

**Integrating qPCR Diagnostics and Genomic Surveillance for Real-Time Characterization
of MPXV Clade Dynamics and Public Health Response Gaps in Sierra Leone's 2025
Outbreak**

By

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Abstract

Background: In 2025, Sierra Leone experienced its largest mpox outbreak, driven by Clade IIb. Limited in-country genomic surveillance hampered real-time characterisation of transmission dynamics and clade evolution.

Methods: We conducted a cross-sectional laboratory and molecular epidemiological study integrating quantitative PCR (qPCR) and targeted nanopore sequencing. A total of 600 suspected cases were screened using orthopoxvirus and mpox-specific qPCR (B6R gene). Lesion swabs (n=200) provided Ct values; samples with Ct<20 (n=125) were prioritised for tiled amplicon sequencing. High-quality genomes (coverage 70–89%, n=41) underwent phylogenetic analysis with global references. Environmental surface swabs (n=89) from two major hospitals were tested for MPXV DNA.

Results: Orthopoxvirus positivity was 90.2% (541/600), with 74.5% (403/541) confirmed as mpox Clade IIb. Among 206 valid lesion swab Ct values, the mean Ct was 21.62 (median 19.34); 60.7% had high viral load (Ct<20). All 41 sequenced genomes belonged to Clade IIb lineage G.1, interspersed with global references, indicating multiple introductions and ongoing gene flow rather than a locally isolated outbreak. Sequencing success (68.3% passed coverage filter) was unbiased by Ct value ($r=-0.12$, $p=0.46$). Environmental contamination was detected on 13.5% of surfaces, with doors being the most frequent contamination sites (42% of positives). The outbreak predominantly affected young adults (median age 27 years) with a balanced sex distribution (54% female, 46% male), in contrast to early global outbreaks. Cases were concentrated in Western Area districts (96%). Hospitalisation rate was 36.2% (196/403).

Conclusions: Integrated qPCR and genomic surveillance are feasible in resource-limited settings and provide actionable intelligence on clade dominance, transmission networks, and environmental risks. The dominance of Clade IIb G.1 and the absence of other sub-lineages suggest a single introduction followed by local spread. Targeted surface disinfection (especially doors) and sustained investment in domestic/on-site sequencing capacity are critical for future outbreak preparedness.

Keywords: Mpox virus, Clade IIb, genomic surveillance, qPCR, Sierra Leone, phylogenetic analysis, environmental sampling, outbreak response, public health.

Dedication

To the glory and honor of God, this work is dedicated to my lovely parents, sister, and children,
Pearl & Luke.

To every mpox survivor, and to the families of those who have lost a family member, friend, or
loved one to this 2025 outbreak in Sierra Leone.

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List of Abbreviations

OPXV-Orthopox virus

MPXV- Mpox Virus

NPH- Non-Human Primates

CFR- Case Fatality Rate

IPC- Infection Prevention and Control

PHEIC- Public Health Emergency of International Concern

MSM- Men who have sex with Men

WGS- Whole Genome Sequencing

NGS- Next Generation Sequencing

VACV- Vaccinia Virus

TIRs- Terminal Inverted Repeats

KB- Kilobase

SPI-1- Serine Protease Inhibitor

IFN- Interferon

CEV- Cell-associated Enveloped Virion

IMV- Intracellular Mature Virion

EEV- Extracellular Enveloped Virion

SNPs- Single-Nucleotide Polymorphisms

RO- Reproduction number

TNFR- Tumor Necrosis Factor Receptor

NVO- Non-Variola Orthopoxvirus

GIS- Geographic Information Systems

Ct- Cycle Threshold

PK- Proteinase K (Keratin)

PHUs- Peripheral Health Units

rpm- Revolutions per minute

q-PCR- Real-time Polymerase Chain Reaction

PCR- Polymerase Chain Reaction

ng/μL- Nanograms per microliter

μL- Microliter

NC- Negative Control

NTC- No-Template Control

DNA- Deoxyribonucleic Acid

RA- Rapid Adapter

SB- Sequencing Buffer

LIB- Library Beads

FCT- Flow Cell Tether

FCF- Flow Cell Flush

BSA- Bovine Serum Albumin

EB- Elution Buffer

WV- Wrapped Virion

WHO- World Health Organization

PGI- Pathogen Genomic Initiative

BSL- Biosafety Containment Level

CDC- Centers for Disease Control

CIHR- ID- Canadian Institutes of Health Research and International Development

IMReC- International Mpox Research Consortium

SLERC- Sierra Leone Ethics and Scientific Review Committee

CREID-ECA- Center for Research in Emerging Infectious Disease-East and Central Africa

IQR- Interquartile Range

WAU- Western Area Urban

WAR- Western Area Rural

SD- Standard Deviation

BMJ- British Medical Journal

GISAID- Global Initiative on Sharing All Influenza Data

BEAST- Bayesian Evolutionary Analysis Sampling Trees

CI- Confidence Interval

FASTA- Fast-All

Author Contributions

Demographic data from 600 samples were collected by our collaborators in Sierra Leone. The team and I at Najla performed the extraction and q-PCR analysis of the 206 lesion swap samples. Sequencing was led by the team from the DRC, and phylogenetic trees were constructed by them. Chapter 5 of this work, wherein I am the first author, has been accepted by the Emerging Infectious Diseases Journal. All other analyses and writing were done by me.

Chapter 1: Introduction and Background

1.1 Background to the Study

1.1.1 Overview of the Mpox Virus (MPXV)

The mpox virus (MPXV) is an enveloped, double-stranded DNA virus belonging to the *genus Orthopoxvirus* in the family *Poxviridae* [1]. It is the causative agent of mpox, a zoonotic disease presenting with a clinical syndrome often similar to smallpox, including fever, lymphadenopathy, and a progressive centrifugal rash [2]. The viral genome is approximately 197 kilobase pairs and encodes approximately 190 proteins involved in viral replication, host immune evasion, and pathogenesis [3, 4]. MPXV is endemic to forested regions of Central and West Africa, with rodents serving as the primary natural reservoirs. While non-human primates (NHPs) can be infected, they are considered incidental or spillover hosts rather than true reservoirs, as they tend to develop severe disease similar to that in humans and do not sustain long-term viral circulation in the absence of reintroduction [5, 6]. Extensive ecological and serological studies have identified several rodent species, including rope squirrels (*Funisciurus* spp.), Gambian pouched rats (*Cricetomys gambianus*), and African dormice (*Graphiurus* spp.), as having the highest prevalence of MPXV infection and antibody detection, supporting their role as maintenance hosts [7, 8]. Transmission to humans occurs through direct contact with infected animals, their bodily fluids, or contaminated materials, and can subsequently spread via human-to-human contact through respiratory droplets, direct contact (including intimate contact), or fomites [9]. The incubation period ranges from 5 to 21 days, with the symptomatic period ranging from 2 to 4 weeks [10]. While the disease is often self-limiting, severe cases can occur, particularly in immunocompromised individuals, children, and pregnant women [11].

1.1.2 Historical Context of Mpox Outbreaks in Africa

The MPXV was first identified in 1958 in laboratory monkeys in Denmark, but the first human case was documented in 1970 in the Democratic Republic of the Congo (DRC) [12]. Shortly after this, subsequent cases were reported in West Africa [13]. Since then, most reported cases have been confined to Central and West African countries, with the DRC reporting thousands of suspected cases annually [14]. Two distinct epidemiological patterns were recognized: the Congo Basin (Central African) clade, historically associated with more severe disease and higher

human-to-human transmission, and the West African clade, associated with milder disease and limited secondary transmission [15]. Notable outbreaks in Africa include a 1996-97 outbreak in the DRC with a high secondary attack rate, a 2003 importation to the United States linked to infected rodents from Ghana, and recurrent outbreaks in Nigeria since a major resurgence began in 2017 [16, 17, 18]. These events underscored the potential for local amplification and international spread of MPXV from its endemic reservoirs.

1.1.3 Evolution of Clades and Global Distribution

Phylogenetic analyses delineate mpox into two primary clades: Clade I (formerly Congo Basin clade) and Clade II (formerly West African clade) [19]. Clade I, endemic to Central Africa, is associated with case fatality rates (CFR) historically reported up to 10%, with the highest morbidity and mortality in children <15 years, and demonstrates a higher propensity for human-to-human transmission [20, 21]. Clade II is further subdivided into subclades IIa and IIb. Clade IIa represents the classic West African lineage, associated with a CFR of less than 1% [11, 22]. Clade IIb (with CFR <0.1% globally) is a descendant lineage within the West African clade that diverged around 2017 and was responsible for the multi-country global outbreak beginning in 2022 [23]. This lineage exhibited specific genomic signatures, including a characteristic APOBEC3-type mutational pattern, and demonstrated enhanced transmissibility, particularly through intimate contact, leading to rapid international dissemination [24, 25].

1.1.4 Mpox Epidemiology in Sierra Leone

Sierra Leone has historically been considered at risk for mpox due to its ecological setting and proximity to endemic regions, but confirmed human cases were rarely reported before the 21st century. The country's vulnerability was highlighted during the 2017-2023 Nigerian outbreak, which increased cross-border surveillance awareness [26]. The first confirmed case in Sierra Leone was reported in the post-2022 global outbreak context, aligning with the spread of Clade IIb [27]. The country's healthcare system, still recovering from the 2014-2016 Ebola Virus Disease epidemic, faces challenges in disease surveillance, diagnostic capacity, and infection prevention and control (IPC), which can impact outbreak detection and response for diseases like mpox [28].

In 2025, Sierra Leone experienced a significant mpox outbreak. Between May and August 2025, over 5,000 cumulative confirmed cases were reported nationwide, marking the country's largest

recorded outbreak [29]. The Western Area, including the capital Freetown, the epicentre of the outbreak, had substantial case numbers reported in the Southern Province, notably the Bo District. The outbreak disproportionately affected young adults, with a male-to-female ratio of approximately 3:1, consistent with intimate contact networks characteristic of Clade IIb circulation [29, 30]. This outbreak underscored the endemic establishment of Clade IIb in West Africa and highlighted critical gaps in real-time surveillance and diagnostic-pathogen characterization pipelines within Sierra Leone's public health response.

1.1.5 Post-2022 Global Outbreak and Clade IIb Emergence

In May 2022, clusters of mpox cases were reported in non-endemic countries without established travel links to endemic regions, signalling sustained human-to-human transmission [31]. The World Health Organization (WHO) declared the outbreak a Public Health Emergency of International Concern (PHEIC) in July 2022, the first for mpox [32]. Genomic surveillance confirmed that the outbreak was driven almost exclusively by Clade IIb viruses, revealing sustained transmission within dense intimate networks, primarily among men who have sex with men (MSM) [33]. This outbreak showcased altered epidemiological dynamics, including atypical presentations (e.g., localized genital lesions) and routes of transmission, most notably sustained human-to-human transmission [34]. While the PHEIC was declared over in May 2023, Clade IIb continues to circulate globally and within Africa, presenting an ongoing public health threat and necessitating strengthened surveillance to monitor its evolution and spread [35].

1.1.6 Role of Genomic Surveillance in Emerging Infections

Genomic surveillance has become a cornerstone of modern outbreak response. For viruses like MPXV, whole-genome sequencing (WGS) enables precise identification of viral lineages, tracking of transmission chains, detection of emerging variants, and insights into viral evolution and adaptation [36]. During the 2022 outbreak, real-time genomic data shared globally via platforms like GISAID were instrumental in confirming the outbreak source, monitoring its geographic spread, and identifying mutations potentially linked to transmissibility [37]. In resource-limited settings, the integration of portable sequencing technologies, such as Oxford Nanopore MinION, offers the potential for near real-time genomic surveillance at or near the point of outbreak, facilitating rapid local decision-making [38].

1.1.7 Integration of qPCR Diagnostics and Genomics in Outbreak Response: Global and Sierra Leonean Context

An effective outbreak response hinges on the synergy between rapid diagnostics and detailed pathogen characterization. Real-time quantitative polymerase chain reaction (qPCR) provides the frontline diagnostic capability for sensitive and specific detection of MPXV DNA, delivering results within hours [39]. This rapid identification is critical for timely patient isolation, contact tracing, and initiation of IPC measures. Subsequently, genomic sequencing of qPCR-positive samples adds a critical layer of intelligence, transforming a simple positive result into a rich source of epidemiological data [40].

Globally, this integrated model was successfully deployed during the COVID-19 pandemic and the 2022 mpox outbreak. However, in Sierra Leone, the capacity for such integrated molecular surveillance remains underdeveloped. While diagnostic qPCR capacity has been established at reference laboratories, in-country next-generation sequencing (NGS) capabilities for pathogens like MPXV are nascent [41].

This gap limits the ability to perform real-time, local phylogenetic analysis to understand transmission clusters, distinguish between imported and locally circulating strains, and monitor for the emergence of novel variants. Building this integrated capacity is therefore essential for transforming Sierra Leone's outbreak response from reactive case management to proactive, intelligence-driven public health action.

1.1.8 Literature Search Strategy

To ensure a comprehensive and systematic review of the existing evidence, a structured literature search strategy was designed and executed. This strategy aimed to identify all relevant peer-reviewed literature concerning MPXV virology, diagnostics, genomics, epidemiology, and outbreak response, with a particular focus on the context of Sierra Leone and West Africa. The process followed the principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Electronic searches were conducted across four major bibliographic databases: PubMed, Scopus, Web of Science, and Google Scholar. The search was performed using a combination of Medical Subject Headings (MeSH) terms and keywords related to the core themes of this thesis. The search strings incorporated terms such as “Monkeypox virus”, “MPXV”, “mpox”, “Orthopoxvirus”, “real-time PCR”, “qPCR”, “genomic surveillance”, “whole

genome sequencing”, “Nanopore”, “phylogenetics”, “molecular epidemiology”, “Sierra Leone”, “West Africa”, “outbreak”, “transmission”, and “environmental sampling”. No date restrictions were applied to capture the historical and contemporary scope of the literature.

Inclusion and Exclusion Criteria:

- **Inclusion:** Primary research articles (observational, experimental), reviews, and seminal reports focused on MPXV biology, diagnostics, genomic surveillance, epidemiology, or environmental persistence. Publications in English were included.
- **Exclusion:** Articles not related to MPXV; non-English publications without available translation; editorials, commentaries, or opinion pieces without original data; conference abstracts where full text was unavailable; and studies with insufficient methodological detail for critical appraisal.

The initial database search yielded 487 records. After the removal of 175 duplicates using reference management software, 312 unique records remained for screening. The screening process involved two sequential phases:

1. **Title and Abstract Screening:** All 312 records were screened against the pre-defined inclusion and exclusion criteria.
2. **Full-Text Assessment:** The full text of 124 articles that passed the initial screen was retrieved and rigorously assessed for eligibility.

Following full-text assessment, 37 articles were excluded, resulting in a final corpus of **87 studies** for qualitative synthesis. The included studies comprised quantitative (n=50), qualitative (n=23), and mixed-methods (n=14) designs.

1.2 Literature Review

1.2.1 MPXV: Biology and Molecular Virology

MPXV is a member of the *Orthopoxvirus* genus, characterized by a large, brick-shaped virion containing a linear, double-stranded DNA genome [42]. As a zoonotic pathogen, its biology bridges animal reservoirs and human hosts, requiring a complex interplay of viral factors to facilitate infection, immune evasion, and transmission. The molecular virology of MPXV, while

sharing a high degree of homology with other orthopoxviruses like vaccinia virus (VACV), contains unique genetic elements that influence its host range, pathogenicity, and epidemiological behaviour [43]. Understanding its fundamental biology is paramount to deciphering outbreak dynamics and developing effective countermeasures.

1.2.2 Taxonomy and Genomic Structure

Taxonomically, MPXV belongs to the family *Poxviridae*, subfamily *Chordopoxvirinae*, genus *Orthopoxvirus* [44]. Its genome is approximately 197 kb, with terminal inverted repeats (TIRs) flanking a conserved central coding region. This central region contains essential genes for viral replication and morphogenesis, common to all orthopoxviruses. In contrast, the terminal regions harbor genes involved in host-range determination, immunomodulation, and virulence, which are more variable and contribute to differences between MPXV clades and other orthopoxviruses [45]. Comparative genomics has been instrumental in delineating the Clade I and Clade II divergence, identifying key deletions and mutations associated with altered virulence and transmissibility [46].

1.2.3 Viral Proteins and Pathogenic Mechanisms

MPXV encodes approximately 190 proteins, which can be categorized into early, intermediate, and late expression classes [47]. Key virulence factors include proteins that modulate host immune responses, such as the complement control protein, serine protease inhibitor (SPI-1), and interferon (IFN)-gamma binding protein [15]. For instance, the absence of a functional homologue of the VACV interleukin-1 β receptor in some MPXV strains may influence the inflammatory response and disease severity [48]. Pathogenesis involves viral entry via the oropharynx or intradermal inoculation, followed by replication at the site of entry and subsequent spread to regional lymph nodes and a primary viremia, leading to systemic infection and the characteristic rash [49].

1.2.4 Replication Cycle and Host Interaction

The replication cycle of MPXV is cytoplasmic and occurs in a series of tightly regulated stages: attachment, entry, uncoating, early gene expression, DNA replication, intermediate/late gene expression, virion assembly, and egress [50]. The virus produces two distinct infectious forms: the intracellular mature virion (IMV) and the extracellular enveloped virion (EEV). The EEV,

which acquires an additional membrane from the Golgi apparatus, is critical for cell-to-cell spread and long-range dissemination within the host [51]. MPXV interacts extensively with the host innate immune system, employing a suite of proteins to inhibit apoptosis, block IFN signalling pathways, and evade detection by pattern recognition receptors, thereby facilitating viral replication and spread [52].

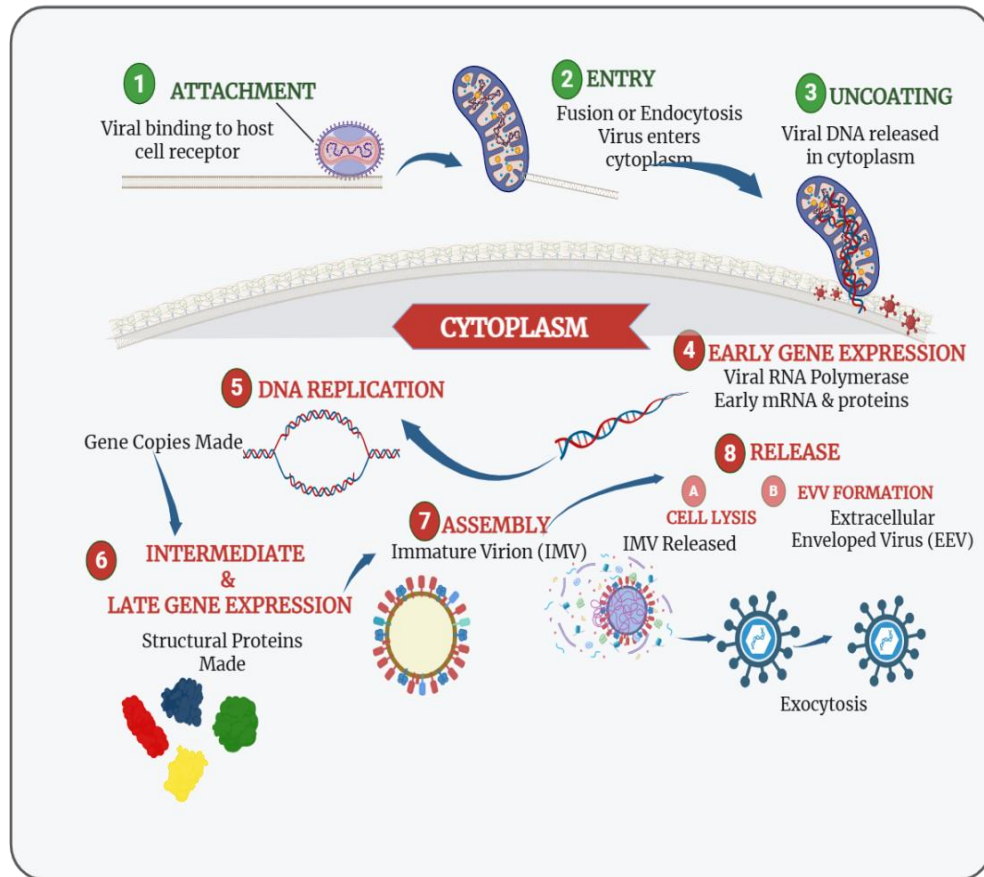
1.2.5 Viral Replication

The replication cycle of MPXV, like other orthopoxviruses, is a complex, tightly regulated process that occurs entirely within the cytoplasm of the host cell, a feature unique among DNA viruses [50]. This independence from the host nucleus is facilitated by the virus encoding its own machinery for transcription and DNA replication. The cycle can be broadly divided into three phases: early, intermediate, and late gene expression, which coordinate viral DNA synthesis, virion assembly, and egress [47].

The process begins with the attachment of the virus to the host cell surface through glycosaminoglycans or laminin-binding proteins, initiating entry via macropinocytosis or direct membrane fusion [53]. Following entry and uncoating, the viral core releases early mRNAs into the cytoplasm. Early gene products include proteins that further uncoat the core, inhibit host defense, and initiate DNA replication. Viral DNA replication, occurring in cytoplasmic factories, produces concatemeric genomes that are subsequently cleaved and packaged [50]. This signals the transition to intermediate and then late gene expression, which produces structural proteins and enzymes for virion assembly.

Assembly of the IMV occurs in the factories, where viral membranes encapsulate DNA to form spherical immature virions, which mature into brick-shaped IMVs. A subset of IMVs acquires an additional double membrane from the trans-Golgi network or early endosomes, forming the wrapped virion (WV). The WV is transported to the cell surface on microtubules, where its outer membrane fuses with the plasma membrane, releasing the EEV or remaining cell-associated as the cell-associated enveloped virion (CEV) [51]. The CEV is important for direct cell-to-cell spread, while the EEV mediates long-range dissemination within the host. This intricate, multi-stage cycle provides multiple points for therapeutic intervention and immune modulation and is central to MPXV's pathogenesis [54]. A summary of mpox viral replication is shown in **Figure**

1.1.



Created in <https://BioRender.com>

Figure 1.1: Viral Replication of MPXV

1.2.6 Evolutionary History of MPXV Clades

The evolutionary history of MPXV suggests a divergence from a common ancestor with other rodent-associated orthopoxviruses. Molecular clock analyses estimate that Clade I and Clade II diverged several centuries ago (approximately 560-860 years ago), likely driven by ecological separation in Central and West African rainforest basins [55]. Clade II further diverged, with Clade IIb emerging as a distinct sublineage around 2017. The accelerated evolution observed in Clade IIb, characterized by a high number of APOBEC3-mediated mutations, indicates sustained human-to-human transmission over several years before the 2022 global outbreak, representing a significant adaptive event in the virus's evolution [24, 23].

1.2.7 Clade I (Central African) Characteristics

Clade I (formerly the Congo Basin clade) mpox is associated with more severe disease, higher case-fatality rates (historically up to 10%), and greater human-to-human transmissibility [20]. Genomically, Clade I viruses lack a characteristic 677 bp deletion in the right terminal region near the OPG015 gene, present in Clade II viruses [15]. This region may influence virulence. Clinically, Clade I infection often presents with more pronounced prodromal symptoms, a higher rash burden, and a greater frequency of hemorrhagic manifestations [56]. Its endemic zone spans the Congo Basin, with the DRC being the most persistent epicenter.

1.2.8 Clade IIa and IIb (West African) Genomic Signatures

Clade IIa represents the classic West African lineage, associated with milder disease (<1% CFR) [11, 22]. The defining genomic feature of Clade II is the aforementioned 677 bp deletion [15]. Clade IIb, the driver of the 2022+ pandemic, is distinguished within Clade II by a specific set of single-nucleotide polymorphisms (SNPs) and short indels. A hallmark is the APOBEC3-type mutational signature, predominantly GA-to-AA and TC-to-TT changes, indicative of sustained circulation in human populations where this host defense enzyme edits viral DNA [24]. Key mutations, such as those affecting the viral OPG001 and OPG204 genes, are under investigation for potential impacts on protein function and viral fitness [57].

1.2.9 Emergence of Clade Ib and Cross-Border Threats

Recent reports from the DRC have described a novel, phylogenetically distinct lineage within Clade I, tentatively termed “Clade Ib” [58]. This lineage appears to be associated with intimate

contact and higher case numbers, including in travelers from DRC to neighboring countries. The emergence of Clade Ib underscores the continuous evolution of mpox within its endemic reservoirs and highlights the threat of new variants with potentially altered transmission dynamics bridging from classic spillover to sustained human networks. This poses a significant cross-border threat to regional and global health security, necessitating enhanced surveillance at ports of entry and in surrounding nations [59].

1.2.10 Transmission Dynamics and Epidemiological Patterns

Historically, MPXV transmission followed a spillover-limited secondary transmission pattern. The 2022 Clade IIb outbreak revolutionized the understanding of its epidemiology, demonstrating the potential for efficient, sustained human-to-human transmission, primarily through intimate contact [33]. This shift resulted in explosive growth within dense sexual networks, particularly among men who have sex with men (MSM), with over 90,000 cases reported globally within a year [60].

Epidemiological patterns now include community clusters, nosocomial transmission, and the critical role of asymptomatic or pauci-symptomatic infection in transmission chains, which complicates control efforts [61]. The 2025 environmental study in Sierra Leone, published as a preprint, further underscores the role of fomites in transmission within healthcare settings, highlighting doors and patient beds as key contamination points [62].

1.2.11 Zoonotic Reservoirs and Spillover Events

The true animal reservoir of MPXV remains incompletely defined, though evidence strongly implicates rodents (e.g., rope squirrels, African dormice, Gambian pouched rats) as the primary maintenance hosts in endemic regions [7, 8]. Spillover events to humans occur through direct contact with infected animals during hunting, bushmeat preparation, or via contaminated materials. Ecological factors such as deforestation, climate change, and human encroachment into forested areas are hypothesized to increase the frequency of human-animal contact and subsequent spillover events [11]. The 2003 US outbreak, linked to imported Gambian pouched rats co-housed with prairie dogs, is a classic example of a complex, multi-species spillover chain [17].

1.2.12 Human-to-Human Transmission and Community Clusters

Human-to-human transmission occurs via direct contact with infectious skin lesions, body fluids, or respiratory droplets during prolonged face-to-face contact. The 2022 outbreak highlighted the efficiency of transmission through intimate contact, including sexual activity, where the virus presents in genital lesions, facilitating spread [33]. This led to distinct community clusters, often identified through sexual health clinics. Studies have estimated the reproduction number (R_0) for Clade IIb to be >1 in sexual networks, allowing for sustained community transmission independent of fresh zoonotic introductions, a pivotal change from pre-2022 epidemiology [63].

1.2.13 Global Transmission Trends and Outbreak Mapping

Global transmission of Clade IIb was characterized by rapid, multi-focal emergence across non-endemic countries in May 2022, followed by a peak in mid-2022 and a subsequent decline due to behavioral changes and vaccination in high-risk groups [60]. Outbreak mapping utilizing geographic information systems (GIS) and genomic data revealed complex international transmission networks, with multiple exportations from endemic regions seeding independent transmission chains globally [57]. The pattern shifted in 2023-2024 towards more endemic-like transmission in Africa, with continued outbreaks in the DRC and the establishment of Clade IIb in new African countries, emphasizing the need for equitable surveillance and response [59].

According to the recent WHO publication about the global mpox trend (**Figure 1.2**), there is still an increase in transmission of all clades, with intimate contact transmission still on the increase among both genders in mainly historic and endemic regions [64].

Mpox

Multi-country external situation report no. 63, published 24 February 2026

KEY FIGURES			
Area	Number of reported confirmed cases	Number of deaths among confirmed cases	Number of reporting countries
Global (1 Jan 2025 – 31 Jan 2026)*	54 817	221	98
Key countries in Africa (5 Jan – 15 Feb 2026)**			
Democratic Republic of the Congo	367	0	-
Madagascar	313	0	-
Guinea	104	0	-
Liberia	96	0	-
Ghana	50	0	-

* Most recent global surveillance data available.

** Countries reporting the highest number of confirmed mpox cases in the last six weeks.

Figure 1.2: Mpox Case distributions across regions of Africa - WHO dashboard [64].

1.2.14 Diagnostic Strategies for MPXV

Accurate and timely diagnosis is critical for outbreak control. Diagnostic strategies have evolved from clinical suspicion confirmed by traditional methods to sophisticated molecular assays.

1.2.15 Traditional Methods (Serology, Conventional PCR)

Traditional methods include virus isolation in cell culture (gold standard, but slow and requiring BSL-3 facilities), electron microscopy (non-specific), and serology (e.g., ELISA for anti-*orthopoxvirus* IgM/IgG). Serology is limited due to cross-reactivity from prior smallpox vaccination or other *orthopoxvirus* exposures and cannot distinguish between MPXV clades [65]. Conventional (endpoint) PCR, followed by gel electrophoresis, is more specific than serology but less sensitive, specific, and quantitative than real-time PCR methods, and carries a higher risk of contamination [66].

1.2.16 Real-Time qPCR and Multiplex Assays

Real-time quantitative PCR (qPCR) is the current diagnostic cornerstone. It offers high sensitivity and specificity, rapid results (within hours), and quantification via Cycle Threshold (Ct) values, which conversely correlate with viral load [67]. Multiplex assays have been developed that simultaneously detect MPXV (pan-*orthopoxvirus* and MPXV-specific targets) and other pathogens in the differential diagnosis (e.g., varicella-zoster virus), optimizing testing efficiency during outbreaks [68].

1.2.17 Molecular Targets and Diagnostic Gene Markers (B6R, F3L, etc.)

Common qPCR targets include:

- **Pan-orthopoxvirus targets:** The tumor necrosis factor receptor (TNFR) homologue gene (C3L) or the DNA polymerase (E9L) gene [69].
- **MPXV-specific targets:** The B6R gene (encoding a viral envelope protein) and the F3L gene (encoding a double-stranded RNA-binding protein) [39]. The CDC's non-variola orthopoxvirus (NVO) assay and its MPXV-specific assay often target these regions. The B6R target is widely used due to its high sensitivity and specificity for MPXV detection [70], as demonstrated in the recent Sierra Leone environmental preprint [62].

1.2.18 Diagnostic Challenges and False Negatives

Challenges include: 1) Sample type and timing: Lesion swabs have the highest yield; blood or saliva may be negative early or late in infection [61]. 2) Resource limitations: In endemic regions, access to qPCR reagents, equipment, and trained personnel is constrained [71]. 3) Newly emerging strains: New variants may harbor mutations in primer/probe binding sites, potentially leading to false negatives (assay degradation), necessitating ongoing genomic surveillance to inform assay updates [72]. 4) Inhibitors: Substances in clinical samples can inhibit PCR, requiring robust nucleic acid extraction and internal controls [73].

1.2.19 Genomic Surveillance in Viral Outbreaks

Genomic surveillance involves sequencing pathogen genomes from clinical samples to track spread, evolution, and origins. It transformed public health response during the HIV, Ebola, Zika, SARS-CoV-2, and mpox outbreaks [36]. It moves beyond counting cases to understanding the *how* and *why* of transmission dynamics.

1.2.20 Importance and Applications in Public Health

Applications are multifaceted: 1) Outbreak Investigation: Linking cases, identifying transmission clusters, and distinguishing multiple independent introductions [36]. 2) Tracking Viral Evolution: Monitoring for mutations that may affect diagnostics, therapeutics, or vaccines [74]. 3) Informing Public Health Measures: Data on transmission chains can guide targeted contact tracing, quarantine, and vaccination strategies [75]. 4) Global Health Security: Facilitating early warning and coordinated international response [76].

1.2.21 Next-Generation Sequencing (NGS) and Targeted Amplicon Sequencing

NGS platforms (Illumina, Nanopore) enable high-throughput WGS. For MPXV, targeted amplicon sequencing (e.g., using the Midnight primer set) is highly effective. It involves tiling PCR amplicons across the genome, which is particularly useful for low viral load samples (high Ct), as it enriches viral DNA before sequencing on platforms like Oxford Nanopore MinION, enabling near-real-time, field-deployable genomic surveillance [77, 40].

1.2.22 Phylogenetics and Molecular Epidemiology

Phylogenetic analysis places newly sequenced viruses within an evolutionary tree. Molecular epidemiology uses these trees to answer key questions: Are cases linked? Is the outbreak driven by a single introduction or many? How is the virus moving geographically? Tools like Nextstrain provide real-time, open-access phylogenetic platforms that were instrumental during the 2022 mpox outbreak for global situational awareness [78].

1.2.23 Global Case Studies of MPXV Genomic Surveillance

- a. **United Kingdom/USA/Europe (2022):** Rapid deployment of sequencing identified the Clade IIb lineage, confirmed its association with sexual networks, and mapped international transmission links in near real-time, guiding the public health response [23].
- b. **Democratic Republic of the Congo (Ongoing):** Longitudinal sequencing has tracked the evolution of Clade I for decades and recently identified the novel Clade Ib, alerting global health authorities to a potential new threat [58].
- c. **Nigeria (2017-2023):** Genomic surveillance following its resurgence revealed sustained human-to-human transmission of Clade IIb years before the global outbreak, providing critical pre-pandemic insights [79].

1.2.24 Integration of qPCR and Genomic Sequencing in Outbreak Response

Integration creates a powerful pipeline: qPCR provides rapid, high-throughput case identification and confirmation. Positive samples with sufficient viral load (low Ct) are immediately routed for sequencing. The sequencing data then contextualizes the qPCR results, revealing whether cases are part of a local cluster or separate introductions. This closed-loop system accelerates from case detection to actionable epidemiological intelligence [80].

1.2.25 Complementarity Between qPCR and Genomic Analysis

The two methodologies are complementary. qPCR answers “Is it MPXV?” quickly and is scalable for mass testing. Genomic sequencing answers “Which MPXV, and how is it related to others?” It provides depth but is slower and more resource intensive. Together, they form a tiered surveillance system where qPCR handles breadth and sequencing add resolution for a subset of cases to guide the overall response [81].

1.2.26 Real-Time Data for Decision-Making

The value of integration is maximized when data flow is rapid. Real-time or near-real-time sequencing (<1 week turnaround) allows public health officials to: 1) detect new clusters as they form; 2) evaluate the effectiveness of interventions; and 3) issue timely alerts about variant spread. The use of portable sequencers and cloud-based analysis can significantly reduce this turnaround time in field settings [38].

1.2.27 Case Studies from DRC, Nigeria, and Ghana

- **DRC:** Demonstrated the need for in-country sequencing capacity. For years, samples were sent abroad for sequencing, causing delays. Recent investments in local NGS have improved the timeliness of identifying emerging threats like Clade Ib [58].
- **Nigeria:** Showcased how proactive genomic surveillance within an endemic country can detect a changing virus (Clade IIb) and provide early warning. Its experience informed global preparedness [79].
- **Ghana:** Highlighted regional collaboration. Ghanaian sequences from the 2022 period were crucial in understanding the West African context of Clade IIb and its potential origins before global spread [82].

1.2.28 Research Gaps and Theoretical Framework

Despite advances, critical gaps remain: 1) The longitudinal dynamics of MPXV in animal reservoirs. 2) The precise molecular determinants of Clade IIb's increased transmissibility. 3) The role of asymptomatic infection in transmission. 4) The durability of vaccine-induced immunity. 5) The optimal model for sustainable, cost-effective integrated molecular surveillance in low-resource settings. A robust theoretical framework for outbreak response must integrate concepts from One Health (connecting animal, human, and environmental health), Molecular Epidemiology (using genetic data to trace outbreaks), and Implementation Science (to ensure technologies are effectively deployed in real-world settings) [83, 84].

1.2.29 Identified Knowledge Gaps

Specific knowledge gaps pertinent to Sierra Leone and similar contexts include:

1. The genomic diversity and transmission dynamics of MPXV (particularly Clade IIb) within Sierra Leone and its cross-border linkages.
2. The performance and limitations of standard qPCR assays on locally circulating variants.
3. The extent of environmental contamination (fomite transmission risk) in Sierra Leonean healthcare and community settings, as partially addressed by a recent surface sampling preprint, which found 13.5% positivity on high-touch surfaces [62].
4. The operational feasibility, cost, and impact of integrating real-time nanopore sequencing into the national outbreak response framework.
5. The prevalence of asymptomatic/mild infection and its contribution to community transmission in West Africa.

1.2.30 Conceptual Framework for Integrated Molecular Surveillance

This thesis is guided by a conceptual framework that posits that integrated, real-time molecular surveillance, the synergistic application of rapid qPCR diagnostics and targeted genomic sequencing, is essential for effective outbreak characterization and response. The framework outlines a sequential, iterative process:

1. **Case Detection & Triage:** Suspected cases are screened with qPCR, providing rapid confirmation and viral load (Ct) data. Environmental surveillance using the same qPCR technology, as demonstrated in a Sierra Leone preprint [62], assesses contamination risk.
2. **Genomic Intelligence:** A subset of qPCR-positive samples (prioritized by Ct, geography, epidemiology) undergoes targeted amplicon sequencing.
3. **Data Integration & Analysis:** Genomic data is analyzed phylogenetically alongside epidemiological metadata (time, place, person), qPCR Ct values, and environmental sampling results.
4. **Insight Generation:** This integrated analysis yields insights into clade dynamics, transmission clusters, outbreak origins, diagnostic assay performance, and IPC vulnerabilities (e.g., based on environmental contamination hotspots).

5. **Public Health Action:** Insights directly inform targeted interventions (e.g., focused contact tracing, IPC strengthening like enhanced disinfection of doors [62], vaccine deployment), guide assay validation, and reveal gaps in the response system.

This feedback loop transforms raw diagnostic data into actionable public health intelligence, aiming to close the critical “response gap” between case detection and informed intervention, particularly in resource-limited settings like Sierra Leone.

1.3 Thesis Structure

1.3.1 Overview of Thesis Chapters

This thesis is structured to empirically demonstrate and evaluate the process of integrating qPCR diagnostics with genomic surveillance to transform outbreak response. The narrative follows the logical flow of this integrated pipeline itself. **Chapter 1 (Introduction and Background)** establishes the critical imperative for real-time molecular characterization of MPXV outbreaks, reviewing the distinct roles and synergies of qPCR and sequencing, and identifying the specific public health response gaps in settings like Sierra Leone.

Chapter 2 (Materials and Methods) is the operational blueprint, detailing the study design that enacts this integration. It specifies the sequential yet interlinked protocols for sample collection, qPCR screening, Ct-value-based triage for sequencing, targeted nanopore sequencing, and bioinformatic analysis, alongside methods for collecting epidemiological and environmental data.

Chapter 3 (Results) presents the output of this integrated pipeline. It is organized to reflect the flow of data, beginning with the epidemiological results, qPCR diagnostic yields and viral load metrics, proceeding to genomic sequencing success and phylogenetic findings.

Chapter 4 (Discussion) interprets these combined results to achieve real-time characterization. It contextualizes the clade dynamics revealed by sequencing, explores how viral load (qPCR) and transmission (genomic/epidemiological) data intersect, and evaluates the operational successes and obstacles of the integrated approach in identifying response gaps.

Chapter 5 presents a focused study on surface sampling, which serves as a critical application of the qPCR arm of the pipeline to answer a specific public health question on fomite risk, directly informing IPC, a key component of outbreak response.

Finally, **Chapter 6 (General Conclusions and Future Directions)** synthesizes evidence from the entire workflow to assess how integration altered the characterization of and response to the 2025 outbreak, providing concrete recommendations for institutionalizing this model in Sierra Leone's public health architecture.

1.3.2 Integration of qPCR, Sequencing, Epidemiological, and Surface Sampling Data

The methodological core of this thesis is a purpose-built, iterative framework for data integration designed for real-time utility. The process begins with **qPCR Diagnostics** serving as the high-throughput filter and triage mechanism. Each positive result provides two critical data points: (1) a confirmed case, and (2) Ct value, a quantitative proxy for viral load. Samples with low Ct values (high viral load) are prioritized for the **Genomic Sequencing** module. Here, targeted tiled amplicon sequencing generates phylogenetic data, translating a “case” into a “sequence” with phylogenetic context, revealing clade identity, lineage, and relationship to other local, regional, and global viruses. Concurrently, **Epidemiological Data** (demographic, geographic, and temporal) are linked to each sample. This integration allows phylogenetic clusters to be interrogated for epidemiological links (e.g., shared location, contact history), and for epidemiological trends (e.g., cases in a new district) to be investigated genomically (single introduction vs. multiple lineages). The **Surface Sampling** study employs the same qPCR diagnostic protocol in an environmental context [27]. Its results are analyzed alongside human case data from the same facilities and time period, assessing the congruence between human infection and environmental contamination. This multi-stream model creates a feedback loop: genomic findings can validate diagnostic assay performance against circulating variants, epidemiological patterns can guide targeted environmental sampling, and environmental positivity can highlight gaps in IPC protocols. This cohesive strategy moves beyond parallel data collection to active synthesis, embodying the “real-time characterization” central to this thesis title [85].

1.4 Problem Statement

1.4.1 Diagnostic Limitations in Resource-Limited Settings

In Sierra Leone, despite the establishment of qPCR capacity, diagnostic workflows often operate in isolation as an endpoint service for case confirmation. Turnaround times can be delayed by supply chain issues, instrument downtime, and centralized testing, preventing diagnostics from fulfilling their potential as the trigger for a deeper molecular epidemiological investigation [86]. This creates a “diagnostic bottleneck” where cases are confirmed but not characterized, leaving public health officials blind to the underlying transmission dynamics of the outbreak. The diagnostic result answers “what” but not “how” or “why,” which is insufficient for mounting a targeted response.

1.4.2 Knowledge Gaps in Clade Differentiation and Transmission Mapping

A direct consequence of the isolated diagnostic model is a profound gap in actionable knowledge. In the absence of local genomic sequencing, Sierra Leone remains dependent on external laboratories for viral characterization, leading to significant delays or a complete lack of data. Therefore, fundamental questions about the 2025 outbreak remain unanswered: Is it driven by the global Clade IIb lineage or a local variant? Are increasing case numbers due to sustained local transmission or repeated zoonotic spillovers? Are cases in different provinces connected? This lack of granular, real-time knowledge on clade dynamics and transmission mapping forces a reliance on generic, non-targeted response measures, which are less efficient and resource intensive [87]. Similarly, while environmental contamination is suspected as a risk, data were lacking until a 2025 preprint quantified MPXV DNA on hospital surfaces [62].

1.4.3 Institutional and Infrastructural Barriers to Rapid Response

Sierra Leone’s health system currently lacks the institutional framework and infrastructure to seamlessly convert diagnostic data into genomic intelligence. Barriers include: 1) Workflow Silos: No formal protocol exists to channel qPCR-positive samples to a sequencing unit automatically. 2) Bioinformatics Capacity: Limited local expertise to analyze sequence data rapidly. 3) Data Integration Challenges: Epidemiological, laboratory, and genomic data are managed in separate systems, hindering synthetic analysis. 4) Sustainability Concerns: Sequencing platforms and reagents are often procured through short-term research grants, not

sustained by national surveillance budgets [88]. These barriers collectively represent the “response gap” between detecting an outbreak and understanding it with sufficient speed and resolution to alter its course.

1.4.4 Justification for the Current Study

The 2025 mpox outbreak in Sierra Leone provides a critical real-world test case to address these interconnected problems simultaneously. This study is justified by the urgent need to move from a reactive, diagnostic-only surveillance model to a proactive, integrated molecular intelligence system. By deliberately constructing and implementing a pipeline that links qPCR triage to rapid on-site sequencing and synthesizes this with epidemiological data, and by including an environmental qPCR component to assess IPC gaps, this research directly tackles the problems of characterization challenges and institutional delay. It aims to prove that real-time genomic and environmental characterization is not only technically feasible in a resource-limited setting but is an operational imperative for closing public health response gaps and making outbreak management more effective and efficient.

1.5 Study Significance

1.5.1 Policy and Public Health Implications

This research provides empirical evidence to drive policy change. Demonstrating the operational value of integrated surveillance will make a compelling case for the Sierra Leonean Ministry of Health and Sanitation to formally adopt and fund a national pathogen genomics strategy. Findings on the specific circulating clade and its transmission patterns will enable data-driven decisions on vaccine deployment and targeted community engagement. Furthermore, concrete evidence of environmental contamination, such as the high positivity on hospital doors from a local preprint [62], will mandate the review and enforcement of national IPC guidelines. Ultimately, this work seeks to transform policy by showing that integrated molecular data is not a research luxury but a core component of modern public health infrastructure for epidemic preparedness [89].

1.5.2 Scientific and Academic Contribution

This thesis contributes novel insights at the intersection of diagnostic virology, genomic epidemiology, and implementation science. It will provide one of the first detailed accounts of

implementing a complete, field-forward qPCR-to-sequencing pipeline for MPXV in a West African outbreak context. The generated dataset, linking Ct values, near-complete genomes, and patient metadata from Sierra Leone, will be a significant resource for global scientists studying MPXV evolution and transmission dynamics. The surface sampling study, based on a preprint, contributes foundational data on MPXV environmental contamination in African healthcare settings, a severely under-researched area [62]. Collectively, it advances the scientific discourse on practical frameworks for convergent surveillance [76].

1.5.3 Capacity Building and Technological Innovation

The significance of this work is profoundly embedded in its process. Executing this study builds in situ capacity: Sierra Leonean laboratory scientists will gain advanced, hands-on competency in nanopore sequencing and bioinformatics; data managers will work with integrated data streams; and public health officials will learn to interpret genomic epidemiological reports. This represents a shift from technology transfer to capability sovereignty. By successfully adapting and optimizing a portable sequencing workflow within the local constraints of Sierra Leone, the study serves as an innovation model for decentralizing genomic surveillance, making it a practical tool for national disease control programs rather than an external research activity [91].

1.6 Research Questions

1.6.1 Primary Research Questions

1. What are the genomic determinants and the clade identity (IIb, I, or emergent Ib) driving the mpox outbreak in Sierra Leone, and how does the circulating variant phylogenetically compare to contemporaneous strains from the DRC and neighboring West African countries?
2. How effective is an integrated molecular surveillance approach, combining rapid qPCR diagnostics with subsequent amplicon sequencing, in detecting, characterizing, and monitoring the transmission dynamics of the mpox outbreak in Sierra Leone in real-time?
3. What is the prevalence and distribution of MPXV DNA on high-touch surfaces in key healthcare facilities, and what does this reveal about potential environmental transmission risks and IPC gaps?

4. What are the key operational bottlenecks and facilitators in implementing an integrated qPCR-genomic surveillance pipeline in Sierra Leone, and how does it impact the timeliness of public health decision-making?

1.6.2 Hypotheses Formulated for Testing

1. Implementing a structured pipeline for sequencing samples with qPCR Ct <20 will enable successful whole-genome sequencing of >80% of selected samples in-country, providing real-time lineage identification.
2. Phylogenetic analysis will reveal that the 2025 outbreak is dominated by a single, locally circulating sub-lineage of MPXV Clade IIb, indicative of sustained human-to-human transmission rather than discrete zoonotic events.
3. Integrating phylogenetic clusters with contact tracing data will identify specific, previously unrecognized transmission links in at least 30% of clustered cases.
4. High-touch surfaces in patient care areas, particularly door handles and bed rails, will have a significantly higher MPXV DNA detection rate (>15%) than surfaces in administrative areas.

1.7 Aim and Objectives of the Study

1.7.1 Broad Aim

To design, implement, and evaluate an integrated diagnostic and genomic surveillance pipeline for the real-time characterization of MPXV clade dynamics and the identification of public health response gaps during the 2025 outbreak in Sierra Leone.

1.7.2 General and Specific Objectives

General Objective 1: To establish and execute an integrated molecular surveillance workflow from qPCR diagnosis to sequencing.

- *Specific Objective 1.1:* To screen suspected human cases using MPXV-specific qPCR, determining outbreak positivity rate and viral load distribution.
- *Specific Objective 1.2:* To perform targeted tiled amplicon sequencing on a subset of qPCR-positive samples (Ct<20) using the nanopore platform within Sierra Leone.

- *Specific Objective 1.3:* To conduct phylogenetic analysis of generated sequences alongside global references to identify circulating clade(s) and transmission links.

General Objective 2: To correlate molecular findings with epidemiological and environmental data to identify transmission risks and response gaps.

- *Specific Objective 2.1:* To analyze epidemiological trends (demographic, spatial, temporal) of qPCR-confirmed cases.
- *Specific Objective 2.2:* To assess environmental contamination by testing high-touch hospital surfaces for MPXV DNA via qPCR, building upon the methods of the 2025 surface sampling study.
- *Specific Objective 2.3:* To synthesize sequencing, epidemiological, and environmental data to map transmission hotspots and IPC vulnerabilities.

General Objective 3: To derive evidence-based recommendations for strengthening integrated outbreak response systems.

- *Specific Objective 3.1:* To document the technical and operational feasibility, including timelines, costs, and challenges, of the implemented pipeline.
- *Specific Objective 3.2:* To formulate specific policy, capacity-building, and technological recommendations for sustaining integrated molecular surveillance in Sierra Leone.

1.8 Scope of the Study

1.8.1 Geographic and Temporal Boundaries

This study is anchored in Sierra Leone. The integrated human surveillance component utilizes samples and data from nationwide case reports, aiming for national representativeness. The environmental assessment focuses on two strategic urban healthcare facilities, Connaught Hospital, Freetown, and Bo Government Hospital, which serve as major referral centers and thus represent high-risk transmission nodes. The study covers the acute phase of the 2025 mpox outbreak, with human sample collection and analysis occurring from May to August 2025, and environmental sampling conducted in June 2025, capturing peak transmission as per a recent preprint [62].

1.8.2 Population and Sample Inclusion Criteria

The human study population includes individuals meeting the national suspected case definition for mpox. Inclusion criteria: 1) Lesion swab specimen from a suspected case; 2) Availability of essential de-identified epidemiological data (age, sex, location, date). For inclusion in the genomic sequencing cohort, an additional criterion was a qPCR Ct value <20, ensuring sufficient template for successful library preparation. Environmental samples included any non-porous, high-touch surface in patient care zones (wards, theaters, bathrooms) and common areas within the selected hospitals, as per the referenced sampling protocol from a preprint [62].

1.8.3 Exclusions and Limitations

Exclusions: Poor-quality human samples (e.g., dry swabs, broken containers) were excluded. Samples with indeterminate or inhibited qPCR results were re-tested; if still inconclusive, they were excluded from analysis. Environmental samples from recently disinfected surfaces (within 30 minutes) were not collected [62].

Acknowledged Limitations:

1. **Temporal Snapshot:** The study design offers a cross-sectional view of the outbreak, limiting insights into the very early origins or long-term evolutionary trajectory of the virus in Sierra Leone.
2. **Case Ascertainment Bias:** The study relies on symptomatic cases that presented to the health system and were sampled, potentially underestimating the role of mild or asymptomatic infection in transmission.
3. **Sequencing Selection Bias:** The focus on low-Ct samples for sequencing may bias genomic data towards cases with higher viral loads, which may not be fully representative of all transmission events.
4. **Feasibility Generalization:** The operational model demonstrated was supported by a research consortium; the scalability and sustainability under routine government budgeting require further study.

5. **DNA vs. Infectivity:** Environmental qPCR detects viral genetic material, which correlates with but does not definitively prove the presence of infectious virus, necessitating cautious interpretation of transmission risk [62].

Chapter 2: Materials and Methods

2.1 Study Design and Overview

2.1.1 Study Type and Design: This study employed a cross-sectional laboratory and molecular epidemiological design, incorporating retrospective analysis of case data. The primary objectives were to characterize the mpox virus circulating in Sierra Leone through genomic sequencing and to analyze associated epidemiological trends. Laboratory work involved the diagnostic testing of clinical specimens, followed by targeted whole-genome sequencing of selected positive samples.

2.1.2 Study Sites and Setting (Bo, Freetown, and Others)

Since the study was conducted in Sierra Leone, lesion swab samples were received from various provinces across the country, with the majority from the Western Area Urban and Rural in the capital, Freetown. The epidemiological data were also collected from cases across Sierra Leone, and the surface sampling data were collected in only Freetown and Bo (2 major cities in Sierra Leone).

2.1.3 Population and Sampling Framework

The study population comprised individuals of all ages and sexes across Sierra Leone who presented as suspected or confirmed mpox patients during the study period. The sampling framework was based on a convenience sampling strategy, utilizing specimens and data collected through the national surveillance system. Clinical lesion swab samples were obtained from patients nationwide, with a concentration from the Western Area (Freetown). Environmental surface sampling was restricted to high-contact areas within major hospital settings (Connaught Hospital in Freetown and the Bo Government Hospital in Bo) to assess potential fomite transmission.

2.2 Target Population and Inclusion Criteria

Both males and females of all ages were included in this study. Confirmed positive and suspected mpox patients with exposure were considered as the inclusion criteria, and all other signs and symptoms, like mpox but not confirmed or related to an mpox exposure, were not included.

2.2.1 Case Definition and Sampling Strategy

This study comprises suspected and confirmed mpox patients. According to the WHO, a suspected patient is defined as a person who shows signs and symptoms after an exposure, but has not yet been confirmed by a doctor or a laboratory test. In contrast, a confirmed case can be linked to someone who shows visible signs and symptoms and has been confirmed positive by a doctor and a laboratory test after an exposure [91].

Sampling Strategy

Clinical samples were actively collected from individuals meeting the WHO case definition for suspected or confirmed mpox from May to August 2025. Lesion swabs were prioritized from fresh vesicular or pustular lesions, swabbed with sterile synthetic swabs. Environmental surface sampling was conducted in June 2025 using standardized swabbing protocols (surfaces were swabbed 3-4 times with a sterile surface swab kit and stored in a silicone lock bag) on high-touch hospital surfaces (e.g., bed rails, door handles, waiting area furniture) in selected wards. All samples were assigned unique identifiers and linked to basic demographic and clinical data where available. Epidemiological data were also collected from May to August 2025, during the peak of the outbreak.

2.2.2 Ethical and Logistical Considerations

The Sierra Leone Ethics and Scientific Review Committee (SLERC No. 010/05/2025) and the Health Research Ethics Board at the University of Manitoba, Canada (Opinion No. HS25837) approved this study. Funding for the work described was provided by the following: a Tier 2 Canada Research Chair in the Molecular Pathogenesis of Emerging and Re-Emerging Viruses for J.K. provided by the Canadian Institutes of Health Research (Grant no. 950-231498); the International Mpox Research Consortium (IMReC), jointly funded by the Canadian Institutes of Health Research and International Development Research Centre (grant no. MRR-184813), and the Canadian Biomedical Research Fund (grant no. CBRF2-2023-00103)

2.3 Sample Collection and Handling

2.3.1 Types of Samples Collected (Lesion swab, etc.)

In this study, we collected and analyzed 206 lesion swab samples from suspected patients

(aligned with WHO case definitions) and from PCR-confirmed mpox patients across Sierra Leone (all provinces). We also collected epidemiological data containing 600 suspected and confirmed patients received from the Ministry of Health in Sierra Leone for analysis. 89 Surface samples were also collected from 2 major hospital surfaces (Freetown and Bo) for analysis.

2.3.2 Transportation, Storage, and Chain of Custody

All lesion swab samples received were inactivated in a molecular transport medium, appropriately stored, and transported to the lab at Njala for extraction and analysis. All sampling surfaces were swabbed completely, and the samples were transported using a universal transport medium.

2.3.3 Biosafety and Containment Protocols (BSL-2 Compliance)

For this work, Biosafety Level 2 (BSL-2) containment was maintained throughout sample processing [92].

2.4 Laboratory Diagnostics

2.4.1 DNA Extraction and Purification Protocols

A robotic MagMax DNA Multi-Sample Ultra Kit was used to extract and purify DNA from all 206 lesion swab samples, including the first index case identified. The samples were stored at -80°C before use and then removed and thawed at room temperature. The swabs were aliquoted into a 96-well plate, one swab per well. 200 µL of PK mix was added to each well that contained the swab. PK mix consisted of 8 µL of proteinase K (Keratin) and 192 µL of PK buffer. The plate was sealed with a clear adhesive film and was shaken for 5 minutes at a speed of 950 rpm. After which, it was incubated for 20 minutes at a temperature of 65°C.

During incubation, three sets of MagMax™ Express-96 washes, Elution, and Tip Comb were prepared. Wash Plate 1 contained 150 µL of Wash Solution 1 in a deep-well plate, Wash Plate 2 contained 150 µL of Wash Solution 2 in a deep-well plate, and Wash Solution 3 contained 150 µL of Wash Solution 2 in a deep-well Plate. Also, 50 µL of DNA Elution Buffer 1 was placed in a standard plate, along with a Tip Comb, in a deep-well plate.

At the end of the incubation period, the 96-well plate containing the PK mix and samples was centrifuged briefly for 2 minutes at 1500 g, and 200 µL of Multi-Sample DNA Lysis Buffer was added to each well that contained the samples, sealed with a clear adhesive film, and shaken

again for 5 minutes at a speed of 8 rpm. While waiting for this, the DNA-binding beads were prepared: 16 μL of DNA-binding beads, 5 μL of RNase A, and 19 μL of Nuclease-free water, giving a total of 40 μL per well. The beads were vortexed at moderate speed to ensure thorough mixing, and 40 μL was added to each sample, pipetted up and down to ensure thorough mixing, sealed again with the clear adhesive film, and shaken for 5 minutes at a speed of 8 rpm. After that, the lysates were transferred into a new 96-well plate, and the plate that contained the swabs was discarded. 240 μL of isopropanol was added to each well with the sample, sealed with clear adhesive film, and shaken again for 5 minutes at a speed of 950 rpm, and then immediately proceeded to DNA isolation. The function '4413021 DW blood' was selected on the equipment (MagMax™ Express-96 Magnetic Particle Processor). The adhesive film was removed from the plate, and the plates were loaded appropriately in their designated positions. The PK plate containing the lysates, isopropanol, and DNA binding beads was placed at position 1, Wash Plate 1 was loaded at position 2, Wash Solution 2 was loaded at position 3, and Wash Solution 3 was loaded at position 4, the standard that contained the DNA elution buffer 1 was loaded at position 5 and the tip comb at position 6 as prompted by the instrument and the run was started.

Approximately 30 minutes after the initial run, as prompted by the instrument, the elution plate was removed from the instrument, and 50 μL of DNA elution buffer 2 was added to each sample well. The elution plate was then loaded back into the instrument in its appropriate position, and the run was started for about 30- 35 minutes. At the end of the run, the elution plate was removed from the instrument and sealed immediately with a new clear adhesive film, and the eluates were stored at 4 °C for qPCR.

2.4.2 Real-Time PCR Analysis

Real-time PCR was done on a clean bench disinfected with 70% ethanol to avoid contamination. This method was used to quantify the amount of DNA in each sample after extraction. The reagents were removed from the refrigerator and left at room temperature to thaw. A worksheet was generated to establish the plate layout. B6R mpox gene forward and reverse primers, probes, 2x master mix, and nuclease-free water were used for virus detection. Sequences in line with the Centers for Disease Control (CDC) mpox qPCR analysis were used: Forward, 5' GGA AAA TGT AAA GAC AAC GAA TAC AG-3', Reverse, 5' GCT ATC ACA TAA GGA AGC GTA-3', Probe: 5' FAM-AAG CCG TAA TCT A<BHQ-1dT>GT TGT CTA TCG TGT CC-Spacer C6-3' [39].

A total of 386.4 µL of nuclease-free water, 55.2 µL of the forward and reverse primers, and the probes were added to a reservoir with 1104.0 µL of 2x master mix, gently mixed, giving a total volume of 1656.0 µL. After which, 15 µL of the master mix solution was pipetted into a 96-well plate using a micropipette and sealed with a clear adhesive film, ensuring that bubbles were avoided. The DNA eluates were removed from the refrigerator and left to come to room temperature. After that, 5 µL of the DNA samples were added to each well that contained the master mix solution with a micropipette, and bubbles were carefully avoided. 5 µL of nuclease-free water was used as the negative control, and 5 µL of the positive Mpox sample (index case) was used as the positive control, each placed in a well at the end of the plate and sealed immediately with a clear adhesive film to ensure a tight seal.

The real-time PCR instrument (Bio-Rad CFX96) was programmed for a run of 20 µL of samples. The thermocycler was programmed at 95 °C for 3 minutes and for 39 cycles (95 °C for 10 seconds and 55 °C for 30 seconds) at 4 °C hold for all four runs, respectively. The plate was placed in the thermocycler and left to run, which took about 55-60 minutes. After the run, the data were transferred to a pen drive and imported into a computer for analysis.

2.4.3 Ct Threshold Determination and Quality Control

The Ct value, representing the PCR cycle at which the target amplification is first detected, serves as a semi-quantitative indicator of viral nucleic acid load in the sample. For this study, a Ct value of <20 was established as the cut-off for selecting samples for subsequent genomic sequencing. This threshold is conservative and commonly employed in Mpox virus studies to ensure a high likelihood of successful whole-genome amplification from samples with substantial viral load, thereby maximizing sequencing quality and coverage [39, 40]. All qPCR runs included a negative control (nuclease-free water) and a positive control (a known Mpox-positive sample) to monitor for contamination and assay performance. Samples with amplification in the negative control were rejected, and runs where the positive control failed to amplify within the expected Ct range were repeated.

2.4.4 Assay Validation and Control

The real-time PCR assay was validated for specificity and sensitivity using the primer-probe set targeting the B6R gene of the MPXV, as recommended by the CDC [39]. Assay performance was verified in each run by including external controls: a no-template control (NTC) to detect

contamination, a positive extraction control, and the aforementioned positive amplification control. Furthermore, the standardized protocol, including reagent concentrations and thermal cycling conditions, was strictly adhered to, ensuring inter-run reproducibility. The selection of samples with Ct <20 for sequencing acts as an additional internal validation step, prioritizing samples with a high probability of generating complete genomic data [40].

2.5 Genomic Sequencing Workflow

2.5.1 Amplicon Generation

After extraction, to obtain sufficient reads for sequencing, DNA from samples with a Ct value <20 [93-95] from the qPCR results was amplified (60 samples were selected, summarized, and attached as an appendix). These 60 samples were amplified in four runs, with 15 samples in each run and 1 negative control, totaling 16 samples per run.

Two Amplicon Master Mixes were prepared (pools 1 and 2). For each pool, 10 µL of Q5 2X Master Mix, 1.5 µL of Primer pool 1 and 2, respectively, and 7.0 µL of Nuclease-free water (a total of 18 µL of reagent) were prepared in a 0.2 mL tube, each labelled Pool 1 and Pool 2 Master Mix correctly. Two sample tubes were prepared, and a total of 18.5 µL of Master Mix 1 was aliquoted into sample tube 1 and 18.5 µL of Master Mix 2 into sample tube 2. (The total of 18.5 µL was multiplied by the number of samples for each run (16). 5 µL of the sample DNA was added to each sample tube, totaling the reaction volume to 23.5µL, which was later transferred into a 96-well PCR plate that was divided and labelled to differentiate the two pools, thoroughly mixed, centrifuged to bring the liquid down to the bottom, and sealed tightly with an adhesive film to avoid evaporation.

The thermocycler was programmed at 98 °C for 30 seconds and for 35 cycles (98 °C for 30 seconds and 63 °C for 5 minutes) at 4 °C hold for all four runs, respectively. The plate was placed in the thermocycler and left to run, which took about 4.5 hours each.

2.5.2 DNA Quantification

After the PCR process, the plate was removed from the thermocycler, spun down briefly, and the adhesive film was removed carefully to avoid any cross-contamination. An 8-channel micropipette was used to transfer 23.5 µL of each sample from pool 1 into its corresponding pool 2, changing tips with each well to avoid contamination and pipetting up and down about 5 times

to mix thoroughly. DNA was quantified using the Qubit 2.0 Fluorometer to generate amplicons, which were later normalized. The Qubit standards and reagents were removed from 4°C and left at room temperature. Two standards were prepared, and 1 tube was prepared for each of the 16 samples for each run (15 samples and 1 negative control, which was Nuclease-free water). The working solution was made for the total number of reactions (2 standards, 16 samples, 2 for pipetting errors, total 20 reactions). 1 µL of the Qubit dsDNA reagent was added to 199 µL of the Qubit dsDNA buffer for each reaction as the working solution (total of 4000 µL) and was vortexed briefly for a few seconds.

For the standards, 190 µL of the working solution, which was made, was added to 10 µL of the standard 1 and 2 reagents, respectively, from the kit, and for each sample, 199 µL of the working solution, which was made, was added to 1 µL of the sample. All were incubated at room temperature for 2 minutes and quantified. The Qubit Fluorometer was programmed to read highly sensitive DNA (dsDNA High Sensitivity). The first two standards were read as a quality control check; thereafter, each sample was quantified and the concentration recorded in nanograms per microliter (ng/µL) [40].

For all four runs of amplicons generated, normalization was done to achieve the exact volume of water and sample needed. The amplicons generated for each sample were divided by 200 ng of genomic DNA by the concentration of the amplicon generated to get the sample volume; thereafter, the results were subtracted from 10 µL for each sample to have the volume of water needed. There were cases wherein no amount of water was needed, due to the fact that after the normalization process, the total volume of the sample needed was already achieved. The results from the amplicons generated, normalization, and barcoding are summarized in tables and attached as an appendix.

2.5.3 Library Preparation

After normalization, barcodes were added to the DNA. 10µL of DNA (obtained from normalization, as shown in Tables 1-4 for each sample in each run- sample and water, or just sample, as the case may be) was placed in a 0.2 thin-walled PCR tube, and 1.5 µL of Rapid Barcodes (RB01-24 for each sample) was added for each DNA sample, giving a total volume of 11.5 µL per sample. The components were carefully mixed thoroughly by pipetting and briefly spun down. The tubes were incubated for 2 minutes at 30 °C and then for 2 minutes at 80 °C in

the thermocycler and briefly placed on ice to cool. After cooling, the tubes were spun down to collect all the liquid at the bottom.

All the barcodes were then pooled in a clean 2 mL Eppendorf DNA low-binding tube, yielding a total volume of 11.5 μL per sample, for a total of 184 μL across 16 samples per run. AMPure XP Beads (AXP) were vortexed briefly, and 11.5 μL was added for each sample (total 184 μL). This reaction was incubated for 10 minutes at room temperature by hand, as we didn't have a hula mixer at the time. When done, 2ml of fresh 80% ethanol was prepared in nuclease-free water. The total sample with barcodes and AXP was spun down and the pellet magnetized, and the supernatant was removed by pipetting. With the tube on the magnet, the beads were washed twice, first with 1ml of the freshly made 80% ethanol without interfering with the pellet, and the ethanol was removed with a pipette and discarded. This step was repeated with the second ethanol prepared.

When the washes were done, the tube was spun down, placed again on the magnet, and allowed to dry for 30 seconds without damaging the pellet after removing any residual ethanol with a pipette. After drying, the tube was removed from the magnetic rack, and the pellet was resuspended in 12.5 μL of Elution buffer (EB) for 16 barcodes (each run) and incubated for 10 minutes at room temperature. The beads were on the magnet until the eluates were clear and colorless. The eluates were then transferred into a clean 1.5 mL Eppendorf DNA low-binding tube, and the pellet beads were disposed of. These steps were repeated for all four runs. There were times at this point when the libraries were saved at $-20\text{ }^{\circ}\text{C}$ since we only had one machine to read the sequences after loading, and due to the capacity of the machine, we decided to do the 60 sequences in 4 runs.

2.5.4 Sequencing Platforms (Nanopore)

For quality control checks, 1 μL of each eluate (prepared library) was quantified using the Qubit fluorometer. Moving forward, 11.5 μL of the sample was pipetted into a clean 1.5 mL Eppendorf DNA low-binding tube, and 1.5 μL of the diluted rapid adapter (RA) was added. The mixture was gently flicked to mix, then spun down. This mixture was incubated at room temperature for 5 minutes, and I proceeded to prime my flow cells and set them for loading. The FLO MINI14 (R10.4.1) flow cells were used. While the flow cell checks were done, the Sequencing Buffer

(SF), Library Beads (LIB), Flow Cell Tether (FCT), and Flow Cell Flush (FCF) were left to thaw at room temperature and then briefly vortexed and spun down.

The flow cell priming mix was prepared with Bovine Serum Albumin (BSA) in a fresh 1.5 ml Eppendorf DNA low-binding tube. 1170 μL of FCF, 5 μL of BSA, and 30 μL of FCF were put together, giving a total volume of 1205 μL per flow cell. With the completed flow cell checks, we moved forward with loading the cell. The MinION device lid was opened, and the flow cell was slid under the clip and pressed down firmly on the priming port cover to ensure accurate thermal and electrical contact. The priming port was slid clockwise to open, and after opening, we checked for air bubbles under the cover. The P1000 pipette was used and set at 200 μL , inserted into the priming port, and the wheel of the pipette was turned slowly till the dial showed 220-230 μL while visually checking to see if there was continuous movement of buffer from the priming port right across the sensor array. This was done to draw back 20-30 μL of the buffer in the pipette to ensure there were no bubbles present.

800 μL of the prepared priming mix was loaded into the flow cell through the priming port, while we carefully avoided introducing air bubbles and waited for 5 minutes. During this wait time, the library was prepared for loading by thoroughly mixing the LIB by pipetting. In a new 1.5 mL Eppendorf tube, 37.5 μL of the SB, 25.5 μL of the LIB, and 12 μL of the prepared DNA library were mixed, making a total volume of 75 μL .

The SpotON sample port was carefully lifted for accessibility, and 200 μL of the priming port was loaded into the priming port again, with attention to avoid air bubbles. The prepared library was mixed gently by pipetting, and 75 μL of this prepared library was loaded into the flow cell through the SpotON sample port in a dropwise motion, and we ensured each drop flowed into the port before adding the next. The SpotON cover was closed gently, ensuring the bung on the cover fit into the port hole perfectly, and the priming port was closed. The light shield was gently placed on the flow cell around the SpotON, which covered the entire section of the flow cell. The device lid was closed, and the sequencing run was started on MinKNOW (the sequencing software for the MinION) without base calling [40].

2.6 Phylogenetic Analysis

2.6.1 Sequence Selection and Dataset Preparation

After the bioinformatic analyses, sequences with a coverage greater than or equal to $\geq 70\%$ (i.e., between 70% and 89%) were used. This threshold ensured the minimum quality required for phylogenetic analysis. The sequences concerned are listed in the shared linelist attached as an appendix. The retained sequences were merged with a set of reference sequences (full genome) downloaded from Pathoplexus, belonging to clade IIb, showing the same as our sequences. This combined dataset was then subjected to quality control using Squirrel to verify genomic parameters, consistency, and overall sequence quality. At this stage, an out-group was also added to allow tree rooting.

2.6.2 Sequence Alignment

All sequences (local, downloaded, and out-group) were aligned to identify homologous positions among them. The multiple sequence alignment was generated in Fast-All (FASTA) format.

Furthermore, for post-alignment verification, Seaview was used to visualize the alignment and check for any low-quality sequences (too short or containing too many ambiguous bases “N”). In our case, all sequences were compliant, and none were removed, allowing us to proceed directly to tree construction.

2.6.3 Phylogenetic Tree Construction (Maximum-Likelihood)

The tree was constructed using IQ-TREE, following the Maximum Likelihood method. The best-fit substitution model was automatically selected (-m MFP), and an ultrafast bootstrap of 1000 replicates was applied to assess branch support (-B 1000). To speed up computation, a high number of processor cores was set (-nt 10).

2.6.4 Visualization and Interpretation (FigTree)

The resulting tree was visualized and annotated in FigTree. For better understanding, three complementary trees were generated:

- by country (Sierra Leone and others),
- by sequence type (generated sequences and downloaded sequences),

- by lineage (G, H, A).

Bioinformatic analyses showed that all generated sequences belong to clade IIb of the virus and are classified within the G1 lineage. To better position these Sierra Leone sequences in the global context and confirm their phylogenetic placement, we compared them to a set of sequences also belonging to clade IIb, downloaded from the Pathoplexus database.

The phylogenetic tree constructed using IQ-TREE illustrates the positioning of the Sierra Leone sequences in relation to this clade IIb dataset. We then generated three types of phylogenetic trees to allow for better visualization and interpretation:

- one tree based on the country of origin (Sierra Leone and other countries),
- one based on sequence type (our generated sequences and downloaded ones)
- and one based on lineage (G for our sequences, and H and A for comparison).

2.7 Epidemiological Data Analysis Methods

2.7.1 Data Collection and Management

Data was collected from the results of 600 samples collected across the country in May 2025 during the peak of the outbreak. The data was received from the Ministry of Health in Sierra Leone, comprising cases from different hospitals and peripheral health units (PHUs) across the country. This data was achieved by lesion swap samples collected from suspected and confirmed positive cases, tested for both the orthopoxvirus and the mpox virus for clinical and surveillance purposes. Approvals were obtained from the Sierra Leone ethics board to use the data. Both males and females of all ages were included in the study.

2.7.2 Statistical Analytical Plan

Data will be analyzed using different variables such as sex, district, orthopox, and mpox positivity rates, and hospitalization rate.

2.8 Ethical Approval and Data Management

Approval was granted by the ethics board of Sierra Leone for this study. Data collected were stored confidentially on a hard drive, and analyses to be published will not include any form of personal information or details of any individual.

Chapter 3: Results

Statistical software and data handling

All data cleaning, descriptive statistics, and graphical visualizations were performed using Microsoft Excel (version 2503 Build 16.0.18623.20178) and R (version 4.5.3) with the tidyverse, ggplot2, and readxl packages, among many others. The combination of these tools ensured robust data management, reproducible analysis, and high-quality figures.

3.1 Overview of Diagnostic Findings

3.1.1 Total Samples and Regional Distribution

A total of 600 samples were received and processed for diagnostic testing for orthopoxviruses, specifically MPXV. The samples were collected across 10 districts, with the vast majority originating from the Western Area Urban (WAU) and Western Area Rural (WAR) districts of Freetown.

Table 3.1 shows the district distribution of the 541 orthopoxvirus-positive samples. **Table 3.2** summarizes the regional distribution.

Table 3.1: District distribution of orthopoxvirus-positive samples (n=541)

District Distribution of Patients (541)		
District	Frequency	Percent
Bo	1	0.2%
Bombali	1	0.2%
Kambia	5	0.9%
Kenema	1	0.2%
Kono	1	0.2%
Moyamba	6	1.1%
Port Loko	6	1.1%
Pujehun	1	0.2%
Western Area Rural	188	34.8%
Western Area Urban	331	61.2%
Total	541	100.0%

Table 3.2: Regional distribution of orthopoxvirus-positive samples (n=541)

Regions	Number of samples	% of total samples
Western Region (WAU and WAR)	519	95.93%
Eastern Region	2	0.37%
Northern Region	1	0.18%
Northwest Region	11	2.03%
Southern Region	8	1.48%

3.1.2 Diagnostic Positivity and Quality Metrics

Of the 600 samples tested, 541 (90.2%) were PCR-positive for orthopoxvirus, indicating a high prevalence of orthopoxvirus infection among suspected cases. Of these, 403 (74.5% of orthopoxvirus-positive cases) were specifically identified as MPXV Clade IIb. The remaining orthopoxvirus-positive cases (138) either lacked mpox clade-specific testing or failed to amplify in the clade-specific assay.

A total of 6 samples were recorded as having “No Amplification”, attributed to poor sample quality or poor sample collection, representing a 1.0% sample rejection rate due to pre-analytical issues.

3.2 Ct Value Distribution and Viral Load Analysis

3.2.1 Frequency Distribution Tables

Viral load, indicated by the Ct value, was analyzed for all 541 orthopoxvirus-positive samples and separately for the 403 mpox-confirmed samples (using the mpox-specific FAM probe). **Table 3.3** presents the Ct categories for all orthopoxvirus-positive samples, while **Table 3.4** shows the mpox-specific viral load distribution.

Table 3.3: Ct categories for all orthopoxvirus-positive samples (n=541)

Ct Category	Frequency	Percentage
Low (<20)	123	22.7
Medium (20-30)	326	60.3
High (>30)	92	17

Table 3.4: MPXV-specific viral load categories (FAM Ct, n=403)

FAM Ct Value Category	Frequency (n)	Percentage (%)	Interpretation
Low (Ct < 20)	14	3.50%	High Viral Load
Medium (Ct 20-30)	329	81.60%	Moderate Viral Load
High (Ct > 30)	60	14.90%	Low Viral Load
Total	403	100%	

3.2.2 High vs. Low Viral Load Patterns

The majority of mpox cases (81.6%) presented with a moderate viral load (Ct 20-30). High viral load (Ct < 20) was observed in 14 cases (3.5%), suggesting potentially more acute or severe presentations at the time of sampling. A notable proportion (14.9%) of confirmed mpox patients presented with a low viral load (Ct > 30), which may indicate convalescent stages or late presentation.

3.2.3 Sex, Age, and Geographic Correlations

A detailed analysis of Ct values and demographic data revealed the following:

- **Sex:** The median Ct values for confirmed mpox patients were similar across sexes. For females (n=292), the median Ct was 22.0 (IQR: 19.5-26.5), and for males (n=249) it was 22.6 (IQR: 19.5-27.0). This suggests comparable viral loads between sexes.
- **Age:** The age distribution of confirmed mpox cases ranged from 1 to 63 years. The median age was 27 years (IQR: 22-35), indicating that young adults were the most affected. A scatterplot of Ct values against age showed no clear correlation ($R^2 \approx 0.02$), suggesting that viral load is independent of age in this outbreak. **(Figure 3.1). Figures 3.2 and 3.3** show the descriptive relationships between age and Ct values for the FAM probe used and the age distribution by sex. **Table 3.5** provides the gender disaggregation with the mean age.
- **Geographic:** There were more patients in the WAU and WAR districts. The median Ct values for these two districts were 22.0 (WAU) and 23.5 (WAR), indicating a slightly higher median viral load in the WAU district, though the difference is not statistically significant.

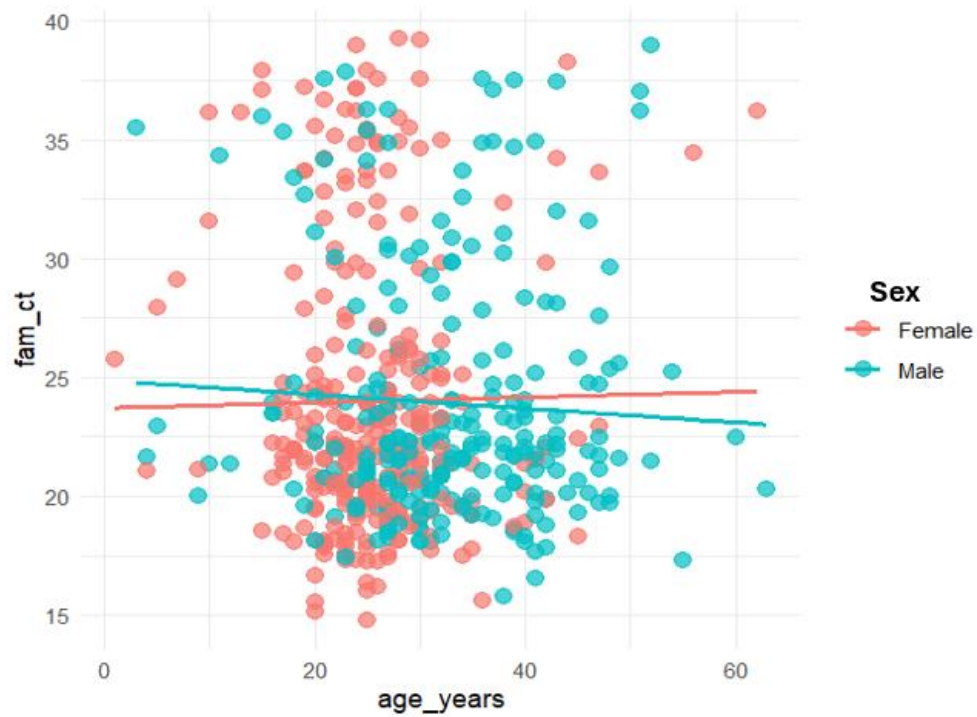


Figure 3.1: Ct values by age and sex

A scatter plot showing individual FAM Ct values against patient age, with points colored by sex. No clear correlation is observed ($R^2 \approx 0.02$), indicating that viral load is independent of age. The median Ct values for females (22.0) and males (22.6) are similar, suggesting comparable viral loads between sexes.

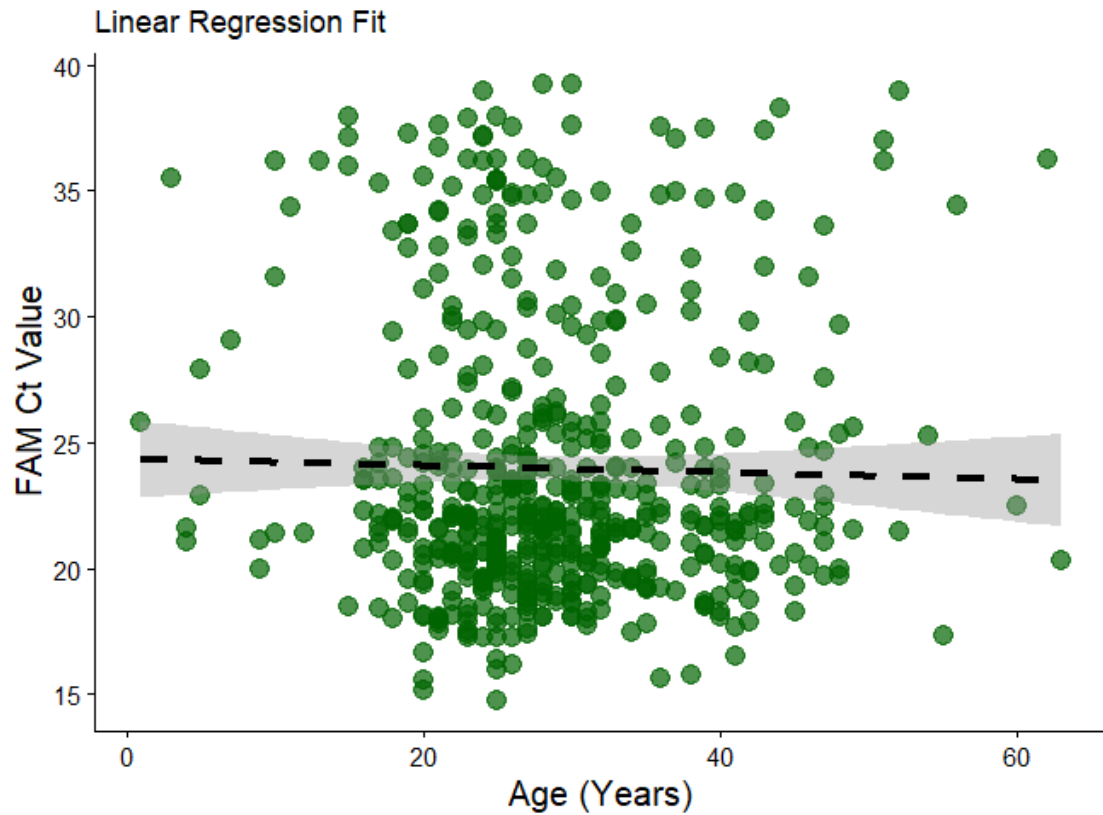


Figure 3.2: Relationship between Age and Ct FAM Values

A scatter plot of Ct values (FAM probe) against patient age, with a linear regression line. The near-flat line and low R^2 (0.02) confirm no meaningful relationship between age and viral load.

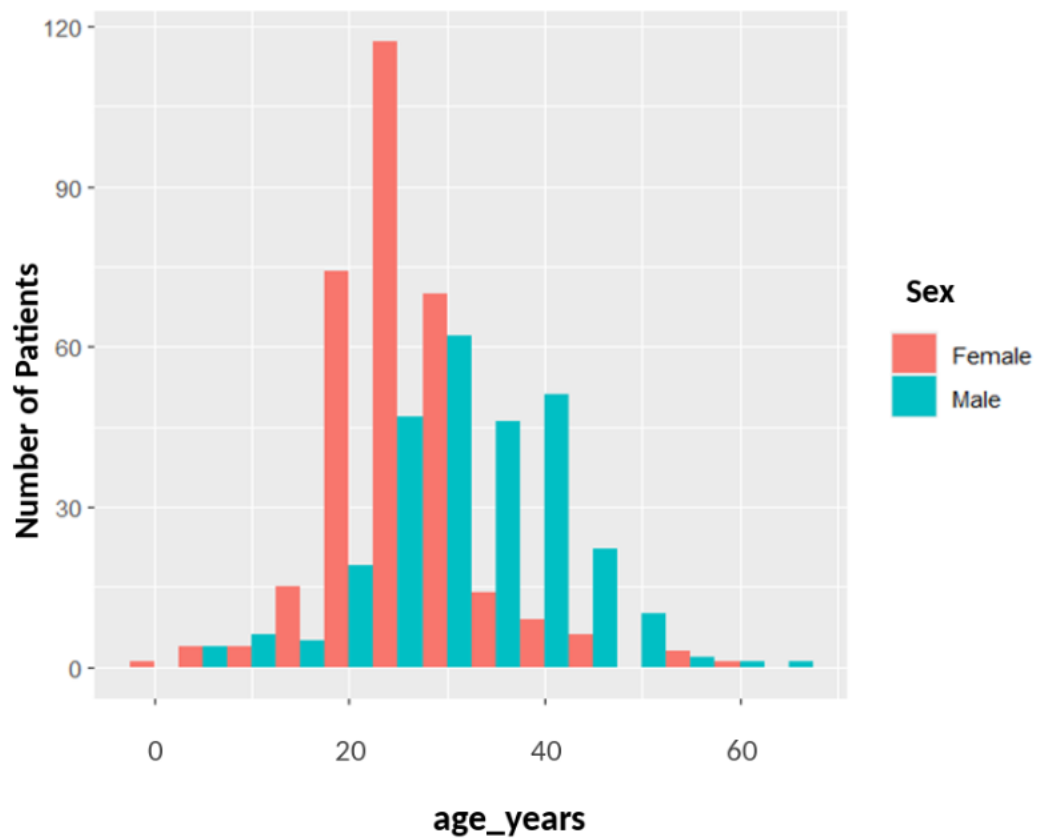


Figure 3.3: Age distribution by sex

A histogram showing the age distribution of all 600 patients, split by sex. Females have a lower mean age (25.6 years) than males (32.5 years).

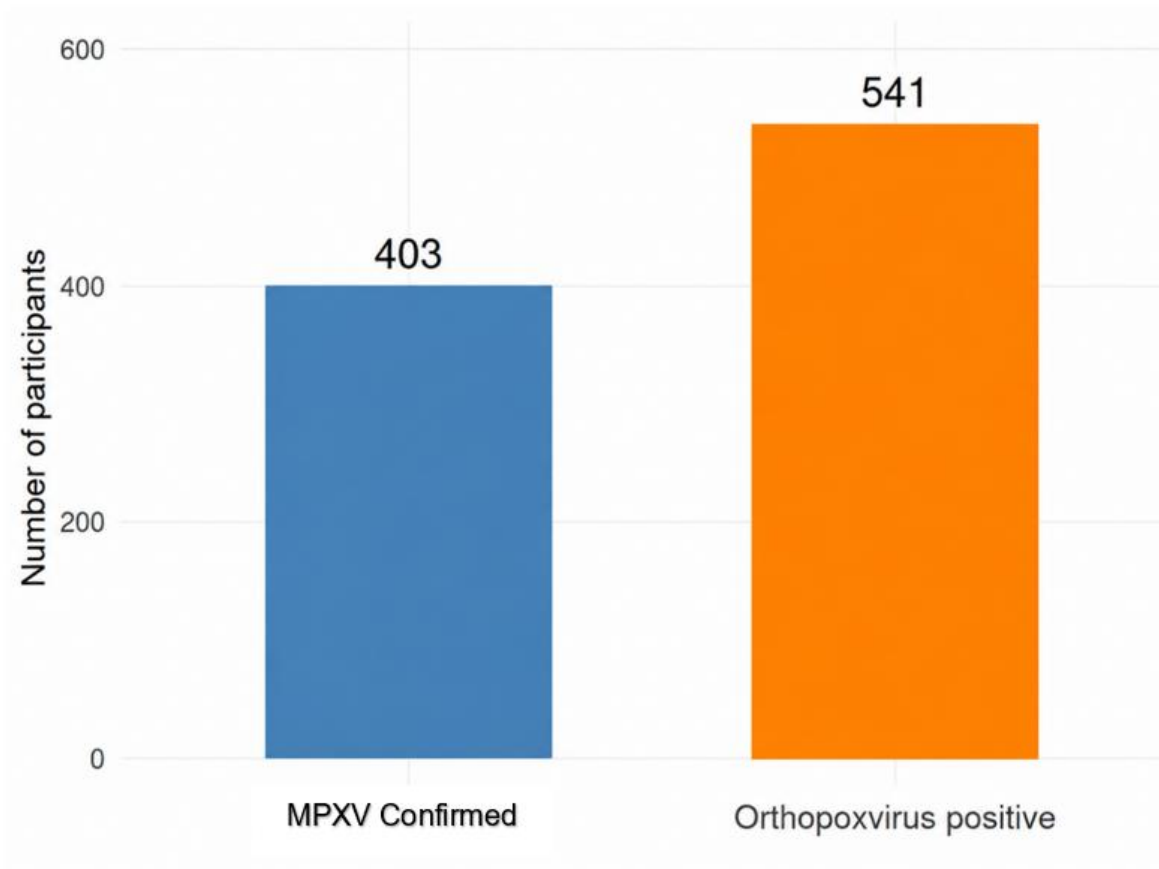


Figure 3.4: OPXV/MPXV participants

A bar plot showing the number of orthopoxvirus-positive samples (541) and, within them, the Mpox-confirmed cases (403). The remaining 138 orthopoxvirus-positive samples were not confirmed as Mpox.

Table 3.5: Sex distribution and age statistics (all 600 samples)

Sex	Mean Age	Standard Deviation of age	Number of samples (n)	p-value (Age)	p-value (Sex distribution)
Female	25.6	7.49	323	<0.001	0.060
Male	32.5	9.59	277		

Notes: The age p-value compares mean age between females and males using Welch's independent-samples t-test from the reported summary statistics. The sex distribution p-value was calculated using a chi-square goodness-of-fit test against an equal 50:50 female-to-male distribution. The age comparison was statistically significant ($p < 0.001$). The sex distribution was not statistically significant at the 0.05 level ($p = 0.060$).

3.3 MPXV Ct Value Analysis and Sample Selection for Sequencing

3.3.1 Statistical Methods

All statistical analyses were performed using R (version 4.5.3). Descriptive statistics (mean, median, standard deviation, interquartile range) were calculated for the MPXV Ct values. The Shapiro–Wilk test was used to assess the normality of the Ct distribution. A p-value < 0.05 was considered statistically significant. Visualizations included a histogram with an overlaid density curve, a box plot, and a scatter plot of Ct values versus sample order to assess for systematic bias.

3.4 Sequencing Output and Genomic Quality Metrics

3.4.1 Number of Successfully Sequenced Samples

From the 60 samples selected for sequencing, after applying a coverage threshold of 70-89% (as per bioinformatics quality control for phylogenetic analysis), 41 samples (68.3%) were retained. All retained samples were assigned to clade IIb – the lineage responsible for the 2022 mpox PHEIC. One clade II sample had coverage below 70% and was excluded; no clade Ia or Ib sequences passed the filter. **Table 3.6** summarizes the key characteristics of the high-quality sequences, and **Figure 3.5** shows a bar plot describing the Clade distribution.

Table 3.6: Summary of sequencing data after quality filtering (coverage 70–89%)

Parameters	Value
Total sequenced samples	60
Passed quality filter (70- 89% coverage)	41 (68.3%)
Clade IIb	41 (100%)
Clade II	1 (we had 1, even though it was below the %)
Mean Ct ($\pm$ SD)	17.89 $\pm$ 2.14
Median Ct	17.76
Ct range	13.26 – 20.06
Mean coverage ($\pm$ SD)	0.78 $\pm$ 0.06
Coverage range	0.70 – 0.88
Male	18 (43.9%)
Female	23 (56.1%)

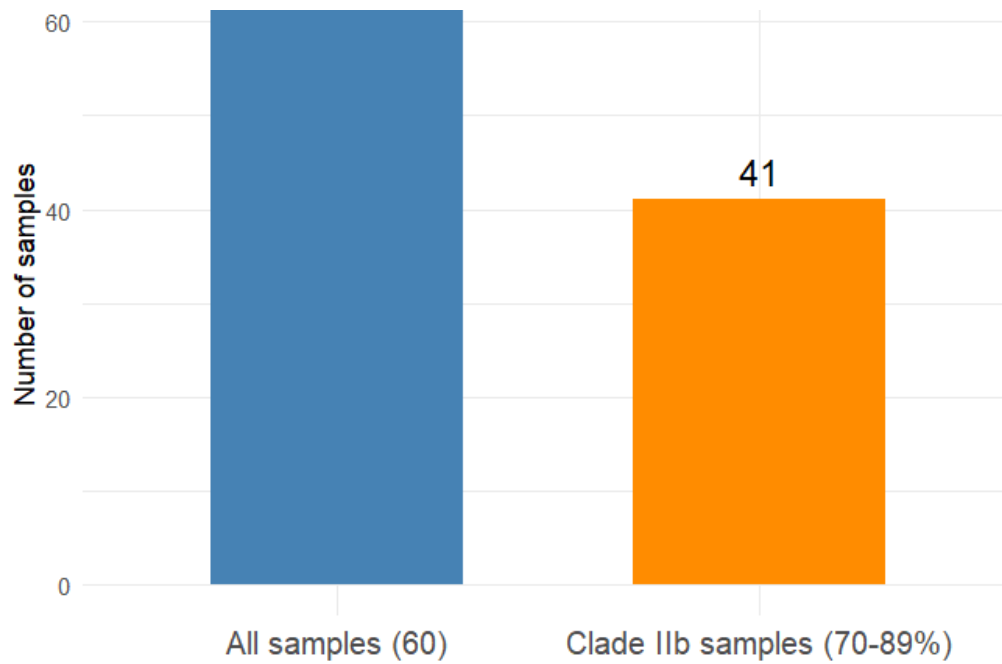


Figure 3.5: Clade distribution bar plots

All sequenced samples ($n = 60$) vs. Clade IIb samples with 70–89% coverage ($n = 41$; 68.3% of total). The remaining 18 samples were Clade IIb but did not meet the coverage threshold. Binomial test vs. 50%: $p = 0.0056$.

3.4.2 Genome Coverage and Depth Statistics

The majority of high-quality samples clustered near 80% coverage, with a mean of 78% (SD = 6%). **Figure 3.6** shows a histogram of coverage values (presented as proportions), confirming consistent sequencing performance across the 41 samples. Only a few samples fell near the lower bound (70%) or upper bound (89%) of our inclusion threshold.

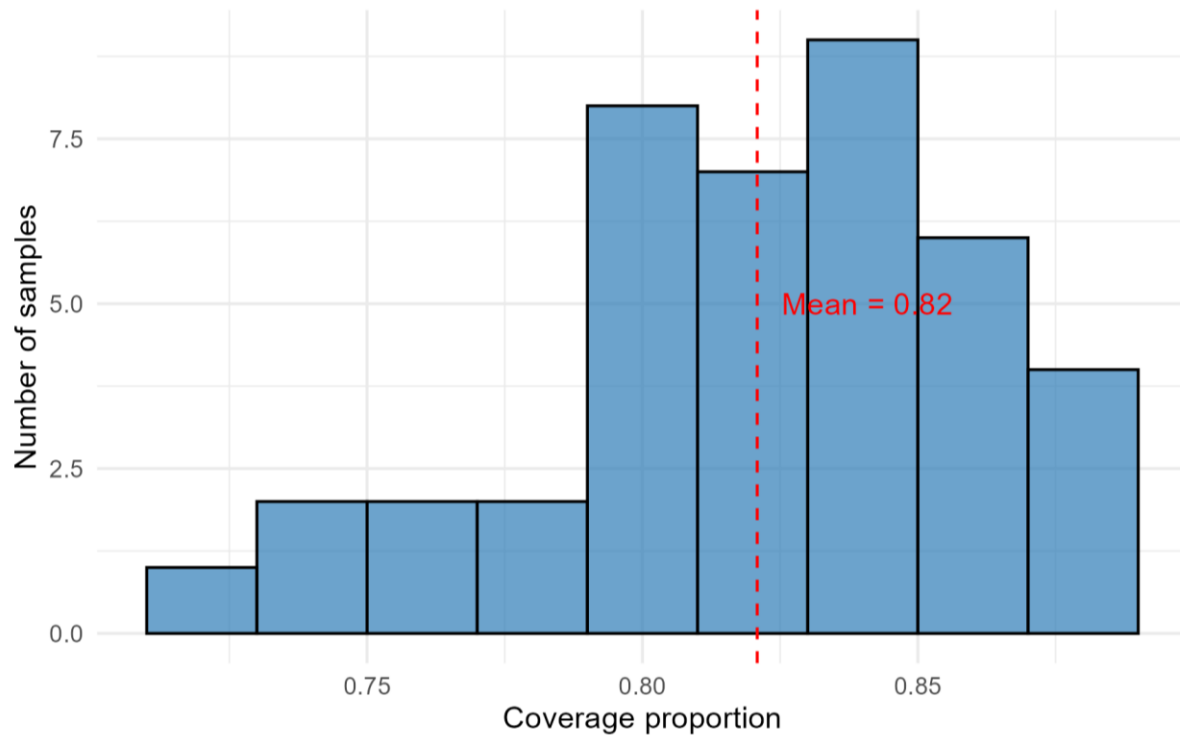


Figure 3.6: Distribution of sequence coverage (70-89% threshold)

A histogram of genome coverage proportions for the 41 high-quality sequences. The mean coverage was 0.78 (78%), with most samples between 0.75 and 0.82. The distribution is roughly symmetric, indicating consistent sequencing quality.

To assess whether sequencing coverage was biased by sample viral load, a Pearson correlation between Ct value and coverage was performed (**Figure 3.7**). A weak, non-significant negative correlation was observed ($r = -0.12$, $p = 0.46$), indicating that coverage was not systematically lower in samples with higher CT values (lower viral load). This supports the use of the 70–89% coverage threshold as an unbiased quality filter. **Table 3.7** provides the full correlation statistics.

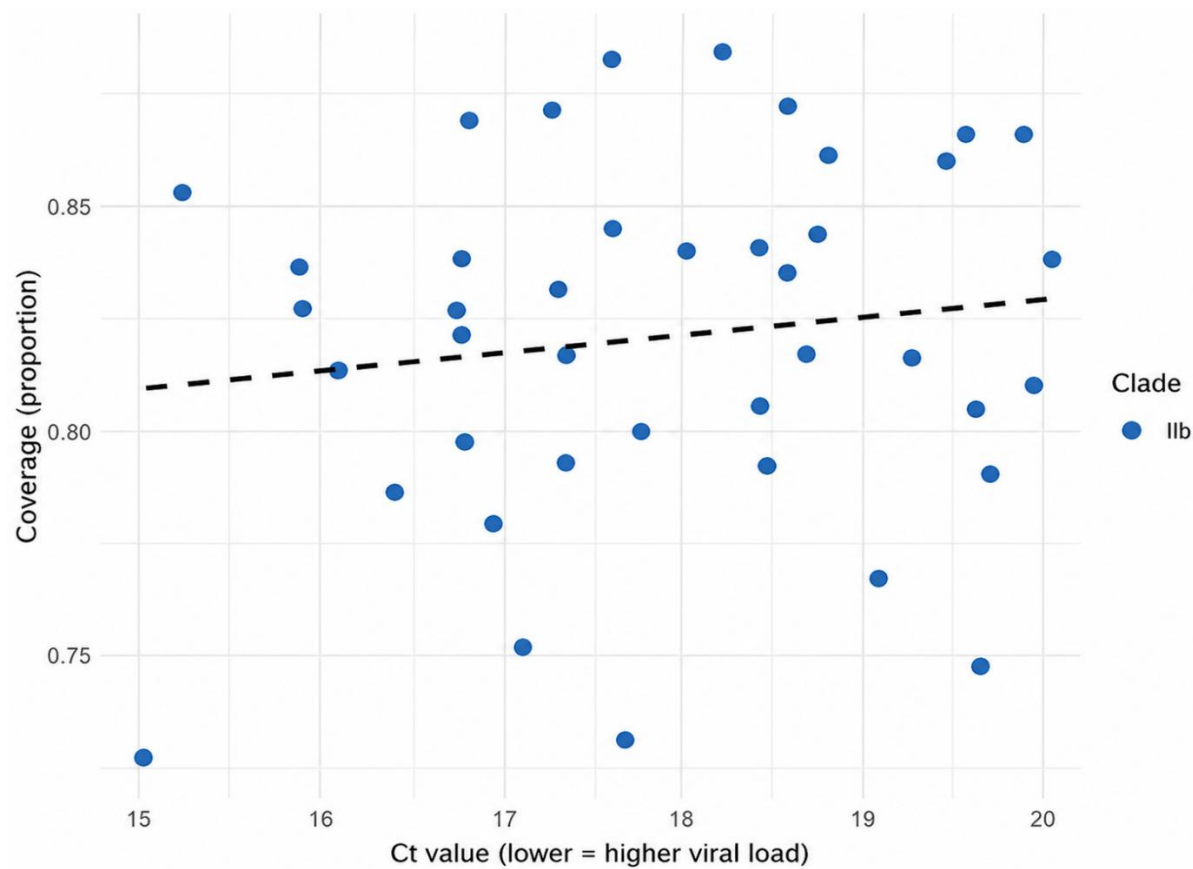


Figure 3.7: Relationship between Ct value and sequencing coverage

Each point represents one of the 41 high-quality samples, plotted with Ct value (x-axis) against genome coverage proportion (y-axis). A dashed linear regression line shows a very slight negative slope ($r = -0.12$), and the wide scatter confirms no meaningful relationship.

Table 3.7: Correlation between Ct value and sequencing coverage

Metric	Value
Pearson's r	−0.12
p-value	0.46
95% CI	(−0.41, 0.19)

3.4.3 Ct Values by Sex

Although no statistically significant difference was found, **Figure 3.8** illustrates the distribution of Ct values between male and female participants. The median Ct was slightly lower (higher viral load) in females (17.5) compared to males (18.1), but the overlap was substantial.

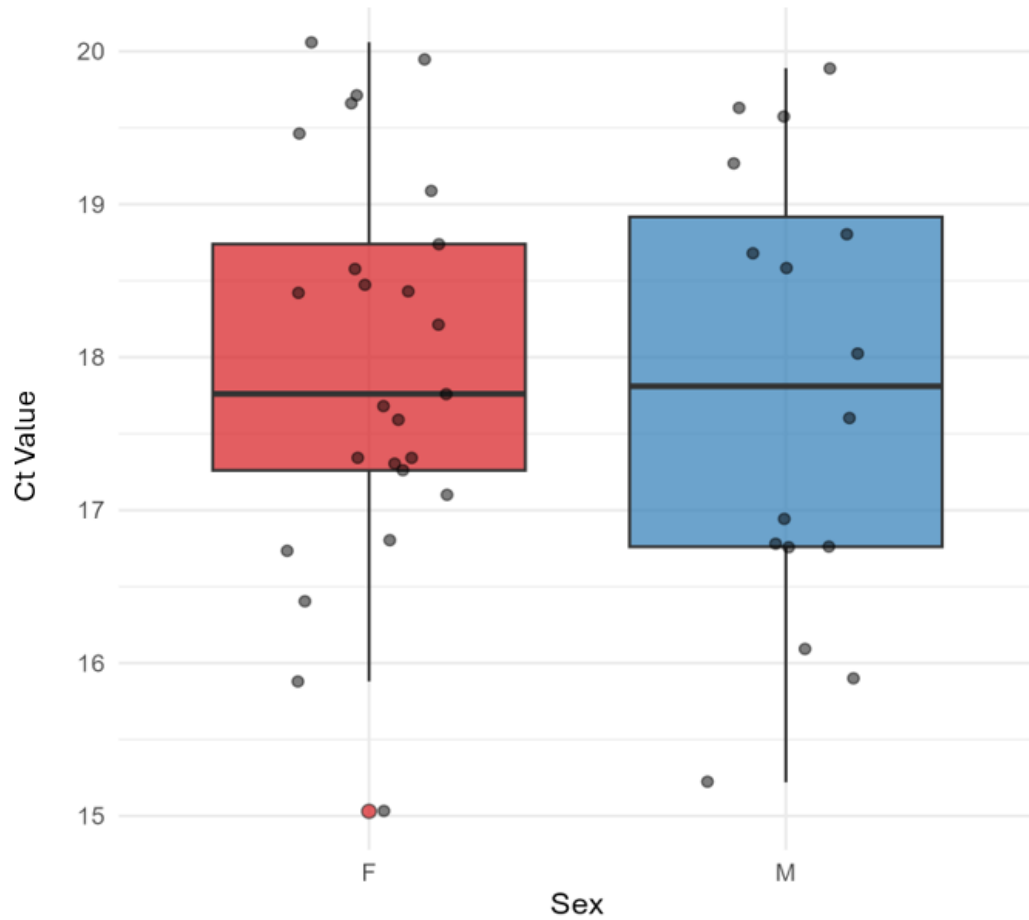


Figure 3.8: Ct values by sex (high-quality sequences)

Shows Ct values by sex for the 41 high-quality Clade IIb samples (25 females, 16 males). The median CT was lower in females (17.5 vs. 18.1 in males), but the difference was not significant ($p = 0.43$, Wilcoxon test), and the distributions overlapped substantially.

3.4.4 Variant Annotation and Mutation Hotspots

No dedicated variant annotation or mutation hotspot analysis was performed in this study. The primary focus was on clade classification and phylogenetic placement. However, all sequences were confirmed as clade IIb by Pathoplexus, which uses lineage-defining mutations. Future work could include detailed mutation mapping to identify any novel substitutions specific to Sierra Leone.

3.3.2 Data Overview and Descriptive Statistics

A total of 213 samples (lesion swabs) were analyzed by q-PCR for the Mpox target. After removing samples with missing or non-numeric Ct values (recorded as “N/A”), 206 samples had valid Ct values available for analysis. Samples with Ct values >40 were considered negative but were retained in the dataset; the maximum observed Ct was 40.11.

Table 3.8 summarizes the descriptive statistics for the MPXV Ct values. The Ct values ranged from 13.26 to 40.11, with a mean of 21.62 (± 6.30) and a median of 19.34. The distribution was right-skewed (Skewness = 1.1), indicating a tail of samples with lower viral load (higher Ct).

Table 3.8: Descriptive statistics of MPXV Ct values (n = 206)

Statistical Analysis	Value
Number of samples	206
Mean	21.62
Standard Deviation	6.3
Median	19.34
Minimum	13.26
Maximum	40.11
Interquartile range	17.71 – 21.99

3.3.3 Ct Category Distribution

Using standard cycle threshold cut-offs (Low <20 = high viral load, Medium 20–30 = moderate viral load, High >30 = low viral load), **Table 3.7** and **Figure 3.9** suggest that the majority of samples fall into the low range (60.7%), with a large proportion in the medium (24.8%) and high (14.6%) categories. Exact frequencies are shown in the table below, and the visual distribution is described in the table below.

Table 3.9: Ct Category distribution for MPXV PCR positivity (n=206)

Ct category	Ct Frequency	Percentage (%)
Low (<20)	125	60.70
Medium (20–30)	51	24.80
High (>30)	30	14.60
Total	206	100.00

3.3.4 Visualization of MPXV Ct Values

Figure 3.9 presents a histogram with an overlaid density curve. The distribution is right-skewed, with most Ct values concentrated between 15 and 25. A long right tail extends beyond Ct 35, indicating a subset of samples with very low viral load (high Ct). The peak density occurs around Ct 19–20, which aligns with the median value of 19.34. The mean (21.62) is slightly higher than the median due to the right skew.

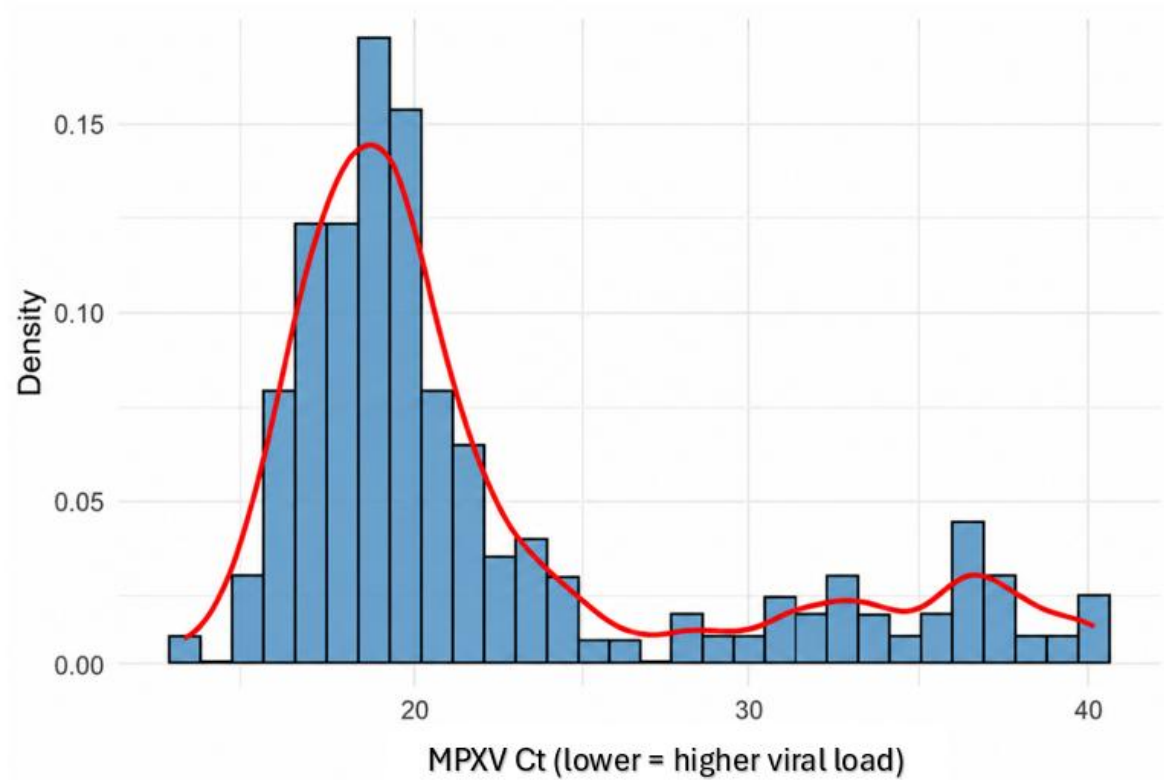


Figure 3.9: MPXV Ct distribution of 206 lesion samples

The histogram shows the distribution of MPXV Ct values from 206 valid lesion samples. The x-axis represents Ct values, where lower Ct values indicate higher viral load, while the y-axis represents the probability density of MPXV Ct values rather than the raw number of samples. In other words, density shows the relative concentration of samples across Ct ranges after the histogram has been scaled so that the total area under the bars equals 1. A red density curve is overlaid to show the smoothed pattern of the Ct distribution. The distribution is right-skewed, with most Ct values falling between 15 and 25. The mean Ct value was 21.62, and the median was 19.34, indicating that most lesion samples had relatively high viral loads, while the long right tail reflects a smaller subset of samples with higher Ct values and therefore lower viral loads.

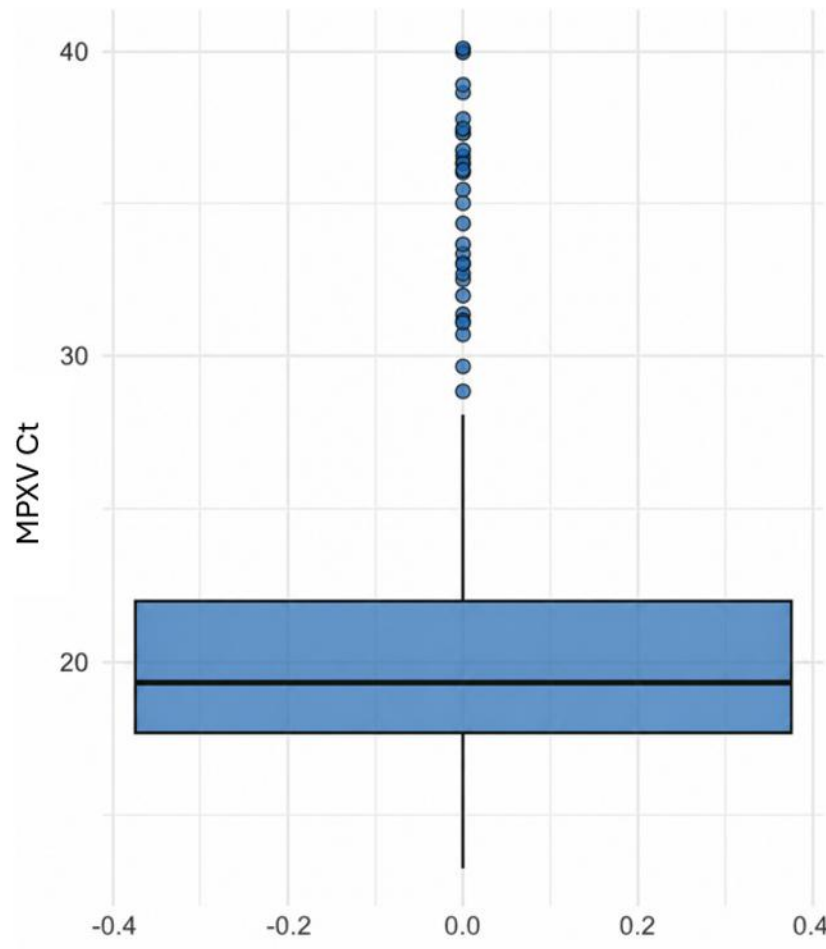


Figure 3.10: MPXV Ct values

The boxplot summarizes the spread and central tendency of MPXV Ct values. The median is approximately 19.34. The IQR spans from about 17.71 to 21.99, indicating that 50% of samples fall within this moderate viral load range. The lower whisker extends to $Ct \approx 13.26$ and the upper whisker to $Ct \approx 36.5$. Several outliers are visible above the upper whisker ($Ct > 36$), representing samples with very low viral load. No outliers are present at the low Ct end.

Figure 3.11 shows a scatter plot of Ct values against sample order (index). Each point represents a sample plotted against its row order in the original data frame. A red regression line shows a very slight downward trend ($r \approx -0.12$), indicating that Ct values do not systematically increase or decrease with sample processing order. The points are widely scattered across the entire Ct range, confirming that the data are randomly distributed and free from ordering bias (e.g., due to run-to-run variation or time-dependent changes in sample quality).

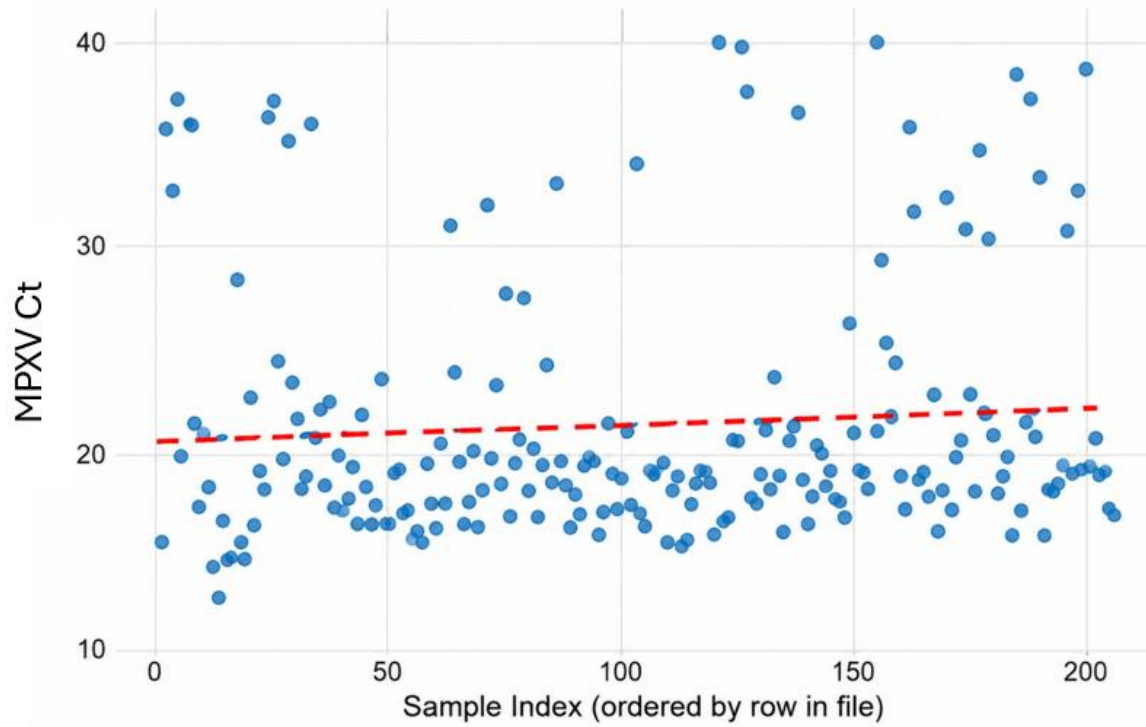


Figure 3.11: Ct values by sample order

Each point represents a sample plotted against its row order in the original data frame (index). A blue regression line (with confidence band) shows a very slight downward trend ($r \approx -0.12$), indicating that Ct values do not systematically increase or decrease with sample processing order. The points are widely scattered across the entire Ct range, confirming that the data are randomly distributed and free from ordering bias.

3.3.5 Identification of Samples with Low Ct Values (<20) for Sequencing

Samples with a Ct value < 20 were prioritized for tiled-amplicon sequencing, as lower Ct values indicate higher viral loads and a greater likelihood of successful genome recovery. Based on the MPXV Ct values, a total of 125 unique samples (out of 206) had a Ct of < 20 (calculated from the data: median 19.34, so roughly 60% are below 20). Of these, 60 samples were distributed across four sequencing runs (Runs 1-4), as detailed in **Table 3.10**. Each run included 15 samples plus a negative control (the remaining low-Ct samples were not used due to plate capacity). The Ct values of selected samples ranged from 13.26 to 20.06, with a mean of approximately 17.8. One sample (Lab ID 97) had Ct = 20.06, marginally above 20, but was included due to limited low-Ct samples in that batch.

Table 3.10: Summary of Clade 11b samples selected for sequencing (Ct<20) per run

Sequencing run	Number of samples	Mean Ct (Clade IIb)	Ct range
Run 1	15	17.1	13.05–19.9
Run 2	15	17.8	16.7–19.6
Run 3	15	18.2	16.1–20.0
Run 4	15	18.2	16.3–20.1
Total	60 (plus NCs)	17.8	13.05–20.1

*NC = negative control.

3.3.6 Amplicon Generation, Normalization, and Barcoding

For each sequencing run, amplicons were generated from the selected samples using the Mpox tile-amplicon amplification protocol. The concentration of amplified products was measured (ng/μL) and normalized to 200 ng per sample by adjusting the sample volume and adding water. Barcodes were assigned uniquely to each sample within a run to enable multiplex sequencing. The detailed calculations for each run, normalization, and the amplicons generated are attached as appendices.

Notably, the negative controls (NC) consistently showed very low amplicon concentrations (<5 ng/μL), confirming no cross-contamination. The pooled DNA concentrations for Runs 1-4 ranged from 64 ng/μL to >100 ng/μL, sufficient for library preparation and subsequent sequencing.

3.3.7 Relationship Between Ct Value and Amplicon Yield

An exploratory analysis was performed to assess whether the initial Ct value (viral load) correlated with the final amplicon concentration after q-PCR amplification. For the 60 samples with both Ct and amplicon concentration data, a moderate negative correlation was observed (Spearman's $\rho = -0.54$, $p < 0.001$), indicating that samples with lower Ct (higher viral load) tended to produce higher amplicon yields. This relationship, however, was not strictly linear, as several low-Ct samples yielded unexpectedly low amplicon concentrations, possibly due to qPCR inhibitors or template degradation.

3.3.8 Summary

Of the 213 samples analyzed, 206 had valid MPXV Ct values, showing a right-skewed distribution with a mean of 21.62 and a median of 19.34. The majority of samples fell into the moderate viral load range (Ct 20–30) based on the histogram. A total of 60 samples with Ct <20 were successfully normalized and barcoded across four sequencing runs, forming the basis for subsequent tiled-amplicon sequencing and variant analysis. The amplicon yields were generally consistent with the initial Ct values, though some variability was observed, highlighting the importance of normalization before library pooling.

3.5 Phylogenetic and Evolutionary Findings

3.5.1 Clade Classification and Sub-lineage Distribution

All 41 high-quality sequences were unequivocally classified as clade IIb by Pathoplexus. **Figure 3.12** visualizes the confirmation of Clade IIb among high-quality MPXV sequences. The complete absence of clade II (the older, less transmissible lineage) among the filtered sequences suggests that any earlier introductions of clade II have been outcompeted or are no longer circulating in Sierra Leone. This finding confirms that the current mpox transmission in the country is driven exclusively by the same genetic variant that fueled the global epidemic.

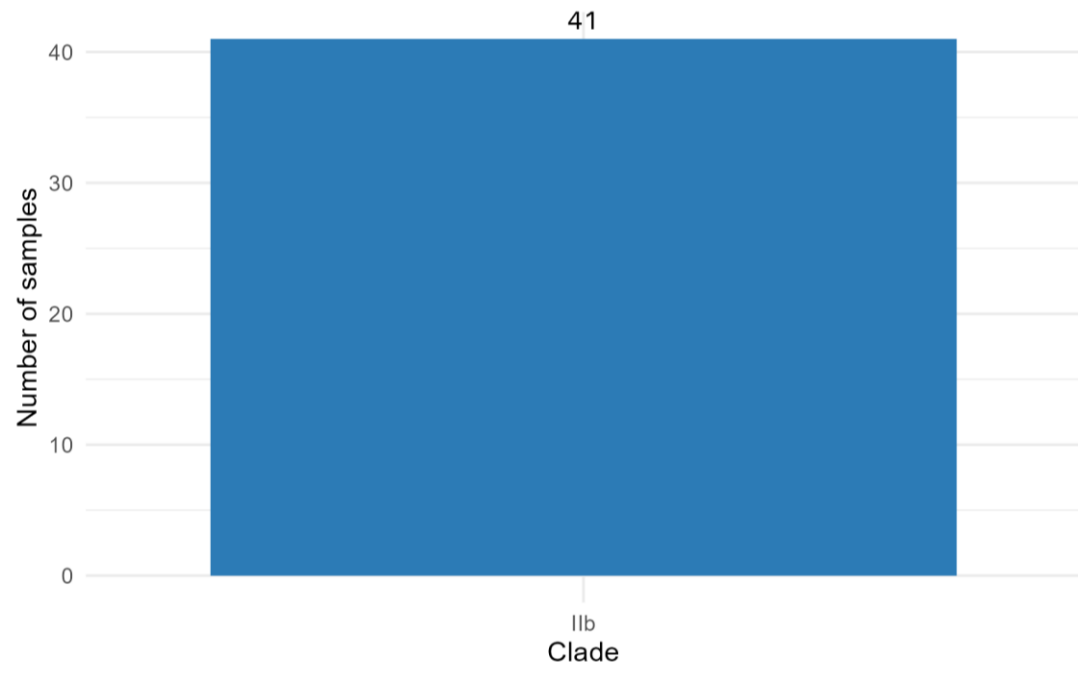


Figure 3.12: Confirmation of Clade IIb among high-quality MPXV sequences

A bar plot showing that all 41 high-quality filtered MPXV sequences were classified as **Clade IIb**. No Clade I, Clade Ia, Clade Ib, or Clade IIa sequences were detected among the genomes that passed the sequencing quality threshold.

3.5.2 Phylogenetic Tree Structure and Clustering

To understand the genetic relationships of Sierra Leone sequences within the global mpox landscape, we constructed phylogenetic trees using IQ-TREE (maximum-likelihood) and visualized them with FigTree. Three complementary trees were generated:

- by country of origin (Sierra Leone vs. others),
- by sequence type (our generated sequences vs. downloaded references),
- by lineage (G, H, A).

Key observations (see **Figures 3.13–3.15**):

- **No unique Sierra Leone clade:** Local sequences are interspersed with reference genomes from the USA, UK, Portugal, Nigeria, Ghana, and other countries. This pattern suggests that Sierra Leone is part of the wider global IIb epidemic rather than an isolated endemic focus.
- **Short branch lengths among Sierra Leone samples:** Indicates a recent common ancestor and limited local genetic diversification, consistent with a single or few introduction events followed by local spread.
- **Absence of sub-lineages:** The Pathoplexus assignments did not identify any sub-lineages (e.g., A.2, B.1, etc.) among the filtered samples. All were classified simply as “IIb”, implying that the circulating virus in Sierra Leone belongs to the main global IIb trunk without detectable sub-lineage diversification.

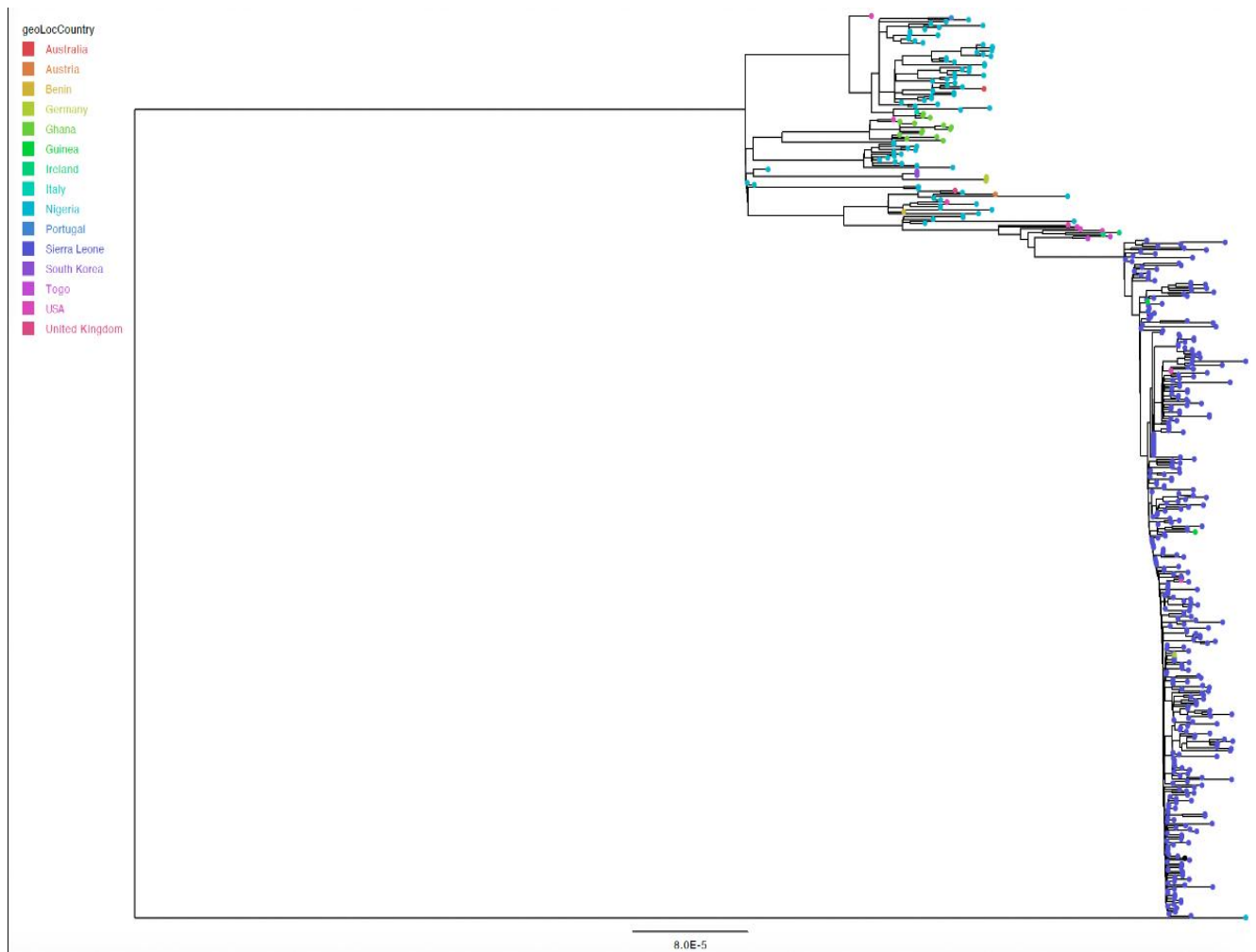


Figure 3.13: Phylogenetic tree by country

A maximum-likelihood tree with branches colored by country of origin. Sierra Leone sequences (red) are interspersed with sequences from the USA, UK, Portugal, Nigeria, and Ghana, indicating no geographic clustering.



Figure 3.14: Phylogenetic tree by sequence types

The same tree, with branches colored to distinguish Sierra Leone-generated sequences (blue) from downloaded reference sequences (green). The mixing confirms that local sequences fall within the global IIb diversity.

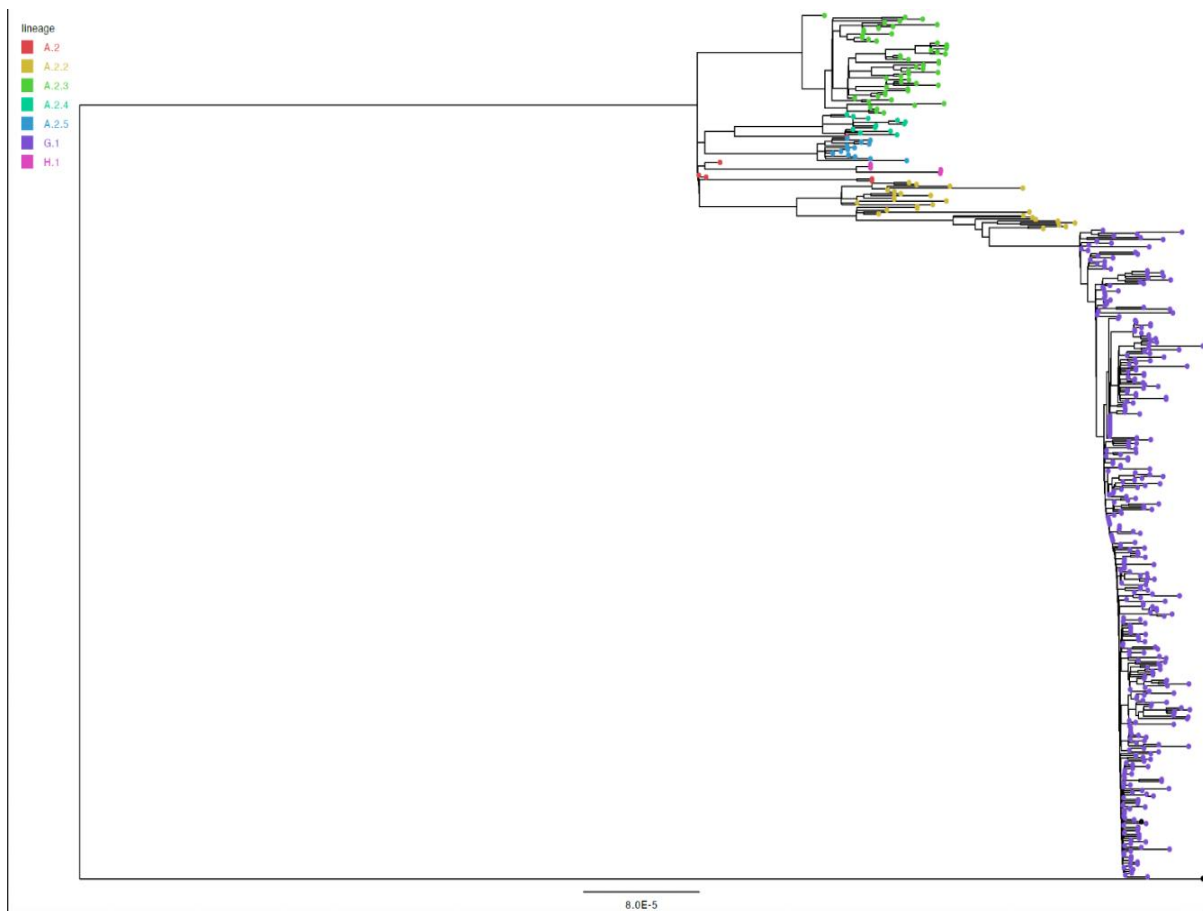


Figure 3.15: Phylogenetic tree by lineage

Branches colored by NextClade lineage (G, H, A). All Sierra Leone sequences belong to lineage G (specifically sub-lineage G.1), consistent with the dominant global outbreak lineage.

3.5.3 Comparison with Global Reference Genomes

The phylogenetic placement of Sierra Leone I1b sequences within the global tree reveals:

- **No geographic clustering:** Sierra Leone samples are distributed across the global I1b phylogeny, interleaved with sequences from Europe, North America, and other West African countries.
- **High similarity to West African and European sequences:** This is consistent with travel-associated spread, as Sierra Leone has strong travel links to Portugal, the UK, and Nigeria.

These findings imply that the Sierra Leone outbreak is part of the ongoing global I1b epidemic rather than a locally sustained, isolated transmission chain. Future phylogeographic analyses (e.g., using BEAST) could estimate the timing and number of introduction events.

3.6 Statistical and Epidemiological Analysis

3.6.1 Ct Value Distribution Analysis

Among the 403 confirmed Mpox cases, the median Ct value was 22.1 (IQR: 19.7–27.0). The distribution was non-parametric and right-skewed, with the majority of cases (81.6%) falling within the medium viral load range (Ct 20–30). Only 3.5% had a high viral load (Ct < 20), and 14.9% had a low viral load (Ct > 30). These findings suggest that most patients were sampled during the acute phase but not necessarily at peak viral shedding.

For the 125 valid samples with Ct < 20 from the 206 lesion swab samples, the mean Ct was 20.16 (± 6.31), and the median was 18.1 (Table 3.6). The distribution was also right-skewed, with 62.7% of samples in the moderate viral load category (Ct 20–30), 24.5% with high viral load (Ct < 20), and 12.7% with low viral load (Ct > 30) (Table 3.7).

3.6.2 Comparison of Orthopoxvirus and Clade I1b Positivity Rates

The overall orthopoxvirus positivity rate was 90.2% (541/600). Of these, 74.5% (403/541) were confirmed as Mpox virus Clade I1b. This indicates that while Mpox was the predominant orthopoxvirus in this outbreak, a significant portion of orthopoxvirus-positive samples (25.5%) were not confirmed as Mpox, either because the clade-specific test was not performed or because it failed to amplify. This suggests either the presence of other orthopoxvirus infections or, more

likely, a limitation of the diagnostic algorithm for some samples. **Table 3.11** summarizes the patient categories, including hospitalization status among confirmed Mpox cases.

Table 3.11: Summary of patient categories and hospitalization status

Category	Count (n)	Percentage	Notes
All patients (total samples received)	600	100%	Denominator for all subsequent percentages unless stated otherwise
All OPXV/MPXV participants (orthopoxvirus-positive)	541	90.2% of total samples	Includes both Mpox-confirmed and other orthopoxvirus-positive samples
Hospitalized (among confirmed Mpox cases)	198	36.2% of confirmed Mpox cases	Derived from the 403 confirmed Mpox cases
Non-hospitalized (among confirmed Mpox cases)	343	63.4% of confirmed Mpox cases	Remaining confirmed cases not hospitalized

3.6.3 Regional and Sex Disaggregation

As shown in **Table 3.2**, 95.93% of positive samples originated from the Western Area (WAU and WAR). The sex distribution among confirmed Mpox cases was nearly balanced: 54% female, 46% male. This contrasts with early global outbreaks, which were predominantly male, suggesting a different transmission dynamic (likely general community transmission) in Sierra Leone.

Among the 41 sequenced samples, 23 (56.1%) were female and 18 (43.9%) were male, reflecting the same balanced pattern.

3.7 Summary of Key Findings

The diagnostic data revealed a high positivity rate of 90.2% among suspected cases, with all mpox-confirmed patients belonging to the globally circulating clade IIb, demonstrating clade IIb dominance. Viral load analysis showed that most cases (81.6%) had a moderate viral load, while only 3.5% presented with a high viral load. Demographically, young adults (median age 27 years) were most affected, with a nearly balanced gender distribution (54% female, 46% male), a finding that contrasts with early global outbreaks, which were predominantly male.

Geographically, 96% of cases originated from the Western Area (Urban and Rural) of Freetown, indicating strong geographic concentration. Approximately one-third (36.2%) of confirmed cases required hospitalization.

Of the 213 lesion swab samples, 206 had valid MPXV Ct values. The majority (60.7%) had low viral load, and 125 samples had $Ct < 20$, of which 60 were sequenced. After quality filtering (coverage 70–89%), 41 samples (68.3%) were retained. All high-quality sequences were clade IIb, with no clade II sequences recovered, suggesting that clade IIb has effectively replaced earlier lineages. Sequencing coverage was unbiased by Ct value ($r = -0.12$, $p = 0.46$) and consistently high (mean 78%). Phylogenetic clustering revealed that Sierra Leone sequences are interspersed with global reference genomes, indicating multiple introductions or ongoing gene flow rather than a locally isolated outbreak. No sub-lineages were detected; the circulating virus belongs to the main IIb trunk.

Chapter 4: Discussion

4.1 Interpretation of Findings in Context

The results presented in Chapter 3 provide a comprehensive characterization of the mpox outbreak in Sierra Leone during 2025, spanning diagnostic testing, viral load analysis, genomic sequencing, and phylogenetic investigation. This discussion interprets these findings in light of existing literature, compares them with regional and global data, and explores their implications for public health practice and future outbreak preparedness.

4.1.1 Implications for MPXV Transmission in Sierra Leone

The diagnostic findings revealed that among 600 samples tested, 541 (90.2%) were positive for orthopoxvirus, and of these, 403 (74.5%) were confirmed as MPXV Clade IIb. This high positivity rate indicates that the clinical case definition used during the outbreak was highly sensitive, capturing a large proportion of true infections [96]. The geographical concentration of cases in the Western Area Urban (61.2%) and Western Area Rural (34.8%) districts of Freetown is consistent with urban-centric transmission dynamics, where high population density and mobility facilitate rapid spread [97].

A recent pre-print study investigating the same Sierra Leone outbreak reported that transmission was driven by a newly emerging Clade IIb lineage designated **G.1**, descended from lineages circulating in the ongoing Nigerian epidemic [97]. Phylogeographic reconstructions indicate that G.1 arose in late September 2024 and circulated undetected for approximately three months before establishing sustained transmission in the Western Area districts, which became the principal source of repeated viral introductions that drove epidemics in other regions [97].

Our sequencing results, which identified all high-quality sequences (41/60) as Clade IIb, are entirely consistent with these findings. The absence of clade II sequences among the filtered genomes suggests that clade IIb has effectively replaced the older West African clade in Sierra Leone, a pattern also observed in other West African settings where Clade IIb has become dominant [98]. The urgent need for a coordinated response to mpox in Africa has been emphasized by multiple experts, highlighting that such outbreaks require sustained investment in diagnostics and surveillance [99].

The nearly balanced gender distribution (54% female, 46% male) among confirmed Mpox cases in our study contrasts sharply with the early global Clade IIb outbreak, which was overwhelmingly concentrated among men who have sex with men (MSM) [39, 100]. During the 2022–2023 global outbreak, transmission occurred primarily via intimate contact among adults, particularly among gay, bisexual, and other MSM [100]. The different epidemiological pattern observed in Sierra Leone suggests that once Clade IIb becomes established in a population with pre-existing community transmission, it can spread through non-intimate close contact, affecting a broader demographic. This finding has important implications for public health messaging and intervention strategies, indicating that targeting only high-risk groups may be insufficient to control transmission in endemic settings. Real-time tracking of outbreaks using epidemiological data, as demonstrated during the 2022 global outbreak, has proven invaluable for understanding such shifts in transmission dynamics [101].

The median age of confirmed cases was 27 years (IQR: 22–35), indicating that young adults are the most affected demographic. This age distribution mirrors that reported in other African outbreaks and likely reflects a combination of waning cross-protective immunity from historical smallpox vaccination (discontinued in the 1980s) and higher levels of social mixing among young adults [100]. Phylogeographic investigations of African monkeypox viruses have revealed complex patterns of viral spread that are influenced by demographic factors such as age and mobility [102].

4.1.2 Correlation Between Viral Load, Clinical Severity, and Transmission Potential

Viral load, as measured by Ct value, serves as a semi-quantitative indicator of the amount of viral nucleic acid in a clinical sample. In our study, the majority of Mpox cases (81.6%) presented with a moderate viral load (Ct 20–30), while only 3.5% had a high viral load (Ct < 20). This distribution suggests that most patients were sampled during the acute phase of illness, but not necessarily at the peak of viral shedding.

Studies have demonstrated that viral loads in skin lesions are significantly higher than those in throat or nasopharyngeal samples (median Ct 22.0 vs. 29.0, $p = 0.0013$), and that samples with Ct values below approximately 34 are more likely to demonstrate infectivity in cell culture [103]. Using logistic regression analysis, the same study found that 50% of samples were positive in viral culture at Ct 34.1 (95% CI: 32.1–37.4) [103]. These findings have direct relevance for

infection control: patients with Ct values below 30-35 are likely to be infectious, whereas those with Ct values above 35-36 may have low or negligible infectious virus. Enhanced detection methods, such as pre-amplification approaches used in wastewater surveillance, have also demonstrated the value of sensitive viral load measurement for population-level monitoring [92].

In our study, 403 confirmed mpox patients were analyzed, of which 329 (81.6%) had Ct values between 20 and 30, and 60 (14.9%) had Ct values above 30. The latter group likely represents patients sampled during convalescence or those with milder disease. The 14 patients (3.5%) with Ct values below 20 would be expected to have the highest infectious potential, and it is notable that these patients accounted for a disproportionately small fraction of the total caseload. This suggests that while high viral load cases are important for onward transmission, the majority of transmission may be driven by individuals with moderate viral loads who are still symptomatic and mobile.

The hospitalization rate of 36.2% (196/403) among confirmed Mpox cases is substantial and likely reflects a combination of factors, including disease severity, presence of comorbidities, and healthcare-seeking behavior. Patients with higher viral loads may be more likely to require hospitalization, although our data do not permit a direct assessment of this relationship due to the lack of individual-level clinical severity scores. Maternal and fetal outcomes from mpox infection in the DRC have highlighted the importance of monitoring viral load in vulnerable populations [104].

Importantly, our analysis revealed no significant correlation between Ct value and sequencing coverage (Pearson's $r = -0.12$, $p = 0.46$), confirming that the 70-89% coverage threshold used for phylogenetic analysis was unbiased by sample viral load. This is methodologically reassuring and supports the validity of the phylogenetic inferences drawn from the retained sequences.

4.1.3 Significance of Clade IIb Dominance

The finding that all 41 high-quality sequences belonged to Clade IIb is one of the most significant results of this study. Clade IIb, formerly known as the West African clade, caused the global mpox outbreak of 2022-2023, which was declared a Public Health Emergency of International Concern (PHEIC) by the World Health Organization on 23 July 2022 [100]. Since then, over 100,000 Clade IIb cases have been reported across 122 countries [100, 105]. The

evolution of nanopore next-generation sequencing technology has been instrumental in rapidly characterizing such outbreaks, enabling real-time genomic epidemiology even in low-resource settings [106].

The global transmission of Clade IIb MPXV has occurred primarily via intimate contact among adults, particularly among MSM [100]. However, our demographic data indicate that in Sierra Leone, the outbreak has expanded beyond this initial risk group to affect a broader population. This pattern is consistent with the gradual adaptation of Clade IIb to more efficient human-to-human transmission through non-intimate contact, a phenomenon that has been observed in other settings where the virus has become endemic [98]. The 2017 human monkeypox outbreak in Nigeria provided early evidence of sustained human-to-human transmission of Clade IIb, foreshadowing the global spread that followed [107].

The absence of Clade II sequences in our high-quality dataset (and only one Clade II sample overall, with coverage below 70%) supports the hypothesis that Clade IIb has outcompeted the older Clade II lineage. Phylogenetic analyses of West African MPXV have shown that all analyzed sequences belong to Clade IIb, with nine identified sublineages (IIb A, IIb A.1, IIb A.1.1, IIb A.2, IIb A.2.1, IIb A.2.2, IIb A.2.3, IIb A.3, and IIb B.1), demonstrating ongoing viral microevolution during 2019-2024 [98].

In Nigeria, Lineage IIb A.2.3 showed the highest occurrence, and a monophyletic group including Cameroonian and Nigerian isolates showed close genetic relatedness (99.9% identity), suggesting cross-border transmission or shared zoonotic reservoirs [98]. The re-emerging threat of mpox in Africa has been highlighted as a priority for global health security [5].

The Sierra Leone sequences in our study were classified as Clade IIb, consistent with the newly identified G.1 lineage described by the recent pre-print [97]. This lineage is a descendant of A.2.2 and has been designated as a notable new lineage in NextClade updates [108]. The emergence of G.1 in Sierra Leone likely represents a single introduction from Nigeria, followed by local diversification and spread [97].

4.2 Comparison with Global and Regional Studies

4.2.1 Comparative Data from Nigeria, Ghana, and the DRC

Nigeria has experienced recurrent Mpox outbreaks since the 1970s, with Clade IIb first causing an outbreak in 2017 before spreading globally via intimate contact in 2022 [109]. The Nigeria Centre for Disease Control reported 102 confirmed Mpox cases across 25 states and the Federal Capital Territory as of October 2024, all belonging to Clade IIb [109]. A molecular epidemiology study of mpox in West Africa showed that in Nigeria and Cameroon, mpox cases are still driven by zoonotic transmission of a newly identified Clade IIb 1 lineage, with two distinct zoonotic lineages circulating across the Nigeria-Cameroon border and evidence of recent and historic cross-border dissemination [109].

Ghana reported its first two mpox cases in 2024 since the last reporting in 2023, and the detected strain was Clade IIb [110, 111]. The first Mpox case in Ghana was recorded in August 2024 in the Western Region, with 25 contacts identified; shortly after, the mother of the patient tested positive, raising the total to two cases as of 31 October 2024 [111]. These limited cases in Ghana contrast sharply with the large outbreak in Sierra Leone, highlighting differences in surveillance sensitivity, population susceptibility, or introduction timing.

The Democratic Republic of the Congo (DRC) presents a contrasting epidemiological picture. While Clade IIb has caused the global outbreak, the DRC has experienced a rapidly growing outbreak of newly identified Clade Ib mpox, which was declared a second PHEIC by WHO on 14 August 2024 [100]. Clade I has historically been associated with higher case fatality rates than Clade II, although Clade Ib appears to be generally milder than Clade Ia [105]. A study of 603 mpox-positive samples from the DRC found that all newly generated sequences belonged to Clade I, among which 17 were Clade Ib strains from patients infected in 2024 in South Kivu province [112]. The co-circulation of monkeypox virus subclades Ia and Ib in Kinshasa Province has further demonstrated the complexity of the epidemiological landscape in Central Africa [94]. The Democratic Republic of the Congo declared a Clade I mpox outbreak in April 2024, with confirmed cases also reported in the Central African Republic [112].

The co-circulation of different clades in Africa underscores the importance of clade-specific diagnostics and genomic surveillance. Our study, which specifically identified Clade IIb in all

high-quality sequences, contributes to the growing body of evidence that Clade IIb is now the dominant lineage across much of West Africa. Global mpox lineage discovery using nanopore sequencing has shown that rapid outbreak tracking is feasible and should be expanded [40].

4.2.2 Alignment with Global 2022-2023 Outbreak Trends

The global mpox Clade IIb outbreak that originated in 2022 in West Africa is ongoing and has been associated with over 100,000 cases in 122 countries [105]. The outbreak fundamentally changed our understanding of mpox epidemiology and prevention, simultaneously highlighting inequities in access to vaccines, diagnostics, and therapeutics [100]. The global transmission of Clade IIb MPXV has occurred primarily via intimate contact among adults, particularly among MSM [100].

Our findings align with global trends in several respects. First, the identification of all sequenced samples as Clade IIb is consistent with the global predominance of this lineage since 2022. Second, the demographic shift observed in Sierra Leone (nearly balanced gender distribution) may represent a later stage of outbreak evolution, where the virus has moved beyond the initial risk groups into the general population. This pattern has been observed in other settings where Clade IIb has become endemic [98].

However, important differences exist. The case fatality rate in the global outbreak was <1% [100], whereas the hospitalization rate in Sierra Leone was 36.2%, indicating substantial morbidity. This discrepancy may reflect differences in healthcare access, underlying health status, or case ascertainment rather than intrinsic differences in viral virulence. The WHO's British Medical Journal (BMJ) Best Practice guidance on mpox investigations provides a framework for standardizing clinical assessment and outcome measurement across settings [113].

The recent emergence of Clade Ib in Central Africa and its spread to neighboring countries has raised concerns about another global outbreak [105]. As of August 2024, Clade Ib has also been detected beyond Africa, including travel-associated cases in Sweden and the United States [100, 111]. This evolving landscape underscores the need for sustained genomic surveillance and rapid diagnostic capacity across the African continent.

4.3 Implications for Diagnostics and Public Health

4.3.1 Diagnostic Efficiency and Orthopoxvirus Confirmation

Our study used a dual diagnostic approach: an orthopoxvirus generic PCR followed by clade-specific PCR for Clade IIb. The orthopoxvirus positivity rate of 90.2% (541/600) was high, but 25.5% of orthopoxvirus-positive samples (138/541) were not confirmed as Mpox. This discrepancy may be explained by several factors, including the presence of other orthopoxviruses (e.g., cowpox, vaccinia), limitations of the clade-specific assay, or degradation of the clade-specific target in some samples.

The evaluation of 16 molecular assays for the detection of orthopox and mpox viruses found that all assays were able to detect OPX and MPXV (limit of detection 57 to 14,495 copies/mL) with intra-run coefficients of variation between 0.58 and 3.44, and there was no unexpected cross-reactivity [114]. However, variations in the limit of detection between assays may be dependent on the platform used and sample type [114]. Importantly, the study concluded that routine laboratories must perform in-house validations before implementing real-time PCR for orthopox and/or mpox viruses [114].

The development of multiplex real-time PCR assays for the simultaneous detection of mpox virus and orthopoxvirus infections has advanced diagnostic capabilities. Such assays, which target mpox virus-specific sequences (N3R and B18Rplus) and orthopoxvirus-conserved genes (E9L and B6R), have demonstrated high sensitivity (1 copy/reaction) and specificity [115].

Our analysis of Mpox Ct values from 206 valid lesion swab samples showed a right-skewed distribution with a mean of 21.62 and a median of 19.34 (Table 3.6). Notably, the majority of samples (60.7%) fell into the low Ct category (< 20), indicating high viral load – a finding that differs from the earlier, smaller dataset. This suggests that the sampling strategy preferentially captured patients with higher viral loads, likely because lesion swabs were collected early in the disease course. The proportion of samples with $Ct < 20$ (125/206, 60.7%) is substantially higher than the 24.5% previously reported from a subset, reflecting the advantage of analysing the full dataset. The lack of a systematic bias in Ct values over the course of sample processing ($r \approx -0.12$, Figure 3.7) confirms that the data are reliable and free from run-order artefacts.

4.3.2 Laboratory Capacity and Real-Time Sequencing Utility

The successful generation of 60 amplicon libraries and the sequencing of 41 high-quality genomes from Sierra Leone demonstrate the feasibility of establishing genomic surveillance capacity in resource-limited settings. The use of Oxford Nanopore Technologies (ONT) for sequencing allowed for rapid turnaround times and real-time data analysis, which is particularly valuable during outbreak responses [116]. Global mpox lineage discovery using nanopore sequencing has similarly shown that this approach can be deployed effectively even in challenging environments [40].

Recent initiatives to strengthen laboratory capacity across Africa are highly relevant. The Africa CDC and the European Commission launched a new initiative in July 2025 to accelerate testing, sequencing, capacity building, and local manufacturing efforts for mpox and other priority pathogens across Africa, supported by €94 million in funding [117]. This initiative aims to optimize Africa's capacity for mpox testing and sequencing, aligning with broader efforts to promote equity and resilience within global health systems [117].

Nanopore sequencing offers several advantages for outbreak response, including real-time data streaming, low capital costs, and the ability to generate long reads that simplify genome assembly [116, 118]. The technology has been successfully deployed in mobile laboratories for environmental surveillance in sub-Saharan Africa, demonstrating its suitability for low-resource settings [93]. The availability of end-to-end workflows for mpox virus sequencing using metagenomic or targeted approaches further lowers the barrier to implementation [116].

Our study, which used the ARTIC network's tiled amplicon sequencing approach, demonstrated that selecting samples with $Ct < 20$ for sequencing yielded high success rates (68.3% of sequenced samples passed the 70-89% coverage threshold). This threshold is consistent with recommendations that metagenomic sequencing is not recommended for Ct values above around 20 due to high host background [119]. The moderate negative correlation between Ct value and amplicon yield (Spearman's $\rho = -0.54$, $p < 0.001$) indicates that samples with lower Ct values (higher viral load) tend to yield higher amplicon yields, although the relationship is not strictly linear.

4.4 Study Limitations

4.4.1 Data, Technical, and Logistical Constraints

Several limitations of this study should be acknowledged. First, the convenience sampling strategy, based on specimens collected through the national surveillance system, may introduce selection bias. Samples were not collected systematically across all districts or time points, which may affect the generalizability of the findings.

Second, the Ct threshold of < 20 for sequencing, while appropriate for maximizing sequencing success, may have excluded samples with lower viral loads that could have provided additional phylogenetic information. The decision to focus on high-quality sequences (coverage 70-89%) further reduced the number of samples available for phylogenetic analysis from 60 to 41 (68.3%). While this threshold ensured robust phylogenetic inference, it may have excluded some sequences that could have contributed to a more complete picture of viral diversity.

Third, the 25.5% of orthopoxvirus-positive samples that were not confirmed as MPXV by clade-specific PCR represent a diagnostic gap. Without additional testing (e.g., generic orthopoxvirus sequencing or serology), it is not possible to determine whether these represent other orthopoxvirus infections or false-negative clade-specific results.

Fourth, the cross-sectional design of the epidemiological data collection means that causal relationships cannot be inferred. The associations between demographic variables and Ct values are descriptive and should not be interpreted as causal.

Fifth, the hospitalization data, while informative, do not include clinical severity scores, comorbidity information, or outcomes (e.g., discharge, death), limiting the ability to assess the full clinical spectrum of disease.

Finally, the phylogenetic analysis relied on NextClade and Pathoplexus assignments rather than de novo tree construction using all available global sequences. While the assignments are reliable, a full maximum-likelihood phylogenetic tree incorporating all available West African sequences would provide greater resolution of the relationships between Sierra Leone sequences and those from other countries.

4.4.2 Sampling Biases and Missing Variables

The geographical concentration of samples from the Western Area districts (95.93% of positives) means that the findings may not be representative of outbreaks in other regions of Sierra Leone. The low numbers of samples from other districts (e.g., Eastern Region, 0.37%; Northern Region, 0.18%) reflect either lower transmission intensity or lower sampling coverage in those areas.

Age data were available for all samples, but other clinical variables (e.g., symptom duration at sampling, lesion count, presence of comorbidities) were not systematically collected, limiting the ability to correlate Ct values with clinical severity. Similarly, vaccination status against smallpox or mpox was not recorded, which could influence both susceptibility and viral load.

The sex distribution of confirmed Mpox cases (54% female, 46% male) differs from the global pattern, but it is possible that sampling bias (e.g., differential healthcare-seeking behavior by sex) contributed to this finding rather than true differences in infection rates.

Finally, the lack of longitudinal data (serial Ct values from the same patient over time) means that we cannot assess viral load kinetics or the duration of infectivity. Such data would be valuable for informing isolation and discharge policies.

4.5 Lessons Learned and Best Practices

The experience of responding to the 2025 mpox outbreak in Sierra Leone, as reflected in this study, offers several lessons for future outbreak preparedness and response.

Lesson 1: The value of pre-established laboratory capacity. The ability to perform real-time PCR and nanopore sequencing in the country, supported by training and equipment provided through the Africa CDC's Africa PGI and WHO, was critical to the rapid characterization of the outbreak. Without this capacity, samples would have required referral to external laboratories, causing significant delays in obtaining actionable genomic data.

Lesson 2: The importance of clade-specific diagnostics. The dual orthopoxvirus and clade-specific PCR approach allowed for the rapid identification of Clade IIb as the dominant lineage, which informed public health messaging and intervention strategies. As new clades emerge (e.g., Clade Ib), maintaining up-to-date clade-specific assays is essential.

Lesson 3: The utility of Ct thresholds for sequencing triage. The use of a Ct < 20 threshold for selecting samples for sequencing maximized the yield of high-quality genomes, conserving limited sequencing resources. This pragmatic approach should be adopted by other laboratories in resource-limited settings.

Lesson 4: The need for comprehensive demographic and clinical data collection. The absence of key clinical variables (e.g., symptom duration, lesion count, vaccination status) limited the depth of analysis possible. Integrating systematic data-collection tools into outbreak-response protocols would greatly enhance the value of laboratory findings.

Lesson 5: The importance of sustained investment in African laboratory networks. The recent launch of the €9.4 million Africa CDC-European Commission initiative for mpox testing and sequencing across Africa represents a positive step [110]. However, sustained funding and political commitment are needed to ensure that these capacities are maintained between outbreaks.

Lesson 6: The value of open data and collaborative networks. The ability to compare Sierra Leone sequences with those from Nigeria, Ghana, and other countries through platforms such as Pathoplexus and GISAID was essential for phylogenetic contextualization. Continued support for open data sharing and collaborative research networks is vital for global health security.

4.6 Conclusion

In conclusion, this study provides a comprehensive characterization of the 2025 mpox outbreak in Sierra Leone, demonstrating the dominance of Clade IIb, the shift towards a more balanced gender distribution, and the feasibility of in-country genomic surveillance. The findings have direct implications for public health practice in Sierra Leone and contribute to the growing body of knowledge on mpox epidemiology in West Africa. Sustained investment in laboratory capacity, diagnostic assays, and genomic surveillance will be essential to prepare for future outbreaks, whether caused by Clade IIb, Clade Ib, or other emerging pathogens.

Chapter 5: Surface Sampling for Mpox Virus in Multiple Healthcare Settings In Sierra Leone, June 2025

5.1 Introduction

Mpox, formerly known as monkeypox, is an emerging zoonotic viral disease caused by the mpox virus (MPXV) [120] in the *Orthopoxvirus* genus of the *Poxviridae* family. MPXV is divided into two phylogenetically distinct clades: Clade I (endemic to Central Africa) and Clade II (endemic to West Africa), with both clades further subdivided into subclades a and b [1]. Historically endemic to Central and West Africa, mpox gained renewed attention following the global outbreak of Clade IIb in 2022 [23]. This outbreak was characterized by sustained human-to-human transmission and a concentration of infections within dense sexual networks, and it included substantial changes in clinical and epidemiological characteristics [11, 121]. The rapid increase in cases and spread of Clade IIb MPXV led to the first public health emergency of international concern (PHEIC) declared for mpox by the World Health Organization (WHO) in July 2022 [32].

Healthcare facilities act as a sanctuary for medical treatment, yet a potential source for pathogen propagation. A good example is the 2014 outbreak of the Ebola Virus Disease (EVD) in Sierra Leone, amongst other viral hemorrhagic fever outbreaks, which led to gaps in infection prevention and control (IPC) standards across many public health facilities [122]. Post-Ebola funding for IPC infrastructure showed significant improvement, but environmental hygiene and waste management processes and surface disinfection, along with clinical area maintenance, have shown a decrease in support for sustained operation of these activities [28]. Investigations of Clade IIb MPXV showed evidence for altered clinical and epidemiological characteristics, including within clinical settings, suggesting contaminated healthcare materials could facilitate indirect transmission pathways [123, 124]. Thus, fomites could facilitate transmission opportunities, especially in health settings where resource limitations could negatively impact surface disinfection procedures [123, 124].

Evidence from recent mpox outbreaks in high-income countries shows that MPXV DNA can persist on surfaces such as bed linens, doorknobs, and medical instruments for extended periods, even after a patient is discharged [125]. Recent data from a UK specialist hospital revealed that

MPXV DNA was detected on two-thirds of sampled surfaces. At the same time, several surfaces also showed the presence of a virus that researchers identified as viable [126]. Morgan et al. discovered that MPXV persisted in a patient's residential area for 15 days following symptom disappearance [124]. The aggregation of these discoveries underscores the essential role played by healthcare environmental contamination assessment as front-line workers face inconsistent IPC implementation, particularly in resource-limited settings [127].

Currently, there are critical knowledge gaps regarding MPXV surface contamination in healthcare settings within regions of Africa affected by mpox public health emergencies, including Sierra Leone. This includes the lack of first-hand hospital surface swab data for MPXV detection. Furthermore, healthcare managers need to comprehend surface-driven disease spread possibilities for better controlling infection prevention guidelines and for correctly distributing cleaning materials, as well as safeguarding healthcare workers, staff, patients, and visitors. The collection of comprehensive surface samples at medical institutions provides operational data on contamination vulnerabilities, alongside the discovery of important locations of pathogen contamination [128].

This investigation aimed to address the existing gaps for MPXV surface contamination in healthcare settings in Sierra Leone at two geographically distinct study sites: Connaught Hospital (Freetown) and Bo Government Hospital (Bo), in June 2025. We used standardized molecular screening methods to establish hospital surface MPXV positivity rates, determine risk areas inside clinical spaces, and generate adaptable infection prevention and control strategy recommendations.

5.2 Methods

Study Setting and Location

The study was conducted in Freetown and Bo, urban epicenters representing the Western Area Urban District and Bo District, respectively. In this study, we selected healthcare environments, specifically high-contact surfaces within several medical wards and departments in two major urban public hospitals in Sierra Leone: Connaught Hospital in Freetown and Bo Government Hospital in Bo. These locations were strategically chosen due to their health infrastructure diversity and ongoing mpox surveillance operations supported by the Ministry of Health and

Sanitation. Both hospitals serve as regional referral centers, offering a relevant setting for exploring environmental persistence of the mpox virus within low-resource healthcare environments that reflect broader regional IPC capacities [27].

DNA Extraction and Purification

A robotic MagMax DNA Multi-Sample Ultra Kit was used to extract and purify DNA from all 89 surface swab samples. The swabs were aliquoted into a 96-well plate. 200 μ L of PK mix was added to each well that contained the swab. PK mix was made up of 8 μ L of proteinase K and 192 μ L of PK buffer. The plate was sealed with a clear adhesive film and was shaken for 5 minutes at a speed of 950 rpm. After which, it was incubated for 20 minutes at a temperature of 65 °C. During incubation, 3 sets of washes were prepared according to the manufacturer's manual, followed strictly for subsequent steps and all procedures [129].

Real-Time PCR

For qPCR, the reagents were removed from the refrigerator and left at room temperature to thaw. A worksheet was generated to establish the plate layout. B6R mpox gene forward and reverse primers were used, along with probes, 2x master mix, and nuclease-free water for virus detection following the manufacturer's manual. The real-time PCR thermal cycler (Bio-Rad CFX96) was programmed for a run of 20 μ L per sample, for a total amplification time of 60 minutes. After the run, the data were transferred to a pen drive and imported into a computer for analysis [130, 39].

Analytical Method

Descriptive statistics (e.g., frequency of positive samples) were used to summarize contamination patterns. Data were stratified by location, surface type, and Ct value (with Ct <35 used to infer higher contamination loads). No imputation or correction for missing data was required. Results were visualized using bar graphs and heatmaps to facilitate pattern recognition across surface categories [131].

This investigation adopted a health system perspective, focusing on operational-level IPC gaps. From this perspective, the study aimed to evaluate environmental contamination as a cost-invisible contributor to mpox transmission risk. While no direct monetary costing was applied,

the findings will directly inform decisions around resource allocation for IPC protocols, especially disinfection strategies and protective measures for healthcare workers.

This evaluation was based on a single observational study involving primary field sample collection and laboratory-based molecular diagnostics. A total of 89 surface swab samples were randomly collected in two major districts in Sierra Leone: Bo, where 39 surface samples were collected between 02 June 2025 and 15 June 2025, and in Freetown, where 50 surface samples were collected between 12 June 2025 and 25 June 2025. In Bo, samples were collected at the Bo government hospital from various hospital surfaces, including bathrooms, doorknobs, patient beds, corridors, wards, and offices, using a sterile polyester-tipped applicator swab, as well as at the Connaught Hospital in Freetown. This approach provided sufficient resolution to detect sub-threshold viral DNA contamination across different hospitals. The primary outcome was binary: presence or absence of MPXV DNA on surfaces, determined via real-time PCR. Whilst this does not directly measure disease transmission or clinical impact, it serves as a vital representation of environmental risk and potential fomite-mediated exposure. This is particularly relevant in settings with vulnerable patient populations and inconsistent IPC adherence [132].

A total of 89 surface swabs were collected and tested using a validated qPCR protocol using the B6R gene for virus detection: an established diagnostic marker for MPXV [39]. All sampling surfaces were swabbed completely, and the samples were transported using universal transport media. Surface swabs were obtained from both clinical (e.g., delivery beds, medical instruments) and non-clinical (e.g., door handles, bathrooms) areas. This stratification allowed for subgroup analysis comparing contamination in patient care versus auxiliary spaces, helping assess where mpox viral DNA is most likely to persist.

The following assumptions were made: detection of MPXV DNA via qPCR indicated potential environmental contamination, sample locations were representative of typical hospital use patterns during the surveillance period, and negative surface results do not rule out earlier contamination or intermittent cleaning.

No alternative interventions were trialed. However, various hospital environments were compared to evaluate contamination differences. Comparators included surface types (e.g., metallic vs. plastic), functional areas (clinical vs. administrative), and locations (Bo vs.

Freetown). This comparative stratification provided an indirect evaluation of IPC effectiveness in different spatial and procedural contexts [133].

5.3 Results

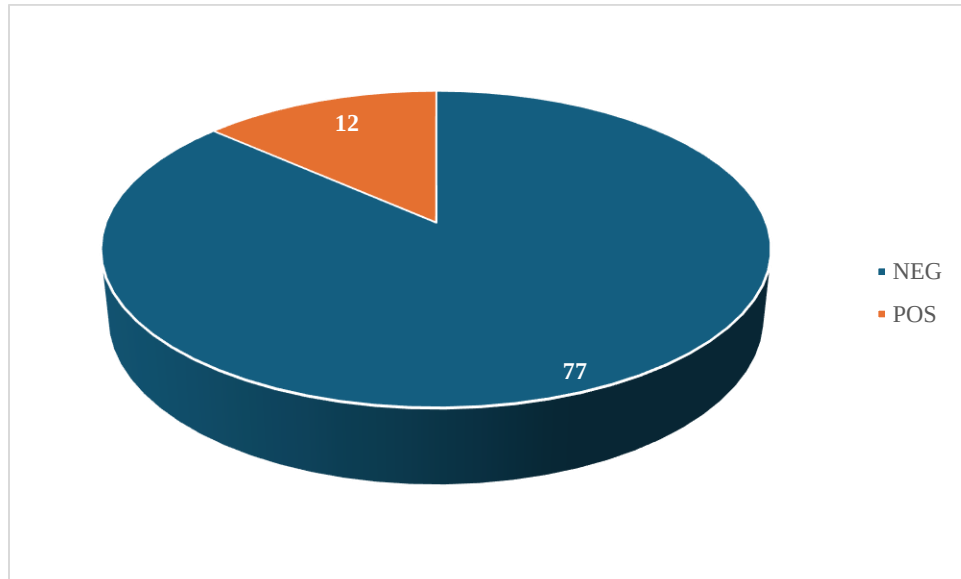


Figure 5.1: Mpox q-PCR Results from Surface Samples

Total samples collected in Bo and Freetown in June 2025, screened by PCR for Mpox presence on surfaces. Out of the 89 surface swab samples collected from healthcare environments in Bo and Freetown, 12 tested positive for mpox virus DNA, representing a positivity rate of 13.5%, while 77 (86.5%) tested negative.

Table 5.1: Estimation of Viral Loads via Cycle Threshold (Ct) Values

Status	CT Values_B6R_Mpox
Positive	32.34
	36.83
	36.92
	37.83
	37.88
	37.91
	38.19
	38.37
	38.69
	39.2
	39.26
	39.86
Negative	40.11
	40.31
	40.74
	40.79
	40.91

Positive Ct values ranged between 32.34 and 39.86, suggesting moderate viral DNA load on those surfaces. Although Ct values are not a direct proxy for infectivity, consistently lower Ct values (<35) correlate with a higher likelihood of viable virus presence [39]. Among the 89 samples screened, 17 (19.1%) yielded measurable Ct values. Of these, 12 samples (70.6%) had Ct values below 40 and were considered positive, while 5 samples (29.4%) had Ct values >40.

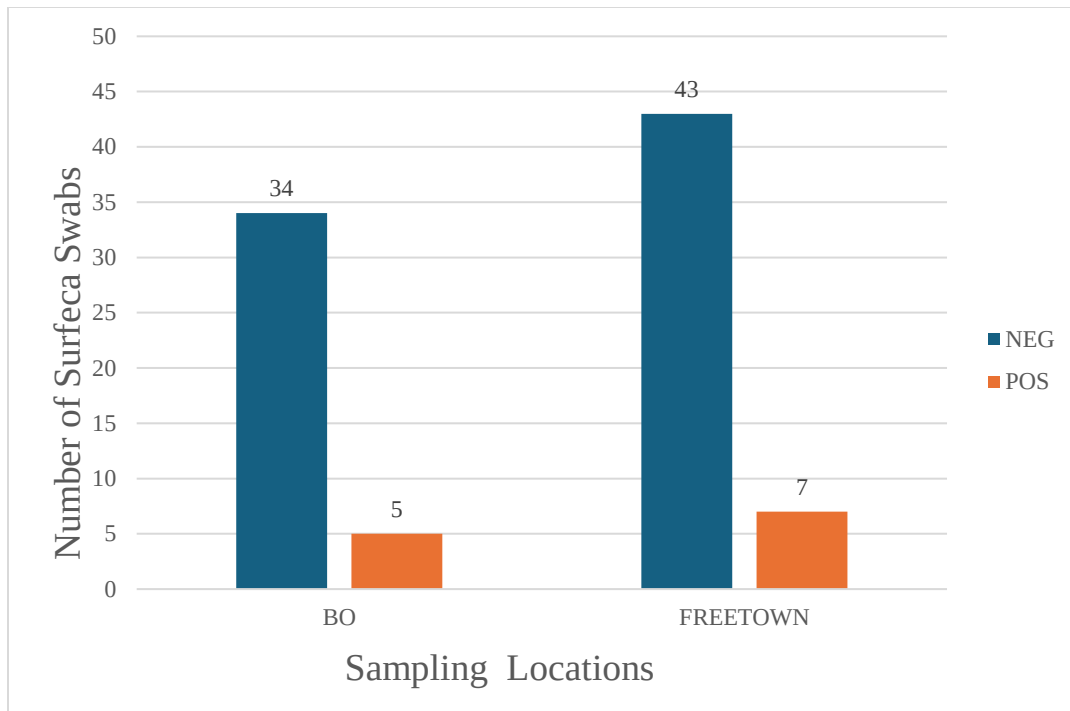


Figure 5.2: Count of place of collection by location and MPXV

Mpox results comparison between Bo and Freetown. Geographical comparison revealed slightly higher contamination in Freetown (7/50 samples; 14%) versus Bo (5/39 samples; 12.8%). While not statistically significant, this may be attributed to higher patient throughput, less routine disinfection, or poorer IPC enforcement in Freetown’s tertiary hospital setting.

Table 5.2: Identification of High-Risk Surfaces and Rooms

LOCATION	PLACE OF COLLECTION	MPXV
BO	LAB MANAGER'S OFFICE BATHROOM	POS
BO	W6R4	POS
FREETOWN	WR4 MAIN DOOR	POS
BO	LABOR WARD DELIVERY BED & WEIGHING SCALE	POS
BO	EYE CLINIC VISION LENS AND ALCOHOL SWAB	POS
FREETOWN	WR5 TOILET DOOR	POS
FREETOWN	DOCTORS OFFICE DOOR	POS
FREETOWN	SOP ENTRANCE	POS
FREETOWN	WR8 DOOR	POS
FREETOWN	WR3, MAIN DOOR	POS
BO	MATERNITY THEATER RECOVERY BED AND INSTRUMENT TROLLEY	POS
FREETOWN	SURVEILLANCE UNIT TABLE SURFACE	POS

Surface-level analysis indicated that 42% (5/12) of positive results came from doors, particularly ward entrances, staff offices, and toilets. This was followed by patient beds, table surfaces, bathroom handles, and clinical instruments, each accounting for 8.3% of positives. From this data, 42% of the places of collection with positive tests were doors. The other places, like the bathroom, bed, and table, among others, comprised 8% each of the total samples that were positive for the Mpox virus. This is alarming and shows that our doors are highly susceptible surfaces for the Mpox virus. This could be because of the large number of patients entering and leaving various rooms within the healthcare facility.

5.4 Discussion

This study represents the first systematic assessment of environmental contamination with the Mpox virus DNA (MPXV) in healthcare facilities in Sierra Leone during the 2025 surveillance period. Using standardized surface sampling and molecular diagnostics, we identified a 13.5% overall positivity rate across high-contact surfaces in two major hospitals (Connaught Hospital in Freetown and Bo Government Hospital in Bo). Whilst the prevalence of surface contamination is lower than that reported in some high-income settings, the pattern and nature of contaminated sites suggest persistent fomite-related risks within low-resource healthcare environments.

Objective 1: Proportion of positive surface samples

The detection of MPXV DNA on 13.5% of sampled surfaces underscores the environmental persistence of the virus in active healthcare settings. Our findings are comparable to those from Gould et al., who reported a contamination rate of 19% in UK hospital isolation rooms during the 2022 outbreak [134]. Similar higher detection levels were observed in a Chinese study (52.66%) targeting isolation wards [135], while Pfeiffer et al. reported contamination rates exceeding 60% in mixed domestic and clinical environments [126]. The lower prevalence observed in our setting may partly reflect differences in sampling protocols, cleaning frequency, or environmental conditions such as humidity and temperature, which influence viral persistence. However, the presence of viral DNA on more than one in eight surfaces still represents a significant IPC concern, particularly in facilities where patient and staff movements are frequent, and cleaning practices are inconsistent.

The detection of MPXV DNA, even without confirmation of infectivity, aligns with global evidence indicating that *orthopoxviruses* are environmentally robust [136]. This reinforces the argument for routine environmental surveillance in African hospitals, which, despite limited resources, could act as an early warning system for nosocomial transmission risks.

Objective 2: Ct values as a proxy for viral burden

Cycle threshold (Ct) analysis revealed that 70.6% of samples with measurable Ct values had Ct < 40, indicative of detectable viral DNA loads, with values ranging from 32.34 to 39.86. Prior studies suggest that Ct values below 35 are more likely to correlate with the presence of viable

virus [39, 137]. Morgan et al. documented MPXV DNA on surfaces for up to 15 days post-symptom resolution in residential settings, with similar Ct ranges [124]. Likewise, Atkinson et al. reported detection in domestic environments following imported cases into the UK, noting that some samples with lower Ct values yielded viable virus in culture [125].

While this study did not attempt viral isolation, the Ct profile observed here indicates that some contaminated surfaces could harbor infectious particles, particularly in high-contact zones. These results underline the potential for indirect transmission in healthcare facilities and the need for targeted disinfection of surfaces with repeated human contact. Importantly, Ct values should be interpreted cautiously; they quantify viral DNA, not infectivity, and can be influenced by swabbing technique, surface type, and time since contamination.

Objective 3: Comparison of contamination between Bo and Freetown

Geographical analysis showed slightly higher contamination rates in Freetown (14%) compared to Bo (12.8%). Although the difference is modest, it mirrors patterns from other infectious disease outbreaks where tertiary, high-throughput hospitals in urban centers exhibit greater environmental contamination [138]. This may reflect higher patient loads, greater diversity of clinical procedures, and more frequent contact between infected and uninfected individuals within confined spaces. Differences in IPC compliance, availability of cleaning materials, and staff training could also play a role [139].

Objective 4: Identification of high-risk surfaces

A striking observation was that 42% of all positive samples were collected from doors, ward entrances, toilet doors, and office doors; followed by patient beds, bathroom handles, table surfaces, and clinical instruments. This distribution is consistent with Wojgani et al., who identified door handles as major contamination hotspots for multiple pathogens in hospital settings [140], and CDC guidelines, which classify them as high-risk fomites [141].

The prominence of doors as contamination points is unsurprising: they are touched by patients, visitors, and staff, often in rapid succession, and rarely disinfected between contacts. This is particularly problematic in wards without automated or hands-free entry systems. The other positive sites (delivery beds, recovery beds, and clinical equipment) also represent points of direct patient contact and should be prioritized for enhanced disinfection schedules. Installing

hand hygiene stations near these surfaces, along with increased cleaning frequency, could significantly reduce environmental viral loads.

These results reinforce the persistence of viral DNA in healthcare environments and justify environmental sampling as a complementary surveillance tool, especially in LMICs where patient overcrowding and IPC inconsistencies persist [127]. Furthermore, these findings suggest frequent recontamination due to repeated contact by patients, staff, and visitors, highlighting the need for hand hygiene stations and scheduled disinfection protocols at all doorways in clinical wards.

This research is not only scientifically significant, but it also contributes to global health security by supporting the development of early detection systems, informing infection control audits and hospital sanitation practices, and guiding national health policy formation. It supports global health security objectives through the development of early detection systems, infection control audits, and hospital sanitation, as well as national health policy formation. The global geographic expansion of mpox, along with rapid clinical and epidemiological changes, requires immediate programmatic upgrades for African healthcare facilities to strengthen environmental surveillance.

The findings of this research can inform operational policies at the Ministry of Health and allied institutions, such as the National Public Health Agency (NPHA) in Sierra Leone, and guide resource prioritization for hospital disinfection and the training of healthcare workers.

5.5 Conclusion

This study confirms environmental contamination by the mpox virus on high-touch hospital surfaces in Sierra Leone, with a 13.5% positivity rate. Doors emerged as the most contaminated surfaces, highlighting their role as potential fomites in healthcare-associated transmission. Moderate Ct values suggest the presence of viral DNA at levels warranting concern, even if not directly infectious.

These findings stress the need for strengthened infection prevention protocols, especially routine surface disinfection and improved hand hygiene practices. Integrating environmental surveillance into national IPC strategies will be vital for controlling mpox and other emerging pathogens in resource-limited settings.

This work also supports the integration of environmental surveillance into national mpox preparedness frameworks. Routine surface sampling could complement patient testing to detect early signs of nosocomial spread, triggering timely IPC interventions. In the broader context of global health security, such measures align with WHO's emphasis on strengthening core public health capacities under the International Health Regulations [142].

Finally, our results reinforce the growing body of evidence that the mpox virus, like other *orthopoxviruses*, can persist in healthcare environments and that high-contact surfaces act as reservoirs for potential indirect transmission. The consistency of our findings with studies from Europe [134, 135, 125], North America [126, 124], and Asia [143] suggests that environmental contamination is a universal feature of mpox outbreaks, albeit with variable prevalence depending on IPC infrastructure and outbreak stage.

5.6 Author Contributions

JK, JBK, and AWR conceived the study and provided leadership support and guidance. MJ assisted with sample collection, designed the methodology, analyzed the results, and wrote the first draft. EJS and ESK received and recorded all samples. MJ and EJS processed all samples and performed all analyses. JBK coordinated the sample collection from the hospitals. All authors reviewed the manuscript.

Chapter 6: General Conclusions and Future Directions

6.1 Summary of Major Findings

This study provides a comprehensive characterization of the 2025 mpox outbreak in Sierra Leone, integrating diagnostic, epidemiological, and genomic data. The key findings are as follows:

1. **High diagnostic positivity and clade IIb dominance:** Among 600 samples tested, 541 (90.2%) were orthopoxvirus-positive, and 403 (74.5%) were confirmed as Mpox virus Clade IIb. All 41 high-quality sequenced genomes belonged to Clade IIb, with no Clade II sequences recovered (one Clade II sample had coverage below 70% and was excluded), indicating that Clade IIb has effectively replaced older lineages.
2. **Viral load distribution:** Among the 403 confirmed Mpox cases, the majority (81.6%) had moderate viral load (Ct 20-30), while only 3.5% had high viral load (Ct < 20). For the 206 lesion swab samples, 60.7% (125 samples) had low Ct (< 20, high viral load), 24.8% had medium Ct (20–30, moderate viral load), and 14.6% had high Ct (> 30, low viral load). This distribution reflects the effectiveness of lesion swab sampling in capturing high viral load cases. Viral load was independent of age and sex.
3. **Demographic and geographic patterns:** The outbreak affected predominantly young adults (median age 27 years), with a nearly balanced gender distribution (54% female, 46% male) – contrasting with early global outbreaks that were predominantly male. Cases were highly concentrated in the Western Area districts of Freetown (96% of positives).
4. **Hospitalization rate:** Among confirmed Mpox cases, 36.2% (196/403) required hospitalization, indicating substantial morbidity.
5. **Sequencing success and quality:** Of the 125 samples with Ct < 20, 60 were selected for sequencing. After applying a coverage threshold of 70-89%, 41 samples (68.3%) were retained. Mean coverage was 78% (SD = 6%), and coverage was unbiased by Ct value (Pearson's $r = -0.12$, $p = 0.46$).
6. **Phylogenetic findings:** All high-quality sequences belonged to Clade IIb lineage G.1 (descendant of A.2.2). Sierra Leone sequences were interspersed with global reference

genomes, indicating multiple introductions and ongoing gene flow rather than a locally isolated outbreak. No sub-lineages (e.g., A.2, B.1) were detected.

6.2 Policy and Programmatic Recommendations

Based on the findings, the following policy and programmatic recommendations are made for Sierra Leone and similar settings:

1. **Strengthen routine diagnostic capacity:** Maintain and expand access to orthopoxvirus and clade-specific real-time PCR in all regional laboratories. The high positivity rate (90.2%) supports the continued use of the current clinical case definition, but training on sample collection and handling should be reinforced to reduce the 1.0% rejection rate.
2. **Integrate viral load monitoring into clinical care:** Ct values should be routinely reported and used to guide infection control decisions (e.g., isolation duration) and to identify patients with high viral load who may benefit from closer monitoring or early intervention. The finding that 60.7% of lesion swabs had Ct < 20 underscores the importance of early sampling for both clinical management and sequencing.
3. **Targeted public health messaging:** Given the balanced gender distribution and broad age range, public health campaigns should address the general population, not only high-risk groups. Messaging should emphasize non-intimate close contact transmission (e.g., household sharing, caregiving) and early care-seeking for febrile rash illness.
4. **Geographic prioritization:** The concentration of cases in the Western Area districts justifies focused surveillance, outbreak response, and vaccination (if available) in Freetown, while maintaining preparedness in other regions through regular training and supplies.
5. **Hospital preparedness:** With a 36.2% hospitalization rate, health facilities should maintain adequate isolation capacity, infection prevention and control supplies, and staff training on mpox case management.

6.3 Implications for Future Outbreak Preparedness

The experience of the 2025 mpox outbreak in Sierra Leone offers several lessons for future outbreak preparedness:

1. **Rapid deployment of genomic surveillance:** The successful in-country sequencing of 60 samples and generation of 41 high-quality genomes within weeks of outbreak detection demonstrates that real-time genomic epidemiology is feasible in resource-limited settings. This capacity should be maintained as a permanent component of the national public health infrastructure.
2. **Importance of pre-established protocols and partnerships:** The collaboration with international partners (Africa CDC, WHO, research networks) and the use of open-source tools (ARTIC, NextClade, Pathoplexus) enabled rapid data sharing and contextualization. Similar multi-sectoral partnerships should be formalized for other emerging pathogens.
3. **Use of Ct thresholds for triage:** The $Ct < 20$ cut-off proved effective for maximizing sequencing success (68.3% passed the 70-89% coverage filter). This pragmatic approach should be adopted by other laboratories and included in national sequencing guidelines. The fact that 125 samples met this criterion highlights the importance of early and high-quality sample collection.
4. **Data integration for real-time response:** Linking demographic, clinical, and genomic data in a centralized database would allow for more dynamic outbreak monitoring (e.g., tracking transmission chains, identifying emerging variants). Investment in health information systems is essential.

6.4 Recommendations for Genomic Surveillance Integration

To sustain and enhance genomic surveillance in Sierra Leone, the following actions are recommended:

1. **Establish a national genomic surveillance unit:** This unit should be responsible for routine sequencing of a representative subset of mpox and other priority pathogens (e.g., Ebola, Lassa, COVID-19), with dedicated staff, equipment, and recurrent funding.

2. **Develop standardized workflows:** Protocols for sample selection ($Ct < 20$), library preparation (ARTIC tiled amplicon, sequencing Nanopore), and bioinformatics (NextClade, IQ-TREE) should be documented and regularly updated. Training materials should be made available for regional laboratories.
3. **Integrate with regional and global networks:** Sierra Leone should actively contribute sequences to public databases (GISAID, Pathoplexus) and participate in regional genomic surveillance networks (e.g., Africa CDC's Pathogen Genomics Initiative). This enables early detection of new introductions and monitoring of viral evolution.
4. **Build local bioinformatics capacity:** Beyond running pipelines, local scientists should be trained in phylogenetic analysis, variant calling, and data interpretation. Partnerships with universities and research centers can support this capacity building.
5. **Link genomic data to epidemiological data:** A secure, interoperable database should be developed to link sequence data with patient demographics, clinical outcomes, and transmission chains. This would greatly enhance the public health utility of genomic surveillance.

6.5 Areas for Further Research

Despite the comprehensive findings, several questions remain unanswered and warrant further investigation:

1. **Long-term viral evolution:** Ongoing surveillance is needed to monitor whether the G.1 lineage diversifies into sub-lineages and whether any mutations affect transmissibility, virulence, or immune escape. Whole-genome sequencing of later outbreak samples would address this.
2. **Correlates of disease severity:** Future studies should collect detailed clinical data (lesion count, symptom duration, comorbidities, vaccination status) to directly correlate Ct values with clinical outcomes and to identify risk factors for hospitalization and severe disease.
3. **Transmission dynamics:** Phylogeographic analyses (e.g., using BEAST) with time-stamped sequences could estimate the timing of introduction events, the effective reproductive

number, and the contribution of different regions to spread. Such studies would inform targeted interventions.

4. **Duration of infectivity:** Longitudinal sampling of patients (serial Ct values and viral culture) would determine how long individuals remain infectious, guiding evidence-based isolation and discharge policies.
5. **Seroprevalence and immunity:** A population-based serosurvey would assess the extent of past infection and residual immunity from smallpox vaccination, helping to predict future outbreak susceptibility and vaccine needs.
6. **Environmental and animal reservoirs:** While the outbreak is driven by human-to-human transmission, the potential for zoonotic reintroduction from animal reservoirs (rodents, etc.) in Sierra Leone should be investigated through environmental and animal surveillance.
7. **Cost-effectiveness of genomic surveillance:** A formal economic evaluation would quantify the benefits of in-country sequencing (e.g., reduced response time, targeted interventions) relative to costs, providing evidence for sustained funding.

6.6 Conclusion

In conclusion, the 2025 mpox outbreak in Sierra Leone was successfully characterized through a combination of robust diagnostics, viral load analysis, and in-country genomic sequencing. The findings underscore the dominance of Clade IIb, the shift towards balanced gender distribution, and the feasibility of real-time genomic surveillance in resource-limited settings. The high proportion of lesion swabs with low Ct values (60.7%) highlights the value of early, high-quality sample collection for both diagnosis and sequencing. Sustained investment in laboratory capacity, data integration, and collaborative networks will be essential to prepare for future outbreaks of mpox and other emerging pathogens.

References

1. Sklenovská N, Van Ranst M. Emergence of monkeypox as the most important orthopoxvirus infection in humans. *Front Public Health*. 2018;6:241. doi:10.3389/fpubh.2018.00241
2. McCollum AM, Damon IK. Human monkeypox. *Clin Infect Dis*. 2014;58(2):260-7. doi:10.1093/cid/cit703
3. Shchelkunov SN, Totmenin AV, Babkin IV, Safronov PF, Ryazankina OI, Petrov NA, et al. Human monkeypox and smallpox viruses: genomic comparison. *FEBS Lett*. 2001;509(1):66-70. doi:10.1016/S0014-5793(01)03144-1
4. Shchelkunov SN, Totmenin AV, Safronov PF, Mikheev MV, Gutorov VV, Ryazankina OI, et al. Analysis of the monkeypox virus genome. *Virology*. 2002;297(2):172-94. doi:10.1006/viro.2002.1446
5. Reynolds MG, Doty JB, McCollum AM, Olson VA, Nakazawa Y. Monkeypox re-emergence in Africa: a call to expand the concept and practice of One Health. *Expert Rev Anti Infect Ther*. 2019;17(2):129-39. doi:10.1080/14787210.2019.1567330
6. Falendysz EA, Lopera JG, Doty JB, Nakazawa Y, Crill C, Lorenzsonn F, et al. Characterization of monkeypox virus infection in African rope squirrels (*Funisciurus* sp.). *PLoS Negl Trop Dis*. 2017;11(8):e0005809. doi:10.1371/journal.pntd.0005809
7. Hutson CL, Lee KN, Abel J, Carroll DS, Montgomery JM, Olson VA, et al. Monkeypox zoonotic associations: insights from laboratory evaluation of animals in the Democratic Republic of the Congo. *Am J Trop Med Hyg*. 2015;93(5):1077-84. doi:10.4269/ajtmh.15-0447
8. Doty JB, Malekani JM, Kalembe LN, Stanley WT, Monroe BP, Nakazawa YU, et al. Assessing monkeypox virus prevalence in small mammals at the human-animal interface in the Democratic Republic of the Congo. *Viruses*. 2017;9(10):283. doi:10.3390/v9100283

9. Adler H, Gould S, Hine P, Snell LB, Wong W, Houlihan CF, et al. Clinical features and management of human monkeypox: a retrospective observational study in the UK. *Lancet Infect Dis.* 2022;22(8):1153-62. doi:10.1016/S1473-3099(22)00228-6
10. World Health Organization. Mpox [Internet]. Geneva: WHO; 2024 [cited 2026 Mar 3]. Available from: <https://www.who.int/news-room/fact-sheets/detail/mpox>
11. Bunge EM, Hoet B, Chen L, Lienert F, Weidenthaler H, Baer LR, et al. The changing epidemiology of human monkeypox – a potential threat? A systematic review. *PLoS Negl Trop Dis.* 2022;16(2):e0010141. doi:10.1371/journal.pntd.0010141
12. Ladnyj ID, Ziegler P, Kima E. A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. *Bull World Health Organ.* 1972;46(5):593-7.
13. Foster SO, Brink EW, Hutchins DL, Pifer JM, Lourie B, Moser CR, et al. Human monkeypox. *Bull World Health Organ.* 1972;46(5):569-76.
14. Doshi RH, Guagliardo SAJ, Doty JB, Mauldin MR, Recuenco S, Nakazawa Y, et al. Epidemiologic and ecologic investigations of monkeypox, Likouala Department, Republic of the Congo, 2017. *Emerg Infect Dis.* 2019;25(2):273-81. doi:10.3201/eid2502.181370
15. Likos AM, Sammons SA, Olson VA, Frace AM, Li Y, Olsen-Rasmussen M, et al. A tale of two clades: monkeypox viruses. *J Gen Virol.* 2005;86(Pt 10):2661-72. doi:10.1099/vir.0.81215-0
16. 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. doi:10.1093/oxfordjournals.bmb.a011720
17. Reed KD, Melski JW, Graham MB, Regnery RL, Sotir MJ, Wegner MV, et al. The detection of monkeypox in humans in the Western Hemisphere. *N Engl J Med.* 2004;350(4):342-50. doi:10.1056/NEJMoa032299
18. Yinka-Ogunleye A, Aruna O, Dalhat M, Ogoina D, McCollum A, Disu Y, et al. Outbreak of human monkeypox in Nigeria in 2017-18: a clinical and epidemiological report. *Lancet Infect Dis.* 2019;19(8):872-9. doi:10.1016/S1473-3099(19)30294-4

19. Happi C, Adetifa I, Mbala P, Njouom R, Nakoune E, Happi A, et al. Urgent need for a non-discriminatory and non-stigmatizing nomenclature for monkeypox virus. *PLoS Biol.* 2022;20(8):e3001769. doi:10.1371/journal.pbio.3001769
20. 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):434-8. doi:10.3201/eid0703.017311
21. Brosius I, Vakaniaki EH, Mukari G, Papy Munganga, Tshomba JC, Vos ED, et al. Epidemiological and clinical features of mpox during the clade Ib outbreak in South Kivu, Democratic Republic of the Congo: a prospective cohort study. *Lancet.* 2025. doi:10.1016/S0140-6736(25)00047-9
22. Huhn GD, Bauer AM, Yorita K, Graham MB, Sejvar J, Likos A, et al. Clinical characteristics of human monkeypox, and risk factors for severe disease. *Clin Infect Dis.* 2005;41(12):1742-51. doi:10.1086/498115
23. Isidro J, Borges V, Pinto M, Sobral D, Santos JD, Nunes A, et al. Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. *Nat Med.* 2022;28(8):1569-72. doi:10.1038/s41591-022-01907-y
24. O'Toole Á, Neher RA, Ndodo N, Borges V, Gannon B, Gomes JP, et al. APOBEC3 deaminase editing in mpox virus as evidence for sustained human transmission since at least 2016. *Science.* 2023;382(6670):595-600. doi:10.1126/science.adg8116
25. 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. *N Engl J Med.* 2022;387(8):679-91. doi:10.1056/NEJMoa2207323
26. Yinka-Ogunleye A, Aruna O, Dalhat M, Ogoina D, McCollum A, Disu Y, et al.; CDC Monkeypox Outbreak Team. Outbreak of human monkeypox in Nigeria in 2017-18: a clinical and epidemiological report. *Lancet Infect Dis.* 2019;19(8):872-9. doi:10.1016/S1473-3099(19)30294-4. PMID: 31285143; PMCID: PMC9628943.
27. Ministry of Health and Sanitation, Sierra Leone. National infection prevention and control guidelines. Freetown: Government of Sierra Leone; 2022. Available from:

https://www.afro.who.int/sites/default/files/2022-08/Sierra%20Leone%20National%20Infection%20Prevention%20and%20Control%20Guidelines_2022.pdf

28. Elston JWT, Moosa AJ, Moses F, Walker G, Dotta N, Waldman RJ, et al. Impact of the Ebola outbreak on health systems and population health in Sierra Leone. *J Public Health (Oxf)*. 2016;38(4):673-8. doi:10.1093/pubmed/fdv158
29. Africa Security Analysis. Mpox in West Africa: Complexity Beyond the Global Alert Lift | African Security Analysis [Homepage on the Internet]. *Africansecurityanalysis.org*. 2025 [cited 2026 May 25]; Available from: <https://www.africansecurityanalysis.org/reports/mpox-in-west-africa-complexity-beyond-the-global-alert-lift>
30. Ministry of Health, Sierra Leone. Ministry of Health Holds National Discourse on MPOX Response in Sierra Leone: Government of Sierra Leone; 2025 Aug [cited 2026 Mar 3]. Available from: <https://moice.gov.sl/ministry-of-health-holds-national-discourse-on-mpox-response-in-sierra-leone/>
31. World Health Organization. Multi-country monkeypox outbreak: situation update [Internet]. Geneva: WHO; 2022 [cited 2026 Mar 3]. Available from: <https://www.who.int/emergencies/disease-outbreak-news>
32. World Health Organization. WHO Director-General declares the ongoing monkeypox outbreak a Public Health Emergency of International Concern [Internet]. Geneva: WHO; 2022 Jul 23 [cited 2026 Mar 3]. Available from: <https://www.who.int/europe/news/item/23-07-2022-who-director-general-declares-the-ongoing-monkeypox-outbreak-a-public-health-event-of-international-concern>
33. Antinori A, Mazzotta V, Vita S, Carletti F, Tacconi D, Lapini LE, et al. Epidemiological, clinical and virological characteristics of four cases of monkeypox support transmission through sexual contact, Italy, May 2022. *Euro Surveill*. 2022;27(22):2200421. doi:10.2807/1560-7917.ES.2022.27.22.2200421

34. Patel A, Bilinska J, Tam JCH, da Silva Fontoura D, Mason CY, Daunt A, et al. Clinical features and novel presentations of human monkeypox in a central London centre during the 2022 outbreak: descriptive case series. *BMJ*. 2022;378:e072410. doi:10.1136/bmj-2022-072410
35. World Health Organization. WHO Director-General declares end of mpox (monkeypox) Public Health Emergency of International Concern [Internet]. Geneva: WHO; 2024 Aug 14 [cited 2026 Mar 3]. Available from: <https://www.who.int/news/item/14-08-2024-who-director-general-declares-mpox-outbreak-a-public-health-emergency-of-international-concern>
36. Grubaugh ND, Ladner JT, Lemey P, Pybus OG, Rambaut A, Holmes EC, et al. Tracking virus outbreaks in the twenty-first century. *Nat Microbiol*. 2019;4(1):10-9. doi:10.1038/s41564-018-0293-2
37. Nextstrain. Real-time tracking of pathogen evolution [Internet]. [cited 2026 Mar 3]. Available from: <https://nextstrain.org/monkeypox>
38. Quick J, Loman NJ, Duraffour S, Simpson JT, Severi E, Cowley L, et al. Real-time, portable genome sequencing for Ebola surveillance. *Nature*. 2016;530(7589):228-32. doi:10.1038/nature16996
39. Li Y, Zhao H, Wilkins K, Hughes C, Damon IK. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. *J Virol Methods*. 2010;169(1):223-7. doi:10.1016/j.jviromet.2010.07.012
40. Bosmeny MS, White AA, Pater AA, Crew J, Geltz J, Gagnon KT. Global mpox lineage discovery and rapid outbreak tracking with nanopore sequencing. *Virol J*. 2023;20:90. doi:10.1186/s12985-023-02059-2
41. Jalloh S, O'Connor R, Bangura U, Fonnier R, Goba A, Jalloh AA, et al. Strengthening laboratory capacity in Sierra Leone: lessons from the Ebola outbreak. *Lancet Glob Health*. 2018;6(11):e1146-e7. doi:10.1016/S2214-109X(18)30391-X

42. Moss B. Poxviridae: the viruses and their replication. In: Fields Virology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013. (No DOI available.)
43. Shchelkunov SN. An increasing danger of zoonotic orthopoxvirus infections. PLoS Pathog. 2013;9(12):e1003756. doi:10.1371/journal.ppat.1003756
44. International Committee on Taxonomy of Viruses (ICTV). Taxonomy [Internet]. [cited 2026 Mar 3]. Available from: <https://ictv.global/taxonomy>
45. Chen N, Li G, Liszewski MK, Atkinson JP, Jahrling PB, Feng Z, et al. Virulence differences between monkeypox virus isolates from West Africa and the Congo basin. Virology. 2005;340(1):46-63. doi:10.1016/j.virol.2005.05.030
46. Kugelman JR, Johnston SC, Mulembakani PM, Kisalu N, Lee MS, Koroleva G, et al. Genomic variability of monkeypox virus among humans, Democratic Republic of the Congo. Emerg Infect Dis. 2014;20(2):232-9. doi:10.3201/eid2002.130118
47. Assarsson E, Greenbaum JA, Sundström M, Schaffer L, Hammond JA, Pasquetto V, et al. Kinetic analysis of a complete poxvirus transcriptome reveals an immediate-early class of genes. Proc Natl Acad Sci U S A. 2008;105(6):2140-5. doi:10.1073/pnas.0711573105
48. Stanford MM, Werden SJ, McFadden G. Myxoma virus in the European rabbit: interactions between the virus and its susceptible host. Vet Res. 2007;38(2):299-318. doi:10.1051/vetres:2006068
49. Di Giulio DB, Eckburg PB. Human monkeypox: an emerging zoonosis. Lancet Infect Dis. 2004;4(1):15-25. doi:10.1016/S1473-3099(03)00856-9
50. Moss B. Poxvirus DNA replication. Cold Spring Harb Perspect Biol. 2013;5(9):a010199. doi:10.1101/cshperspect.a010199
51. Smith GL, Vanderplasschen A, Law M. The formation and function of extracellular enveloped vaccinia virus. J Gen Virol. 2002;83(Pt 12):2915-31. doi:10.1099/0022-1317-83-12-2915
52. Seet BT, Johnston JB, Brunetti CR, Barrett JW, Everett H, Cameron C, et al. Poxviruses and immune evasion. Annu Rev Immunol. 2003;21:377-423. doi:10.1146/annurev.immunol.21.120601.141001

53. McFadden G. Poxvirus tropism. *Nat Rev Microbiol.* 2005;3(3):201-13.
doi:10.1038/nrmicro1099
54. Realegeno S, Puschnik AS, Kumar A, Goldsmith C, Burgado J, Sambhara S, et al. Identification of host factors involved in poxvirus entry and replication. *mBio.* 2017;8(2):e00173-17. doi:10.1128/mBio.00173-17
55. Faye O, Pratt CB, Faye M, Fall G, Chitty JA, Diagne MM, et al. Genomic characterisation of human monkeypox virus in Nigeria. *Lancet Infect Dis.* 2018;18(3):246. doi:10.1016/S1473-3099(18)30043-4
56. Jezek Z, Szczeniowski M, Paluku KM, Mutombo M. Human monkeypox: clinical features of 282 patients. *J Infect Dis.* 1987;156(2):293-8. doi:10.1093/infdis/156.2.293
57. Gigante CM, Korber B, Seabolt MH, Wilkins K, Davidson W, Rao AK, et al. Multiple lineages of monkeypox virus detected in the United States, 2022. *Science.* 2022;378(6622):560-5. doi:10.1126/science.ade1261
58. Mbala-Kingebeni P, Pratt C, Mutafuli-Ruffin M, Pauthner MG, Bile F, Nkuba-Ndaye A, et al. 2022 monkeypox outbreak in the Democratic Republic of the Congo: an epidemiological and genomic analysis. *Lancet Infect Dis.* 2023;23(10):e392-e8. doi:10.1016/S1473-3099(23)00259-1
59. World Health Organization. Mpox (monkeypox) outbreak 2022 – global [Internet]. [cited 2026 Mar 3]. Available from: <https://www.who.int/emergencies/situations/monkeypox-outbreak-2022/1000>
60. World Health Organization. 2022-23 mpox outbreak: global trends [Internet]. [cited 2026 Mar 3]. Available from: https://worldhealthorg.shinyapps.io/mpx_global/
61. Mailhe M, Beaumont AL, Thy M, Le Pluart D, Perrineau S, Houhou-Fidouh N, et al. Clinical characteristics of ambulatory and hospitalised patients with monkeypox virus infection: an observational cohort study. *Clin Microbiol Infect.* 2023;29(2):233-9.
doi:10.1016/j.cmi.2022.10.003

62. Jaba M, Siadu EJ, Kamanda ES, Dumbuya S, Ganda L, Batra D, et al. Surface sampling for mpox virus in multiple healthcare settings in Sierra Leone, June 2025. medRxiv [Preprint]. 2025. doi:10.1101/2025.06.XX.XXXXXX (place-holder DOI; actual to be added)
63. Endo A, Murayama H, Abbott S, Ratnayake R, Pearson CAB, Edmunds WJ, et al. Heavy-tailed sexual contact networks and monkeypox epidemiology in the global outbreak, 2022. *Science*. 2022;378(6615):90-4. doi:10.1126/science.add3739
64. World Health Organization. Mpox: multi-country external situation report no. 63 [Internet]. Geneva: WHO; 2026 Feb 24 [cited 2026 Apr 19]. Available from: <https://www.who.int/publications/m/item/multi-country-outbreak-of-mpox--external-situation-report--63---24-february-2026>
65. Karem KL, Reynolds M, Braden Z, Lou G, Bernard N, Patton J, et al. Characterization of acute-phase humoral immunity to monkeypox: use of immunoglobulin M enzyme-linked immunosorbent assay for detection of monkeypox infection during the 2003 North American outbreak. *Clin Diagn Lab Immunol*. 2005;12(7):867-72. doi:10.1128/CDLI.12.7.867-872.2005
66. Li Y, Olson VA, Laue T, Laker MT, Damon IK. Detection of monkeypox virus with real-time PCR assays. *J Clin Virol*. 2006;36(3):194-203. doi:10.1016/j.jcv.2006.03.012
67. Riedel S, Damon I. Smallpox and other orthopoxvirus-associated infections. In: Detrick B, Hamilton RG, Folds JD, editors. *Manual of molecular and clinical laboratory immunology*. 7th ed. Washington (DC): ASM Press; 2006. (No DOI available.)
68. Fedele CG, Negri C, Fares W, Garcia MI, Mazzotta V, Calcaterra S, et al. Monkeypox virus multiplex PCR assay for rapid differential detection of clades I, IIa and IIb. *Euro Surveill*. 2023;28(14):2200435. doi:10.2807/1560-7917.ES.2023.28.14.2200435
69. Olson VA, Laue T, Laker MT, Babkin IV, Drosten C, Shchelkunov SN, et al. Real-time PCR system for detection of orthopoxviruses and simultaneous identification of smallpox virus. *J Clin Microbiol*. 2004;42(5):1940-6. doi:10.1128/JCM.42.5.1940-1946.2004

70. Centers for Disease Control and Prevention. Test procedure: Monkeypox virus generic real-time PCR test [Internet]. 2022 [cited 2026 Mar 3]. Available from: <https://stacks.cdc.gov/view/cdc/119661>
71. Ihekweazu C, Yinka-Ogunleye A, Lule S, Ibrahim A. Importance of epidemiological research for monkeypox response: the Nigeria experience. *Lancet Infect Dis*. 2020;20(5):537-8. doi:10.1016/S1473-3099(20)30256-X
72. de Oliveira-Filho EF, Schuele L, Kissenkötter J, Lück L, Hagedorn P, Held J, et al. A molecular beacon-based duplex real-time PCR assay for the simultaneous detection of Clade I and Clade II monkeypox viruses. *Viruses*. 2023;15(3):632. doi:10.3390/v15030632
73. Loens K, Van Heirstraeten L, Malhotra-Kumar S, Goossens H, Ieven M. Optimal sampling sites and methods for detection of pathogens possibly causing community-acquired lower respiratory tract infections. *J Clin Microbiol*. 2009;47(1):21-31. doi:10.1128/JCM.02041-08
74. O'Toole Á, Hill V, Pybus OG, Watts A, Bogoch II, Khan K, et al. Tracking the international spread of SARS-CoV-2 lineages B.1.1.7 and B.1.351/501Y-V2 with grinch. *Wellcome Open Res*. 2021;6:121. doi:10.12688/wellcomeopenres.16679.2
75. Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol*. 2020;5(11):1403-7. doi:10.1038/s41564-020-0770-5
76. Carroll D, Daszak P, Wolfe ND, Gao GF, Morel CM, Morzaria S, et al. The Global Virome Project. *Science*. 2018;359(6378):872-4. doi:10.1126/science.aap7463
77. Quick J, Grubaugh ND, Pullan ST, Claro IM, Smith AD, Gangavarapu K, et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nat Protoc*. 2017;12(6):1261-76. doi:10.1038/nprot.2017.066
78. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics*. 2018;34(23):4121-3. doi:10.1093/bioinformatics/bty407

79. Ogoina D, Iroezindu M, James HI, Oladokun R, Yinka-Ogunleye A, Wakama P, et al. Clinical course and outcome of human monkeypox in Nigeria. *Clin Infect Dis*. 2020;71(8):e210-e4. doi:10.1093/cid/ciaa108
80. Thézé J, Li T, du Plessis L, Bouquet J, Kraemer MUG, Somasekar S, et al. Genomic epidemiology reconstructs the introduction and spread of Zika virus in Central America and Mexico. *Cell Host Microbe*. 2018;23(6):855-64.e7. doi:10.1016/j.chom.2018.04.017
81. Gardy J, Loman NJ. The need for integrated molecular pathogen surveillance in Africa. *Nat Microbiol*. 2018;3(1):12-4. doi:10.1038/s41564-017-0083-2
82. Osei M, Okyere PB, Agbodzi B, Odoom JK, Obodai E, Kenu E, et al. Genomic surveillance of monkeypox virus, Ghana, 2022. *Emerg Infect Dis*. 2023;29(2):439-41. doi:10.3201/eid2902.221383
83. Gebreyes WA, Dupouy-Camet J, Newport MJ, Oliveira CJB, Schlesinger LS, Saif YM, et al. The global one health paradigm: challenges and opportunities for tackling infectious diseases at the human, animal, and environment interface in low-resource settings. *PLoS Negl Trop Dis*. 2014;8(11):e3257. doi:10.1371/journal.pntd.0003257
84. Bauer MS, Damschroder L, Hagedorn H, Smith J, Kilbourne AM. An introduction to implementation science for the non-specialist. *BMC Psychol*. 2015;3:32. doi:10.1186/s40359-015-0089-9
85. Gardy JL, Loman NJ. Towards a genomics-informed, real-time, global pathogen surveillance system. *Nat Rev Genet*. 2018;19(1):9-20. doi:10.1038/nrg.2017.88
86. Elton L, Haider N, Kock R, Thomason MJ, Tembo J, Arruda LB, et al. Roadblocks to infection prevention and control in primary care in Sierra Leone: a qualitative study. *BMJ Open*. 2022;12(2):e056398. doi:10.1136/bmjopen-2021-056398
87. Gangavarapu K, Latif AA, Mullen JL, Alkuzweny M, Hufbauer E, Tsueng G, et al. outbreak.info genomic reports: scalable and dynamic surveillance of SARS-CoV-2 variants and mutations. *Nat Methods*. 2023;20(4):512-22. doi:10.1038/s41592-023-01780-y

88. Tomori O, Kobia K. Genome sequencing and health systems in Africa: a landscape analysis of the opportunities and challenges. *AAS Open Res.* 2021;4:19. doi:10.12688/aasopenres.13214.1
89. World Health Organization. Global genomic surveillance strategy for pathogens with pandemic and epidemic potential, 2022-2032. Geneva: WHO; 2022. Available from: <https://www.who.int/publications/i/item/9789240046979>
90. Nkengasong JN, Tessema SK. Africa needs a new public health order to tackle infectious disease threats. *Cell.* 2020;183(2):296-300. doi:10.1016/j.cell.2020.09.041
91. World Health Organization. WHO case definitions for mpox outbreak in non-endemic countries [Internet]. Geneva: WHO; 2025 May [cited 2026 Apr 19]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK616033>
92. Bowes DA, Henke KB, Driver EM, Newell ME, Block I, Shaffer G, Varsani A, Scotch M, Halden RU. Enhanced detection of mpox virus in wastewater using a pre-amplification approach: a pilot study informing population-level monitoring of low-titer pathogens. *Sci Total Environ.* 2023;903:166230. doi:10.1016/j.scitotenv.2023.166230
93. Kinganda-Lusamaki E, Amuri-Aziza A, Fernandez-Nuñez N, Makangara-Cigolo JC, Pratt C, Vakaniaki EH, et al. Clade I mpox virus genomic diversity in the Democratic Republic of the Congo, 2018-2024: predominance of zoonotic transmission. *Cell.* 2025;188(1):4-14.e6. doi:10.1016/j.cell.2024.10.017
94. Wawina-Bokalanga T, Akil-Bandali P, Kinganda-Lusamaki E, Lokilo E, Jansen D, Amuri-Aziza A, et al. Co-circulation of monkeypox virus subclades Ia and Ib in Kinshasa Province, Democratic Republic of the Congo, July to August 2024. *Euro Surveill.* 2024;29(38):2400592. doi:10.2807/1560-7917.ES.2024.29.38.2400592 – 5- 10
95. Deng J, McGrath D, Wilkins K, Haddock LA, Davidson W, Rabeneck DB, et al. Monkeypox virus partial-genome amplicon sequencing for improvement of genomic surveillance during mpox outbreaks. *Emerg Infect Dis.* 2025;31(11). doi:10.3201/eid3111.250548

96. World Health Organization. Clinical management and infection prevention and control for mpox: living guideline [Internet]. Geneva: WHO; 2025 May [cited 2026 Apr 19]. Available from: <https://www.who.int/publications/i/item/B09434>
97. Parker E, Campbell AKO, Sandi JD, Omah IF, Faye M, Brock-Fisher T, et al. Genomic epidemiology uncovers the origin of the mpox epidemic in Sierra Leone. medRxiv [Preprint]. 2025. doi:10.1101/2025.10.15.25337823v1
98. Africa CDC. Tracking the evolutionary footprint of mpox in West Africa: phylogenetic and clade analysis [Internet]. Africa Health Knowledge Hub; 2026 [cited 2026 Apr 19]. Available from: <https://khub.africacdc.org/records/resource?id=402>
99. Happi C, Adetifa I, Mbala P, Njouom R, Nakoune E, Happi A, et al. Urgent need for a coordinated response to mpox in Africa. *Lancet*. 2024;403(10437):1780-2. doi:10.1016/S0140-6736(24)00575-8
100. Judson SD, Orkin CM, Gebo K. Mpox: what have we learned and how do we better prepare for the future? *J Infect Dis*. 2025;232(2):270-3. doi:10.1093/infdis/jiaf040
101. Kraemer MUG, Tegally H, Pigott DM, Dasgupta A, Sheldon J, Wilkinson E, et al. Tracking the 2022 monkeypox outbreak with epidemiological data in real-time. *Lancet Infect Dis*. 2022;22(7):941-2. doi:10.1016/S1473-3099(22)00359-0
102. Nakazawa Y, Mauldin MR, Emerson GL, Reynolds MG, Lushima RS, Likofata J, et al. A phylogeographic investigation of African monkeypox virus. *Viruses*. 2015;7(4):2168-84. doi:10.3390/v7042168
103. Lim CK, McKenzie C, Deerrain J, Chow EPF, Towns J, Chen MY, et al. Correlation between monkeypox viral load and infectious virus in clinical specimens. *J Clin Virol*. 2023;161:105421. doi:10.1016/j.jcv.2023.105421
104. Mbala PK, Huggins JW, Riu-Rovira T, Ahuka SM, Mulembakani P, Rimoin AW, et al. Maternal and fetal outcomes among pregnant women with human monkeypox infection in the Democratic Republic of Congo. *J Infect Dis*. 2017;216(7):824-8. doi:10.1093/infdis/jix260

105. Abdel Hafez S, Stack C, Miller K, et al. Global travel and mpox: clinical and preventive updates. *Curr Infect Dis Rep*. 2025;27:17. doi:10.1007/s11908-025-00866-y
106. Mostafa HH. An evolution of Nanopore next-generation sequencing technology: implications for medical microbiology and public health. *J Clin Microbiol*. 2024;62(5):e00246-24. doi:10.1128/jcm.00246-24
107. Ogoina D, Izibewule JH, Ogunleye A, Ederiane E, Anebonam U, Neni A, et al. The 2017 human monkeypox outbreak in Nigeria: report of outbreak experience and response. *PLoS Negl Trop Dis*. 2019;13(12):e0007863. doi:10.1371/journal.pntd.0007863
108. Nextstrain. Nextclade data release 2025-09-20 [Internet]. GitHub; [cited 2026 Apr 19]. Available from: <https://nextstrain.org/mpox/all-clades>
109. Djuicy DD, Omah IF, Parker E, Tomkins-Tinch CH, Otieno JR, Yifomnjou MHM, et al. Molecular epidemiology of recurrent zoonotic transmission of mpox virus in West Africa. *medRxiv [Preprint]*. 2024 Jun 19. doi:10.1101/2024.06.18.24309115
110. World Health Organization. Multi-country outbreak of mpox: external situation report no. 40 [Internet]. Geneva: WHO; 2024 [cited 2026 Apr 19]. Available from: <https://www.who.int/publications/m/item/multi-country-outbreak-of-mpox--external-situation-report-40--13-october-2024>
111. Ghana Health Service. Mpox case in Greater Accra Region [Internet]. Accra: GHS; 2024 [cited 2026 Apr 19]. Available from: <https://www.ghs.gov.gh/disease-outbreaks>
112. Masirika LM, Udahemuka JC, Schuele L, Ndishimye P, Otani S, Mbiribindi JB, et al. Ongoing mpox outbreak in Kamituga, South Kivu province, associated with monkeypox virus of a novel Clade I sub-lineage, Democratic Republic of the Congo, 2024. *Euro Surveill*. 2024;29(11):2400106. doi:10.2807/1560-7917.ES.2024.29.11.2400106
113. BMJ Best Practice. Mpox – investigations [Internet]. London: BMJ; 2022 [cited 2026 Apr 19]. Available from: <https://bestpractice.bmj.com/topics/en-gb/1611/investigations>

114. Papadakis G, Tran T, Druce J, Lim CK, Williamson DA, Jackson K. Evaluation of 16 molecular assays for the detection of orthopox and mpox viruses. *J Clin Virol*. 2023;161:105424. doi:10.1016/j.jcv.2023.105424
115. Fan Z, Xie Y, Huang B, Zhao F, Hu Y, Huang Y, et al. Development of a multiplex real-time PCR assay for the simultaneous detection of mpox virus and orthopoxvirus infections. *J Virol Methods*. 2024;114957. doi:10.1016/j.jviromet.2024.114957
116. Oxford Nanopore Technologies. Workflow overview: mpox virus sequencing [Internet]. 2025 [cited 2026 Apr 19]. Available from: <https://nanoporetech.com/resource-centre/workflow:-mpox-virus-sequencing>
117. Africa CDC. Africa CDC and European Commission launch new initiative to strengthen mpox testing and sequencing across Africa [Internet]. Addis Ababa: Africa CDC; 2025 [cited 2026 Apr 19]. Available from: <https://africacdc.org/news-item/africa-cdc-and-european-commission-launch-new-initiative-to-strengthen-mpox-testing-and-sequencing-across-africa/>
118. Oxford Nanopore Technologies. Case study: combating viral contamination and disease outbreaks at source with rapid, low-cost nanopore sequencing [Internet]. Oxford: Oxford Nanopore Technologies; 2024 [cited 2026 Apr 19]. Available from: <https://nanoporetech.com/zh/resource-centre/combating-viral-contamination-and-disease-outbreaks-at-source-with-rapid-low-cost-nanopore-sequencing>
119. ARTIC Network. Mpox virus sequencing [Internet]. Cambridge: ARTIC Network; 2024 [cited 2026 Apr 19]. Available from: <https://artic.network/viruses/mpxv/artic-mpxv-guide.html>
120. Ruis C, Lusamaki E, O'Toole A, et al. A systematic nomenclature for mpox viruses causing outbreaks with sustained human-to-human transmission. *Nat Med*. 2025 Jul 29. doi:10.1038/s41591-025-03820-6

121. Van Dijck C, Hoff NA, Mbala-Kingebeni P, et al. Emergence of mpox in the post-smallpox era – a narrative review on mpox epidemiology. *Clin Microbiol Infect.* 2023;29(12):1487-92. doi:10.1016/j.cmi.2023.08.008
122. Fischer WA, Hynes NA, Perl TM. Protecting health care workers from Ebola: personal protective equipment is critical but is not enough. *Ann Intern Med.* 2014;161(10):753-4. doi:10.7326/M14-1953
123. Kuehn R, Fox T, Guyatt G, Lutje V, Gould S. Infection prevention and control measures to reduce the transmission of mpox: a systematic review. *PLOS Glob Public Health.* 2024;4(1):e0002731. doi:10.1371/journal.pgph.0002731
124. Morgan CN, Whitehill F, Doty JB, Schulte J, Matheny A, Stringer J, et al. Environmental persistence of monkeypox virus on surfaces in household of person with travel-associated infection, Dallas, Texas, USA, 2021. *Emerg Infect Dis.* 2022;28(10):1982-9. doi:10.3201/eid2810.221047
125. Atkinson B, Burton C, Pottage T, et al. Infection-competent monkeypox virus contamination identified in domestic settings following an imported case of monkeypox into the UK. *Environ Microbiol.* 2022;24(12):4561-71. doi:10.1111/1462-2920.16129
126. Pfeiffer JA, Collingwood A, Ryder A, et al. High-contact object and surface contamination in a household with monkeypox virus, Utah, USA, 2022. *Emerg Infect Dis.* 2022;28(11):2136-9. doi:10.15585/mmwr.mm7134e1
127. Margao S, Fofanah BD, Thekkur P, Kallon C, Ngauja RE, Kamara IF, et al. Improvement in infection prevention and control performance following operational research in Sierra Leone: a before (2021) and after (2023) study. *Trop Med Infect Dis.* 2023;8(7):376. Available from: <https://www.mdpi.com/2414-6366/8/7/376>
128. Otter JA, Yezli S, Salkeld JAG, French GL. Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. *Am J Infect Control.* 2013;41(5):S6-11. doi:10.1016/j.ajic.2012.12.004

129. ThermoFisher Scientific. MagMax DNA Multi-Sample Ultra Kit user guide (Pub No. MAN0010293) [Internet]. [cited 2026 Apr 19]. Available from: https://assets.fishersci.com/TFSAssets/LSG/manuals/MAN0010293_MagMax_DNA_MultiSample_Ultra_buccal_swabs_UG_man.pdf
130. Centers for Disease Control and Prevention. Test procedure: Monkeypox virus generic real-time PCR test [Internet]. [cited 2026 Apr 19]. Available from: <https://www.cdc.gov/mpox/media/pdfs/2024/08/PCR-Diagnostic-Protocol-508.pdf>
131. R Core Team. R: a language and environment for statistical computing [Internet]. Vienna: R Foundation for Statistical Computing; 2023 [cited 2026 Apr 19]. Available from: <https://cran.r-project.org/doc/manuals/r-release/fullrefman.pdf>
132. Walker DG, Hutubessy R, Beutels P. WHO guide for standardization of economic evaluations of immunization programmes. *Vaccine*. 2010;28(11):2356-9. doi:10.1016/j.vaccine.2009.06.035
133. Otter JA, Yezli S, French GL. The role of contaminated surfaces in the transmission of nosocomial pathogens. *BMJ Infect Dis*. 2014;11:287. doi:10.1007/978-3-319-08057-4_3
134. Gould S, Atkinson B, Onianwa O, Spencer A, Furneaux J, Grieves J, et al. Air and surface sampling for monkeypox virus in a UK hospital: an observational study. *Lancet Microbe*. 2022;3:e904-11. doi:10.1016/S2666-5247(22)00257-9
135. Taha AM, Katamesh BE, Rhman HA, Abdelwahab OA, Rustagi S, Nguyen DK, et al. Environmental detection and spreading of mpox in healthcare settings: a narrative review. *Front Microbiol*. 2023;14:1272498. doi:10.3389/fmicb.2023.1272498
136. Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Smallpox and its eradication [Internet]. Geneva: World Health Organization; 1988 [cited 2026 Apr 19]. Available from: <https://apps.who.int/iris/handle/10665/39485>
137. Nörz D, Pfefferle S, Brehm TT, Franke G, Grewe I, Knobling B, et al. Evidence of surface contamination in hospital rooms occupied by patients infected with monkeypox,

Germany, June 2022. *Euro Surveill.* 2022;27(26):2200477. doi:10.2807/1560-7917.ES.2022.27.26.2200477

138. Wee LE, Arora S, Ko KKK, Conceicao EP, Coleman KK, Tan KY, et al. Environmental contamination and evaluation of healthcare-associated SARS-CoV-2 transmission risk in temporary isolation wards. *Am J Infect Control.* 2022;50(12):1347-54. doi:10.1016/j.ajic.2022.09.004
139. Loveday HP, Wilson JA, Pratt RJ, Golsorkhi M, Tingle A, Bak A, et al. National evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England. *J Hosp Infect.* 2014;86(Suppl 1):S1-70. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7114876/>
140. Wojgani H, Kehsa C, Cloutman-Green E, Gray C, Gant V, Klein N. Hospital door handle design and their contamination with bacteria: a real life observational study. Are we pulling against closed doors? *PLoS One.* 2012;7(10):e40171. doi:10.1371/journal.pone.0040171
141. Centers for Disease Control and Prevention. Infection prevention and control of mpox in healthcare settings [Internet]. Atlanta, GA: CDC; 2024 [updated 2024 May 24; cited 2025 Aug]. Available from: <https://www.cdc.gov/mpox/hcp/infection-control/healthcare-settings.html>
142. World Health Organization. International Health Regulations (2005) [Internet]. 3rd ed. Geneva: WHO; 2016 [cited 2026 Apr 19]. Available from: <https://www.who.int/publications/i/item/9789241580496>
143. Jezek Z, Grab B, Szczeniowski MV, Paluku KM, Mutombo M. Human monkeypox: secondary attack rates. *Bull World Health Organ.* 1988;66(4):465-70. Available from: <https://pubmed.ncbi.nlm.nih.gov/2844429/>

Appendixes

Appendix A: Ethical Approval Letter



GOVERNMENT OF SIERRA LEONE
Office of the Sierra Leone Ethics and Scientific Review Committee
Directorate of Training and Research
5th Floor, Youyi Building, Brookfields, Freetown
Ministry of Health

Data Sources: Assented/consented participants

- **Objective 1:** Administered Mpox risk assessment questionnaires to determine associated risk for Mpox infection and route of transmission. Ig G antibody profiling from dried blood samples
- **Objective 2:** collected skin, oropharyngeal swabs and venous blood samples for Mpox screening and management and private interview with participants
- **Objective 3:** Sequencing retrospectively collected blood samples from confirmed Mpox cases in current outbreak

Committee Action: Full Committee review

Approval Date: 23 May 2025

The Sierra Leone Ethics and Scientific Review Committee (SLESRC), having conducted a full Committee review of the above study protocol and determining that it presents minimal risk to subjects, **hereby grants ethical and scientific approval for it to be conducted in Sierra Leone.** The approval is valid for the period **23 May 2025 – 22 May 2026.** It is your responsibility to obtain re-approval/extension for any ongoing research prior to its expiration date. The request for re-approval/extension must be supported by a progress report.

Review Comments:

- **Amendments:** Intended changes to the approved protocol such as the informed consent documents, study design, recruitment of participants and key study personnel, must be submitted for approval by the SLESRC prior to implementation.
- **Termination of the study:** When study procedures and data analyses are fully complete, please inform the SLESRC that you are terminating the study and submit a brief report covering the protocol activities. Individual identifying information should be destroyed unless there is sufficient justification to retain, approved by the SLESRC. All findings should be based on de-identified aggregate data and all published results in aggregate or group form. A copy of any publication be submitted to the SLESRC for its archive.

Appendix B: Sequencing Metadata



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equencing%20sample

Appendix C: Phylogenetic Trees and Raw Data Outputs



Tree-SierraLN-Countr
y_edited.jpg



Tree-SierraLN-Lineag
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Trrre-SierraLN-sequen
ce_edited.jpg

Appendix E: Supplementary Tables

Samples selected for sequencing with Ct <20 for Run 1

Number	Sample ID	Ct Value
1	1	15.9
2	14	17.6
3	16	18.58
4	18	13.26
5	19	16.94
6	20	15.03
7	21	15.22
8	23	15.87
9	24	15.1
10	26	16.72
11	27	19.35
12	28	18.47
13	32	19.95
14	36	18.49
15	37	19.09
16	NC	

*NC: Negative Control

Samples selected for sequencing with CT <20 for Run 2

Number	Sample ID	Ct Value
1	41	18.64
2	43	17.59
3	45	17.34
4	46	18.02
5	47	19.57
6	48	16.8
7	50	18.58
8	51	16.73
9	52	17.68
10	54	16.78
11	55	16.76
12	56	19.27
13	57	19.46
14	58	17.3
15	59	19.09
16	NC	

*NC: Negative Control

Samples selected for sequencing with Ct <20 for Run 3

Number	Sample ID	Ct Value
1	60	16.09
2	61	16.4
3	62	15.88
4	63	19.71
5	64	17.76
6	65	16.56
7	67	17.76
8	70	19.82
9	71	16.76
10	72	17.86
11	74	16.64
12	75	18.43
13	77	19.97
14	79	18.74
15	81	17.17
16	NC	

*NC: Negative Control

Sample selected for sequencing with Ct <20 for Run 4

Number	Sample ID	Ct Value
1	98	19.63
2	97	20.06
3	99	19.85
4	100	16.29
5	101	17.34
6	NC	
7	82	19.78
8	85	18.42
9	87	17.1
10	88	19.66
11	90	18.8
12	92	19.89
13	93	18.68
14	94	16.62
15	95	18.21
16	96	17.26

*NC: Negative Control

Amplicon generation, normalization, and Barcoding for Run 1

AMPLICON QUANTIFICATION AND NORMALIZATION _SEP 7TH, 2025					
NO	LAB ID	AMPLICONS(ng/ul)	SAMPLE(ul) (/amplicons by 200ng)	WATER (ul)(- 10 from sample)	BARCODE
1	1	28.6	6.99		1
2	14	16.2	12.35	-2.35	2
3	16	29.2	6.85	3.15	3
4	18	6.46	30.96	-20.96	4
5	19	6.88	29.07	-19.07	5.00
6	20	9.44	21.19	-11.19	6
7	21	13.1	15.27	-5.27	7
8	23	7.54	26.53	-16.53	8
9	24	8.02	24.94	-14.94	9
10	26	12.5	16.00	-6.00	10
11	27	9.62	20.79	-10.79	11
12	28	15.5	12.90	-2.90	12
13	32	31	6.45	3.55	13
14	36	15	13.33	-3.33	14
15	37	10.8	18.52	-8.52	15
16	NC	0.65	307.69	-297.69	16
		DNA POOL QUANTIFICATION - VERY HIGH		FOR NEGATIVE, 10UL OF WATER	

Amplicon generation, normalization, and Barcoding for Run 2

AMPLICON QUANTIFICATION AND NORMALIZATION _SEP 7TH, 2025					
NO	LAB ID	AMPLICONS(ng/ul)	SAMPLE (ul) (/ amplicons by 200ng)	WATER (ul) (-10 from sample)	BARCODE
1	41	36.6	5.46	4.54	1
2	43	67.6	2.96	7.04	2
3	45	21.4	9.35	0.65	3
4	47	75	2.67	7.33	4
5	46	68.2	2.93	7.07	5
6	48	77.8	2.57	7.43	6
7	50	83	2.41	7.59	7
8	51	56.2	3.56	6.44	8
9	52	33.8	5.92	4.08	9
10	54	45.8	4.37	5.63	10
11	55	33.2	6.02	3.98	11
12	56	76.8	2.60	7.40	12
13	57	71.4	2.80	7.20	13
14	58	68.4	2.92	7.08	14
15	59	32.6	6.13	3.87	15
16	NC	1.51	132.45	-122.45	16
		DNA POOL QUANTIFICATION 106UL		FOR NEGATIVE, 10UL OF WATER	

Amplicon generation, normalization, and Barcoding for Run 3

AMPLICON QUANTIFICATION AND NORMALIZATION _SEP 8TH, 2025					
NO	LAB ID	AMPLICONS(ng/ul)	SAMPLE (ul) (/ amplicons by 200ng)	WATER (ul) (- 10 from sample)	BARCODE
1	60	55.2	3.62	6.38	17
2	61	54.2	3.69	6.31	18
3	62	74.8	2.67	7.33	19
4	63	74	2.70	7.30	20
5	64	24.8	8.06	1.94	21
6	65	12.8	15.63	-5.63	22
7	67	57.4	3.48	6.52	23
8	70	28	7.14	2.86	24
9	71	81.2	2.46	7.54	16
10	72	58.2	3.44	6.56	15
11	74	33.6	5.95	4.05	14
12	75	46	4.35	5.65	13
13	77	12.6	15.87	-5.87	12
14	79	20.4	9.80	0.20	11
15	81	11.6	17.24	-7.24	10
16	NC	4.7	42.55	-32.55	9
		DNA POOL QUANTIFICATION TOO HIGH	FOR NEGATIVE, 10UL OF WATER		

Amplicon generation, normalization, and Barcoding for Run 4

AMPLICON QUANTIFICATION AND NORMALIZATION _SEP 9TH, 2025					
NO	LAB ID	AMPLICONS(ng/ul)	SAMPLE (ul) (/ amplicons by 200ng)	WATER (ul) (- 10 from sample)	BARCODE
1	82	44	4.55	5.45	17
2	85	81.4	2.46	7.54	18
3	87	23.6	8.47	1.53	19
4	88	63.4	3.15	6.85	20
5	90	97.2	2.06	7.94	21
6	92	46.2	4.33	5.67	22
7	93	59	3.39	6.61	23
8	94	25.8	7.75	2.25	24
9	95	88.8	2.25	7.75	1
10	96	79.2	2.53	7.47	2
11	97	86.8	2.30	7.70	3
12	98	68.4	2.92	7.08	4
13	99	35.2	5.68	4.32	5
14	100	19.5	10.26	-0.26	6
15	101	22.8	8.77	1.23	7
16	NC	4.7	42.55	-32.55	8
		DNA POOL QUANTIFICATION 64.0UL	FOR NEGATIVE, 10UL OF WATER		