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# Nepal's First Whole Genome Sequencing of the Monkeypox Virus: Imported Cases from Saudi Arabia with Evidences of Secondary Transmission

Shivendra Kumar Jha,[1] Priya Karna,[1] Gaurab Pandey,[2] Pratik Kunwar,[2] Ranjan Raj Bhatta,[2] Nirajan Bhusal[2]

[1]Sukraraj Tropical and Infectious Diseases Hospital, Teku, Kathmandu, Nepal, [2]National Public Health Laboratory, Teku, Kathmandu, Nepal.

## ABSTRACT

Monkeypox (Mpox) is an infection caused by a zoonotic orthopoxvirus that is being reported in non-endemic regions. We present here 4 confirmed and probable cases managed at Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu. Three patients were Nepalese men returning from Saudi Arabia with fever, malaise, and papulopustular rashes. The fourth case, the wife of one patient, developed fever, myalgia, lethargy, headache, and later vaginal pustular lesions progressing to ulcers, indicating secondary transmission. Lesion swabs and crust samples underwent real-time PCR at the National Public Health Laboratory (NPHL), Teku, Kathmandu, Nepal targeting G2R and F3L genes, all testing positive (Ct ≤35). Positive samples were sequenced in-country at NPHL for the first time using the PrimalSeq protocol on an Illumina MiSeq platform. Genome analysis confirmed Clade IIb A.2, matching strains circulating in Europe and the Middle East. NPHL's clade identification was concordant with WHO reference laboratories, validating Nepal's genomic capacity.

**Keywords:** Clade IIb A.2; genome sequencing; Monkeypox; Mpox; Nepal

## INTRODUCTION

Mpox, a zoonotic orthopox DNA virus related to smallpox, was first identified in humans in the Democratic Republic of the Congo in 1970.[1] The 2022 outbreak disproportionately affected gay, bisexual, and other men who have sex with men, with frequent genital, oral, and perioral lesions suggesting sexual transmission.[2] On July 14, 2022, Saudi Arabia announced the first laboratory-confirmed human case of Mpox, and by August of that year, five cases had been verified.[3] The first confirmed case in Nepal occurred in 2023.[4]

Here we report three imported Mpox cases in Nepalese migrant workers who had recently returned from Saudi Arabia, along with one secondary transmission case.

## CASE REPORT

CASE 1

A 36-year-old male from Tanahun, Nepal, employed as a plumber in Saudi Arabia for over 10 years, presented to Sukraraj Tropical and Infectious Disease Hospital (STIDH), Kathmandu, with a one-month history of progressive papular and pustular skin lesions. He reported sexual contact with three sex workers in Saudi Arabia, with condom use except for one episode of breakage. Three to four days post-sexual exposure, he developed low-grade fever, fatigue, sore throat, and myalgia, followed by erythematous, painful penile lesions that progressed to pustules and crusts. The rash subsequently spread to the hands, forearms, trunk, back, scrotum, scalp, face, soles, and intergluteal region. Examination revealed >50 well-defined papular/pustular lesions (0.1–1 cm), asymmetrically distributed, without mucosal involvement or lymphadenopathy. Systemic examination was unremarkable. Blood tests and serology for HIV, hepatitis B, and syphilis were negative.

CASE 2

A 44-year-old male from Sindhuli, Nepal, employed as a driver in Saudi Arabia for 16 years, presented to Sukraraj Tropical and Infectious Disease Hospital, Kathmandu, with a two-week history of progressive papular and pustular rash. The patient denied a history of sexual exposure; however, given the involvement of genital lesions, sexual transmission could not be excluded. Seventeen days before presentation, he

**Correspondence: Shivendra Kumar Jha, Sukraraj Tropical and Infectious Diseases Hospital, Teku, Kathmandu, Nepal,Email: shivendrakjha@gmail.com, Phone: +9779841567712.**

developed acute-onset fever with chills, headache, sore throat, myalgia, fatigue, and painful bilateral inguinal swelling. Two days later, lesions appeared on the right leg, progressing sequentially to the genitals, hands, face, arms, trunk, scalp, and left shin.

On examination, >40 well-defined papulopustular lesions with crusting were distributed over extremities, trunk, scalp, palms, and genitalia. Genital swelling prevented prepuce retraction; the glans showed multiple superficial ulcers with crusting. No oral or ocular involvement was noted. Systemic examination was unremarkable. Routine blood tests and serology for HIV, hepatitis B, and syphilis were normal.

CASE 3

A 37-year-old male Nepalese migrant worker residing in Saudi Arabia returned to Nepal and presented to Sukraraj Tropical and Infectious Disease Hospital, Kathmandu, with multiple grouped crusted papules over the suprapubic region for several days. The lesions, approximately 15–16 in number, erythematous, and crusted, with 6–8 discrete urticated papules over the abdomen, bilateral thighs, and fingers. The patient reported a history of close intimate contact approximately 12 days prior to lesion onset and a subsequent intimate contact after arriving in Nepal with his wife (Case 4), who later developed compatible symptoms. On palpation, lesions were warm and mildly tender without regional lymphadenopathy. Systemic examination was unremarkable. Given the clinical appearance and history, Mpox was suspected.

CASE 4

The wife of Case 3, a 34-year-old female, presented three days after close contact with her partner, reporting feverish sensation, myalgia, lethargy, and headache.

Two days later, she developed pustular lesions at the vaginal orifice, which ruptured to form two ulcers (1 cm × 0.5 cm) located at the 5 and 7 o'clock positions of the labia minora. The ulcers were oval, with well-defined margins, fibrinous bases, and normal surrounding mucosa. On palpation, the ulcers were soft, tender, non-indurated, and bled mildly on touch. No regional lymphadenopathy was detected. Based on the temporal association with a confirmed case and clinical findings, secondary Mpox transmission was suspected.

In all four cases, Mpox infection was confirmed by real-time PCR from lesion samples (fluid, crust, or swabs), and patients were managed with isolation and supportive care, including wound care, emollients, analgesics, antibiotics when indicated, and symptomatic treatment, with favorable clinical outcomes.

Mpox PCR testing was performed at the National Public Health Laboratory (NPHL) in Teku, Kathmandu, and confirmed positive for all four patients. After confirmation, the NPHL conducted next-generation sequencing (NGS) on the clinical samples. The viral genome data obtained were analyzed using bioinformatics pipelines to identify viral lineage and genetic similarities to other global Mpox strains. Phylogenetic analysis showed that both cases were infected with the Clade IIb A.2 strain, which has been frequently reported in recent outbreaks across Europe and the Middle East.

Notably, NPHL's internal validation assessments demonstrated that their clade identification results were fully concordant with those obtained from WHO collaborating reference laboratories.

## DISCUSSION

With considerable outbreaks in non-endemic areas, Mpox has recently resurfaced as a major worldwide health problem. This case of a 36-year-old man (Case 1) with Mpox provides insight into the natural history of the disease, including patterns of transmission, progression of clinical manifestations, and diagnostic confirmation, consistent with findings from previous studies. The patient had a history of high-risk sexual encounters, which led to the development of skin lesions that were initially papular and pustular before becoming crusted. The findings of Adler et al., who reported comparable lesions in a group of Mpox patients in the UK, are in line with this clinical trajectory.[5] Although lymphadenopathy, a common prodromal symptom of Mpox, was absent in our patient, research conducted by McCollum et al. has shown that it is not always present, especially in instances with mild systemic involvement.[6]

This case's geographic setting is noteworthy. The patient lived in Saudi Arabia, a non-endemic country, but during the 2022 outbreak, there were five confirmed cases of Mpox there by August 2022. This is consistent with research by Bunge et al. showing that Mpox is spreading to non-endemic regions globally.[7] The global spread of Mpox is driven primarily by international travel and close-contact transmission, particularly within high-risk social networks. While many cases in the current outbreak have been associated with non-

sexual transmission routes, the role of sexual exposure warrants close attention. In our series, three patients (Cases 1, 3, and 4) reported sexual exposure as the likely route of acquisition. Furthermore, Case 2 presented with a lesion located exclusively on the genitalia. Given the established requirement for intimate skin-to-skin contact for transmission, this finding strongly suggests sexual or very close genital contact occurred, regardless of the patient's reported history. Consequently, we highlight that close-contact transmission, including sexual exposure, remains a critical mechanism driving the spread in specific patient cohorts, and this should be incorporated into public health messaging and surveillance strategies.

In this instance, PCR testing of lesion samples confirmed the presence of Mpox virus, as PCR is widely recognized as the gold standard for Mpox diagnosis.[8]

To stop additional transmission, the supportive management in this instance concentrated on wound care, hydration, and isolation. These treatments are similar to those described in earlier research, including those conducted by Rao et al., which emphasizes isolation as a vital public health strategy to stop the virus's transmission.[9] Additionally, the use of topical and oral antibiotics to prevent secondary bacterial infections is a standard approach documented in existing Mpox management protocols. According to Reynolds et al., the inability to obtain some antivirals, including tecovirimat, in this instance emphasizes the necessity of these medications being more widely available worldwide.[10]

## CONCLUSIONS

However, given the patient's favourable outcome with supportive care, this case demonstrates the effectiveness of early diagnosis and appropriate symptomatic management in uncomplicated Mpox cases.

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