Monkey pox Virus: Genomic Evolution and Transmission

[1]Miss Ifshana Shafi, [2]Mr Peerzada Mohammad Idrees, [3]Mr Shaikh Anaitullah
[1,2,3] *Sher-i-Kashmir Institute of Medical Sciences*
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***Abstract*—Monkeypox virus (MPXV), recently termed Mpox virus, is a zoonotic double-stranded DNA virus belonging to the genus *Orthopoxvirus* within the family *Poxviridae*. Historically endemic to Central and West Africa, MPXV re-emerged as a global public health concern during the 2022 multicounty outbreak, marking the largest recorded spread outside Africa. This outbreak highlighted significant changes in viral epidemiology, transmission dynamics, and genomic evolution. Unlike most RNA viruses, MPXV possesses a relatively stable DNA genome of approximately 197 kb; however, recent genomic surveillance has revealed an accelerated accumulation of mutations compared to earlier strains. Notably, mutational signatures consistent with host APOBEC3 cytidine deaminase activity suggest strong host-driven evolutionary pressure during sustained human-to-human transmission.**

Phylogenetically, MPXV is classified into two principal clades: Clade I (formerly Congo Basin clade), associated with higher virulence and case fatality rates, and Clade II (formerly West African clade), which includes the Clade IIb lineage responsible for the 2022 global outbreak. Comparative genomic analyses demonstrate gene loss, gene duplication, and point mutations—particularly within regions encoding immune modulating and host-range proteins—indicating ongoing viral adaptation to human hosts. These genomic changes may influence transmissibility, immune evasion, and viral fitness, though their precise phenotypic consequences remain under investigation.

This review synthesizes current knowledge on MPXV genomic architecture, evolutionary mechanisms, mutation patterns, and transmission pathways, while discussing their implications for public health surveillance, vaccine effectiveness, antiviral strategies, and outbreak preparedness. Continuous genomic monitoring and integrated epidemiological studies are essential to anticipate future evolutionary trajectories and to mitigate the global impact of this re-emerging orthopoxvirus.

***Index Terms*—Monkeypox virus (MPXV), genomic evolution, APOBEC3, zoonotic transmission, orthopoxvirus, viral mutation, Clade IIb, emerging infectious disease.**

I. INTRODUCTION

Mpox virus (formerly known as monkeypox virus) is a zoonotic, double-stranded DNA virus belonging to the genus *Orthopoxvirus* in the family *Poxviridae*. It is closely related to other orthopoxviruses, including Variola virus, the causative agent of smallpox, as well as cowpox and vaccinia viruses. First identified in 1958 during outbreaks among laboratory monkeys, and subsequently recognized in humans in 1970 in the Democratic Republic of the Congo, mpox has since emerged as a significant public health concern, particularly in Central and West Africa (Ahmed *et al.,* 2024).

Mpox is primarily transmitted through close contact with infected animals or humans, including exposure to bodily fluids, respiratory droplets, skin lesions, or contaminated materials. The natural reservoir is believed to involve small mammals such as rodents, although definitive reservoir species have not been fully confirmed. Clinically, mpox presents with symptoms resembling smallpox, including fever, lymphadenopathy, and a characteristic vesiculopustular rash. While generally less severe than smallpox, case fatality rates have varied depending on viral clade, host factors, and access to healthcare (Guimarães *et al .,*2023).

Historically, mpox cases were largely confined to endemic regions; however, international travel and changing ecological dynamics have facilitated its global spread. A notable multicountry outbreak occurred in 2022, with sustained human-to-human transmission reported across multiple continents. In response, the World Health Organization declared the outbreak a Public Health Emergency of International Concern, underscoring the virus's capacity for rapid dissemination beyond traditional endemic zones (Moss, 2024).

The cessation of routine smallpox vaccination following the eradication of smallpox in 1980 has contributed to increased susceptibility in younger populations, as cross-protective immunity against orthopoxviruses has waned. Current preventive strategies include vaccination with newer-generation smallpox vaccines, surveillance, contact tracing, and public health education. Ongoing research focuses on viral evolution, transmission dynamics, immune responses, and the development of targeted antiviral therapies (Dumonteil *et al.,* 2023).

Given its re-emergence, expanding geographic distribution, and potential for sustained human transmission, mpox represents a critical area of study in emerging infectious diseases. A comprehensive understanding of its virology, epidemiology, clinical manifestations, and control measures is essential for strengthening global preparedness and response strategies (Guimarães *et al .,*2023).

Transmission of MPXV occurs through zoonotic spill over from infected animal reservoirs, as well as through direct human-to-human contact via skin lesions, bodily fluids, respiratory droplets, contaminated fomites, and vertical transmission. The 2022 outbreak revealed sustained transmission within close-contact networks, emphasizing the evolving epidemiological patterns of the virus. Reduced population immunity following the cessation of smallpox vaccination has further contributed to increased susceptibility worldwide (Moss, 2024).

Mpox virus is an enveloped, brick-shaped, double-stranded DNA virus classified within the genus *Orthopoxvirus* of the family *Poxviridae*. The viral genome is approximately 197 kilobase pairs in length and encodes more than 190 open reading frames involved in viral replication, host immune modulation, and virulence. Similar to other orthopoxviruses, mpox virus replicates entirely within the cytoplasm of infected host cells, utilizing its own transcriptional machinery. This cytoplasmic replication strategy distinguishes poxviruses from many other DNA viruses and contributes to their complex life cycle (Ahmed *et al.,* 2024).

Phylogenetically, mpox virus has been categorized into two major clades: Clade I (formerly Congo Basin clade), historically associated with higher transmissibility and case fatality rates, and Clade II (formerly West African clade), generally linked to milder disease. Genomic surveillance during recent outbreaks has revealed ongoing viral evolution, including mutations that may influence transmissibility and host adaptation. These findings highlight the importance of molecular epidemiology in tracking viral spread and assessing emerging risks therapies (Dumonteil *et al.,* 2023).

The pathogenesis of mpox involves initial viral entry through broken skin, mucous membranes, or the respiratory tract, followed by local replication and dissemination to regional lymph nodes. A primary viremia phase enables systemic spread to distant organs, leading to secondary viremia and the characteristic cutaneous eruption. Unlike smallpox caused by Variola virus, mpox is typically associated with pronounced lymphadenopathy, which serves as a distinguishing clinical feature. The disease course generally includes a prodromal phase with fever, malaise, headache, and myalgia, followed by a centrifugal rash progressing through macular, papular, vesicular, pustular, and crusting stages (Yadav *et al .,*2025).

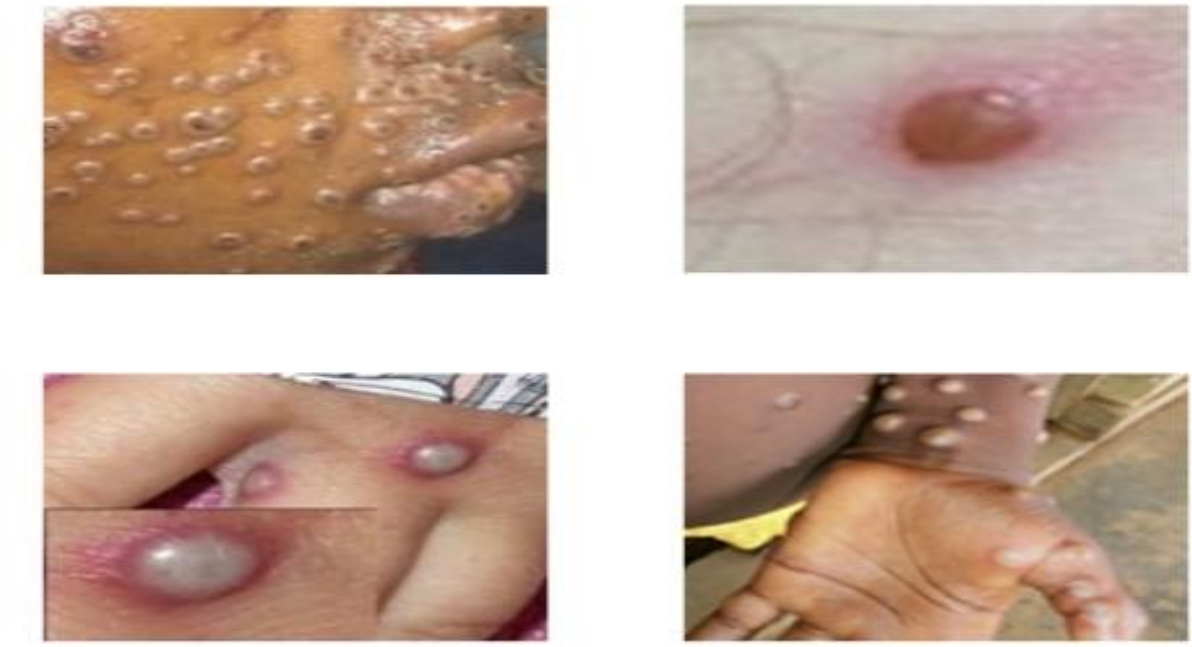
Transmission dynamics have evolved over time. Historically, zoonotic spillover events from wildlife to humans were the primary mode of infection, particularly in forested regions of Central and West Africa. However, sustained human-to-human transmission became evident during the 2022 global outbreak, with cases reported in non-endemic countries across Europe, the Americas, Asia, and Oceania. In July 2022, the World Health Organization declared mpox a Public Health Emergency of International Concern, reflecting the scale and international spread of the outbreak. Epidemiological data indicated that close physical contact, including sexual contact, played a significant role in transmission networks during this period (Tiecco *et al*., 2022).

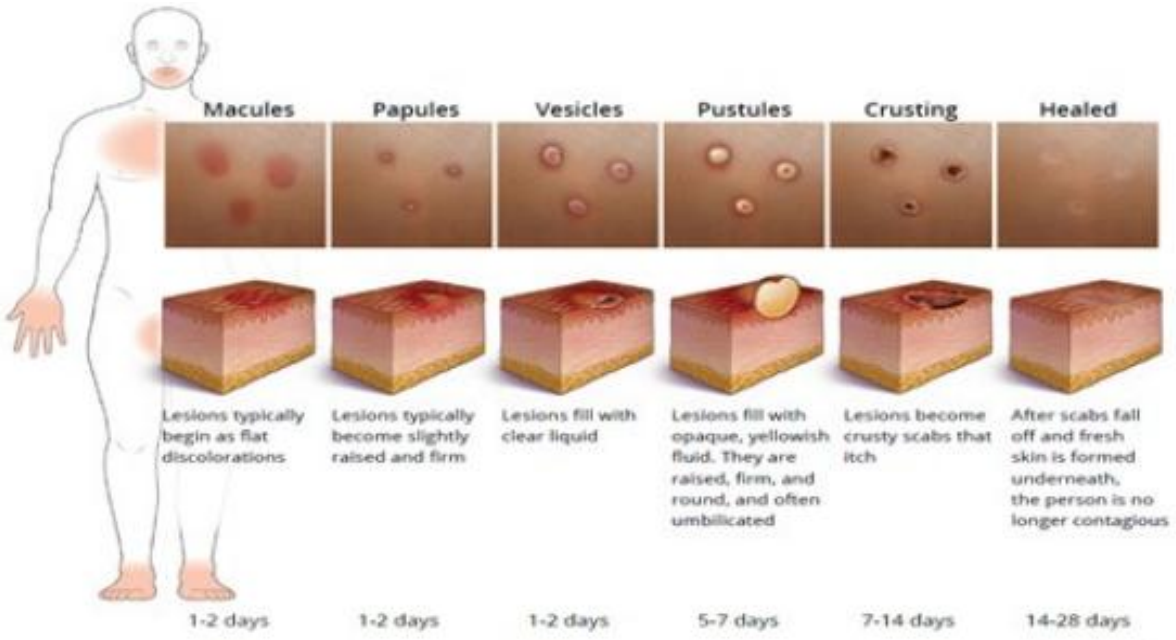
The global re-emergence of mpox has been partly attributed to declining population immunity following the discontinuation of routine smallpox vaccination after the eradication of smallpox in 1980. Vaccines originally developed against smallpox have demonstrated cross-protective efficacy against mpox due to antigenic similarities among orthopoxviruses. Additionally, antiviral agents such as tecovirimat have been deployed under expanded access protocols for severe or high-risk cases, though further clinical trials are required to establish definitive therapeutic guidelines (Tiecco *et al*., 2022).

From a public health perspective, mpox underscores the interconnectedness of human, animal, and environmental health, aligning with the One Health framework. Deforestation, wildlife trade,

urbanization, and increased global mobility may facilitate zoonotic emergence and international dissemination. Consequently, strengthening surveillance systems, improving laboratory diagnostic capacity, enhancing risk communication, and ensuring equitable vaccine access remain critical components of global preparedness strategies(Yadav *et al .,*2025).

In summary, mpox represents a re-emerging zoonotic infection with evolving epidemiological patterns and significant implications for global health security. Continued interdisciplinary research into viral genomics, host–pathogen interactions, transmission networks, and prevention strategies is essential to mitigate future outbreaks and enhance pandemic preparedness.





II. MATERIALS AND METHODS

1. Study Design and Specimen Collection

This section describes standardized laboratory procedures for the detection of Mpox virus in clinical and environmental specimens. The protocol is designed for use in diagnostic, surveillance, and research laboratories operating under appropriate biosafety conditions (minimum BSL-2 with BSL-3 practices for viral culture).

Clinical specimens:

Specimens were collected from suspected cases presenting with characteristic vesiculopustular lesions and systemic symptoms. Recommended samples included:

- Lesion swabs (dry synthetic swabs or swabs in viral transport medium)
- Lesion crusts or scabs
- Vesicular or pustular fluid
- Oropharyngeal or nasopharyngeal swabs (where indicated)
- Whole blood or serum (for serology)

Lesion material was prioritized due to high viral load. Samples were placed in sterile, leak-proof containers, labelled appropriately, and transported to the laboratory under cold chain conditions (2–8°C) within 24–48 hours (Araf *et al .,* 2024).

2. Biosafety and Laboratory Precautions

All procedures involving potentially infectious materials were conducted in a Class II biological safety cabinet. Personnel used appropriate personal protective equipment (PPE), including gloves, gowns, N95 respirators, and eye protection.

Specimen handling and nucleic acid extraction is performed in separate designated areas to prevent cross-contamination. Viral culture, when conducted, followed enhanced containment procedures consistent with national and international biosafety guidelines (Jadhav *et al .,*2025).

3. Molecular Detection by Real-Time PCR

3.1 Nucleic Acid Extraction

Viral DNA is extracted from clinical specimens using commercially available silica column–based or magnetic bead–based extraction kits according to the manufacturer's instructions. Extracted DNA is eluted in nuclease-free water or elution buffer and stored at −20°C until analysis.

3.2 Real-Time Polymerase Chain Reaction (qPCR)

Detection of mpox viral DNA was performed using real-time polymerase chain reaction (qPCR) assays targeting conserved regions of the orthopoxvirus genome and mpox-specific gene sequences (e.g., F3L, B6R, or E9L genes).

Each reaction mixture (25 µL total volume) contained:

- 12.5 µL of 2× PCR master mix
- Forward and reverse primers (final concentration 0.4–0.5 µM each)
- Fluorescent probe (0.2 µM)

- 5 µL of extracted DNA template
- Nuclease-free water to volume

Thermal cycling conditions typically included:

1. Initial denaturation at 95°C for 2–3 minutes
2. 40–45 cycles of:
   - Denaturation at 95°C for 10–15 seconds
   - Annealing/extension at 55–60°C for 30–60 seconds

Amplification was performed using a real-time PCR detection system. A cycle threshold (Ct) value ≤38–40 was interpreted as positive, depending on assay validation criteria (Araf *et al.,* 2024).

3.3 Controls

Each run included:

- Positive control (known mpox DNA template)
- Negative extraction control
- No-template control (NTC)
- Internal control (to detect PCR inhibition) (Tiecco *et al*., 2022).

4. Conventional PCR and Sequencing

For confirmatory testing and molecular characterization, conventional PCR targeting orthopoxvirus-specific genes was performed. Amplified products were visualized using agarose gel electrophoresis (1.5–2% agarose gel with ethidium bromide or safe dye).

Positive amplicons were purified and subjected to Sanger sequencing. Sequence data were analyzed using bioinformatics software and compared with reference sequences available in genomic databases to confirm virus identity and determine clade classification (Jadhav *et al.,* 2025).

5. Viral Culture (Where Applicable)

Virus isolation was performed in selected high-containment laboratories. Clinical specimens were inoculated into susceptible cell lines such as Vero cells and incubated at 37°C with 5% CO_2.

Cells were monitored daily for cytopathic effects (CPE), including cell rounding, detachment, and syncytia formation. Confirmation of viral growth was achieved through PCR testing of culture supernatants or immunostaining methods.

Due to biosafety concerns and limited additional diagnostic benefit, viral culture was not routinely performed for primary diagnosis (Kumar *et al.,* 2025).

6. Serological Testing

Serological assays were conducted to detect anti-orthopoxvirus IgM and IgG antibodies in serum samples using enzyme-linked immunosorbent assay (ELISA). IgM positivity indicated recent infection, while IgG positivity suggested past exposure or prior smallpox vaccination.

Cross-reactivity with other orthopoxviruses was considered during interpretation. Serology was used primarily for epidemiological studies rather than acute diagnosis.

7. Electron Microscopy (Optional)

Transmission electron microscopy (TEM) was employed in specialized laboratories for visualization of characteristic brick-shaped orthopoxvirus particles in lesion material. While not species-specific, TEM provided rapid presumptive identification of orthopoxvirus infection.

8. Quality Assurance and Data Analysis

All assays were validated prior to implementation. Laboratory quality assurance included:

- Calibration of equipment
- Use of certified reference materials
- Participation in external quality assessment (EQA) programs
- Strict documentation and traceability

Data were analyzed using appropriate statistical software. Diagnostic sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated where applicable (Kumar *et al.,* 2025).

9. Case Definition and Inclusion Criteria

Suspected cases were identified based on clinical presentation consistent with mpox infection, including acute rash illness with vesicular or pustular lesions, fever, lymphadenopathy, and epidemiological risk factors (e.g., contact with a confirmed case or travel to affected regions). Confirmed cases required laboratory confirmation by nucleic acid amplification testing (NAAT).

10. Sample Processing and Storage

Upon receipt in the laboratory, specimens were logged into a laboratory information management system (LIMS). Samples were processed as follows:

- Lesion swabs/crusts: Rehydrated (if dry) in sterile phosphate-buffered saline (PBS), vortexed, and centrifuged to obtain supernatant.
- Fluid samples: Directly subjected to nucleic acid extraction.
- Blood samples: Centrifuged at 1,500–2,000 × g for 10 minutes to separate plasma/serum.

Aliquots were prepared to avoid repeated freeze–thaw cycles and stored at −20°C (short-term) or −80°C (long-term) (Karagoz *et al ., 2023).*

11. Multiplex PCR Assays

To differentiate mpox from other rash-causing pathogens, multiplex real-time PCR assays were employed. These assays simultaneously targeted:

- Orthopoxvirus genus–specific gene
- Mpox virus–specific gene
- Internal amplification control

Multiplex assays improved diagnostic efficiency and reduced turnaround time. Analytical sensitivity was determined using serial dilutions of quantified viral DNA standards, with limits of detection typically ranging from 10–100 copies/reaction, depending on assay optimization (Yadav, *et al* ., 2025).

12. Digital PCR (dPCR)

For quantitative viral load assessment and research applications, digital PCR (dPCR) was performed. Extracted DNA was partitioned into thousands of micro-reactions using droplet-based or chip-based systems.

Thermal cycling conditions were similar to qPCR; however, absolute quantification was achieved without reliance on standard curves. Viral load was expressed as copies per microliter of sample. dPCR was particularly useful for low viral load detection and monitoring treatment response in severe cases (Yadav, *et al* ., 2025).

13. Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) assays were evaluated as a rapid, field-deployable diagnostic alternative. LAMP reactions were performed at a constant temperature (60–65°C) for 30–60 minutes using specifically designed primer sets targeting conserved mpox genomic regions.

Results were interpreted by fluorescence detection or colorimetric change. Although slightly less specific than qPCR, LAMP provided rapid screening capability in resource-limited settings) (Karagoz *et al ., 2023).*

14. Whole Genome Sequencing (WGS)

For genomic characterization and outbreak investigation, whole genome sequencing (WGS) was conducted on selected positive samples.

15.1 Library Preparation and Sequencing

Extracted DNA underwent library preparation using standard protocols compatible with next-generation sequencing (NGS) platforms. Sequencing was performed using short-read or long-read technologies depending on availability.

15.2 Bioinformatics Analysis

Raw sequence data were quality-filtered, trimmed, and aligned to reference genomes. Variant calling was conducted to identify single nucleotide polymorphisms (SNPs) and insertions/deletions. Phylogenetic trees were constructed to determine clade assignment and transmission clusters.

WGS enabled tracking of viral evolution, detection of mutations associated with transmission efficiency, and differentiation between imported and locally transmitted cases (Guil *et al., 2022).*

16. Immunohistochemistry (IHC)

For tissue-based diagnosis, immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded biopsy samples. Tissue sections were incubated with orthopoxvirus-specific monoclonal or polyclonal antibodies, followed by enzyme-labeled secondary antibodies.

Chromogenic substrates were applied to visualize antigen–antibody complexes under light microscopy. IHC confirmed viral antigen presence in epithelial and dermal tissues and supported histopathological findings (Guil *et al., 2022).*

17. Histopathological Examination

Skin biopsy specimens were stained with hematoxylin and eosin (H&E). Characteristic findings included:

- Epidermal necrosis
- Ballooning degeneration
- Intraepidermal vesicle formation
- Eosinophilic cytoplasmic inclusion bodies (Guarnieri bodies)

Histopathology alone was not diagnostic but provided supportive evidence when correlated with molecular results (Tan, *et al.,* 2022).

18. Environmental and Surface Sampling

Environmental surveillance was conducted in healthcare and household settings with confirmed cases. Swab samples were collected from high-touch surfaces (e.g., bedding, door handles, clothing).

DNA extraction and qPCR were performed as described above. Environmental detection supported infection prevention and control (IPC) assessments but did not necessarily indicate viable virus (Tan, *et al.,* 2022).

19. Validation and Performance Characteristics

Diagnostic assays were evaluated for:

- Analytical sensitivity: Determined using serially diluted viral DNA standards.
- Analytical specificity: Assessed against related orthopoxviruses and other common viral pathogens (e.g., varicella-zoster virus).
- Precision: Measured through intra- and inter-assay reproducibility testing.
- Accuracy: Confirmed using reference-confirmed clinical samples.

Cross-reactivity studies were essential due to genetic similarity among orthopoxviruses (Qin, P *et al., 2022).*

20. Data Management and Statistical Analysis

Ct values from qPCR assays were recorded and analyzed using instrument software. Descriptive statistics were calculated for viral load distribution, specimen type comparison, and assay performance metrics.

Phylogenetic analyses were conducted using validated bioinformatics pipelines. Confidence intervals for sensitivity and specificity were calculated using standard statistical methods (Srivastava, 2022).

21. Limitations of Diagnostic Methods

- Serological assays may show cross-reactivity with other orthopoxviruses or prior smallpox vaccination.
- Environmental PCR positivity does not confirm infectivity.
- Viral culture requires high biosafety containment and is not routinely feasible.

Improper specimen collection may result in false-negative molecular results (Qin, P *et al., 2022).*

Comprehensive Detection Strategy

An optimal laboratory diagnostic algorithm for Mpox virus includes:

1. Primary confirmation by real-time PCR from lesion material
2. Secondary confirmation and clade identification via sequencing
3. Adjunctive methods (serology, IHC, histopathology) for research or retrospective studies
4. Genomic surveillance through WGS for outbreak tracking

This integrated methodological framework ensures high diagnostic accuracy, supports epidemiological investigations, and contributes to global surveillance and outbreak preparedness efforts (Srivastava, 2022).

Table 01.

Specimen Type	Preferred	Notes
Lesion swab	Yes	Best diagnostic yield
Lesion crust	Yes	Good viral DNA concentration
Blood	Limited	Low viral load
Throat swab	Variable	Less reliable

III. TAXONOMY AND GENOMIC STRUCTURE

3.1 Taxonomic Classification

- Family: *Poxviridae*
- Subfamily: *Chordopoxvirinae*
- Genus: *Orthopoxvirus*
- Species: Monkeypox virus (MPXV)

3.2 Genome Organization

1. General Genome Features

The mpox virus possesses a linear, double-stranded DNA genome of approximately 190–200 kilobase pairs (kb), encoding over 190 predicted open reading frames (ORFs). Like other members of the genus *Orthopoxvirus*, the genome is characterized by:

- A central conserved region (~120 kb) containing essential genes for replication and virion assembly
- Variable terminal regions that encode host range and immune evasion factors
- Inverted terminal repeats (ITRs) at both ends of the genome

The GC content is approximately 33%, consistent with other orthopoxviruses (Mills, 2024).

2. Inverted Terminal Repeats (ITRs)

At both ends of the genome are identical but oppositely oriented inverted terminal repeat sequences, typically 6–10 kb in length. These regions contain:

- Tandem repeat sequences
- Hairpin loop structures that form covalently closed ends
- Genes involved in host interaction and virulence

The hairpin termini are critical for viral DNA replication and resolution of concatemeric replication intermediates (Mills, 2024).

3. Central Conserved Region

The central portion of the genome is highly conserved among orthopoxviruses, including Variola virus and vaccinia virus. This region encodes proteins essential for:

3.1 DNA Replication

- DNA-dependent DNA polymerase
- Helicase–primase complex
- DNA ligase
- Topoisomerase
- Processivity factors

Replication occurs entirely in the cytoplasm within specialized structures known as viral factories (Mills, 2024).

3.2 Transcription Machinery

Unlike most DNA viruses, mpox virus encodes its own transcription apparatus, including:

- Multisubunit RNA polymerase
- Transcription factors (early, intermediate, and late stage–specific)
- mRNA capping and polyadenylation enzymes

Gene expression occurs in a temporally regulated cascade:

- Early genes: expressed immediately after entry; involved in immune modulation and DNA replication
- Intermediate genes: regulate late gene transcription
- Late genes: encode structural proteins and enzymes required for virion assembly (Li *et al.,* 2006).

3.3 Virion Structural Proteins

The central region encodes major structural components, including:

- Core proteins
- Membrane proteins
- Scaffold proteins
- Enzymes packaged within the virion

These proteins are critical for assembly of intracellular mature virions (IMVs) and extracellular enveloped virions (EEVs).

4. Terminal Variable Regions

The terminal regions exhibit greater genetic variability and are responsible for differences in virulence, host range, and immune evasion between clades.

These regions encode:

4.1 Host Range Proteins

- Proteins that enable infection of specific mammalian hosts by counteracting host antiviral defences (Li *et al.,* 2006).

4.2 Immune Modulatory Proteins

Mpox virus encodes multiple proteins that interfere with host innate and adaptive immunity, including:

- Cytokine-binding proteins (e.g., interferon-binding proteins)
- Complement control proteins
- TNF receptor homologs
- Chemokine-binding proteins

These proteins reduce inflammation, inhibit apoptosis, and suppress antiviral signalling pathways (Moss, B 2024).

4.3 Apoptosis Inhibitors

Viral Bcl-2–like proteins and caspase inhibitors help prevent premature host cell death, ensuring sufficient time for viral replication.

5. Genome Plasticity and Clade Differences

Comparative genomic analyses have revealed structural differences between Clade I and Clade II strains. Variations include:

- Gene duplications
- Deletions in terminal regions
- Single nucleotide polymorphisms (SNPs)

These differences may influence transmissibility, pathogenicity, and host adaptation. The terminal regions are hotspots for recombination and gene gain/loss events, contributing to viral evolution (Moss, B 2024).

6. Virion Forms and Associated Genes

Mpox virus produces multiple infectious forms:

1. Intracellular Mature Virion (IMV) – Stable form responsible for host-to-host transmission
2. Intracellular Enveloped Virion (IEV)
3. Cell-Associated Enveloped Virion (CEV)
4. Extracellular Enveloped Virion (EEV) – Facilitates long-range dissemination within the host

Distinct gene products regulate membrane wrapping, intracellular transport, and actin tail formation, enabling efficient viral spread (Tiecco &, Degli 2022).

7. Replication-Associated Genomic Features

Replication begins at multiple origins within the genome. Key features include:

- Resolution sequences within ITRs
- Concatemer formation during replication
- Viral resolvase enzymes that cleave and package monomer genomes

The genome is replicated as concatemers that are later resolved into unit-length genomes prior to encapsidation (Tiecco &, Degli 2022).

8. Comparison with Other Orthopoxviruses

Although highly homologous to other orthopoxviruses, mpox virus contains unique gene content in its terminal regions that contributes to differences in:

- Case fatality rates

- Transmission dynamics
- Immune escape capacity

Compared to Variola virus, mpox retains a broader host range but generally exhibits lower human-to-human transmission efficiency (with variation among clades) (Antoni , 2022).

9. Functional Organization Summary

The genome organization of mpox virus can be summarized as:

Table 02.

Genomic Region	Function
Inverted Terminal Repeats	Replication resolution, virulence genes
Central Conserved Core	Replication, transcription, virion assembly
Terminal Variable Regions	Host range, immune evasion, pathogenicity
Structural Gene Clusters	Virion formation and dissemination

IV. GENOMIC EVOLUTION OF MONKEYPOX VIRUS

4.1 Clade Classification

Historically, MPXV has been divided into two major clades:

1. Clade I (Congo Basin clade) – Higher virulence and mortality.
2. Clade II (West African clade) – Lower mortality rate.

The 2022 outbreak was primarily associated with Clade IIb, a lineage derived from the West African clade (Antoni , 2022).

4.2 Mutation Patterns and Evolutionary Dynamics

Unlike RNA viruses, DNA viruses such as MPXV generally evolve more slowly due to proofreading mechanisms in DNA polymerase. However, genomic sequencing during the 2022 outbreak revealed:

- An unexpectedly higher number of single nucleotide polymorphisms (SNPs).
- Strong evidence of APOBEC3-mediated mutagenesis (host cytidine deaminase activity).
- Gene loss and gene duplication events affecting immune-modulating genes.

These findings suggest host-driven evolutionary pressure contributing to viral adaptation in human populations (Vaughan & Aarons 2018).

4.3 Role of APOBEC3 in Viral Evolution

APOBEC3 enzymes induce cytosine-to-thymine (C→T) mutations in viral genomes. Genomic analyses of 2022 outbreak strains demonstrated mutation signatures consistent with APOBEC3 activity, indicating:

- Ongoing human-to-human transmission.
- Host immune system influence on viral evolution.
- Possible enhancement of viral adaptation to human hosts (Ježek & Szczeniowski 1987).

V. TRANSMISSION MECHANISMS

Transmission of mpox virus occurs through multiple interconnected pathways, including zoonotic spillover, direct human-to-human contact, indirect (fomite-mediated) exposure, and, under specific conditions, respiratory spread. The relative contribution of each route varies depending on epidemiological context, viral clade, and behavioral factors.

1. Zoonotic Transmission

Mpox is primarily a zoonotic infection, historically linked to animal-to-human transmission in endemic regions of Central and West Africa.

1.1 Reservoir and Amplifying Hosts

Although the definitive natural reservoir has not been conclusively identified, small mammals—particularly rodents such as rope squirrels, Gambian pouched rats, and dormice—are considered likely reservoirs. Non-human primates may serve as incidental hosts.

1.2 Routes of Animal-to-Human Infection

Zoonotic transmission may occur through:

- Bites or scratches from infected animals
- Direct contact with blood, bodily fluids, or lesions
- Handling or consumption of inadequately cooked bushmeat
- Contact with contaminated animal bedding or excreta

Viral entry occurs through broken skin, mucous membranes, or possibly the respiratory tract (Ježek & Szczeniowski 1987).

2. Human-to-Human Transmission

Human-to-human transmission has become increasingly significant, particularly during recent global outbreaks.

2.1 Direct Contact Transmission

The most efficient route of transmission is direct physical contact with:

- Skin lesions or scabs
- Vesicular or pustular fluid
- Mucosal surfaces of infected individuals

Viral load is highest in lesion material, making close skin-to-skin contact a major driver of spread (Vaughan & Aarons 2018).

2.2 Sexual Transmission

During the 2022 global outbreak, epidemiological data indicated substantial transmission associated with intimate and sexual contact networks. While mpox is not exclusively a sexually transmitted infection (STI), prolonged close physical contact during sexual activity facilitates efficient viral transfer.

Viral DNA has been detected in genital lesions, anorectal swabs, and semen; however, the extent to which semen contributes to infectious transmission remains under investigation. Transmission in this context is primarily attributed to close mucocutaneous contact rather than classical STI mechanisms.

3. Respiratory Transmission

Respiratory transmission may occur through exposure to respiratory droplets during prolonged face-to-face contact. Key characteristics include:

- Larger droplets (>5 μm) requiring close proximity
- Increased risk in household settings
- Potential exposure during healthcare procedures without adequate PPE

Unlike airborne viruses such as measles or varicella, mpox transmission through aerosols appears less efficient and generally requires sustained close interaction (Reynolds 2012).

4. Fomite Transmission

Indirect transmission can occur through contact with contaminated materials, including:

- Bedding
- Towels
- Clothing
- Medical equipment

The virus demonstrates environmental stability due to its double-stranded DNA structure and protective viral envelope. Viral DNA may persist on surfaces for extended periods; however, detection of DNA does not necessarily indicate viable infectious virus (Alakunle *et al., 2024).*

Proper disinfection with approved virucidal agents is essential to interrupt fomite-mediated transmission.

5. Vertical Transmission

Transplacental transmission from mother to fetus has been documented in limited cases. This can result in:

- Congenital infection
- Fetal demise
- Neonatal mpox

The mechanisms likely involve hematogenous spread during maternal viremia. Additional research is required to define transmission risk during pregnancy and breastfeeding (Reynolds 2012).

6. Nosocomial Transmission

Healthcare-associated transmission has been reported in settings where infection prevention and control (IPC) measures were inadequate. Risk factors include:

- Direct contact with lesions without gloves
- Inadequate respiratory protection
- Improper handling of contaminated linens
- Laboratory exposure during specimen processing

Strict adherence to standard, contact, and droplet precautions significantly reduces transmission risk in healthcare facilities.

7. Viral Shedding Dynamics

Viral shedding occurs from multiple anatomical sites:

- Skin lesions (highest concentration)
- Oropharyngeal secretions
- Rectal mucosa
- Blood (during viremia phase)

Infectivity generally persists until all lesions have crusted, scabs have fallen off, and new skin has formed. This prolonged infectious period contributes to household transmission risk (Vaughan & Aarons 2018).

8. Factors Influencing Transmission

8.1 Host Factors

- Lack of prior smallpox vaccination
- Immunocompromised status (e.g., HIV infection)
- Presence of skin barrier disruptions

8.2 Viral Factors

- Clade-specific virulence differences
- Mutations affecting host adaptation
- Efficiency of immune evasion proteins

8.3 Environmental and Behavioural Factors

- Overcrowding
- Close-contact social networks
- International travel
- Wildlife trade and deforestation (Reynolds 2012).

9. Basic Reproductive Number (R_0)

Historically, mpox exhibited a relatively low basic reproductive number ($R_0 < 1$ in many settings), limiting sustained outbreaks. However, recent epidemiological patterns suggest that under certain social and contact conditions, effective reproductive numbers may exceed 1, allowing sustained human-to-human transmission.

Table 03.

Feature	Endemic Regions	Non-Endemic Regions
Primary source	Zoonotic spillover	Human-to-human
Household spread	Common	Common
Sexual network transmission	Less documented historically	Prominent in 2022 outbreak
Environmental exposure	Frequent	Limited

VI. FACTORS INFLUENCING VIRAL SPREAD

Several factors contributed to the 2022 global outbreak:

- Declining population immunity after cessation of smallpox vaccination
- Increased global travel

- Dense social networks
- Viral genetic adaptation

The eradication of smallpox in 1980 and discontinuation of vaccination programs led to reduced cross-protective immunity against MPXV.

1. Viral Determinants

1.1 Genetic Variability and Clade Differences

Mpox virus is divided into distinct genetic clades (Clade I and Clade II), which differ in transmissibility and virulence. Variations in genes located primarily within the terminal regions of the genome influence:

- Host immune evasion capacity
- Viral replication efficiency
- Tissue tropism
- Pathogenicity

Genomic mutations, gene deletions, and duplications may enhance adaptation to human hosts, potentially increasing human-to-human transmission efficiency(Vaughan & Aarons 2018).

1.2 Immune Modulation Mechanisms

The virus encodes multiple proteins that interfere with host innate immunity, including interferon-binding proteins and cytokine decoy receptors. These mechanisms delay immune recognition, prolong viral shedding, and increase the likelihood of transmission (Bunge 2022).

1.3 Environmental Stability

As a large, double-stranded DNA virus, mpox demonstrates relative stability outside the host compared to many RNA viruses. This environmental resilience supports fomite-mediated transmission under favorable conditions.

2. Host Factors

2.1 Population Immunity

The discontinuation of routine smallpox vaccination following global eradication significantly reduced cross-protective immunity against orthopoxviruses. Individuals born after the cessation of vaccination programs lack orthopoxvirus immunity, increasing susceptibility.

2.2 Immunocompromised Status

Individuals with compromised immune systems (e.g., HIV infection, malignancy, immunosuppressive therapy) may experience:

- Prolonged viral shedding
- Higher viral loads
- More severe or atypical disease

These factors can enhance transmission risk within close-contact networks (Bunge 2022).

2.3 Age and Comorbidities

Children, pregnant individuals, and persons with chronic illnesses may be at increased risk for severe disease, potentially influencing healthcare-associated exposure and transmission patterns.

3. Behavioural and Social Factors

3.1 Close-Contact Networks

Transmission efficiency increases in settings involving frequent skin-to-skin contact, including:

- Household environments
- Intimate or sexual networks
- Congregate living settings

High-contact social networks can significantly increase the effective reproductive number (R_e) (Vaughan & Aarons 2018).

3.2 Healthcare-Seeking Behavior

Delayed diagnosis and isolation contribute to prolonged community exposure. Stigma or limited healthcare access may further delay case detection.

3.3 Public Awareness and Risk Communication

Inadequate awareness of symptoms and transmission routes may result in continued contact during infectious periods. Conversely, effective public health messaging reduces high-risk behaviors.

4. Environmental and Ecological Factors

4.1 Urbanization and Population Density

High population density facilitates close-contact transmission, particularly in urban settings where individuals interact frequently in shared spaces.

4.2 Deforestation and Habitat Encroachment

Environmental disruption increases human–wildlife interaction, promoting zoonotic spillover events.

4.3 Climate and Seasonality

Although mpox does not exhibit strict seasonal patterns, environmental conditions affecting animal reservoir behavior may influence spillover frequency (Ježek & Szczeniowski 1987).

5. Globalization and Mobility

5.1 International Travel

Global travel enables rapid geographic dissemination of cases from endemic to non-endemic regions. A single imported case may seed outbreaks if early containment measures are not implemented.

5.2 Trade and Animal Movement

The wildlife trade and movement of exotic pets have historically contributed to cross-border transmission events (Vaughan & Aarons 2018).

6. Healthcare System Factors

6.1 Infection Prevention and Control (IPC)

Insufficient PPE, inadequate isolation facilities, and poor compliance with IPC protocols can result in nosocomial spread.

6.2 Diagnostic Capacity

Limited laboratory access delays confirmation, impeding timely contact tracing and containment.

6.3 Surveillance Systems

Weak surveillance infrastructure may result in underreporting and undetected transmission chains(Bunge 2022).

- Viral Shedding and Infectious Period

The duration and anatomical distribution of viral shedding influence transmission risk. Mpox patients remain infectious from symptom onset until complete lesion resolution. Prolonged lesion healing extends the period of transmissibility.

Higher viral loads in mucosal or genital lesions, particularly during outbreaks characterized by intimate contact transmission, may enhance spread within specific networks.

- Sociocultural and Economic Factors

- Stigma associated with certain transmission routes may discourage testing and reporting.
- Limited access to vaccination or antiviral therapy in resource-constrained settings increases outbreak persistence.
- Occupational exposure (e.g., healthcare workers, laboratory personnel, wildlife handlers) may elevate risk (Bunge 2022).

VII. CLINICAL IMPLICATIONS OF GENOMIC EVOLUTION

Genomic changes may influence:

- Viral transmissibility
- Immune evasion capacity
- Diagnostic sensitivity
- Vaccine effectiveness

Current vaccines such as those based on Modified Vaccinia Ankara remain effective due to strong antigenic conservation across orthopoxviruses.

VIII. PUBLIC HEALTH SIGNIFICANCE

The World Health Organization declared the 2022 outbreak a Public Health Emergency of International Concern (PHEIC). It highlighted:

- The importance of genomic surveillance
- The need for rapid diagnostic capacity
- The significance of international collaboration (Isidro & Borges 2022).

IX. FUTURE PERSPECTIVES

Key research areas include:

- Long-term evolutionary trajectory of Clade IIb
- Identification of definitive animal reservoirs
- Functional analysis of mutated immune-modulating genes

- Improved next-generation vaccines and antivirals

Continuous genomic monitoring is essential to detect adaptive mutations that may alter virulence or transmission efficiency (Ježek & Szczeniowski 1987).

X. CONCLUSION

Mpox virus has transitioned from a historically neglected zoonotic pathogen confined largely to Central and West Africa into a globally recognized public health concern. Its re-emergence in non-endemic regions highlights the dynamic nature of infectious diseases in an era characterized by increased globalization, ecological disruption, and shifting population immunity. The cessation of routine smallpox vaccination, combined with expanding international travel and close-contact transmission networks, has created conditions conducive to sustained human-to-human spread (Ježek & Szczeniowski 1987).

From a virological perspective, mpox virus demonstrates a complex genome organization typical of orthopoxviruses, with a conserved central region encoding replication and structural machinery and variable terminal regions responsible for host range and immune evasion. Its ability to replicate entirely within the cytoplasm using virus-encoded transcriptional machinery reflects a high degree of evolutionary adaptation. Genetic variability between clades contributes to differences in virulence, transmissibility, and epidemiological patterns, underscoring the importance of continuous genomic surveillance (Isidro & Borges 2022).

Clinically, mpox presents with a characteristic febrile prodrome followed by a centrifugal vesiculopustular rash and lymphadenopathy, distinguishing it from infections caused by related viruses such as Variola virus. Although generally less severe than smallpox, mpox can cause significant morbidity, particularly in immunocompromised individuals, pregnant women, and children. The potential for complications—including secondary bacterial infections, respiratory distress, encephalitis, and vertical transmission—necessitates timely diagnosis and appropriate supportive care (Bunge 2022).

Advances in diagnostic technologies, particularly real-time polymerase chain reaction (qPCR) and whole genome sequencing, have strengthened laboratory confirmation and outbreak investigation capabilities. However, disparities in diagnostic infrastructure and healthcare access remain challenges in both endemic and non-endemic regions. Early detection, case isolation, contact tracing, and risk communication remain cornerstones of outbreak control (Isidro & Borges 2022).

The recent global outbreaks emphasize the importance of adopting a One Health approach that recognizes the interconnectedness of human, animal, and environmental health. Zoonotic spillover events are influenced by ecological changes such as deforestation, wildlife trade, and habitat encroachment. Preventing future outbreaks therefore requires coordinated surveillance across veterinary, environmental, and human health sectors (Adler *et al., 2022)*

Vaccination strategies using next-generation smallpox vaccines provide cross-protective immunity against mpox and represent a critical preventive measure, particularly for high-risk populations. In

parallel, antiviral agents and supportive clinical management protocols continue to evolve, though further clinical research is required to establish standardized treatment guidelines. Equitable access to vaccines, therapeutics, and diagnostics is essential to prevent disproportionate disease burden in resource-limited settings (Adler *et al., 2022)*

In conclusion, mpox virus exemplifies the ongoing threat posed by re-emerging zoonotic pathogens. Its evolving transmission patterns, genomic adaptability, and global dissemination reinforce the need for sustained research, strengthened surveillance systems, and coordinated international response mechanisms. Continued investment in virology, epidemiology, immunology, and public health infrastructure will be pivotal in mitigating future outbreaks and enhancing global health security (Bunge 2022).

Mpox virus represents a paradigm shift in the understanding of orthopoxvirus epidemiology in the post-smallpox era. Once considered a rare, geographically restricted zoonosis, mpox has demonstrated its capacity for sustained human-to-human transmission across diverse sociocultural and geographic settings. This transition underscores the broader reality that eradication of one pathogen—such as smallpox—does not eliminate the ecological niche occupied by related viruses. Instead, changing immunity landscapes and human behaviors can facilitate the emergence or re-emergence of related pathogens.

The 21st-century resurgence of mpox highlights the consequences of waning orthopoxvirus immunity following the global cessation of smallpox vaccination. Generations born after eradication lack cross-protective immune memory, creating immunological gaps that enable viral amplification once introduced into susceptible populations. At the same time, increased interconnectedness through international travel, urbanization, and dense social networks accelerates dissemination beyond traditional endemic boundaries (Bunge 2022).

Scientifically, mpox offers valuable insight into viral evolution and host adaptation. Genomic analyses have revealed ongoing microevolution, particularly in genes associated with immune modulation and host interaction. These adaptations may influence transmission efficiency and clinical presentation. Continuous genomic surveillance is therefore essential not only for outbreak tracking but also for detecting mutations that may alter virulence, diagnostic sensitivity, or vaccine effectiveness (Ježek & Szczeniowski 1987).

From a clinical and public health perspective, mpox underscores the importance of rapid diagnostic capacity, standardized case definitions, and integrated surveillance systems. The deployment of molecular diagnostics, contact tracing frameworks, and targeted vaccination campaigns during recent outbreaks has demonstrated that containment is achievable when coordinated responses are implemented promptly. However, inequities in healthcare infrastructure, laboratory capacity, and vaccine distribution remain critical barriers in many low- and middle-income countries (Bunge 2022).

Furthermore, mpox reinforces the necessity of a One Health strategy that bridges human medicine, veterinary science, and environmental monitoring. Zoonotic spillover events are influenced by habitat encroachment, biodiversity loss, climate variability, and wildlife trade practices. Sustainable environmental policies, improved wildlife surveillance, and community education in endemic regions are essential components of long-term prevention (Alakunle *et al., 2024).*
In a broader context, mpox serves as a reminder that emerging and re-emerging infectious diseases will continue to challenge global health systems. Preparedness requires sustained investment in surveillance, laboratory infrastructure, workforce training, and equitable access to medical countermeasures. Lessons learned from mpox outbreaks should inform preparedness strategies for other zoonotic threats with pandemic potential (Adler *et al., 2022)*

Ultimately, the global experience with Mpox virus illustrates that infectious disease control is not solely a biomedical endeavor but a multidisciplinary effort integrating science, policy, social behavior, and environmental stewardship. Strengthening these interconnected systems will be critical to mitigating future outbreaks and safeguarding global health security in the decades ahead (Adler *et al., 2022)*

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