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Short Communication

Mpox Outbreaks in South Sudan: Genomic Characterization of Clade 1b Mpox Virus, 2025

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Abstract

Objectives: To detect and genetically characterize Mpox virus (MPXV) cases in South Sudan using both real-time PCR and GeneXpert assays.

Methods: Active Mpox surveillance was conducted at border sites, sentinel hospitals and in community in early 2025. Clinical samples collected from suspected cases were tested using real-time PCR and GeneXpert assays, followed by whole-genome sequencing (WGS) using Illumina MiSeq and analysis via the DRAGEN pipeline.

Results: Eight suspected cases were reported. Three (37.5%) tested positive via PCR and GeneXpert. All three cases were from Juba, with one individual reporting travel to Uganda. Sequencing confirmed all three belonged to Clade 1b, with high genome coverage (94.6% to 99.4%). Phylogenetic analysis indicated close relatedness to strains from Uganda and the DRC.

Conclusion: This study confirms the presence of Clade 1b MPXV in South Sudan, suggesting local transmission. Strengthened genomic surveillance and regional coordination are vital to contain the spread and inform public health responses.

Keywords: *Monkeypox Virus; Clade 1b; Genomic Surveillance; South Sudan; PCR; GeneXpert; Illumina*

Introduction

Mpox is a zoonotic disease caused by Monkeypox virus (MPXV), a member of the Orthopoxvirus genus [1]. First identified in 1958 in laboratory monkeys, human Mpox was documented in the Democratic Republic of the Congo (DRC) in 1970 [2]. Direct contact with skin lesions, respiratory droplets, contaminated fomites, and sexual contact with infected individuals are the primary means by which the disease is transmitted from person to person [3]. Clinically, mpox symptoms include fever, rash, and lymphadenopathy; these can develop into complications such pneumonitis, encephalitis, and bacterial infections [4]. The notable increase in Mpox cases emphasizes the necessity of enhanced diagnostic techniques with high sensitivity, precision, and rapidity. This expanding medical concern is being successfully addressed by conventional and real-time PCR and serological testing-based methods [5].

Two main genetic clades exist: Clade I (Central African) and Clade II (West African). Clade I sublineage Ib causes more severe disease than Clade II and has been associated with outbreaks in the DRC and Uganda [6]. In Africa, additional cases have been reported in Kenya, Liberia, Nigeria, Rwanda, South Africa, Uganda, Burundi, Cameroon, Central African Republic, Congo, Cote d'Ivoire, and the DRC in 2024 [7]. In response to rising cases in neighboring countries, South Sudan launched targeted surveillance in early 2025, particularly in areas bordering South Sudan and Uganda, to mitigate the risk of cross-border spillover on public health. Coordination of research and policy initiatives is crucial for going ahead to close knowledge gaps and improving resistance to future outbreaks.

Purpose of the Study

The purpose of this study was to detect and genetically characterize Mpox virus infections in South Sudan in early 2025, with a specific focus on identifying the circulating clade and evaluating evidence of local transmission. The study was conducted as a component of the viral pathogen surveillance.

Methods

Case identification and sample collection: Eight suspected Mpox cases were identified between January and February 2025 through active surveillance. Patients presented with fever, rash, lymphadenopathy, and vesicular lesions. Swab samples (lesions) were collected and transported to the National Public Health Laboratory (NPHL) of South Sudan under a cold chain.

PCR and GeneXpert testing: DNA was extracted using the QIAamp DNA Mini Kit and tested using FlexStar® Monkeypox virus PCR Mix 1.5 (Altona Diagnostics). Real-time PCR was performed on a Bio-Rad CFX96 platform. GeneXpert® Mpox assay was run using 300 µL of sample on the GeneXpert Xpress system (Cepheid) to detect MPXV DNA.

Whole-genome sequencing: Positive samples underwent target enrichment WGS using the Illumina MiSeq platform and the Viral Surveillance Panel v2. The analysis was performed using the UVRI in-house metagenomics pipeline, supplemented by the DRAGEN Microbial Enrichment tool. Phylogenetic trees were generated to determine clades and genetic relatedness.

Results

Of eight suspected cases (5 females, 3 males), three were confirmed positive for MPXV. Confirmed cases included two males (ages 31 and 35) and one female (age 30), all residing in urban neighborhoods of Juba, in table 1. Only one patient had a recent travel history to Uganda.

Ct values for MPXV PCR ranged from 15.14 to 20.15; GeneXpert values ranged from 21.0 to 24.5. WGS yielded genome coverage of 94.6% to 99.4%. All three genomes belonged to Clade 1b.

Sex/Age	Sample Type	Residence	Travel History	Symptom Onset	MPXV PCR Result	GeneXpert Result	NGS Coverage (%)	Clade
M/31	Swab	Luri, Juba	Frequent to UG	Jan 25, 2025	Positive (Ct:20.15)	Positive (Ct: 24.5)	99.4%	Clade 1b
F/30	Swab	Munuki C	None	Feb 5, 2025	Negative	Negative	N/A	N/A
F/19	Swab	UNS, POC	None	Feb 5, 2025	Negative	Negative	N/A	N/A
F/30	Swab	Nyakuron	None	Feb 2, 2025	Positive (Ct:15.14)	Positive Ct: 21.0)	94.6%	Clade 1b

F/39	Swab	Gurei	None	Feb 9, 2025	Negative	Negative	N/A	N/A
M/35	Swab	Munuki Bk B	None	Feb 4, 2025	Positive (Ct: 18.7)	Positive Ct:23.6)	99.4%	Clade 1b
M/59	Swab	WES	None	Feb 8, 2025	Negative	Negative	N/A	N/A
M/31	Swab	J. Nabari	None	Feb 4, 2025	Negative	Negative	N/A	N/A
Key: UNS: Upper Nile State, POC: Protection of Civilian Site, N/A: Not Applicable, UG: Uganda								

Table 1: *Demographic and laboratory results of Mpox cases in South Sudan, 2025.*

Discussion

The recent re-emergence of Monkeypox virus (MPXV), particularly the virulent Clade 1b sublineage, has heightened public health concerns across Central and East Africa [4]. Countries neighboring South Sudan, notably Uganda and the Democratic Republic of the Congo (DRC), have reported outbreaks associated with this clade last year 2024. Despite South Sudan's geographic vulnerability, genomic surveillance data on MPXV in the country have been lacking, limiting early detection, preparedness, and response planning. Through targeted surveillance and laboratory confirmation via PCR, GeneXpert, and next-generation sequencing, we identified three MPXV-positive cases out of eight suspected patients in Juba. All three positive samples were genetically confirmed as Clade 1b. Notably, two of the three cases had no travel history, suggesting the possibility of undetected, community-based transmission.

Phylogenetic analysis further revealed that the detected strains were closely related to Clade 1b sequences from Uganda and the DRC, as shown in figure 1, providing evidence for regional virus movement and underscoring the importance of cross-border surveillance efforts. These findings point to a shifting epidemiological landscape where MPXV may be silently spreading in areas with limited diagnostic capacity [8]. The combination of real-time PCR, GeneXpert, and whole-genome sequencing proved effective in confirming Mpox cases and assigning them to a clade with high confidence [9-11]. The use of Illumina MiSeq and the DRAGEN pipeline enabled high-quality genomic coverage (94.6-99.4%), providing robust data for regional comparisons. Among the five suspected but PCR-negative cases, clinical symptoms were consistent with Mpox, raising the possibility of other etiologies such as varicella-zoster virus or enteroviral exanthems. This suggests a need to incorporate broader viral diagnostic panels and consider differential diagnoses in future surveillance efforts. This investigation demonstrates the value of integrating genomic tools into national surveillance systems, especially in countries with limited prior sequencing experience. Genomic surveillance not only aids in case confirmation and outbreak tracking but also contributes to the global understanding of MPXV evolution and transmission dynamics. However, the small sample size and absence of contact tracing reduce the ability to establish firm transmission chains. Additionally, serological testing was not performed, which may have helped identify asymptomatic or past infections. Future studies should incorporate sero-epidemiological assessments and integrate socio-behavioral data to characterize transmission dynamics and risk factors better.

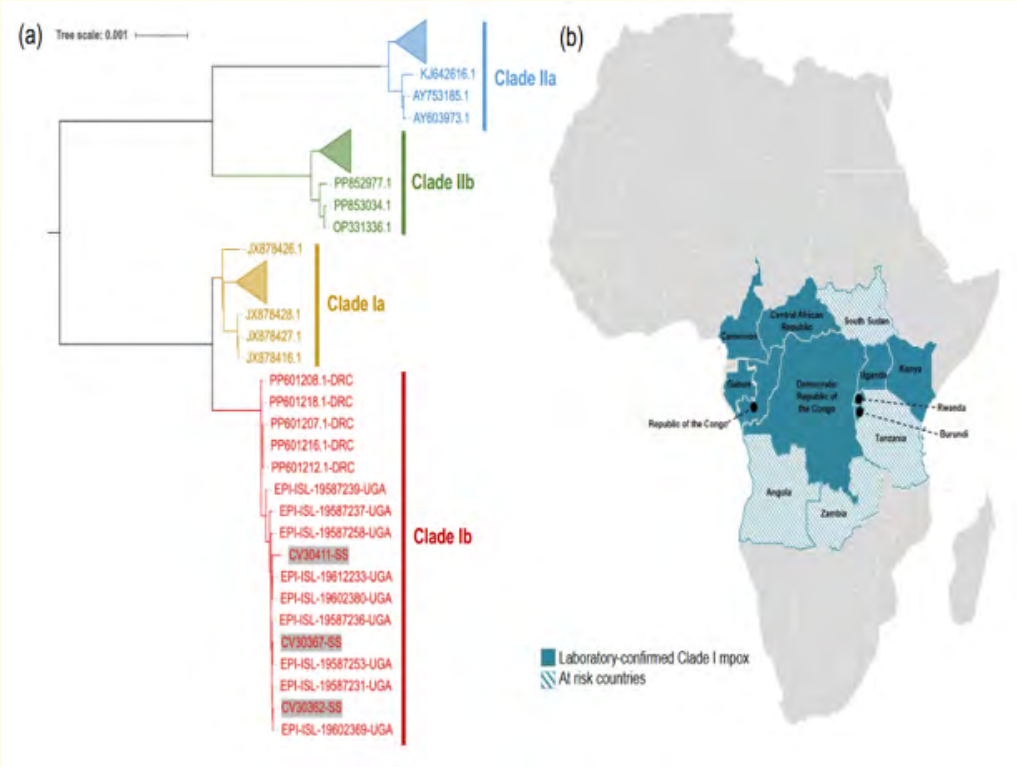

Figure 1: *(a) Maximum likelihood phylogenetic tree showing the evolutionally relationship among different clades of Monkeypox virus. Sequences from South Sudan highlight with a gray background cluster within Clade Ib, showing a closer relationship to Ugandan sequences than to other Clade Ib sequences from DRC. (b) African Region countries reporting laboratory confirmed clade I Mpox or where there may be a risk of clade I Mpox exposure in 2025 (Source: The UK Health Security Agency).*

Conclusion

This study confirms the presence of Clade 1b Monkeypox virus (MPXV) in South Sudan and provides evidence of possible community-based transmission. The application of real-time PCR, GeneXpert, and whole-genome sequencing enabled reliable detection and genetic characterization of MPXV, with phylogenetic analysis revealing close relatedness to strains from Uganda and the DRC. This finding underscores the urgent need to strengthen genomic surveillance, enhance molecular diagnostic capacity, and improve regional coordination for early detection and containment of Mpox outbreaks.

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Author Contributions

Andrew A. Othow and James Ayei were responsible for conceptualization, study design, data analysis, Laboratory analysis, data validation, and writing the original draft. Yona Kenyi: Data collection, Field coordination, and Community engagement. Deogratius

Ssemwanga was responsible for Genomic sequencing and bioinformatics phylogenetic analysis, Data interpretation. Gregory Wani, Abe Gordon, and Lul Lojok were responsible for the critical review of the manuscript.

Conflict of Interest

The authors declare no competing interests.

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Ethical Approval Statement

The study was done as part of the Viral Pathogen Surveillance and Discovery study, approved by the MOH Research Ethics Committee MOH/RERB/P/D.06/2025.

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