

Mpox Virus (MPXV): Comprehensive Analysis of Pandemic Risks, Pathophysiology, Treatments and mRNA Vaccine Development.

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ABSTRACT

Mpox, formerly known as monkeypox, is a zoonotic disease caused by the Mpox virus (MPXV), which has recently attracted global attention due to its potential for widespread outbreaks. Initially identified in 1958, MPXV primarily spreads to humans through contact with infected wild animals, particularly rodents. Historically confined to Africa, the virus has expanded beyond endemic regions, with notable outbreaks in Europe and North America in 2022, especially among men who have sex with men (MSM). The World Health Organization (WHO) has declared the current Mpox outbreak a Public Health Emergency of International Concern. This review explores the epidemiology, pathophysiology, and clinical manifestations of MPXV, along with current treatment strategies and the role of mRNA vaccines. It emphasizes the importance of understanding the changing dynamics of Mpox transmission, which are influenced by factors such as waning immunity from smallpox vaccinations and increased global interconnectedness. The potential for developing multi-epitope vaccines that can stimulate robust immune responses is highlighted, showcasing how bioinformatics can facilitate the identification of immunogenic antigens. Continued research and investment in vaccine development are crucial to address the urgent need for effective candidates that can protect at-risk populations. In summary, this review underscores the necessity for proactive public health measures and collaborative efforts among healthcare authorities, researchers, and communities to mitigate the impact of Mpox and enhance global preparedness for future outbreaks.

Keywords: Mpox Virus (MPXV), Epidemiology, Pathophysiology, mRNA Vaccines, Vaccine Development.

Introduction

Mpox, formerly monkeypox, caused by the MPXV, is a zoonotic disease that has attracted significant global attention due to its potential to cause widespread outbreaks. First identified in 1958 during outbreaks of a smallpox-like disease in monkeys in Copenhagen, the virus has since been recognized as primarily affecting humans through transmission from wild animals, predominantly rodents, which are considered its natural reservoirs (von Magnus et al., 1959). Despite its initial discovery in non-human primates, evidence suggests that rodents play a more crucial role in the natural history of MPXV, which belongs to the Orthopoxvirus genus, alongside other notable pathogens like variola virus (the causative agent of smallpox) (Cho and Wenner, 1973, Cohen, 2022).

Recent studies indicate that the classification of Mpox as a primate-centric disease is misleading, as rodents are more critical in its transmission dynamics (Letafati et al., 2023). This aligns with emerging evidence that suggests zoonotic diseases like Mpox may see increased outbreaks due to environmental changes such as deforestation and human encroachment on wildlife habitats (Charostad et al., 2023).

Historically, Mpox was considered an exclusively African disease, with sporadic outbreaks mainly reported in West and Central African countries (Lourie et al., 1972). The first human case was recorded in the Democratic Republic of Congo (DRC) in 1970, amidst efforts to eradicate smallpox (Beer and Rao, 2019). Human cases were sporadic and largely limited to rural areas of Africa, until a significant shift occurred in the 21st century. The virus began to spread beyond Africa, with notable outbreaks in the United States in 2003, linked to the importation of infected rodents, and more recent outbreaks in non-endemic regions such as Europe and North America in 2022 (Reed et al., 2004, Bunge et al., 2022, Mauldin et al., 2022). This sudden global spread, characterized by human-to-human transmission and a high number of cases among men who have sex with men (MSM), led the World Health Organization (WHO) to declare the Mpox outbreak (2022) a Public Health Emergency of International Concern (Likos et al., 2005, Otu et al., 2022).

The epidemiological patterns observed in the 2022 outbreak have prompted discussions on the impact of waning immunity due to the discontinuation of smallpox vaccination programs, which once conferred cross-protection against MPXV (Karami et al., 2022). Additionally, the role of global travel and interconnectedness in accelerating virus transmission has been highlighted (Masmejan et al., 2020, Khan et al., 2023).

The rising incidence of Mpox cases worldwide is attributed to several factors, including waning immunity from the discontinued smallpox vaccination programs, which previously provided cross-protection against MPXV (Chen et al., 2005, Bunge et al., 2022). Environmental changes such as deforestation, increased human encroachment on wildlife habitats, and greater global interconnectedness also contribute to the increased frequency of outbreaks (Fenner et al., 1988, Haller et al., 2014). The current epidemiological pattern of Mpox shows significant differences from past outbreaks, with variations in clinical presentation, routes of transmission, and demographic impact, highlighting the need for updated surveillance, diagnostic, and therapeutic strategies (Fine et al., 1988, Esposito et al., 2006, Rimoin et al., 2010, Shchelkunov, 2013).

Messenger RNA (mRNA) is a vital molecule in the process of protein synthesis, acting as the intermediary between the genetic information stored in DNA and the protein-making machinery of the cell (Rohner et al., 2022). Transcribed from a DNA template, mRNA carries instructions from the nucleus to the cytoplasm, where ribosomes read its sequence and translate it into a chain of amino acids,

eventually forming a protein. Essentially, mRNA is the translator that enables the cell to understand and use the genetic code in DNA, making it a crucial link in the creation and functioning of living organisms (Barbier et al., 2022).

Recent advancements in mRNA technology, inspired by the rapid development of COVID-19 vaccines, suggest a potential for the use of mRNA vaccines in combating Mpox outbreaks (Karami et al., 2022, Letafati et al., 2023). Such innovative strategies could enhance global response capabilities, especially in rapidly curbing future zoonotic threats like Mpox (Khan et al., 2023).

This review aims to provide a comprehensive overview of the MPXV, focusing on its epidemiology, pathophysiology, clinical manifestations, and current and potential treatment strategies, including vaccine development with a particular emphasis on mRNA technology. Given the resurgence of Mpox in 2022 and the potential for future outbreaks, this review will explore the factors contributing to the virus's spread and discuss the necessary preparedness measures to mitigate the risk of a second pandemic. The aim is to outline key actions that should be implemented by 2024 to enhance global response capabilities, including surveillance systems, rapid diagnostic protocols, and the development of effective vaccines and therapeutics to prevent and control future outbreaks of Mpox.

By understanding the unique characteristics of the Mpox virus and its recent epidemiological trends, public health authorities can better prepare for and respond to future outbreaks, potentially averting a crisis similar to the COVID-19 pandemic (Karcioglu Batur and Hekim, 2021). This includes advancing research on the MPXV, enhancing international collaboration, and ensuring that robust public health strategies are in place to protect vulnerable populations and prevent widespread transmission. In particular, early diagnostic protocols and targeted vaccination campaigns could be pivotal in controlling Mpox, as evidenced by recent public health strategies against other zoonotic viruses such as Ebola and Zika (Charostad et al., 2023, Letafati et al., 2023).

Comprehensive Analysis of the MPXV

History and classification of the MPXV

Mpox virus (MPXV) belongs to the family *Poxviridae*, subfamily *Chordopoxvirinae*, and genus *Orthopoxvirus*, which includes other significant poxviruses such as variola (smallpox), vaccinia, cowpox, and camelpox. These viruses are characterized by their large, double-stranded DNA genomes, which enable them to replicate in the cytoplasm of host cells (Doty et al., 2017). The Orthopoxviruses share considerable genetic and antigenic similarities, a factor that contributes to the cross-immunity observed among them. Notably, the cessation of the global smallpox vaccination program in 1980, which had previously provided some degree of protection against MPXV, resulted in a decline in population-level immunity, thereby facilitating the re-emergence of Mpox in human populations (Buchmeier, 2007, Cardeti et al., 2017, Doty et al., 2017).

The Mpox virus is currently classified into two main genetic clades: the **Central African (Congo Basin) clade** and the **West African clade**. The Central African clade is known to be more virulent, associated with higher mortality rates and increased human-to-human transmission compared to the West African clade (Doty et al., 2017). In the recent 2022 outbreaks, a new lineage, referred to as **clade 3**, emerged, primarily linked to the West African lineage (Bunge et al., 2022). This newly identified clade exhibited significant differences in transmission dynamics, particularly in non-endemic regions such as Europe and North America, suggesting adaptations that may have facilitated its rapid spread in these populations (Mauldin et al., 2022, Otu et al., 2022).

Recent phylogenetic analyses have shown that the Mpox virus has undergone genetic mutations that may affect its virulence and transmission capabilities (Letafati et al., 2023). The emergence of clade 3 indicates ongoing viral evolution, likely driven by increased human-to-human transmission, especially in densely populated urban centers (Masmejan et al., 2020).

Historically, the first recognized human case of Mpox was reported in the Democratic Republic of Congo (DRC) in 1970 during the smallpox eradication campaign (Beer and Rao, 2019). Sporadic outbreaks continued in the 1980s and 1990s, primarily in rural parts of West and Central Africa. However, with increased global travel, the virus eventually spread to non-endemic regions. For example, the 2003 outbreak in the United States was traced to infected pet rodents imported from West Africa (Reed et al., 2004).

Recent studies indicate that MPXV may have been underreported in Africa for decades due to limited surveillance and diagnostic capabilities (Charostad et al., 2023, Khan et al., 2023). With the recent surge in cases in non-endemic areas, particularly among men who have sex with men (MSM), the virus's transmission patterns have shifted, suggesting the need for updated surveillance measures and vaccination strategies (Karami et al., 2022).

In light of the recent outbreaks, there is an urgent need to reconsider vaccination policies. The use of second-generation smallpox vaccines, along with the potential development of targeted mRNA vaccines, could help control future outbreaks by enhancing cross-protection against emerging MPXV strains (Masmejan et al., 2020, Letafati et al., 2023). Additionally, advances in genomic sequencing are providing valuable insights into the evolutionary trajectory of MPXV, highlighting the necessity for continuous genomic surveillance to detect potential mutations that could impact virulence and transmissibility (Charostad et al., 2023).

By understanding the historical and genetic evolution of MPXV, public health authorities can better prepare for future outbreaks, focusing on enhanced surveillance, rapid diagnostic protocols, and effective vaccination strategies to mitigate the risk of a second pandemic (Karcioglu Batur and Hekim, 2021, Chen and Bibby, 2022).

Structure of the MPXV and genomic epidemiology

The Mpox virus genome comprises approximately 200 genes, with the Central African MPXV Zaire strain having at least 190 open reading frames (ORFs) and a genome size of approximately 197,000 base pairs (Rabaan et al., 2023). These genes, particularly those involved in immune evasion, are mainly located in the central region of the genome. The virus exists in two infectious forms: the intracellular mature virus and the extracellular enveloped virus, which differ in their surface glycoproteins and mechanisms of entry into host cells. Although the entry receptors for MPXV are not fully characterized, it is believed that multiple receptors, including chondroitin sulfate and heparan sulfate, may play a role. The virus's ability to evade the immune response and spread within the host is a crucial factor in its pathogenicity. Figure 1 illustrates the structure of the virus, providing a schematic and TEM images of the viral particles. To further understand the genetic diversity and evolution of the virus, sequence alignment and visualization of available sequences on NCBI Genbank are regularly performed by Nextstrain. This analysis uses both historical sequences and those associated with the 2022-24 global mpox outbreak. In August 2022, WHO, in consensus with scientists worldwide, renamed the MPXV clades: The Congo Basin clade became Clade I, and the West African clade became Clade II, which includes two subclades, Clade IIa and Clade IIb. Currently, all sequences from the ongoing global mpox outbreak are associated with Clade IIb, predominantly from the B.1 lineage. Although a few sequences belong to the related A.2 lineage, these findings suggest that the current outbreak is sustained through human-to-human transmission rather than multiple zoonotic spillover events. Understanding the

sequence diversity from earlier periods in regions with continuous mpox circulation is critical to determine when sustained human-to-human transmission began. Phylogenetic trees are visualized using R with ggtree and treeio packages, with further details and interactive visualizations available on the Nextclade website and GitHub. As of August 2024, 314 sequences have been visualized, excluding those submitted to GISAID, another major platform for sharing mpox genomic data. These analyses provide insights into the ongoing transmission patterns and evolution of the Mpox virus (Shchelkunov et al., 2002, Weaver and Isaacs, 2008, Kugelman et al., 2014, Moss, 2016).

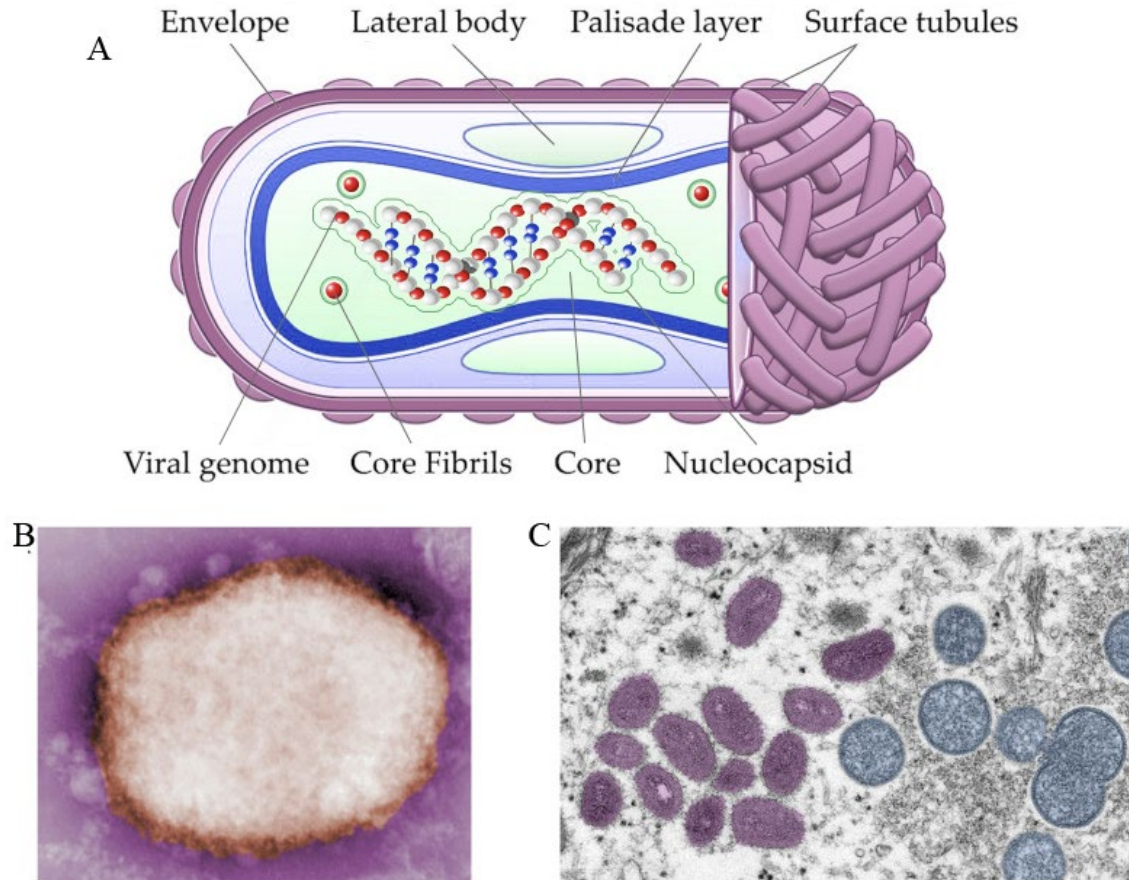


Figure 1. (A) Schematic representation of the Mpox virus structure, showing the viral genome, core fibrils, core, nucleocapsid, lateral body, palisade layer, and surface tubules enclosed by the envelope. (B) Transmission electron microscopy (TEM) image displaying the external morphology of the Mpox virus with a distinct envelope structure (Harapan et al., 2022). (C) Thin-section TEM image showing cross-sectional views of Mpox virus particles, highlighting the viral cores and surrounding structures. The purple structures represent the viral cores of Mpox particles, while the blue structures highlight the surrounding viral envelopes or external components (Harapan et al., 2022).

Transmission methods of the MPXV

Mpox is a zoonotic disease that primarily spreads from animals to humans through bites, scratches, or handling contaminated animal products. Natural hosts of the MPXV include various rodents and primates in Central Africa, with species like tree squirrels and Gambian pouched rats suspected to be the main reservoirs, although this has not been definitively proven. Human infections often arise from direct contact with these animals, involving exposure to mucous membranes, body fluids, tissues, or eating undercooked meat. Transmission of Mpox between humans typically occurs through direct contact with skin lesions, bodily fluids, or contaminated items like clothing or bedding. Respiratory droplets can also spread the virus, but usually require prolonged face-to-face contact. Additionally,

vertical transmission is possible, meaning an infected mother can pass the virus to her newborn during pregnancy. While Mpox can spread between humans, it typically requires close and sustained contact, making it less easily transmissible compared to airborne viruses like the flu or COVID-19. (26-29). (Harris, 2022, Walter and Malani, 2022, Beeson et al., 2023a, Frew, 2023) Figure 2 illustrates these transmission pathways, highlighting the zoonotic and human-to-human modes of spread. Recent outbreaks in areas where the virus is not usually found have raised concerns about its potential to spread widely among humans, especially as smallpox immunity has waned and human activity increasingly disrupts wildlife habitats (Adler and Taggart, 2022).

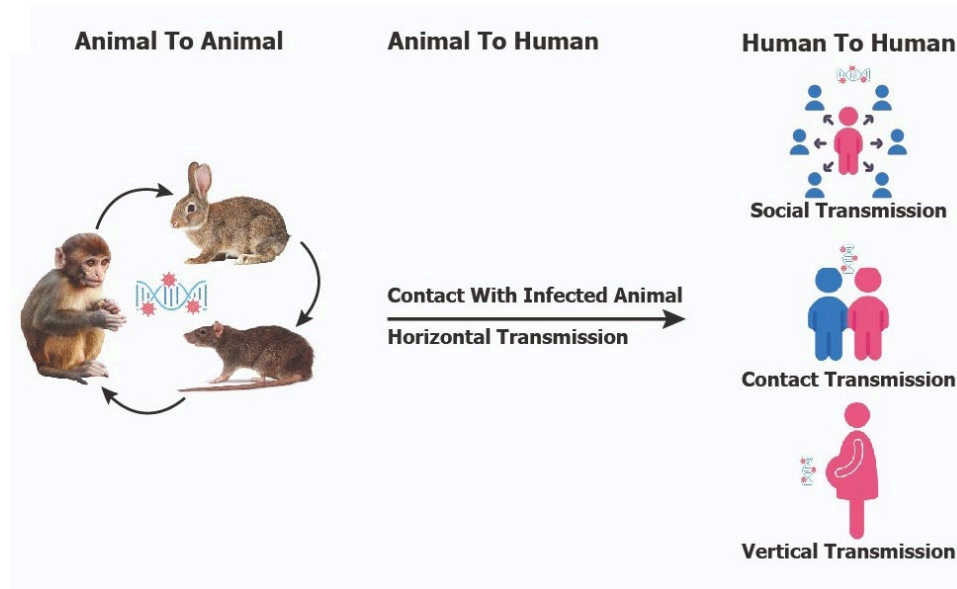


Figure 2: Modes of Transmission and Factors Contributing to the Spread of Mpox Disease. Mpox transmission occurs via several pathways, including animal-to-animal, animal-to-human, and human-to-human, showing the virus's potential for extensive spread through different channels (Lu et al., 2023a).

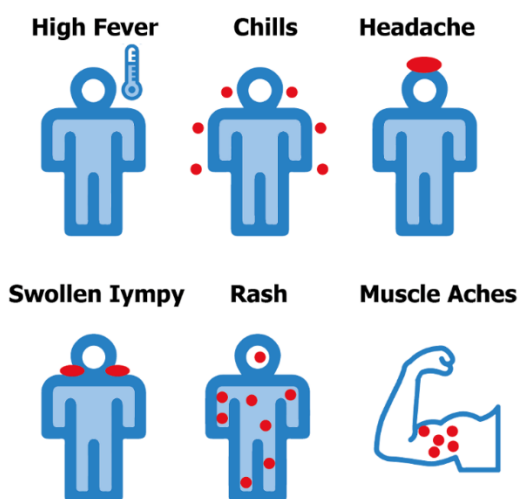
The current Mpox outbreak has become the largest recorded epidemic outside of Africa, differing significantly from past outbreaks. Previously, Mpox cases were mainly identified following contact with infected animals or travel to endemic regions (Durski, 2018, Kumar et al., 2023, Wang et al., 2023). In contrast, the ongoing epidemic has seen a significant number of cases linked to sexual contact, particularly among homosexual and bisexual men. Research indicates that 98% of recent cases were among this group, with 41% co-infected with HIV, and most lesions appearing in the anal and genital areas (Del Rio and Malani, 2022, Velavan and Meyer, 2022, Aden et al., 2023). Mpox has an incubation period of about 7-14 days, with symptoms lasting between 14-21 days (Nolen et al., 2016, Guarner et al., 2022). This prolonged incubation period can complicate diagnosis and delay treatment, increasing the risk of disease spread (McFadden, 2005, Nakazawa et al., 2015, Doty et al., 2017, Howley and Knipe, 2020).

Pathogenesis and clinical symptoms of Mpox Disease

Mpox is generally a self-limiting disease, with the severity of the infection varying based on several factors, including the viral strain, the individual's immune system status, and the potential for complications (Mahmoud and Nchasi, 2023). Early symptoms commonly associated with Mpox infection include pain, fever, fatigue, and significant lymphadenopathy, especially in the inguinal region (Altindis et al., 2022, Candela et al., 2023, Gaspari et al., 2023, Liu et al., 2023). Lymphadenopathy is a distinguishing feature of Mpox, setting it apart from other orthopoxvirus infections (Shafaati and Zandi, 2022).

Understanding how Mpox is transmitted is crucial for developing effective prevention strategies. The virus enters the body through mucous membranes (such as those of the eyes, respiratory tract, mouth, urethra, and rectum) or through broken skin, typically after exposure to the respiratory secretions or body fluids of an infected person (Alakunle et al., 2020, Kumar et al., 2022), once inside, it spreads through tissue-resident immune cells and draining lymph nodes (Kumar et al., 2022, Martínez-Fernández et al., 2023). This marks the latent period of Mpox infection, which usually lasts up to two weeks. During this time, individuals infected with Mpox typically do not show symptoms and do not have lesions. Following this latent period, the infected individuals may begin to exhibit non-specific symptoms such as fever, chills, headaches, muscle pain, and lymphadenopathy, which usually last about three days. Afterward, a rash appears, initially on the head and face, and then spreading across the body. The rash progresses through stages from papules to vesicles and pustules, eventually forming crusts that heal, often leaving scars. This phase of rash development lasts for approximately 2-4 weeks (Alakunle et al., 2020, Malik et al., 2023, Zahmatyar et al., 2023). In recent outbreaks among men who have sex with men (MSM), rashes have been predominantly seen around the genital and anal areas before spreading more widely (Srivastava et al., 2023, Wang et al., 2023). Common symptoms of mpox include a high fever, chills, headaches, swollen lymph nodes, muscle pain, and a painful skin rash. This rash generally appears as raised bumps on the skin, primarily affecting the face, genital area, and extremities, as shown in Figure 3 (Weinstein et al., 2005, Gupta et al., 2023a). The rash typically progresses swiftly from macules to papules, then to vesicles, followed by pustules, umbilicated pustules, and finally forming crusted lesions (Barnes et al., 2022, Gupta et al., 2023a). Notably, mpox patients may not exhibit all stages of lesions simultaneously during an outbreak, and not all lesions will necessarily be at the same stage of development (Barnes et al., 2022). Interestingly, a study conducted (Barnes et al., 2022) in Spain in 2022, which analyzed 185 cases of mpox, found that the initial lesions were uniform and papular (pseudo-pustules) rather than pustular (Català et al., 2022).

Severe Mpox cases can lead to complications like hemorrhagic and necrotic conditions, obstructive diseases, inflammation of vital organs, and septicemia. In 2022, the case fatality rate of Mpox in non-endemic regions was estimated at about 0.04% (Billieux et al., 2022, Maqbool et al., 2023, Rao, 2023). Individuals with compromised immune systems, such as children, the elderly, and those with conditions like HIV or on immunosuppressive drugs, are at a higher risk of severe disease (Fink et al., 2023, Harris, 2023, Laurenson-Schafer et al., 2023, Zahmatyar et al., 2023). These individuals are also more likely to facilitate the virus's adaptation to human hosts, potentially leading to increased transmission and broader outbreaks (Huhn et al., 2005, Harris, 2023).



Clinical symptoms typically manifest after Mpox infection

- Central nervous system: Headache, encephalitis
- Circulatory system: Sepsis
- Urogenital System: Infection
- Throat: Laryngoplegia
- Muscle: Myalgia
- Digestive system: Lymphomegaly, gastrointestinal edema
- Skin: Rashes, pustules and scars
- Eyes: Infection
- Mouth: Oral infection
- Lymph node: Lymphadenopathy
- Cardiac: Myocardium, pericarditis

Figure 3: Common symptoms of monkeypox infection. After infection with the MPXV, clinical symptoms typically develop. Initial symptoms may include fever, headaches, muscle aches, back pain, swollen lymph nodes, chills, and fatigue. These are often followed by a distinctive rash that evolves from flat lesions (macules) to raised bumps (papules), fluid-filled blisters (vesicles), pus-filled lesions (pustules), and eventually scabs (Gupta et al., 2023b, Lu et al., 2023a).

The manifestation of Mpox symptoms can vary depending on the immune status of the individual. Treatment options range from general supportive care to the use of antiviral medications. As outlined in **Table 1**, the chosen treatment approach is influenced by the infection's severity and the patient's overall health, with those who are immunocompromised potentially requiring more comprehensive care (Lu et al., 2023b).

Table 1: Treatment options of Mpox disease according to the individual's immune status (Lu et al., 2023b).

The available clinical treatment options based on the individual's immune status*	
<u>Normal Population</u> - Self-healing - Supportive therapies - +/-	<u>Immunodeficiency population</u> (HIV positive or immunosuppressive therapy) - Supportive therapies - Intravenous Tecovirimat - +/- Local use - +/- Immunomodulator - +/- VIGIV (Vaccinia Immune Globulin Intravenous) - +/- Brincidofovir or Cidofovir - +/- Other antiviral drug
<u>Mild Symptoms</u> - Fever - Fatigue - Headache - Myalgia - Cough - Pain - Skin rash - Lymphadenopathy - Mucosal injury - Pneumonia - Conjunctivitis	<u>Severe</u> - Central nervous system symptoms - Inflammation of large organs - Superinfection - Obstructive edema - Ocular lesions
<u>Immunocompromised population</u> (older, children, pregnant, barrier damage, comorbidities basic diseases) - Supportive therapies - +/- Local use - Oral and intravenous Tecovirimat - +/- VIGIV - +/- Brincidofovir or Cidofovir	<u>Tends Towards Terminal Illness</u> - Hemorrhagic terminate - Necrotic disease - Obstructive disease - Sepsis

*Symptoms of Mpox infection can vary depending on the individual's immune status.

Diagnosis of Mpox Disease

Accurate diagnosis of Mpox is crucial for controlling outbreaks and effectively managing patient care. Mpox, caused by MPXV, presents with symptoms that overlap with many other viral diseases, making precise diagnostic techniques essential for distinguishing it from similar conditions such as smallpox and chickenpox. Effective diagnostic strategies integrate clinical assessments with laboratory tests to confirm the presence of the virus. This section explores the key diagnostic methods currently in use for Mpox, providing a comprehensive understanding of their strengths and limitations.

Clinical Evaluation

The initial step in diagnosing Mpox typically involves a detailed clinical examination, as the disease often presents with nonspecific symptoms such as fever, headaches, and muscle aches, followed by the characteristic rash that differentiates it from other orthopoxvirus infections. According to Adler and Taggart studies, the progression of Mpox rashes from macules to papules, vesicles, and eventually scabs is a crucial diagnostic feature that helps distinguish it from other conditions. However, clinical diagnosis alone can be challenging due to overlapping features with chickenpox and other poxvirus infections, necessitating the use of laboratory confirmation (Adler and Taggart, 2022).

Laboratory Testing

- **Polymerase Chain Reaction (PCR):** The gold standard for diagnosing Mpox is laboratory confirmation via PCR, a molecular technique that allows for the detection of MPXV DNA in patient samples. PCR is highly sensitive and specific, targeting the conserved regions of the MPXV genome, thereby minimizing false positives. The test typically involves the collection of swab samples from lesions, with vesicular or pustular fluid being ideal due to the high viral load present in these samples (Guarner et al., 2022).

A recent cohort study involving 70 male mpox patients who had sex with men found that more than one-third of the participants experienced proctitis (Yakubovsky et al., 2023). Interestingly, in about two-thirds of these cases, the typical rash associated with mpox was absent, and in one-fifth of the cases, there were no visible rash symptoms at all, complicating the diagnostic process. However, using PCR testing on a rectal swab can aid in identifying mpox infection (Maronese et al., 2023). In the 2003 US outbreak, Huhn and colleagues investigated 34 individuals with confirmed mpox infections (Huhn et al., 2005). The most commonly reported symptoms were rash (97%), fever (85%), chills (71%), swollen lymph nodes (71%), headache (65%), and muscle pain (56%) (Huhn et al., 2005). About 68% of these patients had a monomorphic rash, while 29% displayed a pleomorphic rash. This distinction is significant when compared to smallpox, where patients typically show monomorphic pustules or crusting lesions. Moreover, a generalized centrifugal rash distribution was more frequently observed (48.4%) than a localized rash distribution (25.8%) (Huhn et al., 2005).

Timely and precise testing for mpox infection is crucial to disrupt transmission chains and control the outbreak (Djuicy et al., 2024). Healthcare providers should advise relevant laboratory tests for any individuals suspected of having mpox. Diagnostic testing involves using real-time or traditional PCR, either alone or with sequencing, to identify specific viral DNA sequences (Beeson et al., 2023b). Samples for testing can include swabs of the lesion surface, exudate, roofs from multiple lesions, or crusts from the lesions (Organization, 2022). To ensure accurate sample collection and transfer, swabs made from dacron, nylon, or polyester should be used, rather than cotton. Those handling the samples, including both the collectors and laboratory personnel, should proceed with caution, adhering to a risk-based protocol. A test result may indicate non-variola orthopoxvirus detected, which, given that no other orthopoxviruses are currently of public health concern, is considered a presumptive diagnosis of mpox. The scientists advises that all test results, regardless of whether they are positive or negative, should be reported to national health authorities (Djuicy et al., 2024)

Differential diagnosis

Mpox, chickenpox, and smallpox diseases exhibit several similar clinical symptoms (Weinstein et al., 2005, Barnes et al., 2022, Gupta et al., 2023a, Long et al., 2023). Table 2 presents a comparative analysis of these diseases, highlighting their specific signs and symptoms.

Table 2: Differences between mpox, chickenpox, and smallpox infections (Gupta et al., 2023b).

Characteristic			Monkeypox	Chickenpox	Smallpox (eradicated)
Pathogen			Monkeypox virus (<i>orthopox-virus</i> family)	Varicella zoster virus	Variola virus (<i>ortho-poxvirus</i> family)
Incubation period (days)			7-17	12-14	7-17
Prodrome period (days)			1-4	0-2	2-4
Desquamation (days)			14-21	6-14	14-21
Death rate			1-10% of cases	Rare	About 30% of cases
Symptoms	Fever		Moderate	Mild or None	Severe
	Headache		Moderate	Mild	Severe
	Malaise		Moderate	Mild	Moderate
	Lymphadenopathy		Moderate	None	None
	Lesions	Depth (diameter in mm)	Superficial to deep (4-6)	Superficial (2-4)	Deep (4-6)
		Distribution	<u>Prior outbreaks:</u> centrifugal <u>2022 outbreak:</u> Mainly penile, anorectal, and oral-pharyngeal lesions	Centripetal	Centrifugal
		Evaluation	<u>2022 outbreak:</u> Usually polymorphic rash	Polymorphic rash	Monomorphic rash
		Frequency on palms or soles of the feet	<u>Prior outbreaks:</u> Common <u>2022 outbreak:</u> Less common	Rare	Common

Serological Testing

In addition to PCR, serological tests are used to determine whether a patient has developed antibodies against MPXV, indicating a recent or past infection. However, serological assays have limitations in their specificity due to cross-reactivity with other orthopoxviruses, such as vaccinia virus, which is used in smallpox vaccines. Consequently, while serological testing can provide supportive information, it is not used for definitive diagnosis (Mattiuzzi et al., 2024).

Electron Microscopy

Historically, electron microscopy has been utilized to identify the characteristic brick-shaped virions of orthopoxviruses. However, this technique is primarily useful in research or in resource-limited settings where molecular methods are unavailable (Asif et al., 2024). Its high cost and requirement for specialized equipment make it impractical for routine diagnostics.

Advanced Diagnostic Approaches

Recent advancements in diagnostics are exploring the use of next-generation sequencing (NGS) and CRISPR-based diagnostics to enhance the accuracy and speed of Mpox diagnosis (Bourner et al., 2024). NGS enables whole-genome sequencing, which not only helps in identifying the virus but also in understanding the epidemiology of different strains. CRISPR-based diagnostics, a more recent innovation, offers a rapid point-of-care solution that can provide results in under an hour, which is particularly advantageous in outbreak settings. However, these methods are still under validation for widespread clinical use and are not yet widely accessible, particularly in low-resource areas (Chauhan et al., 2023).

Current Global Prevalence and Geographic Distribution

The Mpox virus was first reported in 1958 in laboratory monkeys employed for research purposes at the State Serum Institutes in Copenhagen, Denmark, as well as in Africa (Brasil et al., 2022, Kaler et al., 2022). Humans in Sub-Saharan Africa have been infected with Mpox through intimate contact with diseased animals, suggesting that the disease has been present for thousands of years. Mpox was formally recognized as a different illness in 1970, when the smallpox-eradication campaign revealed a continuing occurrence of smallpox-like disorders in rural regions (Weinstein et al., 2005, Farahat et al., 2022). Imported human Mpox-virus infections beyond the African continent have been infrequent in the last 50 years. Mpox has gained attention as a disease of global public-health significance since the first outbreak in the United States in 2003, which was linked to an infected pet prairie dog (Weinstein et al., 2005, Chen and Bibby, 2022). It was believed that native prairie dogs housed alongside rats from Ghana introduced in Western Africa were the main source of the pandemic. This is because most infected individuals became ill after coming into contact with pet prairie dogs (Weinstein et al., 2005).

Since then, Mpox has been identified globally, including significant outbreaks in Nigeria in 2017 and isolated cases in countries like the United Kingdom, Singapore and the United States (Vaughan et al., 2018, Yinka-Ogunleye et al., 2018, Ng et al., 2019, Silenou et al., 2020, Hobson et al., 2021). By January 2023, the WHO had reported over 85,000 suspected or confirmed Mpox cases across 110 countries, resulting in 86 fatalities (Núñez et al., 2023). The majority of cases have occurred in Europe and the Americas, with substantial numbers also reported in Africa. Epidemiological models indicate a high potential for Mpox to spread, with basic reproduction numbers ranging from 1.10 to 2.40 in regions with little prior exposure to Orthopoxvirus species, suggesting the possibility of a widespread outbreak if the virus is introduced into these populations (Grant et al., 2020, Hussain et al., 2020, Okyay et al., 2022). Despite a recent decrease in weekly reported cases, the Americas remain the most affected region, followed by Africa. The WHO currently assesses the global risk of Mpox as moderate, with higher risks in specific regions such as the Americas (Ramírez-Soto and Arroyo-Hernández, 2024).

Mpox is endemic in Central and West African countries, with the DRC being the most severely affected. The disease has also been reported sporadically in other countries, including Nigeria and the DRC (Yinka-Ogunleye et al., 2019, Lapa et al., 2022, 2023). Since 2022, the virus has spread to nonendemic regions, leading to outbreaks in countries such as the United States and the United Kingdom (Kantele et al., 2016, Yinka-Ogunleye et al., 2019, Kozlov, 2022, 2023). These outbreaks are often linked to international travel or the importation of infected animals. The global increase in Mpox cases reflects the combined effects of reduced smallpox immunity,

ecological changes, and increased global connectivity (Sale et al., 2006, Patrocinio-Jesus and Peruzzu, 2022, Sah et al., 2022, 2023).

In the second half of 2024, the WHO has reiterated its declaration of the mpox outbreak as a Public Health Emergency of International Concern, due to rising cases in the DRC and the virus's spread to neighboring countries. This resurgence, driven by two distinct outbreaks—clade Ia in Equateur and endemic areas, and a new clade Ib in the South and North Kivu provinces—poses a significant public health risk globally. The WHO's latest assessment rates the mpox risk as high in eastern DRC and surrounding regions, high in endemic areas within the DRC, moderate in other endemic regions across Africa, and moderate globally. These developments underscore the potential for a worldwide resurgence of Mpox, necessitating a coordinated international response (Cevik et al., 2024).

Mpox Prevention and Protection Strategies

Preventing mpox infection, particularly among healthcare workers and clinicians, poses challenges due to their frequent close contact with infected patients (Guarner et al., 2022). To mitigate risks, healthcare professionals must take several precautionary measures: they should avoid direct contact with lesions or materials used by infected patients, wear personal protective equipment (PPE) such as gowns, gloves, eye protection, and a fitted N95 mask when treating patients with skin rashes, and promptly mask patients who are suspected or confirmed to have mpox (Guarner et al., 2022). Skin lesions should be covered with a gown or cloth, and patients should be isolated in a single room or a negative-pressure room if hospital care is necessary. If hospitalization is required, proper handling of laundry from infected patients, using gloves, is also crucial (Guarner et al., 2022).

For the general public, preventive strategies include staying home if symptoms appear, reducing contact with others, avoiding intimate or sexual interactions with infected individuals, and practicing good hand hygiene and respiratory etiquette, such as wearing masks and disinfecting frequently touched surfaces after visitors (Gupta et al., 2023a, Suher et al., 2023). Safe sex practices, including condom use and limiting sexual partners, further help in reducing transmission (Gupta et al., 2023a).

On a larger scale, pre-exposure prophylaxis through smallpox vaccination offers substantial protection, with studies suggesting up to 85% cross-protection against mpox (Control and Prevention, 2022). The discontinuation of routine smallpox vaccinations after 1980 has been linked to the resurgence of mpox (Rimoin et al., 2010). In the United States, two vaccines—ACAM2000 and JYNNEOSTM are licensed for smallpox prevention and may also protect against mpox (Control and Prevention, 2022, Yazdanpanah, 2022, Gupta et al., 2023a).

Currently, antiviral agents, vaccines, and therapeutic drugs effective against the orthopoxvirus family, which includes smallpox, are utilized to manage mpox infections (Meo et al., 2022, Raccagni et al., 2022). Although specific treatments for mpox in humans are not fully confirmed, these existing options serve as the primary defense until a vaccine specifically approved for mpox is developed. Preventive measures are vital to containing outbreaks. Health authorities, such as the WHO and CDC, recommend strategies like avoiding contact with animals known to carry the virus, minimizing interactions with infected individuals, and maintaining proper hygiene through regular handwashing and disinfection (Stittelaar et al., 2006, Ajman et al., 2022, Chakraborty et al., 2022, Kumar et al., 2022, Laurence, 2022).

For those exposed to the virus, both pre-exposure and post-exposure prophylaxis are recommended, especially for high-risk groups like healthcare workers, laboratory personnel, and scientists handling clinical samples (Mahase, 2022, Payne, 2022, See, 2022). Additionally, public health policies focusing on raising awareness about infection risks, preventive measures, and available treatments are essential. Community engagement and education can significantly reduce the virus's spread, ensuring prompt intervention and effective case management (Payne, 2022). A coordinated effort between healthcare systems and the public, guided by scientific recommendations, remains critical to curbing the impact of mpox on global health.

Approved Treatment Medication

Antiviral Treatment Options for Mpox

Brincidofovir (CMX001)

Brincidofovir, also known as CMX001 or hexadecyloxypropyl-cidofovir, is a lipid-conjugated form of cidofovir designed to enhance bioavailability when administered orally. This broad-spectrum antiviral drug is metabolized intracellularly into its active form, cidofovir diphosphate, which inhibits DNA polymerases and prevents viral DNA replication. Notably, brincidofovir has shown efficacy against dsDNA viruses, including the Mpox virus, in both in-vitro and in-vivo studies (Fowotade et al., 2018). Unlike cidofovir, brincidofovir is less nephrotoxic, making it safer for patients with renal issues, and it is only available in oral form (Florescu and Keck, 2014). It has demonstrated antiviral effects in studies with prairie dogs, highlighting its potential in treating poxvirus infections (Hutson et al., 2021). Brincidofovir is also under the EA-IND protocol for managing Mpox, similar to tecovirimat (Guarner et al., 2022).

Cidofovir

Cidofovir, primarily approved by the FDA for treating cytomegalovirus retinitis, is effective against a range of DNA viruses, including orthopoxviruses like Mpox (Rizk et al., 2022). As a prodrug, cidofovir is converted into its active diphosphate form within host cells, where it incorporates into viral DNA, hindering its replication. By mimicking viral nucleotides, cidofovir inhibits DNA polymerase, thus preventing viral DNA synthesis (Magee et al., 2008, Siegrist and Sassine, 2023). However, its nephrotoxic effects necessitate the concurrent use of probenecid, which helps mitigate kidney damage. Despite its toxicity, cidofovir's structural similarity to viral nucleotides makes it a potent antiviral, albeit with caution advised for use in children, pregnant women, and those allergic to sulfa drugs (Stittelaar et al., 2006). Although there is limited evidence of its teratogenic effects in humans, the risk–benefit ratio suggests careful consideration in specific patient populations (Meaney-Delman et al., 2022).

Ribavirin

Ribavirin is a broad-spectrum antiviral known for its efficacy against both RNA and DNA viruses. It has been employed to treat various viral infections, including respiratory viruses, hepatitis, and other severe viral diseases. Ribavirin works by inhibiting inosine monophosphate dehydrogenase (IMPDH), leading to reduced guanosine monophosphate production, which is essential for mRNA translation (Nyström et al., 2019). Additionally, ribavirin induces mutations in viral RNA, causing incorrect placement of ribavirin triphosphate (RTP) for guanosine triphosphate (GTP), thus impairing viral replication. Animal models have

demonstrated ribavirin's effectiveness against orthopoxviruses, and it has shown synergistic effects when used with cidofovir (Baker et al., 2003). Its ability to inhibit helicase activity in viral RNA polymerase and increase viral mutation rates suggests it could be a valuable option for managing Mpox infections (Jahrling, 2011).

Tecovirimat (TPOXX) (ST-246)

Tecovirimat (ST-246), approved by the FDA for smallpox treatment, has shown promise in both in-vitro and in-vivo studies for Mpox. It targets the viral p37 protein, essential for the maturation and release of the virus, thus preventing its spread within the host. Approved by the FDA in 2018 for symptomatic smallpox, tecovirimat is available as an oral capsule or intravenous vial through the Strategic National Stockpile (Rizk et al., 2022, Dhapola et al., 2024). Its use against nonvariola orthopoxviruses, including the Mpox virus, is permitted under the EA-IND protocol by the CDC (Duraffour et al., 2015, Guarner et al., 2022). Tecovirimat's precise mechanism, thought to involve the inhibition of virion wrapping, has been studied in various animal models, showing improved survival rates in Mpox-infected subjects (Duraffour et al., 2015). The drug is well tolerated, non-toxic, and poses no significant teratogenic or embryotoxic risks, making it a viable option for managing Mpox (Dashraath et al., 2022).

A Group of Drugs Predicated for the Treatment of Mpox Undergoing Clinical Trials

The following drugs are currently undergoing clinical trials for the treatment of Mpox:

(S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine (HPMPA)

HPMPA is an acyclic nucleoside phosphonate prototype known for its broad-spectrum antiviral activity, demonstrated in in-vitro studies on PRK (Primary Rabbit Kidney) cell cultures. It has shown efficacy against a wide range of viruses, including poxviruses, adenoviruses, herpesviruses, iridoviruses, and hepadnaviruses. HPMPA operates independently of viral thymidine kinase, becoming active in its diphosphate form through cellular enzyme activation and ultimately inhibiting viral DNA synthesis. Its cytosine analog, which has fewer toxic effects, has demonstrated activity against all DNA viruses, such as poxviruses, papillomaviruses, polyomaviruses, adenoviruses, and herpesviruses (De Clercq, 2001, Groaz and De Jonghe, 2021). Additional in-vitro studies on Vero and LLC-MK2 cells showed HPMPA's effectiveness against Mpox and other orthopoxviruses by inhibiting viral DNA polymerase. HPMPA is similar in its antiviral mechanism to cidofovir (Baker et al., 2003). Esterification of HPMPA has led to increased antipoxviral efficacy in both in-vitro and in-vivo studies, highlighting its potential for managing MPXV infections (De Clercq, 2011).

Tiazofurin

Tiazofurin, a synthetic nucleoside inhibitor, is recognized for its broad-spectrum antiviral capabilities. It acts as a potent inhibitor of inosine monophosphate dehydrogenase (IMPDH) (Nair and Wenzel, 2004). As previously discussed, IMPDH is crucial for purine nucleotide synthesis, facilitating the conversion of inosine-5'-monophosphate (IMP) into xanthosine-5'-monophosphate (XMP), thereby disrupting the transfer of essential components for viral RNA and DNA synthesis (Panattoni et al., 2007). Tiazofurin is a synthetic analog of ribavirin and must be converted into its active metabolite, thiazole-4-carboxamide adenine dinucleotide, to exert antiviral effects (Franchetti et al., 1996, Jager et al., 2002, Panattoni et al., 2007). An in-vitro study confirmed tiazofurin's antiviral activity against MPXV, vaccinia, and cowpox

viruses by inhibiting IMPDH in cell lines such as Vero 76, Vero A6, BSC-40, and LLC-MK2. These findings support the potential effectiveness of tiazofurin against Mpox (Baker et al., 2003).

Adenosine N1 Oxide

Adenosine N1 oxide (ANO), derived from royal jelly, has demonstrated specific inhibitory effects on vaccinia virus replication. The drug's biologically active form is thought to be ANO-triphosphate. Studies on BSC40 cells infected with the vaccinia virus showed complete inhibition of DNA replication and viral translation, indicating ANO's antipoxvirus effects (Kane and Shuman, 1995). ANO inhibits poxvirus mRNA translation by incorporating adenosine analogs into viral mRNA (Harvey et al., 2015). In-vitro studies on Vero cells confirmed the antiorthopox virus properties of ANO, which inhibited the replication and mRNA translation of MPXV (Baker et al., 2003). Further studies on Vero and LLC-MK2 cells demonstrated that ANO and its derivatives were highly effective against Mpox and mousepox viruses, and also inhibited other viruses, including cowpox and vaccinia, at higher doses (Khandazhinskaya et al., 2006). S-adenosylmethionine (SAM) acts as the methyl group donor in cellular methylation processes, which produce S-adenosyl-L-homocysteine (SAH) as a byproduct. The accumulation of SAH due to SAH hydrolase inhibition hinders cellular and viral transmethylation processes, making SAH hydrolase inhibitors a promising antiviral therapy against Mpox (Baker et al., 2003). ANO is considered an analog of hydrolases, suggesting that ANO analogs might be effective in vivo without causing significant side effects.

(Matyugina et al., 2010). Additionally, two adenosine analogs (C-ca3-Ado and C3-Npc A), known to inhibit the SAH hydrolase enzyme in host cells, were found to significantly reduce orthopoxviral replication.

The Role of Bioinformatics in Mpox Drug Research

The re-emergence of Mpox in 2022, which has spread to 94 countries and resulted in over 50,000 cases, highlights the urgent need for innovative research approaches to develop effective treatments. Although no treatments are specifically approved for Mpox, antiviral drugs like tecovirimat, initially developed for smallpox, have shown promise for severe Mpox cases due to their effectiveness against dsDNA viruses (Fowotade et al., 2018, Adler et al., 2022). Bioinformatics has emerged as a critical tool in these efforts, leveraging computational techniques to identify and repurpose existing FDA-approved drugs as potential treatments (Kumar et al., 2022).

Understanding the genetic variations of the Mpox virus is crucial for developing targeted therapies. Bioinformatics tools help map these variations by analyzing viral genome sequences, which can reveal mutations that may influence the virus's transmissibility and resistance to antiviral drugs (Jacob et al., 2012, Vijayaraghavan and Sriramkumar, 2021, Adler et al., 2022, Kumar et al., 2022). Tracking the evolution and spread of Mpox through bioinformatics tools like phylogenetic analysis provides real-time insights into how the virus is moving and adapting, enabling more effective public health responses (Baker et al., 2003, Lu et al., 2023b).

One of the significant challenges in treating Mpox is the virus's ability to mutate, potentially leading to resistance against single-target antiviral drugs. Therefore, bioinformatics-driven approaches focus on multi-target drug development to enhance treatment efficacy. Using methods such as molecular docking and dynamic simulations, researchers have identified

several promising drug candidates, including Tipranavir, Cefiderocol, Doxorubicin, and Dolutegravir. These drugs interact with key viral proteins, such as thymidylate kinase and the D9 decapping enzyme, which are essential for Mpox replication (Shchelkunov et al., 2002, Weaver and Isaacs, 2008, Moss, 2016).

Furthermore, bioinformatics is expanding the search for antiviral agents beyond conventional pharmaceuticals. This includes exploring natural products and nanomaterials for their potential antiviral properties (El-Beeh et al., 2022, Ramadan et al., 2022). Techniques like Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) and Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) are used to evaluate the binding affinities between these agents and viral proteins, optimizing the development of novel therapeutics (Talevi, 2015). By harnessing the power of bioinformatics, researchers aim to advance our understanding of Mpox, predict future outbreaks, evaluate vaccine efficacy, and ultimately develop effective treatments to mitigate the global impact of this virus (Bansal et al., 2022).

Through these comprehensive bioinformatics approaches, there is hope to not only control but eventually eradicate the Mpox virus, addressing the critical public health challenges posed by its ongoing spread (Bansal et al., 2022).

Bioinformatics Analysis

Mpox protein sequences.

Mpox is believed to have multiple phylogenetic branches. Studies have been conducted using 15 distinct genetic sequences to analyze the evolutionary relationships between different strains of the virus. These phylogenetic sequences represent the virus's genetic material, allowing researchers to trace how various strains have evolved and diverged over time. (Isidro et al., 2022). Immune Epitope Database and Analysis Resource (IEDB; <http://www.iedb.org/>) was used to collect Mpox-specific epitopes. We selected protein sequences based on their antigenicity, immunogenicity, conservedness, MHC binding properties and IFN- γ stimulation. The antigens selected for this study are: Protein OPG190 (UniProt: P0DTN2), Protein OPG161 (UniProt: A0A7H0DND2), Entry-fusion complex associated protein OPG095 (UniProt: M1LBP0), Virion membrane protein OPG141 (UniProt: A0A7H0DNB3), DNA polymerase (UniProt: A0A7H0DN44), and Protein OPG154 (UniProt: P0DTN3). These proteins were chosen based on their relevance to key viral functions, such as viral entry, replication, and structural integrity, as well as their potential to elicit an immune response. The protein amino acid sequences were obtained from the UniProt database (<https://www.uniprot.org/>), a comprehensive resource where protein sequences are curated based on extensive literature studies. Further analyses were performed using these sequences.

Prediction of cytotoxic T lymphocytes epitope

In silico tools are available to identify CTL epitopes for subunit vaccine manufacture. To identify potential CTL epitopes, the amino acid sequence was analyzed using the NetCTL 1.2 server (<https://www.cbs.dtu.dk/services/NetCTL/>) (Larsen et al., 2007). Epitope prediction is based on three key features: first, MHC-I binding; second, proteasomal C-terminal cleavage accomplished using artificial neural networks (ANN); and third, TAP (Transporter Associated

with Anti-gene Processing) transport activity estimated using the weight matrix. Thresholds for several metrics such as TAP transport efficiency, proteasomal C-terminal cleavage, and epitope identification were established at 0.05, 0.15, and 0.75, respectively, for CTL epitope prediction purposes. The anticipated epitopes were classified according to their cumulative score. Although the website offers CTL epitope predictions for 54 MHC class I types, only 7 were employed in this work. These 7 MHC class I types were selected based on their prevalence in the population or their relevance to the immune response against the specific antigens being studied. By focusing on these MHC types, the study aims to provide more targeted and potentially impactful insights into immune interactions (Peters et al., 2003).

Prediction of Helper T lymphocytes epitope

To anticipate helper T lymphocyte (HTL) epitopes, the IEDB MHC II server (<http://tools.iedb.org/mhcii/>) was used (Zhang et al., 2008). The type/locus was chosen as Human/HLA-DR, and the human leukocyte antigen (HLA) reference set of seven alleles was used for HTL epitope prediction. Furthermore, epitopes of 9-10mers in length were identified and classified based on percentile value. The peptide score was compared to 5 million 15-mer in the SWISSPROT database and rated in percentile, with compounds with the lowest percentile ranking having significant MHC-II affinity.

Prediction of interferon-gamma inducing epitopes

The IFN-gamma epitope server (<http://crdd.osdd.net/raghava/ifnepitope/scan.php>) was used to test selected HTL epitopes for their ability to trigger an IFN- γ immune response. The server creates overlapping sequences from which IFN- γ epitopes are predicted. Support Vector Machine (SVM) and model-based prediction were performed by choosing IFN- γ versus non-IFN- γ (Dhanda et al., 2013). To create an in silico vaccine, epitopes with favorable IFN- γ responses were chosen.

Prediction of antigenicity of protein sequences

Estimating the antigenicity of vaccine candidates is a critical aspect of vaccine development. ANTIGENpro and VaxiJen v2.0 were used to assess the antigenicity of vaccination candidates. ANTIGENpro (<http://scratch.Proteomics.ics.uci.edu/>) calculates protein antigenicity by analyzing microarray data. Cross-validation trials yielded a 76% accuracy for the server with the combined dataset (Magnan et al., 2010). Antigenic evaluation of the selected genes was performed via a freely accessible online server VaxiJen 2.0 (<http://www.ddg-pharmfac.net/Vaxijen/VaxiJen/VaxiJen.html>) with a threshold value of ≥ 0.4 (Doytchinova and Flower, 2007). The VaxiJen 2.0 server is based on automatic and cross-covariance (ACC) transformation of the main amino acid features of protein sequences into uniform vectors to assess the antigenicity of the vaccine. The VaxiJen algorithm is mainly based on the sequence alignment method and identifies proteins as antigenic by analysing their physiochemical properties (Pandey et al., 2018). Using these tools, putative antigen epitopes were selected for vaccine construction.

Tertiary structure prediction

The homology modeling program I-TASSER (Iterative Treading ASSEmbly Refinement) server (<https://zhanglab.ccmb.med.umich.edu/I-TASSER/>) was used to create a tertiary (3D) model

of the multi-epitope vaccine. The I-TASSER server is an integrated platform for linked protein structure and function prediction that uses the sequence-to-structure-function paradigm to identify homologous structure models in the Protein Data Bank (PDB). I-TASSER produces 3D atom models using multiple thread alignments and iterative structure assembly simulations that begin with an amino acid sequence. A template modelling (TM) value greater than 0.5 shows the right topology model (Shey et al., 2019).

Molecular Docking

The interaction between an antigenic epitope and a specific immune receptor, such as a T-cell receptor (TCR) or an antibody, can be modeled to predict effective immune responses. In this study, the HDock online server (<https://hdock.phys.hust.edu.cn/>) was utilized to model the interactions between the selected antigenic epitope and its corresponding immune receptor (either a T-cell receptor or an antibody, depending on the specific focus of the study).

Docking was performed, and the Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) score was subsequently calculated using the same server. According to the studies by Yan et al., the affinity scores were analyzed, with a lower prediction score indicating a stronger and more favorable binding interaction (Yan et al., 2020).

RESULT

The Mpox amino acid sequences were obtained in FASTA format from the UniProt, Immune Epitope Database, and Analysis Resource databases. These sequences were analyzed to identify functional regions, focusing on potential linear B-cell and T-cell epitopes. This analysis led to the development of a new subunit vaccine targeting Mpox.

Using the NetCTL 1.2 web server, 54 cytotoxic T lymphocyte (CTL) epitopes (9-mer) were initially predicted. Out of these, only seven epitopes were chosen for vaccine development based on their high scores in categories such as MHC-I binding affinity, antigenicity, non-allergenicity, and non-toxicity (see Table 3). For the vaccine design, specific Mpox protein sequences were selected, highlighting epitopes with strong interactions with MHC-I A1, which are both non-toxic and non-allergenic, while also triggering a robust immune response (Table 3).

Additionally, recognition sites for HLA-DRB1*07:01 were evaluated for these sequences, and potential B-cell binding sites associated with the vaccine candidate were predicted (see Tables 4 and 5). This comprehensive approach aimed to optimize the selection of epitopes for an effective and safe vaccine formulation.

Table 3. List of the selected CTL epitopes which have fulfilled all the criteria for antigenicity, nonallergenicity, non-toxicity and could bind efficiently to MHC-I A1-supertype alleles.

Epitope number	Sequence of antigens	MHC binding affinity	Rescale binding affinity	C-terminal cleavage affinity	Transport efficiency	Prediction score
Epitope 1	YTDKFYISK	0.4580	19.446	0.7402	0.2570	20.684
Epitope 2	SIDLYMPKF	0.1629	0.6917	0.9328	26.410	0.9636
Epitope 3	FMTNFSMVY	0.4771	20.255	0.9458	29.620	23.154

Epitope 4	TLATANKIY	0.3279	13.924	0.9497	30.940	16.895
Epitope 5	STAEQLSKY	0.6001	25.478	0.9542	29.430	28.380
Epitope 6	LPDDIEGIY	0.1460	0.6200	0.9712	24.570	0.8886
Epitope 7	GLTNIFGY	0.2303	0.9778	0.9722	27.770	12.625

Highly binding MHC-II epitopes for human HLA DR alleles predicted by the IEDB MHC-II web server were identified as HTL epitopes. A total of two HTL epitopes were nominated for the final vaccine on the basis of binding property, antigenicity, non-allergenicity and non-toxicity as shown in (Table 4). Human alleles and location of predicted epitopes HLA-DRB1*07:01 (31-45) and HLA-DRB1*07:01 (163-177).

Table 4. List of the final selected HTL epitopes which fulfilled all the criteria for antigenicity, non-allergenicity, non-toxicity and could also induce the IFN- γ immune response.

Allele	Start	End	Length	Core Sequence	Peptide Sequence	Score	Percentile Score
HLA-DRB1*07:01	163	177	15	FYISKNIYT	YTDKFYISKNIYTSV	0.7287	0.88
HLA-DRB1*07:01	31	45	15	VAGSTAEQL	NHGVAGSTAEQLSKY	0.7479	0.79

ABCpred server was used to predict B cell epitopes. All predicted B cell epitopes (16-mer) with a binding score >0.8, highly antigenic, allergenic and non-toxic, with a total of two B cell epitopes finally selected for vaccine construction are listed in Table 5.

Table 5. Predicted linear B cell epitopes, a binding score greater than 0.8, are only selected for the final vaccine construct.

Sequence	Start position	Predicted Score
FSMVYTLATANKIYST	23	0.87
ANKIYSTAEQLSKYLP	32	0.82

The I-TASSER web server predicted five tertiary 3D structures for the targeted vaccine, utilizing ten threading templates. The Z-score values ranged from 0.25 to 0.85, and the reliability score (C-score) values ranged from -5 to -2.39. Typically, C-scores range from -5 to 2, with higher values indicating greater accuracy. The structure with the highest C-score of -2.39 was selected for further analysis (Figure 4).

This structure exhibited a predicted TM-score of 0.43 ± 0.14 and an estimated root-mean-square deviation (RMSD) of 8.0 ± 4.4 Å. The TM-score serves as a measure of structural similarity between models, addressing some limitations of RMSD, which can be sensitive to local discrepancies. To refine the predicted protein structure, the I-TASSER ModRefine web server was used to improve structural uniformity. High grade protein was obtained by cycle refinement and energy reduction. Rearranging the projected structure from the first "raw" vaccination model on the ModRefiner web server resulted in five model structures. The root-mean-square

deviation (RMSD 0.225) based on structure quality for all produced structures is 0.225 times more than the mean.

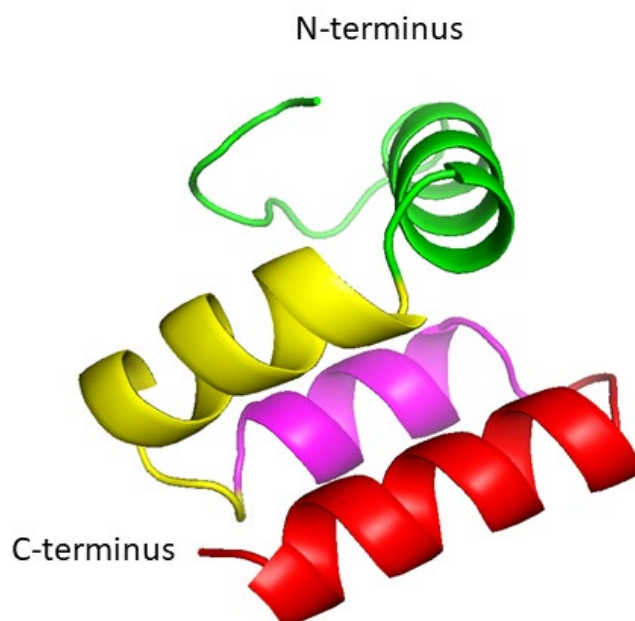


Figure 4: Protein modeling of the vaccine protein. The 3D model of a multi-epitope vaccine was obtained on the I-TASSER server following homology modeling. The folding models of the epitopes identified for the vaccine were determined in silico. According to this modeling, 3 α -helix structures were observed. This model was obtained by modeling the specific epitopes in Table 3. The vaccine candidate protein sequence was then examined for interactions with B cells for in silico examination of immune responsiveness.

There are many programs available for in silico prediction of the vaccine. The most commonly used HDock web-based interface program was used in this study for the prediction of protein-protein docking. Using more than one program increases the accuracy of prediction. However, HDock, which works best with PyMol, was preferred in our study. The docked complexes formed by the HDock server agent were analyzed and visualized with PyMol (Figure 5 and 6). In the knowledge-based iterative scoring system ITScorePP or ITScorePR calculates docking scores. A higher negative docking score indicates a more plausible binding model, but should not be interpreted as the true binding affinity of the two molecules as the score is not calibrated with experimental data. In the docking experiment performed by the HDock host, vaccine docking with TLR-3 had lower binding affinity than docking with HLA-DR3.

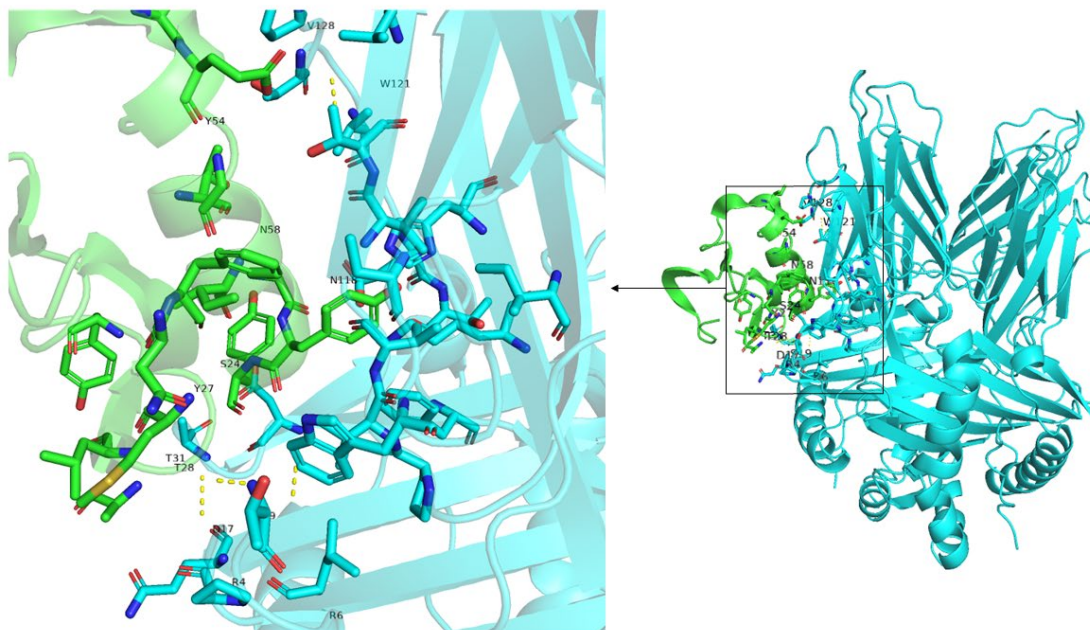


Figure 5: HLD-DR3 docking image of in silico vaccine. Individual HLD-DR3 epitope scores are anticipated in a vaccination including numerous epitope subunits. The ligand protein is illustrated in turquoise, and the receptor protein in green (Docking score: -241.45, Confidence score: 0.8616, Ligand RMSD: 57.49Å).

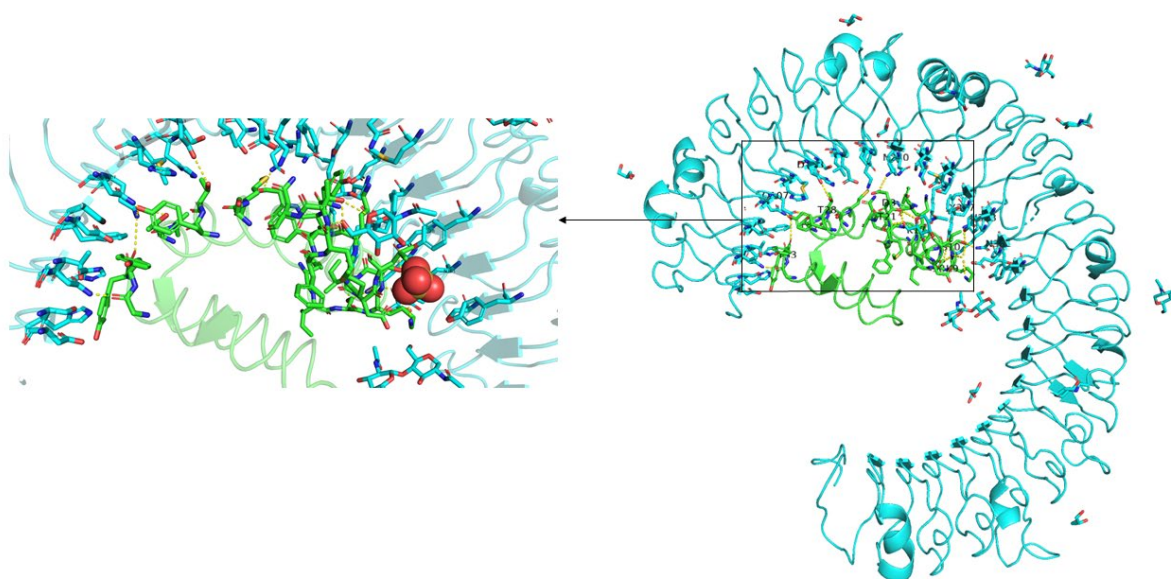


Figure 6: TLR3 docking image of in silico vaccine. Individual TLR3 epitope scores are anticipated in a vaccination including numerous epitope subunits. The ligand protein is illustrated in turquoise, and the receptor protein in green (Docking score: -308.76, Confidence score: 0.9599, Ligand RMSD: 89.23Å).

DISCUSSION AND CONCLUSION

Mpox is a significant disease that poses a serious threat to public health, especially with its resurgence and spread to non-endemic regions. The vaccines currently used against this disease, such as the smallpox vaccine, are expected to provide limited protection, particularly for adults who make up the majority of reported cases. Although it is believed that Mpox and smallpox share a common origin, the exact nature of their relationship remains uncertain. Globally, smallpox was eradicated in the 20th century, meaning that most adults were not vaccinated during their childhood. This has increased the urgency and importance of developing new vaccine candidates for Mpox.

The recent resurgence of Mpox has underscored the critical need for effective public health strategies. Current smallpox vaccines, such as ACAM2000 and JYNNEOS (Li et al., 2023, Titanji and Marconi, 2023), are being repurposed for Mpox prevention, but their efficacy in this context has not yet been fully established. Smallpox vaccines work by inducing immune responses, specifically targeting orthopoxviruses, which include both the smallpox virus and the Mpox virus. However, the rapid emergence of Mpox cases outside traditionally endemic regions has emphasized the need for specific vaccines and therapeutic measures targeting this virus. Recent outbreaks, particularly among high-risk groups such as MSM (Titanji and Marconi, 2023), indicate the changing dynamics of Mpox transmission and necessitate a more tailored vaccine and public health approach.

The spread of Mpox has significantly changed due to factors such as increased international travel, urbanization, and changes in human-animal interactions. Recent outbreaks have disproportionately affected specific groups, particularly MSM. Considering this situation, the need for targeted public health interventions has become even more apparent. Specific public health solutions should be developed for at-risk populations in line with these new patterns of Mpox transmission.

Effectively controlling the Clade Ib Mpox outbreak and preventing future outbreaks require coordinated international efforts. Considering the recommendations put forward by Karami et al. (2024), it is essential to make the vaccine more accessible, enhance surveillance capabilities, and develop programs to raise public awareness. In this context, lessons learned from the COVID-19 pandemic should also be taken into account, establishing a system that emphasizes rapid response, resource allocation, and cross-border cooperation (Karami et al., 2024).

Vaccine development and the use of bioinformatic data provide significant opportunities for developing effective vaccines against Mpox. Bioinformatic approaches offer great advantages in identifying antigens that trigger immune responses. These antigens contribute to triggering an immune response, but they can sometimes also exhibit immunosuppressive effects, complicating the overall immune balance (Galluzzi et al., 2017, Bahrami et al., 2019). In our study, we systematically analyzed two Mpox antigens, predicting B and T cell epitopes that trigger both cellular and non-cellular immunity, forming the basis of subunit vaccines.

B cell epitopes facilitate antigen-antibody interactions in neutralizing pathogens, while T cell epitopes play a critical role in activating adaptive immune responses. The identification of CD4⁺ and CD8⁺ T cell epitopes is particularly important for designing various epitope-based vaccines (EL-Manzalawy et al., 2008, Lu et al., 2018, Eickhoff et al., 2019). These epitopes interact with both MHC Class I and MHC Class II molecules, thereby activating various cellular and non-cellular immune responses.

In silico methods have the potential to address some of the challenges faced by traditional vaccines composed of inactivated microorganisms. New scientific platforms are being developed for the use of DNA/RNA technology, recombinant proteins, and nanomaterials. The aim of these innovations is to achieve a more precise immune response, increase efficacy, and provide long-term protection. However, the limited availability of data on these new technologies may affect their progress. In silico studies can make significant contributions in this field and provide solutions during critical stages of vaccine development, such as platform, adjuvant, formulation, and dosage selection (Hamley, 2022).

The design and formulation of a vaccine are critical factors that determine its overall effectiveness. Adjuvants, in particular, enhance the efficacy and persistence of the immune response while also influencing how the response is triggered (Matić and Šantak, 2022, Thomas, 2022). Therefore, selecting the most suitable vaccine platforms for different populations and maximizing their efficacy is essential for a successful vaccine development process.

Our immunoinformatics analysis identified several MHC Class I, MHC Class II, and B cell epitopes, revealing promising potential for a multi-epitope vaccine that could provide comprehensive protection against Mpox. The proposed vaccine showed higher antigenicity scores on both ANTIGENpro and Vaxijen v2.0 servers, indicating that it could elicit a stronger immune response compared to current options.

Collaboration between public health authorities, researchers, and communities is crucial for effectively addressing the Mpox outbreak. This joint effort will enable the implementation of strategies aimed at mitigating the impact of Mpox and enhancing global preparedness for future outbreaks. By leveraging advances in immunology and bioinformatics, we can identify and develop vaccine candidates that address not only the immediate threats posed by Mpox but also provide more effective responses to future infectious diseases.

In conclusion, the combination of advancements in vaccine research, bioinformatics, and a nuanced understanding of the socio-behavioral factors influencing Mpox transmission is critical in overcoming this disease. Our commitment to comprehensive research and proactive public health initiatives against Mpox will be a significant step towards protecting global public health.

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