evolution.

Procedures:

Baseline: The team in the field, through UNIKIN, will get notified about a case through the surveillance process after which the team will travel to the location and enroll the case. The screening and baseline visits will involve in-person interactions conducted either in a clinical setting, at participants' homes, or clubs, facilitated by a study investigator. During these visits, participants will receive detailed information about the study, and then informed consent will be obtained. Subsequently, a comprehensive interview will be conducted to gather demographic, epidemiological, and clinical data, including any relevant comorbidities and medical history. Photographs of lesions will be taken. The captured images will not only strengthen the dataset for this project but also hold potential for utilization in AI tools for diagnosis. Eligibility will then be verified based on predetermined inclusion and exclusion criteria, with ineligible participants being promptly informed and provided with necessary medical assistance.

Sample collection: For eligible participants, one vesicle fluid sample will be collected and taken to Mbandaka laboratories for GeneXpert rapid within 48 hours to determine the continuation in the study. Additionally, a series of biological samples will be collected, including vesicle fluid from lesions or ulcers, blood for plasma collection, swabs, and first void urine samples and for storage at -80ºC for centralized shipment to Hospital Universitario Germans Trias I Pujol, Barcelona. To ensure sample integrity, swabs will be placed in 3 mL of viral transport medium, blood will be collected as dried blood spots (DBS) on filter paper and the urine is collected in a sterile 8ml tube.

Follow-up visits: Follow-up visits will be conducted on days 0, 7, 14, 28, and 56, using the same baseline procedures for sample collection from skin lesions, blood and oropharynx and shipped to Barcelona for testing.

Data source: Throughout the study, data will be sourced from patient medical health records and laboratory results, forming the primary basis for analysis and interpretation.

2.2 TRACE Study of MPXV Transmission Dynamics (Work Package 6)

Design and population: This will be an observational study evaluating transmission dynamics of MPXV within the context of the outbreak in the DRC The study population comprises individuals who have had close contact with PCR-confirmed Mpox cases within the outbreak setting.

Sample Size: With an indicative sample size of 500 contacts, the study intends to recruit approximately 10 close contacts for each of the 50 Mpox cases identified. This is a convenience sample that falls within the budgetary constraints but is deemed sufficient to determine the SAR.

Inclusion and exclusion criteria: Inclusion criteria include (1) individuals who report close physical contact with a PCR-confirmed Mpox case within 14 days from the onset of the index case's symptoms. Close physical contact is operationally defined as being within 2 meters of an infected person, particularly in enclosed spaces, for at least 5 minutes (CDC 2 meters rule for droplet transmission). (2) Willing to comply with the study protocol and be available for follow-up assessments. (3) Informed consent is a prerequisite for participation, although consent can be provided by a legally authorized representative in case the individual is unable to provide it themselves.
Exclusion criteria include (1) prior vaccination against Mpox, (2) individuals over 40 years old who report having received smallpox vaccination during infancy.

Contact definitions and variables:
'Mpox contact' case definition for TRACE is an individual who reports having had close physical contact with a confirmed Mpox case (within 2 meters in the same enclosed space for at least 5 minutes [CDC, 2 meters rule for droplet transmission]) within 14 days from the symptom onset of the index case.

Variables related to the case: Already described in before in MOVIE study section.

Variables related to the contact:

- ◊ Demographic variables: Age, sex, geographic location.
- ◊ Epidemiological and behavioral variables: Exposure history and contact with other (non-index) known or suspected Mpox cases, personal protective measures and hygiene practices; Exposure setting (household, school, friends, intimate), Exposure risk factor (skin cuts, sores, ulcers before exposure, or none), Date of exposure (range will be acceptable); Occupation; Engagement in sex work; Involvement in sexual activity outside of the partnership; Engaging the services of sex workers in the past 2 weeks.
- ◊ Clinical variables: Site, Location; Comorbidities (measles, TB, malaria); HIV Status: HIV test results; Previous smallpox vaccination. If a contact is confirmed to have Mpox through study procedures, we will collect the variables described for Mpox Case.
- ◊ Laboratory variables: Baseline MPXV serological result of the contact, MPXV DNA detected in the contact specimens (blood, oropharynx, anus), MPXV DNA detected in the index case specimens (i.e., lesion, blood, oropharynx, and urine).

Endpoints:

Primary endpoint:

1)Secondary Attack Rate of Infection (SAR-i), the proportion of contacts who become infected with the pathogen (PCR positivity), regardless of whether they exhibit symptoms.

2)Secondary Attack Rate of Disease (SAR-d), the proportion of contacts who develop symptomatic illness after exposure to the pathogen with PCR confirmation.

In these attack ratios, the denominator includes all individuals who have had close contact with a confirmed case in the past 14 days since the symptom onset of the index case (i.e., higher end of the serial interval range). (26–28), while the numerator includes only those contacts from the denominator who subsequently become infected (SAR-i) or develop the disease (SAR-d).

- Contacts who test positive on PCR at baseline without symptoms will count as contacts.
- Contacts who test positive on PCR at baseline and also exhibit symptoms will be considered as secondary contacts if the index case had symptoms of the disease at least 5 days prior to the contact's onset of symptoms (lower end of the of the serial interval range).(26) If the onset of symptoms was within less than 5 days, they may have been exposed to the same original source of infection and therefore be concomitant cases to the index rather than contacts. These cases will be analyzed separately in secondary analyses.

We will confirm the viral genetic link between index cases and their contacts through genotyping analyses. We will conduct exploratory analysis to examine how the SAR varies by index case viral load, baseline characteristics of the contact and type of case-contact relationship (e.g., household vs. non-household contacts).

Secondary endpoints: consist of factors of the case or the host associated with transmission, measured using all clusters of an index case and their corresponding contacts for which quantitative viral load is available for the index case.

Procedures:

Contact identification: A dedicated field team will be deployed to visit both confirmed Mpox cases and their contacts at their residences, or workplaces on two key days: day 0 (baseline) and day 14. Upon identifying an index case, the team will compile a comprehensive list of close contacts associated with each case in the past 2 weeks.

Baseline visit: Each contact will be visited by the field team to gather essential demographic, epidemiological, co-morbidity, and clinical data at baseline (day 0). During these baseline visit, pertinent epidemiological information will be collected through structured interview. This data will include details such as the time of initial exposure to the index case, the location of contact, the degree of proximity, and the duration of contact.

Sample collection: For eligible asymptomatic participants, a series of biological samples will be collected, including oropharyngeal and blood for PCR and serology testing and swab lesion (if there is). Samples will be collected from 10 contacts for each mpox case (500 contacts) at baseline and 14 days follow-up time points. To ensure sample integrity, swabs will be placed in 3 mL of viral transport medium, blood will be collected as dried blood spots (DBS) on filter paper. Samples will be storage at -80ºC for centralized shipment to Barcelona.

Follow up: We will conduct a follow-up visit on day 14 with systematic swabbing for PCR testing. As part of this follow-up, oropharyngeal swabs and blood samples will be collected from everyone on day 14 for PCR and serological testing.

Management of symptomatic cases: If participants exhibit symptoms indicative of Mpox within the 14 days following enrolment, they will undergo sample collection at the earliest opportunity. These symptomatic individuals will subsequently be enrolled into the MOVIE study for further investigation and will be added to the contact line-list for ongoing monitoring and analysis. This comprehensive approach ensures that all potential cases are thoroughly evaluated and included in the study's analysis, contributing to a more comprehensive understanding of Mpox transmission dynamics.

Data source: Throughout the study, data will be sourced from patient medical health records and laboratory results, forming the primary basis for analysis and interpretation

2.3 Laboratory Assessments

Samples will undergo molecular and genetic analysis in Germans Trias i Pujol Hospital, Barcelona:

PCR testing:

MPXV detection: MPXV GenExpert testing of samples collected from Mpox cases will be performed at a laboratory within the UNIKIN facility to confirm the case and move forward with further analysis. During transport, samples will be kept at 2-8°C; time from sample collection to laboratory receipt will be within 24 h; once received, samples will be processed immediately and/or stored at –80°C until processing. MPXV PCR testing from samples collected at specified time points will be conducted at the microbiology laboratory at the Germans Trias i Pujol Hospital. These samples will be stored at -80ºC at UNIKIN facilities until they are shipped to Barcelona in -20ºC boxes. All samples will be sent at the same time, once the sample collection is finished. All samples will be analyzed for the detection of MPXV DNA by qPCR. Copy number per mL will be determined using a linear dilution series of a quantified MPXV DNA standard. Viral loads from body fluids (i.e., blood and urine) will be expressed in DNA copies per mL, whereas the amount of virus in swab-collected samples (i.e., skin lesions, oropharynx) will be expressed as copies per mL of viral transport medium.

Genotyping analysis

MPXV clade typing: For samples analyzed at the Barcelona laboratory, only those that test positive for Monkeypox virus (MPXV) via quantitative PCR (qPCR) will proceed to clade typing. Clade differentiation is performed using a clade-specific PCR assay that targets genomic regions with known sequence divergence between MPXV clades. The protocol employs primers specifically designed to distinguish between Clade I (Congo Basin lineage, encompassing both Clade Ia and Clade Ib) and Clade II (West African lineage, including Clade IIa and Clade IIb).

Viral Genome Sequencing: MPXV-positive samples with a cycle threshold (Ct) value of ≤25–26 will be selected for whole genome sequencing (WGS) at the microbiology department of Hospital Universitari Germans Trias i Pujol (HUGTiP). High-quality viral DNA will be extracted and prepared for sequencing using Illumina platform.

WGS allows for comprehensive analysis of the entire viral genome, enabling detailed investigation of genetic variation, including point mutations, insertions/deletions, and potential recombination events. This high-resolution genomic data will be used to determine phylogenetic relationships between strains, monitor viral evolution, and assess the emergence of new lineages.

Phylogenetic Analysis: To investigate the evolutionary relationships among the detected MPXV strains, phylogenetic trees will be constructed using full-genome sequences obtained through WGS. Sequences will be aligned using MAFFT tools, and phylogenies will be inferred using Bayesian approaches. Reference genomes representing different MPXV clades (Clade I, IIa, IIb) will be included to contextualize the position of Congolese strains within the global MPXV diversity.

This analysis will provide insights into the origin, diversification, and potential transmission pathways of MPXV in the study region. It will also allow identification of clusters and possible links between cases, contributing to both molecular epidemiology and outbreak control strategies. All phylogenetic outputs will be integrated with epidemiological and geographic metadata to enhance interpretation and guide public health response.

Data management:

Results of laboratory analysis will be collected in a database by laboratory study-staff, with personal system of access by username and password. Clinical data will be collected in an independent database by the investigator or delegated study staff with a personal system of access by username and password. Based on the study ID, databases will be merged to create the final study database. A data management system will be set up and procedures will be implemented to warrant homogenization, traceability, and data quality. Quality control procedures will be put in place for data checking. Consistency checks will be created to reduce errors during data entry.

The data management team and investigators will have access to the database. The backup of the data will be done on a timely basis. The final data for the analysis will not contain any personal identifiable data of the participating patients and those receiving the final data for analysis will not have access to any information that might help to physically identify patients.
Each participant will be assigned a unique study identification number, and all data will be recorded and analyzed with this unique identification number. All information will be stored in a coded fashion in an encrypted password-protected database, using unique patient identifiers. All devices and servers that store protocol data with personal identifiable information will use full drive encryption, require OS-level and application-level authentication, field-level data encryption, and limit authenticated access with user profiles and groups. All transmitted data will be encrypted at source and decrypted at destination over secure channels.

Statistical Methods:

All analyses will be done with the R statistical package, version 4.1 or higher, under a significance level of 0.05.

The demographic and clinical characteristics of study participants will be described using the median and IQR (defined by the 25th and 75th percentiles) or the number and percentage of available data. We will describe the viral load DNA from each 'compartment' (i.e., skin lesions, blood, oropharynx, and urine) using mean and standard deviation and/or median and interquartile range. We will use a linear mixed effect models to describe the log viral load of individuals with Mpox and infer the time to viral clearance and describe the viral load over time. We will assume all bodily fluid compartments (i.e., skin lesions, blood, oropharynx, and urine) to be independent and will fit them separately. Values lower than the limit of detection of the assay will be considered right censored. Time to clearance between the different compartments will be compared with a log-rank test after estimating the Kaplan-Meier.
In exploratory analyses we will evaluate variables associated with time to clearance including clinical features of the current illness (for example extent of skin lesions at enrolment, presence or absence of systemic features) and with demographic and co-morbidity data from participants (for example age and HIV status, co-infection with measles, TB or malaria). We will assess the extent to which clinical indicators of disease resolution (for example cessation of fever, re-epithelization of skin) are associated with clearance of the virus. Associated factors to both acquisition and access to health care will be identify by descriptive statistical techniques including multiple regression analysis. All analyses will be done with the R statistical package, version 4.1 or higher, under a significance level of 0.05.
We will calculate the Secondary Attack Rate (SAR), including symptomatic and asymptomatic SAR. We will conduct exploratory analysis to examine how the SAR varies by increasing index case viral load, baseline characteristics of the contact and type of case-contact relationship. The relationship between characteristics of cases and viral load will be assessed by use of linear regression considering age (in years), sex, number of days from reported symptom onset, number of skin lesions and presence of systemic symptoms. To identify risk factors for transmission, we will use generalized mixed effect models for the risk of transmission to allow for within-cluster variation in the risk of transmission. Factors with potential influence on the risk of transmission include characteristics of the potential transmitter (i.e., age, sex, viral load, the number of skin

lesions, and the presence or absence of systemic symptoms, immunosuppression) and contacts (i.e., age, sex, and the type of contact they had with the index case, immunosuppression).

Limitations:

Limited healthcare infrastructure and transportation challenges: In certain areas, the lack of adequate equipment for sample preservation and transportation may compromise their integrity before reaching Barcelona. Additionally, the international shipment of samples from the DRC to the laboratory in Barcelona can be complex, with potential delays or customs-related issues.

Insufficient sample size: A small or unbalanced number of samples could reduce statistical power, increasing the risk of not detecting significant differences in viral excretion in different body compartments. This limitation may lead to inconclusive or less generalizable results. In addition, variability in the timing of sample collection and individual differences in immune response may further complicate the analysis, potentially affecting the reliability of comparisons between viral load in different compartments.

Difficulty in contact tracing: Incomplete or inaccurate information regarding exposure history may lead to misclassification of cases and contacts, affecting the reliability of SAR estimations. Additionally, logistical challenges in tracking and following up with contacts over time may result in missing data, further limiting the accuracy and generalizability of the findings.

Potential bias in epidemiological data collection and limited access to contacts: Incomplete or inaccurate self-reported data may lead to misclassification or underestimation of certain risk factors. Additionally, difficulties in reaching and enrolling contacts could result in selection bias, limiting the study's ability to capture a comprehensive and representative overview of transmission dynamics.

3. ETHICAL AND LEGAL ASPECTS

3.1 Integration of national or international research activities into the project

The success of the proposed project relies significantly on the integration of previously generated data and research outcomes from national and international activities. The collaborative efforts between the London School of Fight Infections Foundation (FLS), Hygiene & Tropical Medicine (LSHTM) and University of Kinshasa have yielded valuable insights into Mpox, which will serve as a foundation for the proposed research. Our team brings together leading UK, Spanish and African partners (FLS, LSHTM, UNIKIN) with expertise in clinical, epidemiological, and virological research focused on Mpox. Our network has previously undertaken pivotal work on Mpox including the first demonstration that the 2022 clade-II pandemic was due to sexual transmission, the first longitudinal study of viral kinetics of clade-II MPXV, and the largest study in people living with HIV during the 2022 pandemic demonstrating associations between CD4 count and Mpox outcomes. (9–11,13,29).
Key Research Activities that will be integrated:

Clinical Research Expertise: The FLS-LSHTM collaborative group on STIs and NTDs has a longstanding history of collaboration for more than 15 years, with substantial experience in Mpox clinical research over the 2022-2024 period. The University of Kinshasa serves as a key academic and technical partner in the mpox response in the DRC, bridging scientific research, public health training, and operational support to strengthen national outbreak control efforts. The FLS-LSHTM collaboration includes the establishment of a network of clinicians spanning 19 countries, enabling comprehensive data collection on the initial clinical presentation of Mpox, particularly its clade-II sexually transmitted form and severe manifestations in

immunosuppressed patients. These studies have provided invaluable insights into disease epidemiology, risk factors, and clinical management.

Trial Design and Conceptualization: The expertise gained from previous Mpox clinical research forms the cornerstone for trial design and conceptualization in the proposed project. Drawing upon insights from the diverse clinical presentations of Mpox and the nuances of epidemiological studies in different populations, the collaboration between institutes ensures that the upcoming trials are methodologically rigorous, ethically sound, and culturally sensitive.

The integration of national and international research activities in the current proposal is established through regular communication, data sharing, and collaborative decision-making mechanisms to ensure coordination between consortium members. Joint workshops, training sessions, and research meetings are being held to foster knowledge exchange and mutual learning, further strengthening the linkages between national and international research efforts.

3.2 Integration of Expertise and Methods from Different Disciplines

In the context of the proposed work, an interdisciplinary approach is essential for achieving our objectives effectively.

Clinical Epidemiology and Public Health experts will collaborate to design and implement the prospective observational studies, ensuring robust data collection and analysis. Clinicians and epidemiologists will collaborate to characterize the clinical spectrum of clade-I Mpox in DRC, identify risk factors for transmission, and assess disease outcomes. This interdisciplinary approach ensures that clinical observations are systematically analysed and translated into actionable public health recommendations.

Laboratory Sciences and Molecular Biology: Laboratory scientists will employ PCR testing to conduct serial MPXV testing on samples collected from different body compartments of Mpox patients. In addition, viral culture, serology and genomic sequencing will be conducted by international partners to identify genetic variants and chains of transmission. By integrating laboratory findings with clinical data, we can elucidate the biological mechanisms underlying Mpox pathogenesis, time to viral clearance, and transmission dynamics between a case and its contacts.

Data Science and Bioinformatics: Data scientists and biostatisticians will analyse the collected data using statistical methods to determine the time to viral clearance and assess transmission dynamics. They will identify associations between clinical, demographic, and epidemiological factors, providing valuable insights for optimizing Mpox control measures.

3.3 Benefit-risk assessment for research subjects

The studies proposed provide answers on pathogenicity and clinical information on host susceptibility and host immune responses.

Objective 1 - MOVIE Study: by examining the kinetics of viral elimination, the MOVIE study will shed light on the pathogenic mechanisms of MPXV, identifying how the virus interacts with host tissues and immune defences. The study focuses on the kinetics of clade-I MPXV elimination, providing crucial epidemiological data on Mpox viral shedding and the duration of infectiousness. These dynamics are directly related to pathogenicity as they reveal the virus's ability to replicate, persist, and cause damage within the host. Understanding the duration and levels of viral shedding helps in identifying how long and how intensely the virus can affect the host, which is essential for developing effective containment strategies and therapeutic

interventions. The MOVIE study could also contribute to pave the way for future research about clinical trials for therapeutics. It could potentially contribute to establishing a reduction in viral load as an endpoint in treatment RCTs for Mpox.

Objective 2- TRACE Study: The TRACE study focuses on determining secondary attack rates in clade-I MPXV outbreaks. By analysing secondary attack rates, the study assesses host susceptibility within specific populations. Higher secondary attack rates suggest increased susceptibility among contacts, highlighting the vulnerability of certain groups to MPXV infection. This study will identify factors associated with increased susceptibility to MPXV, such as age, health status, and prior smallpox vaccination or natural immunity. This information is crucial for targeting interventions to the most vulnerable populations, including children who have been disproportionately affected. By providing data on host susceptibility, the study will inform public health strategies aimed at protecting high-risk groups and reducing Mpox-related morbidity and mortality. The TRACE study could contribute to provide insights on vaccine efforts as it contributes to assessment of vaccine efficacy endpoints. Phase 3 studies require researchers to assess the vaccine's effectiveness in reducing disease transmission and preventing secondary cases.

Impact of the MOVIE study:

Scientific Impact:

a. **Scientific advancement**: The MOVIE study will significantly advance scientific knowledge by examining viral clearance and duration of infectiousness in various body compartments in people affected by clade-I MPXV. It also aims to elucidate factors influencing faster clearance and immunity related to rapid clearance.
b. **Therapeutic trials**: Beyond its immediate impact on public health, the MOVIE study could also contribute to pave the way for future research studies and clinical trials focused on developing novel therapeutics for Mpox. It could potentially contribute to establishing a reduction in viral load as an endpoint in treatment RCTs for Mpox. Monitoring changes in viral load levels before and after treatment can provide valuable insights into the effectiveness of antiviral therapies and their impact on viral replication and disease progression.
c. **Biomarkers:** Mpox viral kinetics and host factors associated with disease outcomes could provide valuable biomarkers for monitoring treatment response and predicting clinical prognosis.

Societal Impact:

a. **Disease control strategies:** By elucidating the time course of Mpox viral clearance in different body compartments, our aim is to generate new knowledge that can inform public health interventions. Understanding how long an infected individual remains contagious helps public health authorities implement appropriate control measures to prevent further transmission of the disease. For contagious diseases like Mpox, identifying and isolating infectious individuals can help contain outbreaks and reduce the spread of infection within communities.
b. **Reduced disease transmission through isolation**: Knowledge of the duration of infectiousness informs decisions regarding the length of isolation for infected individuals. (30) Public health guidelines may recommend isolating individuals until they are no longer infectious to prevent them from spreading the disease to others.
c. **Contact Tracing and Surveillance**: Duration of infectiousness data aids in contact tracing efforts, allowing public health authorities to identify and monitor individuals who may have been exposed to the disease. (31) Timely identification of contacts enables early intervention, including testing, quarantine, or isolation, to prevent further spread of infection.

d. **Public Health Messaging**: Communicating accurate information about the duration of infectiousness is essential for public health messaging campaigns. Clear guidance on how long individuals should isolate or avoid close contact with others helps promote compliance with preventive measures and reduces the risk of disease transmission in the community.
e. **Increased community resilience**: By generating knowledge that can help mitigate the impact of Mpox outbreaks, MOVIE may contribute to building resilience within affected communities. This may involve empowering individuals with information on disease prevention measures and fostering community engagement in outbreak response efforts.
f. **Reduced morbidity and mortality**: Through evidence-based insights into viral clearance dynamics, the study will inform the development of targeted interventions aimed at reducing Mpox transmission rates and improving infection control measures. This is anticipated to lead to tangible improvements in public health outcomes, including decreased Mpox morbidity and mortality rates in affected communities.
g. **Genomic Surveillance**: Phylogenetic surveillance will help identify the different variants of the virus that may be circulating in the high-risk population. This can be important in distinguishing between local infections and emerging strains that may have different epidemiological behaviors. In addition, genomic data can help detect new waves of outbreaks or unexpected outbreaks by identifying common genetic patterns among cases of infection in different regions.

Economic impact:

a. **Resource Allocation:** By quantifying the risk of Mpox transmission within specific populations or settings, policymakers can make informed decisions regarding resource allocation, intervention strategies, and public health messaging.

Impact of the TRACE study:

Scientific impact:

a. **Scientific advancement**: The study will provide data on secondary attack rates, host susceptibility, and host immune responses in household or school transmission settings. It will contribute to scientific advancements by addressing methodological limitations in previous studies and providing more accurate estimates of SAR in clade-I MPXV outbreaks.
b. **Vaccine efficacy endpoint measurement**: SAR helps establish the baseline risk of disease transmission within specific populations or settings. This information is essential for determining the incidence and prevalence of the disease before vaccine intervention. Moreover, SAR serves as a key endpoint for evaluating vaccine efficacy. By comparing SAR between vaccinated and unvaccinated groups, researchers can assess the effectiveness of the vaccine in reducing disease transmission and preventing secondary cases.
c. **Vaccine efficacy sample size calculation**: SAR data are used to calculate the sample size required for vaccine efficacy studies. Understanding the expected SAR in the target population allows researchers to determine the number of participants needed to detect a significant difference in disease transmission between vaccinated and unvaccinated groups.
d. **Vaccine real-world effectiveness**: SAR data provide real-world evidence of vaccine impact on disease transmission in community settings. By measuring changes in SAR over time following vaccine implementation, researchers can evaluate the long-term effectiveness of the vaccine in reducing overall disease burden and interrupting transmission chains.

Societal impact:

a. **Policy Informing of at-risk populations**: SAR measures the likelihood of an individual contracting Mpox from a known infected individual within a specific setting, such as a household or community. Understanding SAR helps policymakers and public health authorities assess the risk of Mpox transmission in different contexts, identify high-risk settings or populations, and tailor interventions accordingly. For example, if SAR is found to be higher in certain demographic groups or transmission settings, targeted preventive measures, such as vaccination campaigns or enhanced infection control protocols, can be implemented to reduce transmission rates and mitigate the spread of the disease.
b. **Quarantine Policies**: Knowledge of the risk of infection acquisition informs decisions regarding the length of quarantine periods for close contacts. Public health guidelines may recommend quarantining individuals until they are no longer at risk of becoming infectious to prevent them from spreading the disease to others.
c. **Predictive value**: SAR estimates provide valuable information for modelling the impact of different control measures and predicting the trajectory of Mpox outbreaks, thereby guiding the implementation of effective and timely response efforts.
d. **Increased community awareness**: By raising awareness about Mpox transmission and prevention measures, TRACE may empower communities to take proactive steps to protect themselves and reduce the spread of the disease.

Economic impact:

a. **Resource allocation**: By quantifying the risk of Mpox transmission within specific populations or settings, policymakers can make informed decisions regarding resource allocation, intervention strategies, and public health messaging.
b. **Strengthened healthcare systems**: By providing valuable data on Mpox transmission patterns, TRACE may contribute to the strengthening of healthcare systems preparedness in affected regions, improving their capacity to respond to infectious disease outbreaks in the future.

3.4 Scale and significance of contribution

The scale and significance of the MOVIE and TRACE studies' contributions to expected outcomes and impacts are enormous, given the magnitude of Mpox morbidity and mortality in affected regions like the Democratic Republic of Congo (DRC). Successful completion of these studies is expected to result in tangible improvements in public health outcomes, including decreased Mpox transmission rates, improved infection control measures, reduction of morbidity and reduction mortality.

With Mpox outbreaks affecting thousands of individuals in the DRC, successful completion of the MOVIE and TRACE studies could have a considerable impact on reducing transmission rates and enhancing outbreak response.

Given the high mortality rates associated with Mpox outbreaks, any interventions that reduce transmission (i.e., isolation, quarantine, contact tracing, identification of high-risk populations) and improve clinical management (i.e., prioritization of people at risk of severe infection, time of isolation) have significant implications for reducing both mortality and morbidity rates. These two studies could contribute to saving lives and reducing the burden of Mpox-related morbidity in affected populations.

The disproportionate impact of Mpox outbreaks on children in the DRC underscores the relevance and significance of the studies potential impact on reducing morbidity and saving Disability-Adjusted Life Years (DALYs). Given that Mpox outbreaks have resulted in a significant number

of fatalities, particularly among children under the age of 15, addressing the disease burden is of paramount importance.

Another dimension of impact involves enhancing clinical outcomes for Mpox patients, particularly children and pregnant women by identifying factors associated with faster viral clearance.

The information generated to guide future research and clinical trials focused on assessing therapeutics (i.e., time to viral clearance) and vaccines (i.e., SAR) for Mpox is indeed significant. For therapeutics, the study's findings on viral clearance dynamics, factors associated with faster clearance, and immunity related to faster clearance can help establish meaningful endpoints for treatment RCT. For vaccines, data on secondary attack rates, host susceptibility, and immune responses can contribute to assessing vaccine efficacy.

3.5 Research data management

We will develop a comprehensive set of study-specific data management documents. These will guide the processes across sub studies. For each sub-study this will include a study specific data management plan describing how data/research inputs will be set up to ensure compliance with good data management in line with the FAIR principles. A shared database will be set up including the development of electronic case report forms (eCRF), as well as the programming of edit checks and customised study specific reports. The database will be validated before deployment for use. Clinical, demographic, and linked laboratory data will be maintained within these databases. Data management activities will include data review and validation; managing and reconciling data from third parties; data exports to third parties; providing data listings, tables and patients profiles for the purpose of data review; and preparation and review of data before the database lock.

Below, we outline our approach to data management and the management of other research outputs, specifically tailored to the needs and objectives of our project:
Types of Data/Research Outputs and Estimated Size: Our project involves collecting various types of data, including clinical, demographic, and laboratory data. These datasets will comprise observational data, along with images, textual documentation, and laboratory results. The estimated size of these datasets will be substantial, given the comprehensive nature of our study involving 75 Mpox cases and 200 contacts. Careful consideration will be given to data storage and management infrastructure to accommodate the anticipated volume of data generated throughout the project.
Findability of Data/Research Outputs: To enhance the findability of our data and research outputs, each dataset will be assigned persistent and unique identifiers, such as digital object identifiers (DOIs). These identifiers will facilitate the unambiguous identification and citation of our data. We will deposit our data in trusted repositories, ensuring that they are easily discoverable by researchers and stakeholders.
Accessibility of Data/Research Outputs: We are committed to promoting open access to our data and research outputs wherever possible, in line with our commitment to transparency and scientific integrity. However, we recognize the need to balance openness with the protection of intellectual property rights (IPR) and participant confidentiality. We will provide clear guidelines on access to restricted data for verification purposes, ensuring that appropriate safeguards are in place to protect sensitive information.
Interoperability of Data/Research Outputs: Standardization of data formats, metadata, and vocabularies will ensure interoperability and facilitate data integration and reuse. We will adhere to established standards and formats for data and metadata, ensuring compatibility with existing databases and analytical tools.

Reusability of Data/Research Outputs: We will apply appropriate licenses for data sharing and reuse, such as Creative Commons licenses or Open Data Commons licenses, to promote the ethical and responsible reuse of our data. Additionally, we will make available tools, software, and models developed during the project to facilitate data generation, validation, interpretation, and reuse by other researchers.
Curation and Storage/Preservation Costs: We will allocate resources for the curation, storage, and preservation of our data and research outputs throughout the project lifecycle. This includes provisions for data backup, long-term preservation, and access to secure storage facilities. A dedicated person/team will be responsible for data management and quality assurance, ensuring that data integrity and compliance with FAIR principles are maintained.

Community sensitization activities will be carried out in the target study areas, with subject screening for eligibility in the study conducted according to the protocol. Laboratory equipment will be calibrated in accordance with manufacturer's recommendations and will undergo regular QC procedures. All laboratory testing will be performed in facilities implementing a laboratory quality system and following GCLP guidelines. Procedures for data sharing will be set out clearly in the participant information sheet, with current and potential future associated risks explained to research participants as part of the consent process.

3.6 Implementation of Open Science Practices

Open science practices play a pivotal role in ensuring the accessibility, transparency, and impact of our findings, particularly for regions with limited resources and infrastructure. Below, we outline how these practices are tailored to our project's objectives and the needs of LMICs:

Early and Open Sharing of Research: We will adopt a proactive approach to sharing our research findings, recognizing the importance of timely dissemination for informing public health interventions in LMICs. Preliminary findings will be shared through pre-print repositories, allowing for rapid access and feedback from the global scientific community.

Research Output Management: Robust data management protocols will be implemented to ensure the organization and documentation of research outputs. This includes the deposition of datasets in accessible repositories, accompanied by comprehensive metadata records to facilitate discovery and reuse.

Ensuring Reproducibility of Research Outputs: Methodological transparency and reproducibility are paramount in our Mpox research. Detailed documentation of experimental procedures and analysis techniques will be provided to enable replication and verification, enhancing the reliability of our findings.

Open Access to Research Outputs: Recognizing the limited access to scientific literature in LMICs, we are committed to making our research outputs freely available to researchers, healthcare professionals, and policymakers in these regions. Publications will be published in Gold Open Access journals ensuring unrestricted access to vital information.

Participation in Open Peer-Review: We will actively engage in open peer-review processes to enhance the rigor and transparency of our research. By soliciting feedback from a diverse range of stakeholders, including researchers from LMICs, we aim to strengthen the validity and applicability of our findings.

Involving Relevant Knowledge Actors: Engagement with relevant stakeholders, including community members, healthcare providers, and policymakers in the DRC, is integral to the success of our project. Through participatory approaches, we seek to co-create research agendas and solutions that address the specific needs and priorities of Mpox-affected communities in LMICs.

FAIR Data Principles and Data Sharing: Adherence to the FAIR principles ensures that our

data are Findable, Accessible, Interoperable, and Reusable. Metadata-rich datasets will be deposited in accessible repositories, with a commitment to long-term preservation. Each output will be assigned a DOI for citation in reports and publications, promoting transparency and attribution.

Pre-print Servers and Gold Open Access Publication: Manuscripts will be submitted to pre-print servers to accelerate the dissemination of findings, particularly relevant for LMICs where access to subscription journals may be limited. Pursuing Gold Open Access publication ensures that our research outputs are freely available to all, facilitating knowledge exchange and capacity-building in Mpox research globally, including in LMICs like the DRC.

4. PLANS FOR DISSEMINATION AND COMMUNICATION OF RESULTS

4.1 Strategic Communication Approach

To maximize the impact of the MOVIE and TRACE studies, we will implement a comprehensive dissemination and exploitation plan, including strategic communication activities. We will develop a comprehensive communication strategy that includes clear objectives, dissemination and advocacy activities, type of outputs that are appropriate to audience and context.

Objectives and Activities:

Corporate Project Identity: We will develop a unified corporate identity for the project to ensure consistent public perception and recognition.
Key Communication Tools: These will include policy briefs for policymakers, project meetings, and dissemination workshops for partner organizations and the national Mpox response program. Academic and policy audiences will be targeted through peer-reviewed publications and conference presentations, while other materials will be tailored for non-expert audiences.

Target groups:

Scientific Community: We will disseminate our research findings through peer-reviewed publications in high-impact scientific journals, conference presentations, and participation in relevant scientific events and workshops. Additionally, we will share preprints of our manuscripts on open-access repositories to facilitate early access to our research.
Healthcare workers: We will engage with end users through targeted dissemination activities, including workshops, seminars, and webinars. These activities will provide opportunities for direct interaction and knowledge exchange between project researchers and stakeholders, ensuring that our findings are relevant and actionable.
Policy Makers: We will produce concise and informative policy briefs summarizing key research findings, implications, and recommendations for policy makers. These briefs will be designed to present complex scientific information in a clear and actionable format, facilitating evidence-based decision-making.
Public at Large: We will employ various communication channels to reach the broader public, including press releases, social media campaigns, and multimedia content. These efforts will aim to raise awareness about the project, its objectives, and its potential benefits for society, emphasizing the importance of our research in addressing pressing health challenges.
International Agencies: We will ensure results of the project are shared in real-time with the national Mpox response and with other key stakeholders such as the WHO and Africa CDC. Subasequrntly we will ensure data are presented at key academics such as the American Society of Tropical Medicine & Hygiene Meetings.

Dissemination Models:

Innovative models: We will utilize webinars, video diaries, and multimedia outputs to engage diverse audiences.
General Media: We will also engage with the broader community through social media including both institutional and investigator Twitter accounts and other social media platforms. By leveraging the power of social media, we will amplify the reach of our project, foster dialogue, and facilitate knowledge sharing among stakeholders.

4.2 Exploitation measures for real-time data sharing

Open access is vital to research uptake by decision makers and deepening international academic discourse, we will adopt an open access approach to data, research findings and training outputs. In line with EU's Plan S, we will pursue Gold Open Access. All studies will be published in open access format to ensure maximal accessibility. All datasets will be made available, in anonymised form, via open-access repositories to facilitate secondary-analyses of data.

4.3 Stakeholder and policy engagement

Throughout the study, we will continue to meet with key stakeholders and also invite them to visit the study sites to see firsthand the study and the implementation of the interventions. As research is completed, a series of dissemination workshops will be held with local and national stakeholders. We will utilise a combination of strategies to engage with policy makers including: i) Policy briefs; ii) Breakfast meetings where findings and implications can be presented and discussed directly with key policy makers and programme staff; and iii) Stakeholder workshops at local level for government and health service officials and community members.

Based on our experience in research and policy engagement in research and implementation we have identified several factors which increase the probability of translating research into policy and implementation, including: i) research of relevance to local needs in an area of public health importance; ii) delivery of visible health impact; iii) acceptable to local communities and health systems; iv) cost-effective and deliverable at scale; v) informed by and aligned with government policies and health systems. We believe our proposal meets these criteria. We have engaged with members of the Mpox response whilst developing this proposal to to ensure conceptual 'buy-in'. Continuing engagement throughout will maximise the impact of our research.

4.4 Feedback to Policy Measures

We anticipate that the project's findings will contribute to designing, monitoring, reviewing, and rectifying existing policy and programmatic measures. By engaging with policymakers and providing evidence-based recommendations, we aim to support the implementation of new policy initiatives and decisions. The feedback from these studies will be crucial in refining the Mpox response framework, particularly in high-risk populations such as children and immunocompromised individuals.

4.5 Intellectual property

While the project is not expected to generate intellectual property requiring protection, our strategy will focus on ensuring open access to all data and findings. This approach is designed to maximize the dissemination and impact of the research outcomes, facilitating their uptake and application by various stakeholders.

5. SUMMARY

KEY ELEMENT OF THE IMPACT SECTION

SPECIFIC NEEDS	EXPECTED RESULTS
What are the specific needs that triggered this project? This project was triggered by needs of clade-I Mpox-affected communities in LMICs. Firstly, there is an urgent need for comprehensive data on the pathogenicity of the Monkeypox virus (MPXV) to inform the appropriate duration of isolation and specific clinical management practices. Without this information, healthcare providers face challenges in implementing effective interventions. Secondly, there exists a significant gap in understanding the secondary attack rate (SAR) of MPXV, which is crucial for guiding disease control activities such as contact tracing, quarantine implementations, and targeted interventions for vulnerable populations (higher SAR). Accurate SAR data are also essential for evaluating the efficacy and effectiveness of vaccines, which is vital for the successful implementation of vaccination campaigns.	*What do you expect to generate by the end of the project?* The project is expected to yield critical insights into the pathogenicity of the MPXV and its SAR in Mpox-affected communities within the DRC. These findings will inform the optimal duration of isolation and specific clinical management practices, enhancing patient care and public health responses. Additionally, the data on SAR will provide valuable guidance for disease control activities, such as contact tracing and targeted interventions to vulnerable populations and will be instrumental in assessing vaccine efficacy and effectiveness. Ultimately, the results will support the development of evidence-based targeted interventions, improving health outcomes and resilience within these communities.
D & E & C MEASURES	**TARGET GROUPS**
What dissemination, exploitation and communication measures will you apply to the results? The project aims to ensure that its findings reach and benefit diverse audiences, facilitating knowledge exchange and encouraging informed decision-making in the field of infectious disease management. We will develop a comprehensive strategy to maximize de impact of the project. Objectives and Activities: Corporate Project Identity to ensure consistent public perception and recognition and key Communication Tools including policy briefs, project meetings, dissemination workshops, peer-reviewed publications, conference presentations, and engagement through social media channels. Dissemination Models: Innovative Models such as webinars, video diaries, and multimedia outputs to engage diverse audiences: dedicated Programme Website as the primary hub for communication and dissemination activities; dissemination on general Media by using institution and investigator social media profiles to engage with the broader community, amplifying the reach of the project and facilitating dialogue and knowledge sharing among stakeholders.	*Who will use or further up-take the results of the project? Who will benefit from the results of the project?* Scientific Community: Publish in high-impact journals, present at conferences, and share preprints on open-access repositories. Beneficiaries: Researchers and scientists. Healthcare Workers: Conduct workshops, seminars, and webinars for direct interaction and knowledge exchange. Beneficiaries: Medical professionals and frontline healthcare workers. Policy Makers: Create concise policy briefs to support evidence-based decision-making. Beneficiaries: Government and health policy makers. Public at Large: Use press releases, social media, and multimedia to raise awareness and highlight the project's importance. Beneficiaries: General public and affected communities. International Agencies: Share results with national Mpox response teams, WHO, Africa CDC, and present at key academic events. Beneficiaries: Global health organizations and international health agencies.

OUTCOMES	IMPACTS
What change do you expect to see after successful dissemination and exploitation of project results to the target group(s)? We anticipate the following changes to each targeted group: Scientific Community: Better understanding of MPXV pathogenicity and its SAR, leading to more informed and effective research in viral dynamics and epidemiology. Healthcare Workers: More informed isolation duration and improved clinical management strategies for Mpox. Policy Makers: Adoption of evidence-based policies and guidelines for Mpox surveillance, isolation protocols, and vaccination strategies. Public at Large: More informed communities that are better prepared to adhere to public health recommendations. This can result in reduced transmission and better community health outcomes. International Agencies: Strengthened global response to Mpox outbreaks, with coordinated efforts and strategies informed by robust data. Agencies like WHO and Africa CDC will have access to timely and relevant information to support international health initiatives and interventions.	*What are the expected wider scientific, economic and societal effects of the project contributing to the expected impacts outlined in the respective destination in the work programme?* Scientific Impact: We will determine crucial aspects of clade-I MPXV, including viral clearance and duration of infectiousness in various body compartments, factors influencing faster clearance and immunity related to rapid clearance, the Secondary Attack Rate, host susceptibility, exposure risk factors and settings of risk (household or school transmission), and host immune responses. We will address methodological limitations in previous studies and provide more accurate estimates. Economic Impact: Help healthcare systems make informed decisions regarding resource allocation, intervention strategies and public health messaging, and plan accordingly. Societal Impact: Improved public health outcomes and increased community resilience. The project will empower communities with accurate public health messaging, increasing awareness and fostering community engagement and understanding of infectious diseases. Results can lead to targeted interventions to reduce morbidity and mortality rates.

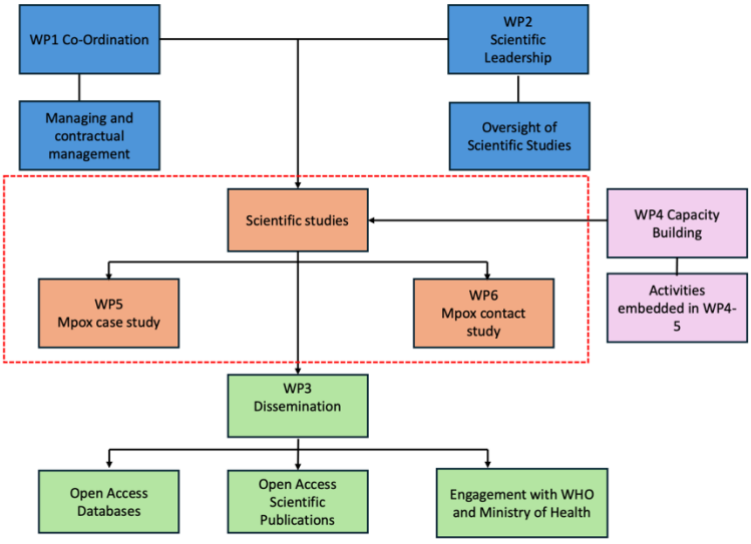
6. QUALITY AND EFFICIENCY OF THE IMPLEMENTATION

6.1 Work plan and resources

Work will be delivered over a 24-month time frame and is divided into 6 inter-linked work packages (Fig. 5). The relationship between these work—packages is shown below. Detailed descriptions of the specific tasks, deliverables, milestones and risks are outlined below.

WPs 1-4: Project Organisation. These three interlinked work packages provide the framework to support the overall delivery of our linked scientific work packages. They cover contractual and budgetary oversight, scientific leadership, and development of a dissemination plan.

WP1 Coordination (Lead: FLS): Project coordination will be overseen by Professor Oriol Mitjà (FLS, SPAIN). This WP will oversee contractual and budgetary elements of the project and ensure the overall scientific work is delivered on time and budget. Along with the Scientific Lead (WP2), the Project Coordinator will direct the Project Steering Group. The Co-ordinator will appoint a dedicated project administrator who facilitate contractual and financial elements of the project.



WP2 Scientific Leadership (Lead: FLS/UNIKIN) Professor Hypolite Muhindo will provide scientific leadership across the consortium. This role will oversee overall coordination of scientific work packages and integration of capacity building across members of the overall consortium. Alongside the Project Coordinator (WP1), the Scientific Lead will direct the Project Steering Group.

Project governance and steering will be performed synergically in both WP-1 and WP-2: The Project Steering Group lead by Professor Oriol Mità (WP1) and Professor Hypolite Muhindo (WP2) and members of all two consortium members (FLS, UNIKIN), including representatives of each work package with administrative support from each of the consortium members. This group will guarantee a rapid and effective decision making and efficient sharing of information across all relevant aspects of the project. Due to the emergency nature of the Mpox response this group will meet once a month and more frequently as required.

WP3 Project Dissemination (Lead: FLS/LSHTM): This WP will oversee both internal communications within the consortium as well as the development and delivery of an external communication strategy and engagement with key stakeholders. This will include knowledge dissemination strategies, ensuring data will be presented at international conferences and WHO meetings, through pre-prints and open access publications in line with the FAIR principles.

WP-4 Capacity Building (Lead: FLS/LSHTM): We have sought input into our project proposal from co-applicants and through engagement with stakeholders involved in the Mpox response in DRC. Although the focus of the project is to support the emergency response to Mpox we aim to use this opportunity to still build critical research and epidemic response skills across the consortium. We will aim to build capacity within collaborating institutions through workshops led by the research team across a range of areas including study design, data collection, and the analysis of quantitative data. Where appropriate, we will provide funds for team members to attend training courses.

Work Packages 5-6: Scientific Work Packages (Lead: UNIKIN): We will deliver two inter-linked scientific studies to address critical questions about transmission of Mpox in the Democratic Republic of Congo. Central to this approach will be the identification of index cases who will be recruited into a longitudinal study of viral shedding and period of infectiousness, and their contacts who will be recruited into an observational study focused on the secondary attack both studies are provided in Section 2 (above) with specific tasks and deliverables outlined below (Section 3).

Project Timeline. We have allowed 24 months for the overall project. Broadly this includes 8 months for study setup, 8 months for recruitment and 8 months for finalizing laboratory work and

analysis (Fig. 6). Given the emergency nature of the call, we anticipate sharing interim results in real-time with the Ministry of Health MPOX response team and other key stakeholders such as WHO and Africa CDC.

			Project Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Workpackage	**Outcome**	**Success Measure**	**Activity**																								
WP1 Project Co-Ordination	Project delivered on time and budget	Contracts signed, Financial and Technical Reporting Completed	Contract Signed																								
			Staff Appointed																								
			Project Steering Group Meetings																								
WP2 Scientific Leadership	Scientific Studies Completed	Ethical approval obtained, scientific studies completed and results translated into policy	Project Steering Group Meetings																								
			Protocol Finalisation																								
			Ethics Approval																								
			Study Training																								
			Study Monitoring																								
WP3 Dissemination, Policy Impact and Community Engagement	Uptake of Results into Policy	Change in National & Regional Policy	Development of a project dissemination plan																								
			Project Website																								
			Data Sharing with MOH mpox response team																								
			Data Deposit																								
			Scientific Publications																								
WP4 Capacity Building	Staff Trained in Research	Staff trained in GCP, GCLP and Researc	Staff Training & Mentorship																								

	and Laboratory Methods	Methodologies. Enhanced laboratory capacity for mpox research																										
WP5 and WP6 Study	Data on the viral load of MPOX Clade 1 in different body tissues.	50 patients and enrolled to analyze viral load in different biological compartments.	Staff training																									
			Participant recruitment and Follow-Up																									
			Laboratory Work																									
			Analysis																									
	Data on secondary attack rate of MPOX virus and determinants of transmission	500 contacts enrolled and completed follow-up with calculation of symptomatic and asymptomatic secondary attack rate	Staff training																									
			Participant recruitment and Follow-Up																									
			Laboratory Work																									
			Analysis																									

6.2 Capacity of participants

6.2.1 Consortium Composition and Objectives Alignment

Our consortium brings together three leading institutions: the Fight Infections Foundation (FLS, Spain), the London School of Hygiene & Tropical Medicine (LSHTM, UK) and University of Kinshasa (UNIKIN, DRC). We have assembled an inter-disciplinary network of investigators with leading expertise and skills in their relative fields and who have access to the infrastructure and skills required to deliver each of our work packages and c conduct this research project.

This consortium is designed to match the project's objectives by integrating diverse disciplinary and interdisciplinary knowledge essential for addressing global public health challenges. FLS brings its strengths in clinical research in infectious diseases and specially its expertise in dermatology and venereology having conducted extensive research on infections of the skins and sexually transmitted infections. LSHTM contributes its extensive expertise in global health and epidemiology ensuring a holistic approach to research challenges related to the current proposal. UNIKIN offers significant capabilities in community-based epidemiological research and laboratory research. The consortium emphasizes open science practices, community-engaged research, and incorporates gender aspects in research and innovation (R&I). This alignment ensures that the research is scientifically robust, culturally relevant, and actionable, involving affiliated entities and associated partners for comprehensive coverage.

6.2.2 Individual Contributions and Roles:

1- Professor **Hypolite Muhindo Mavoko (UNIKIN),** a physician specialized in tropical medicine and full professor at UNIKIN. Since 2013, he has directed numerous clinical trials and observational studies on malaria, Ebola, Covid-19, and Mpox—particularly in resource-constrained settings and among vulnerable populations. He has published extensively on diagnostics, vaccine acceptance, and public health ethics. Professor Muhindo currently leads multiple Mpox research projects, focusing on transmission and vaccination, and actively collaborates with international consortia to build local capacity and reinforce global health preparedness.

2- Professor **Oriol Mitjà (FLS),** is an infectious diseases physician and clinical researcher with significant contributions to science on Neglected Tropical Diseases (NTDs) of the skin including yaws, Haemophilus ducreyi, and syphilis. His team was the first to describe the clinical and virological characteristics of Mpox (The Lancet 2022, Lancet Infectious Diseases 2023); provided a detailed description of the disease presentation in women (The Lancet 2023) and identified an association with a fulminant form of Mpox in individuals with advanced HIV (The Lancet 2023).

3- Dr **Sara Buezo (FLS),** has extensive experience in field research and training in Central Africa in the field of epidemiology of infectious disease transmission.

4- Dr **Camila González-Beiras (FLS),** has extensive experience in conducting field research, training, and capacity building in Papua New Guinea and Western Africa in the field of skin NTDs, including the implementation of large-scale community-based randomized clinical trials on yaws (NEJM 2022), development of point-of-care tools for tropical ulcer conditions (i.e., LAMP test).

5- Professor P**lacide Mbala Kingebeni (IRNB)**, is an Associate Professor at the University of Kinshasa, School of Medicine; he is the Head of the Epidemiology and Global Health Division and Director of the Clinical Research Center at the National Institute of Biomedical Research (INRB in French) in the Democratic Republic of the Congo (DRC). Dr. Mbala has extensive experience in medical biology, with specific training and expertise in microbiology, virology, and outbreak investigations. His research focuses on viral zoonoses with risk factors

for human contamination. As PI and co-investigator for several university and US-funded grants, Dr. Mbala laid the groundwork for his research projects in very remote areas of the DRC where most outbreaks occur.

6- Professor **Michael Marks (LSHTM),** is an Infectious Diseases clinician and epidemiologist with extensive expertise in managing large multinational projects in Africa and the Pacific. He is widely recognized as a world-class scientist in the field of Sexually Transmitted Infections (STIs) and Neglected Tropical Diseases (NTDs), and he serves as an advisor to international institutions and donors in these areas.

6.3 Work package description

Work package number	1
Work package title	Project Co-ordination and Management
Time span	Month 1 to Month 24
WP leader	FLS
Participants	ALL

Objectives

The objective of the Project Co-ordination work package is to ensure effective and appropriate overall leadership of the project to facilitate achievement of the project goals.

Specific objectives are:

1. Implement an organizational structure that will co-ordinate and guide the activities of the project.

2. Coordinate and obtain all ethical and regulatory approvals required from local research ethics committees (RECs).

3. Establish agreements between the sponsor (FLS) and the study sites, including material and data transfer agreements

Description of work

Task 1.1: Project management and coordination, including the establishment of a Project Steering Group (Months 1 to Month 24).

The Co-ordinator (PI), Professor Oriol Mitjà (FLS) will work with the Scientific Leadership team (WP2) and have overall oversight of project activities. The PI will work with the work-package leads to co-ordinate all field and laboratory work and data analysis. Work at each partner sites will be overseen by the relevant PI. The project administrator will assist the project co-ordinator with all administrative tasks including creation and management of project records, organization and minutes for meetings and preparation of reports.

The PI, the work package leaders and the members of the Scientific Steering Committee will constitute the Project Steering Group (PSG). Thus, there will be a bi-directional transfer of information between the PSG and the researchers at each consortium partner. Given the imperative for rapid progress, the PSG will meet weekly initially for evaluation of progress, resolution of problems, negotiating hurdles etc. As the project becomes more established this may move to fortnightly or monthly as required.

The PI and a designated financial administrator at HUGTiP will monitor financial management of the project and regular transfer of funds to the partners. This will be tied into achieving project

milestones and achievement of key deliverables.

Task 1.2: Regulatory issues coordination and clinical research operational design (Month 1 to Month 8)

The PI, in coordination with the Scientific Leadership team will oversight regulatory issues, including the submission of ethics applications at HUGTiP and in DRC, as well as, to register the study on an appropriate study database.

Again, in coordination with the Scientific Leadership, the senior researchers of this study will collaborate to define the overall design of operational clinical procedures on the field seamless adapted to the local surveillance activities ongoing in the outbreak areas.

Task 1.3 Data management (Month 1 to Month 24)

We will develop a comprehensive data management plan (DMP) outlining how data will be transferred between partners, outlining the access rights of consortium partners, and defining the procedures to follow to guarantee Open Data Access without trespassing the GRDP Data transfer agreements will be put in place as appropriate.

Task 1.4: Material and Data Transfer Agreements (Month 4 to Month 8)

The Principal Investigator will coordinate material and data transfer agreements to cover the movement of samples and data between countries and collaborating partners. These agreements will include obtaining, where needed, appropriate national approvals as well as policies on safe transfer and storage of study data.

Work package number	**2**
Work package title	Scientific Leadership
Time span	Month 1 to Month 24
WP leader	FLS/UNIKIN
Participants	ALL

Objectives

The objective of the Scientific Leadership work package is to ensure effective and appropriate overall scientific oversight of the project and ensure scientific goals are achieved.

Specific objectives are:

1. Co-ordinate protocol development

2. With project Co-ordinator (WP1) coordinate and obtain all ethical and regulatory approvals required from local research ethics committees (RECs).

3. Oversee work conducted in WP3 & 4 for delivery of the scientific outputs of the study

Description of work

Task 2.1: Scientific steering and coordination of the project (Month 1 to Month 24)

The Scientific Leadership team (WP) will work with the Co-ordinator (WP1 - Professor Oriol Mitjà) and have overall oversight of project activities. They will work with the work-package leads to co-ordinate all field and laboratory work and data analysis. Work at each partner sites will be overseen by the relevant PI.

The Scientific Leadership team and Co-ordinator in collaboration with the work package leads will constitute a Project Steering Group (PSG). Thus, there will be a bi-directional transfer of information between the PSG and the researchers at each consortium partner. Given the imperative for rapid progress, the PSG will meet weekly initially for evaluation of progress, resolution of problems, negotiating hurdles etc. As the project becomes more established this may move to fortnightly or monthly as required.

Task 2.2: Clinical protocols, ethical clearances, and scientific procedures (Month 1 to Month 8)

The Scientific Leadership Team, in coordination with the Coordinator will be responsible for drafting of clinical trial protocols, the submission of ethics applications and the granting of ethical clearances as well as to draft the standard operational procedures that will rule the homogeneous implementation of the scientific activities of the project

Task 2.3 Scientific Oversight (Month 1 to Month 24)

The Scientific Leadership team will be responsible for overseeing implementation of WPs 3&4. This will include i) training of study teams in relevant processes such as consent, participant recruitment in compliance with Good Clinical Practices (GCP), ii) oversight and conduct of laboratory testing including ensuring compliance with GCLP and maintenance of laboratory record, iii) liaison with field sites, the Ministry of Health and other partners and iv) overseeing integrated analysis of study findings.

Work package number	3
Work package title	Dissemination
Time span	Month 1 to Month 18
WP leader	FLS/LSHTM
Participants	ALL

Objectives

The objective of the Dissemination work package is to ensure results arising from the project are disseminated rapidly to all relevant stakeholders (patients, communities, Ministries of Health) through a variety of mechanisms and that data underpinning these findings are made available to the research and public health community in keeping with FAIR principles. 1) Develop a comprehensive plan for dissemination and exploitation of the project results; 2) Develop a comprehensive plan for sharing of study data and code; 3) Implement the data management and publications plans to ensure timely analysis and reporting of results.

Description of work

Task 3.1 Development of a project dissemination and exploitation plan (Month 1 to Month 2)

We will develop a comprehensive plan for the dissemination and exploitation of both data and results emerging from the project. This plan will include a clear timeline for each dissemination strategy (local and international meetings, publications) and guidance to ensure that all dissemination is in line with Plan-S policies on Open-Access and FAIR guidance on availability of data. The dissemination plan will include clear guidance on recognition of all collaborators to ensure that fair benefit sharing between partners occurs and that study output authorship is representative of the role of all partners in the project.

Task 3.2 Stakeholders meetings (Month 3 to Month 24)

We will hold regular meetings throughout the project where we will bring together academic,

community and ministry of health stakeholders. They will facilitate rapid and regular dissemination of results and ongoing engagement with key stakeholders to ensure study results are utilized to inform national, regional and international guidance on the control Mpox Stakeholders and when leading to changes in the project implementation as decided by the Project Steering Group.

Task 3.3: Academic Publications and presentation at academic conferences (Month 13 to Month 24)

Outputs from the study will be submitted to high-impact open access medical journals to ensure maximum reach within the academic community.

Task 3.4: Data Deposit in an Open Access Repository (Month 16 to Month 18)

We will deposit relevant study data into open access repositories in real-time and in accordance with FAIR principles to ensure that data generated in the study is available to the wider research community to maximise impact of the work.

Work package number	**4**
Work package title	Capacity Building
Time span	Month 2 to Month 18
WP leader	FLS/LSHTM
Participants	ALL

Objectives

The main objective of this work package is to build capacity in research institutions in DRC to respond to future Mpox outbreaks. This will include strengthening laboratory molecular capacity and training in the delivery of epidemic response studies.

Description of work

Task 4.1: Laboratory strengthening (Month 2 to Month 18)

Training will include hands-on in the molecular amplification assays. In addition, the laboratory teams will be trained in GCLP guidelines (WHO, 2009, ISBN 978 92 4 159785 2). The GCLP guidelines are recommended by the WHO's special programme for research and training in tropical diseases diagnostic evaluation expert panel as the standard for clinical laboratories involved in the evaluation of diagnostics for infectious diseases. The training session will include exercises on the application of GCLP in this study and laboratory standard of procedures (SOPs) including SOPs for sampling handling and testing, study relevant documents such as a study analytical plan, and forms to document traceability, transparency and validity of results will be developed. The trained laboratory staff will be assessed at the end of the training to document their competency in performing the assays and training certificates will be provided.

Task 4.2: Research Team Training (Month 4 to Month 12)

The training will cover research methodologies, GCP, GCLP, the study protocol and procedures. This will include approaches to community entry, consent, GCP, sample collection and study documentation. Study staff attending the training sessions will receive training certificates. Following central training site visits will be performed to ensure study procedures are being followed appropriately.

Work package number	5
Work package title	MOVIE Study
Time span	Month 4 to Month 24
WP leader	UNIKIN
Participants	ALL

Objectives

The main objective of this work package is to deliver a longitudinal cohort study to understand the viral load of different body fluids amongst patients with Clade I- MPXV in the context of the current outbreak in the Democratic Republic of Congo.

Description of work

Task 5.1 Protocol Finalization (Month 4 to Month 6)

A comprehensive protocol including all components of the study including recruitment methodology, case record forms, sample tracking documentation and standard operating procedures will be developed. This protocol will form the basis of an ethics submission in both DRC and the EU.

Task 5.2 Study Training (Month 7 to Month 8)

Staff will receive study specific training sessions covering GCP, the study protocol and procedures. This will include approaches to community entry, consent, GCP, ethics, sample collection and study documentation. Study staff attending the training sessions will receive training certificates.

Task 5.3 Participant Recruitment (Month 8 to Month 18)

UNIKIN will identify ~50 patients with PCR-confirmed Mpox. Patients and contacts will undergo serial study visits with specimen collection as outlined elsewhere.

Task 5.4 Laboratory Work (Month 9 to Month 24)

Samples will be stored in the field and transported to Barcelona when the collection of all samples is complete. Quantitative real-time PCR will be performed to measure MPXV viral load in different body tissues. MPXV positive samples will be typed and sequenced for phylogenetic studies. Samples will be stored to allow additional experiments.

Task 5.5 Analysis (Month 20 to Month 24)

Integrated analysis combining clinical meta-data and laboratory analysis. This will result in a detailed report for the Ministry of Health and partners which will provide critical data to define the duration of infectivity of different body tissues and help guide contact tracing and infection control practices.

Work package number	6
Work package title	TRACE Study
Time span	Month 4 to Month 24
WP leader	UNIKIN
Participants	ALL

Objectives

The main objective of this work package is to deliver a contact tracing study to understand i) the secondary attack rate of MPXV ii) the proportion of secondary infections that are symptomatic and asymptomatic and iii) determinants of onward transmission from index cases to their contacts of Clade I. MPXV in the context of the current outbreak in the Democratic Republic of Congo.

Description of work

Task 6.1 Protocol Finalization (Month 4 to Month 6)

A comprehensive protocol including all components of the study including recruitment methodology, case record forms, sample tracking documentation and standard operating procedures will be developed. This protocol will form the basis of an ethics submission in both DRC and the EU.

Task 6.2 Study Training (Month 7 to Month 8)

Staff will receive study specific training sessions covering GCP, the study protocol and procedures. This will include approaches to community entry, consent, GCP, sample collection and study documentation. Study staff attending the training sessions will receive training certificates.

Task 6.3 Participant Recruitment (Month 8 to Month 18)

Using patients identified in WP5 we will generate a line list of contacts for PCR confirmed Mpox patients. We anticipate recruiting ~10 close contacts per patient. Each patient will receive a baseline visit and sample collection followed by two weeks of surveillance and repeat sample collection. If any patients develop Mpox they will be offered recruitment into the case study (WP5) and the contact tracing process repeated.

Task 6.4 Laboratory Work (Month 9 to Month 24)

Samples will be stored in the field and transported to Barcelona when the collection of all samples is complete. Quantitative real-time PCR will be performed to measure MPXV viral load in different body tissues. MPXV positive samples will be typed and sequenced for phylogenetic studies. Samples will be stored to allow additional experiments including serological assessment for both vaccinia and MPXV antibodies.

Task 6.5 Analysis (Month 20 to Month 24)

Integrated analysis combining clinical meta-data and laboratory analysis. This will result in a detailed report for the Ministry of Health and partners which will provide critical data to define the secondary attack rate of Clade I-MPXV, the proportion of secondary infection that are symptomatic and asymptomatic and the determinants of transmission.

7. BIBLIOGRAPHY

1. https://www.cdc.gov/poxvirus/mpox/outbreak/2023-drc.html [Internet]. 2024. 2023 Outbreak in Democratic Republic of the Congo.

2. Jezek Z, Szczeniowski M, Paluku KM, Mutombo M. Human Monkeypox: Clinical Features of 282 Patients. Journal of Infectious Diseases. 1987 Aug;156(2):293–

3. Mitjà O, Ogoina D, Titanji BK, Galvan C, Muyembe JJ, Marks M, et al. Monkeypox. Vol. 401, The Lancet. Elsevier B.V.; 2023. p. 60–74.

4. Ogoina D, Iroezindu M, James HI, Oladokun R, Yinka-Ogunleye A, Wakama P, et al. Clinical Course and Outcome of Human Monkeypox in Nigeria. Clinical Infectious Diseases. 2020 Oct 15;71(8):E210–4.

5. Mandja BAM, Brembilla A, Handschumacher P, Bompangue D, Gonzalez JP, Muyembe JJ, et al. Temporal and Spatial Dynamics of Monkeypox in Democratic Republic of Congo, 2000-2015. Ecohealth. 2019 Sep;16(3):476–87.

6. Centers for Disease Control, (CDC) P. Potential Exposure to Person with Confirmed Human Monkeypox Infection — United States, 2021 [Internet]. Emergency Preparedness and Response. 2021. Available from: https://emergency.cdc.gov/han/2021/han00446.asp

7. Heymann DL, Szczeniowski M, Esteves K. Re-emergence of monkeypox in Africa: a review of the past six years. Br Med Bull. 1998;54(3):693–702.

8. World Health Organization (WHO). Technical Advisory Group on Human Monkeypox: report of a WHO meeting, Geneva, Switzerland, 11-12 January 1999. In: Technical Advisory Group on Human Monkeypox: report of a WHO meeting, Geneva, Switzerland, 11-12 January 1999.

9. Tarín-Vicente EJ, Alemany A, Agud-Dios M, Ubals M, Suñer C, Antón A, et al. Clinical presentation and virological assessment of confirmed human monkeypox virus cases in Spain: a prospective observational cohort study. The Lancet. 2022 Aug 27;400(10353):661–9.

10. Thornhill JP, Palich R, Ghosn J, Walmsley S, Moschese D, Cortes CP, et al. Human monkeypox virus infection in women and non-binary individuals during the 2022 outbreaks: a global case series. The Lancet. 2022 Dec 3;400(10367):1953–65.

11. Mitjà O, Alemany A, Marks M, Lezama Mora JI, Rodríguez-Aldama JC, Torres Silva MS, et al. Mpox in people with advanced HIV infection: a global case series. The Lancet. 2023 Mar 18;401(10380):939–49.

12. Thornhill JP, Barkati S, Walmsley S, Rockstroh J, Antinori A, Harrison LB, et al. Monkeypox Virus Infection in Humans across 16 Countries — April–June 2022. New England Journal of Medicine. 2022 Aug 25;387(8):679–91.

13. Suñer C, Ubals M, Tarín-Vicente EJ, Mendoza A, Alemany A, Hernández-Rodríguez Á, et al. Viral dynamics in patients with monkeypox infection: a prospective cohort study in Spain. Lancet Infect Dis. 2023 Apr 1;23(4):445–53.

14. Ubals M, Tarín-Vicente EJ, Oller X, Mendoza A, Alemany A, Hernández-Rodríguez Á, et al. Evaluating the Accuracy of Self-Collected Swabs for the Diagnosis of Mpox. Clinical Infectious Diseases. 2023 Apr 1;76(7):1311–4.

15. Liu L, Xu Z, Fuhlbrigge RC, Pen V, Lieberman J. Vaccinia Virus Induces Strong Immunoregulatory Cytokine Production in Healthy Human Epidermal Keratinocytes: a Novel Strategy for Immune Vaccinia Virus Induces Strong Immunoregulatory Cytokine Production in Healthy Human Epidermal Keratinocytes : a Novel. 2014 July 79(12):7363-70.

16. Tree JA, Hall G, Pearson G, Rayner E, Graham VA, Steeds K, et al. Sequence of pathogenic events in cynomolgus macaques infected with aerosolized monkeypox virus. J Virol. 2015;89(8):4335–44.

17. Zaucha GM, Jahrling PB, Geisbert TW, Swearengen JR, Hensley L. The pathology of experimental aerosolized monkeypox virus infection in cynomolgus monkeys (Macaca fascicularis). Laboratory investigation. 2001;81(12):1581–600.

18. Saijo M, Ami Y, Suzaki Y, Nagata N, Iwata N, Hasegawa H, et al. Virulence and pathophysiology of the Congo Basin and West African strains of monkeypox virus in non-human primates. Journal of general virology. 2009;90(9):2266–71.

19. Ježek Z, Grab B, Szczeniowski M, Paluku KM, Mutombo M. Clinico-epidemiological features of monkeypox patients with an animal or human source of infection. Bull World Health Organ. 1988;66(4):459.

20. Jezek Z, Marennikova SS, Mutumbo M, Nakano JH, Paluku KM, Szczeniowski M. Human monkeypox: a study of 2,510 contacts of 214 patients. J Infect Dis. 1986 Oct;154(4):551-5.

21. Peiró-Mestres A, Fuertes I, Camprubí-Ferrer D, et al. Frequent detection of monkeypox virus DNA in saliva, semen, and other clinical samples from 12 patients, Barcelona, Spain, May to June 2022. Euro Surveill. 2022 Jul;27(28):2200503.

22. Palich R, Burrel S, Monsel G, Nouchi A, Bleibtreu A, Seang S, et al. Viral loads in clinical samples of men with monkeypox virus infection: a French case series. Lancet Infect Dis. 2022;3099(22):1–7.

23. Hutin YJ, Williams RJ, Malfait P, Pebody R, Loparev VN, Ropp SL, et al. Outbreak of human monkeypox, Democratic Republic of Congo, 1996 to 1997. Emerg Infect Dis. 2001;7(3):434.

24. Pembi E, Omoleke S, Paul H, Augustine T, Cuevas LE. Monkeypox outbreak in a correctional center in North eastern Nigeria. J Infect. 2022 Dec;85(6):702-769.

25. Marshall KE. Health Care Personnel Exposures to Subsequently Laboratory-Confirmed Monkeypox Patients—Colorado, 2022. MMWR Morb Mortal Wkly Rep. 2022;71.

26. Madewell ZJ, Charniga K, Masters NB, Asher J, Fahrenwald L, Still W, et al. Serial Interval and Incubation Period Estimates of Monkeypox Virus Infection in 12 Jurisdictions, United States, May–August 2022. Emerg Infect Dis. 2023 Apr;29(4):818–21.

27. World Health Organization. 2022-24 Mpox (Monkypox) Outbreak: Global Trends. Literature summary & epidemic parameters. 2024. Available from: https://www.who.int/emergencies/situations/monkeypox-oubreak-2022.

28. Marziano V GGLIMS. Incubation period, serial interval, generation time and reproduction number of mpox clade I. medRxiv (preprint). 2024 May 10.

29. Hazra A, Zucker J, Bell E, Flores J, Gordon L, Mitjà O, et al. Mpox in people with past infection or a complete vaccination course: a global case series. Lancet Infect Dis. 2024 Jan 1;24(1):57–64.

30. CDC. Monkeypox. 2022. Isolation and Prevention Practices for People with Monkeypox. Available from: https://www.cdc.gov/poxvirus/monkeypox/clinicians/isolation-procedures.html

31. CDC. https://www.cdc.gov/poxvirus/mpox/clinicians/monitoring.html. 2022. Mpox Monitoring and Risk Assessment for Persons Exposed in the Community.