

Recent Advances in Monkeypox (Mpox): Characterization, Diagnosis, and Therapeutics - A Multidimensional Review

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Abstract

Monkeypox (Mpox) is an uncommon viral infection closely related to smallpox, manifesting as a milder febrile rash in affected individuals. Over the past two decades, the incidence of Mpox has surged, possibly linked to a declining immunity against the smallpox vaccine worldwide. Recent outbreaks of Mpox in multiple countries have sparked concerns regarding altered transmission patterns and the potential for a global menace. In this article, we present a multidimensional review, encompassing the latest scientific discoveries, illuminating the intricate structure of human Mpox virus. Additionally, we explore noteworthy breakthroughs in diagnostic techniques and therapeutic interventions. By shedding light on the molecular aspects, epidemiological trends, and clinical manifestations, our review aims to facilitate a comprehensive understanding of Mpox, providing critical insights for the field of virology and guiding public health efforts in combating this emerging threat.

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Monkeypox (Mpox) is an uncommon viral infection closely related to smallpox, manifesting as a milder febrile rash in affected individuals. Over the past two decades, the incidence of Mpox has surged, possibly linked to a declining immunity against the smallpox vaccine worldwide. Recent outbreaks of Mpox in multiple countries have sparked concerns regarding altered transmission patterns and the potential for a global menace. In this article, we present a multidimensional review, encompassing the latest scientific discoveries, illuminating the intricate structure of human Mpox virus. Additionally, we explore noteworthy breakthroughs in diagnostic techniques and therapeutic interventions. By shedding light on the molecular aspects, epidemiological trends, and clinical manifestations, our review aims to facilitate a comprehensive understanding of Mpox, providing critical insights for the field of virology and guiding public health efforts in combating this emerging threat.

Keywords: Orthopoxvirus, Monkeypox, Zoonotic disease, Vaccine

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1. Introduction

Monkeypox (Mpox) is a rare viral disease and an agent of the genus Orthopoxvirus. The illness, which is primarily seen in Central and West Africa, is comparable to smallpox, which was declared extinct in 1980. As a zoonotic virus, the Mpox virus most frequently infects humans through contact with rodents and primates that have the disease. Fever, chilliness, enlarged lymph nodes, headache, back pain, muscle aches, and fatigue are some of the symptoms of Mpox. A rash typically starts on the face before spreading to other body parts. Mpox can occasionally result in serious disease and even death, especially in people with weakened immune systems. The World Health Organization has recommended adding Mpox to its list of conditions that could cause a public health emergency of global concern (WHO). The disease was identified in humans for the first time in 1970, and periodic outbreaks have been noted. It is common in certain parts of Central and West Africa. Uncertainty still exists over the extent of Mpox transmission in Africa, which may be underreported¹. In recent years few cases of Mpox have been documented in the United States and the United Kingdom, most usually among travelers from the affected African nations. Given that the Mpox virus is a zoonotic disease, it is crucial to understand the role that wildlife plays in its maintenance and dissemination. Hence, to control and halt the spread of the illness, a study on the epidemiology, ecology, and monitoring of Mpox is essential²⁻⁴.

2. Background and Issues

Mpox was initially identified in research laboratory monkeys in 1958. The virus is frequently found in monkeys and rodents in Central and West Africa, where Mpox has proven to be particularly dangerous in children, with death rates as high as 10% in severe outbreaks⁵. Additionally, infected "exotic pets" such as enormous, pouched rats, brush-tailed porcupines, and rope squirrels have spread the Mpox virus outside of Africa. The first Mpox pandemic outside of Africa occurred in the United States of America in 2003, when a caged prairie dog in Milwaukee, Wisconsin, transmitted the virus to a child. As a result of the outbreak, 72 people were ill, which was traced back to Ghanaian-imported Gambian pouched rats⁶. There were a few rare cases recorded in the UK, US, and Singapore between 2018 and 2022; in each of them, the underlying index case was a person who had recently left Nigeria⁷⁻¹⁰.

The Mpox virus can infect humans by animal bites or via direct contact with contaminated human bodily fluids. It can also be spread from person to person by intimate contact, most typically amongst family members; exposure to respiratory droplets may also be a mechanism of transmission¹¹. The illness reveals itself in people two weeks after infection, with fever, headache, general malaise and tiredness, and swollen lymph nodes. A few days later, a rash of elevated pimples appears on the face and torso. They eventually crust and fall off, and the illness takes two to four weeks to run its course. Outbreaks are kept under control by isolating patients and keeping the environment clean¹². To prevent infection, Jynneos (Imvanex or Imvamune), an attenuated live virus vaccine, can be utilised^{2,13}. Smallpox vaccination similarly provides some defense against the Mpox virus; smallpox vaccine injection may help prevent persons who are at risk of being infected with the Mpox virus, such as veterinarians and other animal workers.

A more serious outbreak that included widespread human-to-human spread outside of Africa started in 2022. In May 2022, the outbreak started in the UK and spread quickly in the months that followed, infecting countries in Africa, Asia, Australia, and the Americas. The World Health Organization named the epidemic a Public Health Emergency of Worldwide Concern in late July after more than 18,600 cases had been documented¹⁴⁻¹⁸.

In 1958, human Mpox, which is connected to the smallpox virus, was discovered. The present epidemic in 2022 has been puzzling because there is no epidemiological link and there is a risk that the virus might be spread sexually. In addition, the morphological and pathogenetic mechanisms by which the virus enters host cells are poorly understood. This article seeks to summarise what is now known about this re-emerging virus and speed up the understanding of previously published research findings on the virus of the human Mpox virus.

3. Mpox Virus Classification

The Orthopoxvirus (OPXV) genus of the Poxviridae family contains the Mpox virus, Poxviruses are enclosed

big viruses. 200 kilobase pairs of double-stranded DNA (dsDNA) that are heavily loaded with 200 genes make up their genome^{19,20}. Around half of the genes are well-evolved in vertebrate poxviruses and required for viral replication, while the other half are referred to as "accessory" genes that are mostly involved in virus and host interactions and may not be necessary for viral replication independently²¹.

Many poxviruses infect most invertebrates and vertebrates, affecting a wide range of veterinary and human disorders. The two subfamilies that make up the Poxviridae family are Entomopoxvirinae, which infects insects, and Chordopoxvirinae, which infects vertebrates. One of the 11 genera that make up the subfamily *Chordopoxvirinae* is OPV(**Table 1**) . All OPV species are categorised as zoonotic viruses since they all have animal reservoirs except the variola virus, which only affects humans^{22,23}.

<i>Family</i>	Poxviridae
<i>Subfamily</i>	<i>Chordopoxvirinae</i>
<i>Genus</i>	<i>Orthopoxvirus</i>
<i>Species</i>	<i>Camelpox virus</i> <i>Cowpox virus</i> <i>Ectromelia virus</i> <i>Monkeypox virus</i> <i>Raccoonpox virus</i> <i>Skunkpox virus</i> <i>Taterapox virus</i> <i>Vaccinia virus</i> <i>Variola virus</i> <i>Volepox virus</i>

Table 1. : Orthopoxvirus classification

4. Mpox Genetic Clades and Evolution

Experts in Mpox virology, evolutionary biology, and councils of research institutes from around the world reviewed the phylogeny and nomenclature of known and unknown Mpox virus variations or clades. They discussed the characteristics and evolution of several Mpox virus strains, differences in their apparent phylogenetic and clinical makeup, and any potential repercussions for virologic and evolutionary research as well as public health in the future.^{24,25}

The panel decided on new nomenclature for the virus clades that adheres to professional norms. They agreed on the listing and categorization of virus clades for genomic sequence repository websites. Experts in virology, evolutionary biology, and councils of research institutes from around the world reviewed the phylogeny and nomenclature of known and unknown Mpox virus variations or clades. They discussed the characteristics and evolution of several Mpox virus strains, differences in their apparent phylogenetic and clinical composition, and any potential repercussions for virological and evolutionary research as well as public health in the future. The panel decided on new nomenclature for the virus clades that adheres to professional norms. They reached an agreement on the listing and categorization of virus clades on genomic sequence repository sites²⁶.

In the proper naming system, the clade will be denoted by a Roman number, and the subclades will be denoted by a lower-case alphanumeric character. Hence, Clade I, Clade IIa, and Clade IIb are included in the revised terminology, with Clade IIb denoting the collection of variants that were primarily in use during the global pandemic of 2022²⁷. Lineages will be given the names that the experts have suggested as the pandemic progresses. If further time is required, the experts will convene. The new names for the clades should go into force immediately away, while work on the names of the diseases and viruses continues^{28,29}. Different strains of Monkeypox virus (MPXV) exhibit genetic polymorphism and genomic instability, contributing to strain evolution. MPXV is a DNA virus that undergoes frequent mutations, enabling rapid spread. The emergence of well-adapted variants can have devastating global consequences. Minor genetic changes facilitate host adaptation, with moderate transmission rates being more common. Genetic mutations, both stabilizing and destabilizing, enhance viral fitness and support interhuman transmission. Different strains of Monkeypox virus (MPXV) exhibit variations in their genome sizes, ranging from 190,083 to 206,372 base pairs. These strains are classified into two clades: West African and Congo Basin. Through epidemiological studies, researchers have observed differences in the severity of MPX infection in various regions. By comparing

three West African strains (SL-V70, complement control protein [COP]-58, and WRAIR-61) with a Central African strain (ZAI-96), it was found that there is a nucleotide variance of approximately 0.55% to 0.56%. The primary distinctions between these strains lie in the orthologs of the BR-203C, BR-209, and COP-C3L genes. The BR-203 gene, for instance, encodes a complete protein comprising 221 amino acids. Curiously, the West African strain of Monkeypox virus (MPXV) encodes only a partial N-terminal fragment, consisting of approximately 51 amino acids, for the specific gene under investigation. Intriguingly, this gene is an ortholog of the M-T4 gene that exists in the myxoma virus. The myxoma virus is notorious for causing myxomatosis, a disease affecting European rabbits. Intriguingly, scientific studies have shown that deleting the M-T4 gene results in a heightened inflammatory response. This finding suggests that this gene regulates the immune response to viral infections.

BR-209 acts as an IL-1 β binding protein, preventing the binding of IL-1 β to the IL-1 receptor. The cytokine IL-1, present in forms of IL-1 α , IL-1 β , and IL-1 receptor antagonist, affects the inflammatory response. One of these mechanisms involves the production of vIL-1 β BP. By binding to IL-1 β , vIL-1 β BP prevents IL-1 β from interacting with its cellular receptors, inhibiting its signaling and dampening the immune response. The COP-C3L gene in vaccinia virus codes for a secreted protein called vaccinia virus COP (VCP). The Central African strain of MPXV expresses a shorter protein known as MPX inhibitor of complement enzymes (MOPICE), which is the ortholog of COP-C3L gene. The variola virus, responsible for smallpox, contains the ortholog known as smallpox inhibitor of complement enzymes (SPICE). MPXV strains exhibit genetic diversity, with the Congo Basin clades being more severe and associated with human infections worldwide. Understanding the roles of these genes in MPXV pathogenesis is essential for comprehending the complex interactions and their contributions to the virus's disease-causing mechanisms.

The 2022 outbreak of MPXV is associated with a divergent branch, referred to as lineage B.1, which is derived from lineage A.1. Lineage A.1 has been associated with MPXV exports from Nigeria to the United Kingdom, Israel, and Singapore in 2018-2019. The 2022 MPXV outbreak may be attributed to the ongoing spread and evolution of the virus from the 2017-2018 Nigeria outbreak. Poxviruses, including MPXV, have a lower mutation rate compared to RNA viruses. However, the 2022 MPXV shows a rapid divergence from the 2018 virus, suggesting rapid adaptation to its host. APOBEC3 editing contributes to the mutation rate, with 90% of new nucleotide changes due to this process. Recombination plays a crucial role in poxvirus evolution, with the first natural recombination event in MPXV. Gene loss and amplification are important factors in MPXV's evolution, with the West African clade having larger genomes and more gene content. Monitoring non-synonymous mutations, genome ends, and gene content is crucial for understanding the evolutionary dynamics and possible adaptations of MPXV. The monkeypox virus's development and transmission are influenced by point mutations in several proteins, leading to the multi-country outbreak in 2022. The virus originated from the MPXV/United States/2021/MD virus and has evolved through ten common amino acid alterations. The B.1 viruses, which were transferred from Nigeria between 2018 and 2019, have specific amino acid changes in 22 of the 26 proteins. The study also examined characteristics of codon use and host adaptability, revealing a bias in codon use in genes undergoing nucleotide alterations in the B.1 lineage. Selection pressure, rather than mutation pressure, played a significant role in the evolution of genes expressing nucleotide mutations in the B.1 lineage. Monitoring and analyzing the virus's genetic alterations is crucial for improving our understanding of its behavior and guiding effective preventative and control efforts.

5. Mpox Epidemiology

Sub-Saharan Africa has possibly seen Mpox for several thousands of years since humans initially contracted the virus via close contact with diseased animals³⁰. Mpox was recognised as a distinct sickness after smallpox was eradicated in 1970 and it became clear that smallpox-like infections were still happening in rural areas³⁰. The Mpox virus was first discovered in monkeys used for research in 1958 at State Serum Institutes in Copenhagen, Denmark, and Africa¹². Mpox has received attention as a disease of significant global public health since the outbreak of the first epidemic in the USA in 2003, which was linked with infected pet prairie dogs³¹. It was assumed that the native prairie dogs housed with imported rodents from Ghana in the western region of Africa were the primary source of the outbreak because the bulk of the affected people became

ill after interacting with pet prairie dogs. Mpox has been connected to a rise in cases that began in the Midwest of the US in the summer of 2003³⁰. Mpox cases have been reported in several countries since 2003, with the worst epidemic occurring in Nigeria in 2017³¹.

The Centres for Disease Control and Prevention (CDC) had reported 5783 instances of Mpox as of July 1, 2022, spread over 52 different nations. **Figure 4** shows the geographical distribution of the cases worldwide. Currently, the western hemisphere and parts of Europe are home to the majority of Mpox cases. According to recent studies, the United Kingdom has the most instances in all of Europe. With a median age of 31 years, most cases reported of Mpox currently affect people under the age of 40. This group only emerged after the smallpox vaccination campaign had been abandoned, further illuminating the lack of cross-protective immunity¹². According to the external Situation report issued on June 10, 2023, the World Health Organisation (WHO) received reports of 87,929 laboratory-confirmed cases of monkeypox (mpox) from 111 countries across all six WHO Regions between January 1, 2022, and June 5, 2023. This totals 146 deaths. Since the last situation update, 552 new cases (a 0.6% rise) and six new fatalities have been reported. The number of new cases worldwide fell somewhat in the most recent reporting week. The Americas have recorded the most cases in the last three weeks. Cases have increased in several countries, including Cameroon. Europe and Southeast Asia have also reported additional cases, but with some delay. The number of cases in Africa has dramatically grown, exceeding the previously recorded average. As of June 5, 2023, 15 nations had confirmed new cases within the maximum illness incubation time. The United States, Brazil, and Spain are among the countries with the most cumulative instances. These 10 countries account for 83.9% of all instances globally. The monkeypox outbreak continues, with low levels of transmission in Europe and the Americas, a minor decline in the Western Pacific, and an increase in cases in South-East Asia. The number of cases has recently increased in Africa, where transmission is more constant. Most patients are men, and the age distribution is consistent. Children make up a minor fraction of cases, with the majority being recorded in the Americas region. Transmission occurs largely among homosexual, bisexual, and other males who have intercourse with other men. The predominant mechanism of transmission is through skin and mucosal contact during sex, followed by non-sexual contact between people. The most typically reported exposure contexts are parties involving sexual interaction, however this is changing with time. Rash, fever, systemic rash, and vaginal rash are all common symptoms. However, reliable information on transmission and exposure settings is scarce in the WHO African Region, making it difficult to gain a thorough knowledge of the virus's spread there.

6. Mpox Virus Morphology

The Mpox virus, along with other poxviruses, is one of the biggest and most complicated illnesses. The Mpox virus is large enough to be visible under a fluorescence microscope and has an ultrastructure that can be viewed under an electron microscope. These are brick-like particles with widths ranging from 140 nm to 260 nm with an average particle size of 220 nm to 450 nm³². Hence, to differentiate ultrastructure, stronger amplification afforded by electron microscopy is required. The core, lateral bodies, outer membrane, and outer lipoprotein envelope are the four basic components of the orthopox virion³³.

The center core, encircled by a dense layer of rod-shaped structures known as the palisade layer, contains the viral double-stranded DNA (dsDNA) and core fibrils. The palisade layer, lateral bodies, and central core are enclosed by the outer membrane, which is composed of many surface tubules (**Figure. 1**). In contrast to virions released by cellular rupture, spontaneously generated virions typically contain the outer lipoprotein membrane³⁴. A mature virion seems to have around 80 viral proteins³⁵.

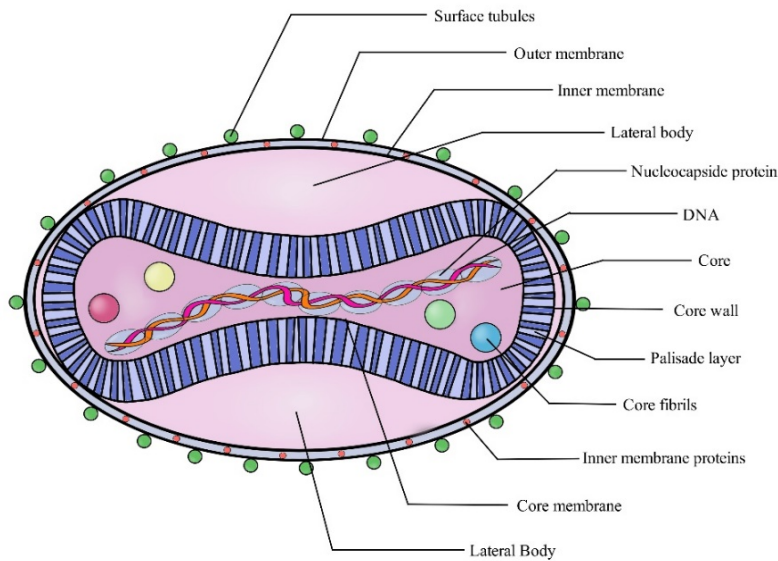


Figure 1: The poxvirus particle

7. Mpx Virus Genome

One of the largest viral genomes, the Mpx genome is a gigantic 197 kb single linear dsDNA molecule [5]. A collection of brief tandem repeats³⁶, terminal hairpin loops³⁷, and identical but oppositely oriented terminal reads of around 6 kbp are present at every end of the genome. About 190 non-overlapping open-reading frames (>180 bp long), each containing 60 or perhaps more amino acid residues, make up the genome. The inverted terminal repeats exhibit four of these¹⁹. Mpx virus DNA contains only around 31.1% guanine and cytosine³⁸. Mpx virus has two separate genetic clades, the West African (WA) clade as well as the Central African (CA) clade³⁹.

It consists of inverted terminal repeats (ITR) of 10kb at either end(**Figure 2**) . Genes are densely packed; intergenic areas longer than 100bp are uncommon. The center conserved area contains genes that perform "housekeeping" functions like transcription, replication, and virion assembly. The genes expressed in the terminal sections of poxviruses differ from one another and encode proteins implicated in host range and pathogenicity⁴⁰. Deep sequencing technology advancements have offered important insights into the standing nucleotide variation (SNV) inherent in poxvirus populations. These variants were previously unappreciated but are now recognised as major contributions to poxvirus evolution. Furthermore, there is an increasing knowledge of the role that genomic architectural alterations play in determining poxvirus evolution. Key drivers of genomic architectural alterations in poxviruses have been identified as homologous and nonhomologous recombination, gene duplications, gene loss, and horizontal gene transfer. A recent study²⁶ found a higher-than-expected incidence of SNV accumulation in recent samples of monkeypox virus . seven SNVs have been seen since the initial epidemic in March 2022, with a total of 50 SNVs discovered since 2018. The increased rate of SNV acquisition in recent monkeypox virus isolates may be due to host adaptability, effective population size, and selection coefficient. Selective sweeps or bottlenecks within individuals or during transmission may limit accumulated diversity, leading to disparities between epidemiological isolates and phylogenetic data. The intergenic region (ITR) of monkeypox viruses has been observed to expand or decrease, with varying clades having varying ITR lengths. Variants in the Z-encoding region of the virus, such as the E3L gene, have been found, potentially affecting PKR. Microsatellite variants, particularly the A26 ortholog, contribute to genetic variability and influence virus-host interactions. Overall, the data highlights the complexities of poxvirus evolution and the various pathways involved in producing genetic variation

within poxvirus populations.

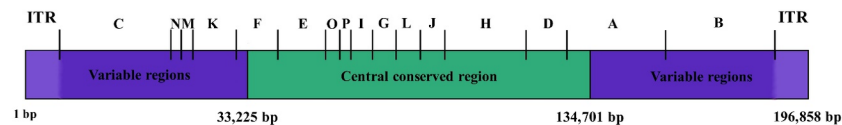


Figure 2: The inverted terminal repeats in the Mpox virus

8. Mpox Virus Replication Cycle

Poxvirus replication is distinct from that of other DNA viruses in that it occurs in the cytoplasm of the host cell. Poxviruses enter cells by an intricate mechanism that includes an attachment, hemifusion, and core penetration within the plasma membrane, or via endocytosis⁴¹. The specific method that poxviruses employ to enter cells is determined by their infectious form, which may either be a mature virion (MV) through one outer membrane or an extracellular enveloped virion (EV) with a different protein composition. In the EV form, the membrane that is particularly EV-specific is eliminated, preserving the underlying membrane affixed to the cell. EV is more specialized for cell-to-cell transmission despite MV's greater prevalence because of its long, mobile projections made by the polymerization of actin which adheres to the cell's surface. When a mature virion enters a cell, it loses its protective coating. Once within the cell's cytoplasm, the virus releases ready-made enzymes and certain other enzymatic components that weaken the cell's defences and start the synthesis of its original genes.

pattern of transmission and the emergence of Mpox in non-endemic locations. Mpox is less likely to inherit changes that speed up its spread since it is a DNA virus, which can detect and correct mutations effectively than RNA viruses such as SARS-CoV-2⁴⁵.

10. Viral Re-Emergence of Mpox

On 18 May 2022, Portugal, Spain, and Canada reported 14, 7, and 13 cases of MPV infection, respectively. On May 19, 2022, Belgium, Sweden, and Italy confirmed their first MPV instances. On May 20, Australia reported two incidents. On May 20, 2022, France, Germany, as well as the Netherlands all reported their first cases. On May 20, the UK's Health Secretary revealed an additional eleven MPV cases, bringing the total to 71. The first nation to impose a 21-day MPV quarantine requirement was Belgium. On May 21, 2022, Israel and Switzerland both verified their initial instances².

On May 18, 2022, Spain confirmed the very first case. The Republic of Spain reportedly reported a spike in cases of 20 on June 3, bringing the overall number of cases to 186. Denmark's first incidence was reported on May 23. This was said by a visitor who had just arrived from the Canary Islands. On May 24, 2022, Quebec in Canada reported the confirmation of 15 instances; the same day, the Czech Republic also announced the confirmation of one case. In Belgium, the putative offender attended a concert event. The country's first confirmed case was a 29-year-old tourist from West Africa who visited the United Arab Emirates at the end of May. Slovenia also verified its initial incident⁴⁶.

19 nations reported MPV cases as of May 24, 2022. The origin of the present MPV outbreak, nevertheless, is yet unknown. According to the MPV's changing nature, it may be transmitted from person to person or from animal to person. Travelers from the endemic areas of Africa to North America and Europe were the ones who initially contracted the illnesses, which subsequently spread⁴⁷(**Figure 4**) . The resurgence of monkeypox has led to debate, with declining immunity being a major theory. The theory is that waning immunity from previous smallpox vaccinations, which conferred some cross-immunity to monkeypox (MPXV), contributed to the rise in monkeypox cases. In the 1980s, the cessation of widespread smallpox vaccination made the population more susceptible to monkeypox, leading to selection pressure on the virus. This selection pressure may have triggered the development of mechanisms to bypass the immune system in MPXV, increasing the transmissibility of the virus. A second theory suggests that nonsynonymous mutations, particularly in coding regions associated with host recognition elements, may facilitate virus adaptation and fitness. These mutations could help the virus evade host immune responses and increase its transmission potential. Because of its affiliation with this phylogenetic branch, the emergence of specific mutations in the genomes of lineage B.1 MPXV compared with related viruses has attracted considerable attention in 2018-2019. These mutations suggest accelerated microevolutionary processes that could lead to an increase in human-to-human transmission. The B.1 MPXV lineage responsible for the current pandemic on the European continent probably first appeared in March 2022. This finding suggests that the B.1 lineage evolved and spread in Europe, resulting in the first MPXV cases that later spread to other continents, such as Oceania and the Americas. Recent analyses also indicate that MPXV has adapted more rapidly in the last two years, suggesting an accelerated process of host adaptation. These results provide evidence for the adaptability of monkeypox virus and its dynamics in human populations. The proposed theories offer possible explanations for the resurgence of monkeypox cases and highlight the importance of monitoring and understanding the evolution of the virus and transmission patterns to support public health.

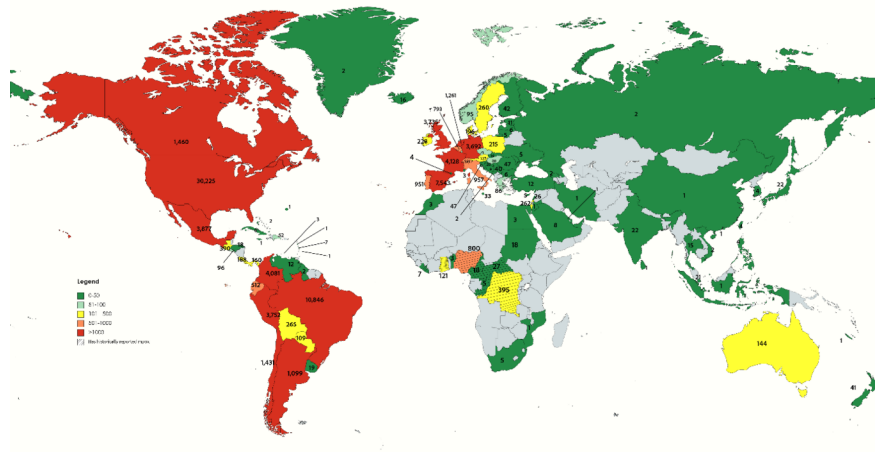


Figure 4: Global Map of Mpox Outbreak in 2022 from CDC. (2022, June 3)

11. Signs and symptoms of Mpox viral infection

Mpox is a self-limiting condition that typically disappears in two to four weeks. The three distinct stages of a less severe form of smallpox include incubation, prodrome, and rash, which are either indicators or symptoms of MPV infection. On average, the incubation phase lasts between seven and fourteen days⁴⁸. Fever and lymphadenopathy are common during the prodrome phase, with the latter characterizing Mpox as opposed to smallpox and chickenpox¹. Compared to many other illnesses, such as the flu, Mpox doesn't initially present with any noteworthy symptoms. In addition to other symptoms, patients may also have a fever, headache, muscular pains, and enlarged lymph nodes. A rash form after a few days, first appearing on the face before moving to other parts of the body. The rash develops in a specific manner, beginning as a macular rash and continuing through papular, vesicular, and pustular phases before crusting over and flaking off. The rash often affects the face, trunk, and limbs, but it may also affect other parts of the body, such as the genitalia⁴⁸.

Mpox symptoms are comparable to smallpox symptoms while being less severe, and most patients recover completely. With a mortality rate between 1 and 10%, mpox can be risky. How severe the infection will depend on many factors, including the virus strain, the accessibility of medical care, the level of exposure, and the patient's condition⁴⁹. Monkeypox virus (MPXV) enters the body and replicates, spreading to lymph nodes, causing primary and secondary viremia. The virus primarily affects the skin, causing rash development, oral mucous membranes, genitalia, and conjunctivae. Anogenital lesions, pharyngitis, and lymphadenopathy are common symptoms. MPXV can also affect the respiratory system, leading to severe breathing difficulties, pneumonia, and lung inflammation. Pulmonary surfactants and lubricants can aid lung function. Oral cavity lesions, such as glossitis, stomatitis, and gingivitis, can spread. Lymphadenopathy is a distinguishing characteristic of MPX, manifesting as lymph node enlargement during primary and secondary viremia. MPXV causes relatively few liver lesions, but viral antigens can disseminate throughout the liver, leading to hepatocyte degeneration and liver lesions.

A recent journal paper⁵⁰ reports the clinical presentation of the first recorded case of monkeypox virus, SARS-CoV-2, and HIV-1 co-infection. Mpox and COVID-19^{51,52} are both airborne viruses that cause symptoms such as fever, lymphadenopathy, headache, sore throat, and exhaustion. This case study's subject was a 36-year-old guy who got symptoms after visiting Spain. The patient tested positive for SARS-CoV-2 and had skin lesions similar to monkeypox. Co-infection with monkeypox virus, SARS-CoV-2, and HIV-1 was verified by diagnostic testing. The case illustrates the overlapping symptoms of monkeypox and COVID-19, emphasising the significance of collecting a complete history and considering sexual behaviours for correct diagnosis. Sexual contact appears to be a significant mechanism of transmission for monkeypox,

necessitating extensive STI screening. Because the patient's HIV-1 status indicates recent infection, care should be maintained even after clinical remission. Because there is presently no widely approved therapy or prevention for monkeypox, healthcare systems must be aware of the likelihood of co-infections and advocate adequate diagnostic testing in high-risk people.

12. Sampling and Diagnosis of Mpox virus

The PCR test can detect the monkeypox virus (MPXV), which causes monkeypox. Personal protective equipment (PPE) must be worn while working within 1 metre of a suspected monkeypox case in order to secure the safety of healthcare workers. This includes droplet and contact precautions, as well as eye protection, if spills are possible during diagnostic procedures such as pharynx biopsies or lesion samples. When gathering a sample for diagnostic testing, it is essential to obtain a viral biopsy from an exposed lesion or the surface of a vesicle (a small fluid-filled abscess). If there are multiple incisions, it is crucial that the sample be obtained from a vesicle, an ulcer, or a vesicle with a crust. Rub the probe gently over the lesion and position it in a tube containing viral culture medium or viral transport medium. Additionally, a pharynx sample should be taken if pharyngeal lesions are present. As unlabeled tubes cannot be processed, designate the collection tube with the patient's name, date of birth, and location of the sample. When high-risk contacts of a confirmed or highly probable case of monkeypox develop systemic symptoms but lack a rash or lesions suitable for sampling, a viral pharynx swab can be taken. Notably, even if the throat biopsy yields a negative result, individuals must continue to monitor their symptoms, adhere to isolation protocols as directed by their local health protection team, and be reevaluated and sampled if additional symptoms develop. It is crucial to package the samples separately when investigating other infections, such as sexually transmitted infections. Samples should ideally be sent to a nearby laboratory for examination. However, if MPXV testing is not available locally, the laboratory should send the samples to the other advance laboratory for MPXV PCR, while continuing to follow their normal local procedures for testing other infections such as HSV, varicella-zoster virus (VZV), or syphilis. For monitoring confirmed or highly probable cases of monkeypox, additional samples for follow-up testing are required. These samples should consist of a lesion swab and a pharynx swab, both of which should be stored in viral transport medium. In addition to obtaining a blood sample using an EDTA tube, a urine sample should be collected in a universally sterile container. It is crucial to submit these samples to a facility capable of conducting MPXV testing. By incorporating these samples into the monitoring procedure, healthcare providers can collect valuable information to evaluate clinical deterioration or determine if a patient should be transferred from isolation to an inpatient setting. In summary, appropriate sampling techniques are essential for accurate diagnostic testing in cases of monkeypox. After collecting the specimens, it is important to store them appropriately for testing. Place the specimens in sterile leak-proof containers, ensuring durability and suitability for shipping and temperature conditions. It is recommended to refrigerate the specimens (2-8°C) or freeze them (-20°C or lower) if testing cannot occur promptly after collection. Specimens such as dry swabs, swabs in viral transport medium (VTM), or lesion crusts stored at 2-8°C can be tested within 7 days from collection. If stored frozen (-20°C or lower), swab specimens in VTM and lesion crusts can be tested within 30 days from collection, while dry swabs stored frozen can be tested within 60 days from collection. When shipping specimens for laboratory testing, it is important to follow certain guidelines. The ongoing Mpox outbreak has been linked to Clade II of the mpox virus, which is now classified as the West African clade. To ship specimens suspected or confirmed to contain Clade II of the Mpox virus, they should be labeled and categorized as Biological Substance. It is recommended to individually bag the specimens to prevent the rejection of all specimens in case of a leak. To maintain the required temperature, specimens should be conveyed on dry ice whenever feasible. It is not recommended to ship specimens at room temperature, as specimens received outside the permissible temperature range will be denied. Even if there are temperature fluctuations because of the refrigerant used, both the primary container and secondary packaging should maintain their integrity. The packaging of dry ice must prevent pressure accumulation and container rupture while allowing for gas discharge. The specimens should only be packaged and shipped by personnel trained in hazardous transportation requirements. The outer package must be marked and labelled with the sender's name and address, the recipient's name and address, a biological substance label, the correct shipping name (Biological substance), an identification

number, and the name and telephone number of the shipper’s responsible party. Following these instructions ensures the safe and correct shipment of mpox samples for laboratory testing.

To confirm Mpox virus infection, diagnostic tests, and clinical and epidemiological data must be correlated. The diagnosis of Mpox(**Table 2**) virus infection is based on clinical signs, laboratory tests, and medical history². By checking for lymphadenopathy at the prodromal stage of the illness, it is possible to clinically distinguish Mpox from chickenpox or smallpox⁵³. The specimen samples are collected and forwarded to the lab to verify the diagnosis in compliance with WHO criteria. PCR is the most widely used laboratory diagnostic method to identify Mpox due to its accuracy and sensitivity⁵⁴. The fluid that is produced by vesicles and pustules as well as dry crusts are the best samples to use in the diagnosis of Mpox. When a lesion sample needs to be kept cool and kept in a dry, sterile tube without a viral transport medium, a biopsy appears to be an alternative in some circumstances. PCR blood tests are often inconclusive and should not be routinely obtained from patients because viremia typically lasts for a short period compared to the time the material is taken after symptoms start⁵⁵. The recommended specimen type for diagnosing monkeypox is skin lesion material, including swabs of lesion exudate, roofs from multiple lesions, or lesion crusts. Testing should be offered to individuals meeting the suspected case definition, considering both clinical and epidemiological factors. It can be challenging to differentiate monkeypox based solely on clinical presentation, and other potential causes of skin lesions should be considered. Safety procedures, including proper personal protective equipment and handling of specimens, must be followed. The collection and storage of specimens, including skin lesion material and additional types for research purposes, are needed. Nucleic acid amplification testing (NAAT), particularly polymerase chain reaction, is recommended for confirming monkeypox infection. Reagents, controls, waste disposal, and biosafety measures should be appropriately managed during testing. Serological testing and genetic sequencing are additional tools for diagnosis and surveillance. Overall, adherence to biosafety measures and risk assessments are crucial in handling specimens and conducting laboratory testing for monkeypox. For confirmation of monkeypox virus (MPXV) infection, clinical and epidemiological data should be considered. Positive detection using an orthopoxvirus (OPXV) polymerase chain reaction (PCR) assay, followed by MPXV confirmation via PCR and/or sequencing, or in suspected cases, positive detection using an MPXV-specific PCR, confirms MPXV infection. Although MPXV-specific confirmatory testing is preferred, a positive OPXV PCR assay result is sufficient for laboratory confirmation. In instances where clinical presentation and epidemiology indicate MPXV infection despite negative PCR results, serological testing can be utilised to investigate prior infection. False-negative results can be influenced by factors such as specimen quality, handling, transportation, and technical issues. Genetic sequence data (GSD), obtained through sequencing positive MPXV specimens, can provide valuable information on the virus’s origins, epidemiology, and characteristics. WHO encourages countries and laboratories to share GSD, including raw data, through public access databases to enhance understanding and collaboration. Sequencing can be performed using Sanger or next-generation sequencing methods. Laboratories must adhere to national reporting regulations. Positive or negative test findings should be immediately reported to national authorities. States Parties to the IHR are reminded of their responsibility to communicate relevant public health information with WHO for occurrences that they have notified WHO. Access to quick and reliable laboratory testing of samples from cases under investigation is critical for diagnosing and monitoring this developing virus. All nations should have access to reliable testing, either locally or by referral to laboratories in other countries willing and capable of diagnosing OPXV or MPXV. WHO’s Regional Offices can help Member States gain access to testing through referral. Inactivation of samples at the local laboratory, where suitable and securely conducted, may improve referral and alleviate logistical issues. Countries are invited to exchange their sequencing data so that the present outbreak can be better understood.

Tests	Description
PCR	It is based on NAAT; the gold standard for detecting monkeypox DNA is present
Viral culture	From a patient sample, the virus is cultured and isolated.
Electron microscopy	Using an electron microscope, pox viruses are morphologically identified.
Immunohistochemistry	Antigens specific to the Orthopoxvirus are evaluated for their presence.

Tests	Description
Anti-Orthopoxvirus IgG and IgM tests	These tests may be used to determine if you have been exposed to Orthopoxvirus

PCR, polymerase chain reaction; NAAT, nucleic acid amplification test; DNA, deoxyribonucleic acid; IgG, immunoglobulin G; IgM, immunoglobulin M

Table 2. : Updated diagnostic tests for monkeypox. Table modified from Cheema, Asfand Yar, et al ⁵⁶.

13. Monkeypox virus and mechanisms of immune evasion

Monkeypox virus (MPXV) has sequence similarities with vaccinia virus (VACV) and uses a variety of strategies to avoid detection by the immune system. The MPXV F3 protein and the VACV E3 protein are homologous, indicating a possible functional relationship. MPXV suppresses the antiviral immune response more effectively than a VACV mutant missing the N-terminal region of its E3 homolog. This suggests that MPXV may prevent symptoms caused by its E3 homolog's lack of the N-terminal region. MPXV cannot downregulate MHC Class I; instead, it employs a method to prevent CD4+ and CD8+ T-cell activation upon interaction with MPXV-infected cells.

By suppressing local T-cell responses, MPXV can evade the immune system while maintaining a viral reservoir. MPXV-encoded immunomodulators play a critical role in blocking antiviral T-cell responses, which are activated by the host. Neutralizing antibodies are crucial for protection against severe MPXV infections, while memory T-cells alone do not provide sufficient protection. The spread of MPXV relies on interactions with circulating monocytes, which may protect the virus from humoral immune responses. Macrophages and other immune cells may also aid in the spread of MPXV. Understanding the immune evasion mechanisms of MPXV and the interactions between various orthopoxviruses, such as VACV, is important for studying immunity to monkeypox and developing effective preventive measures like vaccinations.

14. Clinical management, prevention, and treatment for the Mpox virus

Fortunately, the clinical course of Mpox infections is usually mild and self-limiting. As a result, it infrequently justifies specialised therapy, and instead, treatment is typically supportive. Supportive therapy includes things like analgesics for pain, antipyretics for fever, and antibiotics for subsequent bacterial infections. Yet some people could require a certain kind of treatment. For those with serious illnesses, immunocompromised people, pregnant women, and youngsters, specific therapies can be required ^{57,58}. The treatment and immunisations created to combat smallpox have shown some success against the Mpox virus because of the similarities between Mpox and smallpox. There is, however, little evidence to support this claim ⁵⁹.

Mpox disease is not curable, however, research on treating smallpox indicates that vaccinia vaccinations, tecovirimat, vaccinia immune globulin (IVG), and cidofovir might be beneficial ⁶⁰. Cidofovir is an antiviral drug that inhibits viral DNA polymerase and exerts antiviral activity against a variety of viruses. It is usually administered intravenously with fluid administration and probenecid. Although cidofovir has the potential to cause nephrotoxicity (kidney toxicity), a modified version called CMX-001 has been developed that is not nephrotoxic. In addition, CMX-001 exhibits antiviral activity against numerous orthopoxvirus species. ST-246 is an agent that is administered orally and targets the intracellular release of the virus. It has shown promising results against several orthopoxviruses, including the variola virus that causes smallpox. By inhibiting the release of viral particles from infected cells, ST -246 helps to contain the spread of infection. The European Medicines Agency (EMA) authorised the usage of tecovirimat for treating measles in 2022, reported by the World Health Organization's ⁶¹. It was first created as a smallpox remedy. Because tecovirimat is currently not widely available, any usage of it needs to be carefully supervised. By blocking viral DNA polymerase, cidofovir has antiviral efficacy against some viruses ⁶². It has been observed that tecovirimat is particularly sensitive to several orthopoxviruses, including variola, cowpox, vaccinia, rabbitpox, ectromelia, and Mpox. For the treatment of Mpox, tecovirimat, an oral inhibitor of intracellular viral release, may be beneficial ^{63,64}. These antiviral agents have been studied in various combinations, such as with vaccinia

immunoglobulin, which is used to treat serious adverse events associated with smallpox vaccination clinical trials. The combination of these drugs has the potential to effectively treat orthopoxvirus infections.

Notwithstanding the recommended course of treatment, supportive and symptomatic therapy is the backbone of treating a Mpox virus infection. It's crucial to realize that the only established treatment for Mpox is treating symptoms and preventing complications. Given the outbreak of Mpox in 2022 and the current Mpox cases worldwide, more research must be done before any treatment or vaccine can be created⁶⁵.

To prevent infection with the Mpox virus, certain precautions can be taken. This includes, but is not limited to: (1) consciously avoiding encounters with animals suspected to be Mpox virus carriers, especially in regions where the Mpox virus is prevalent; (2) placing ill people in a room with negative pressure to prevent the virus from spreading from one person to another; (3) isolating and euthanizing the animals suspected to be the virus reservoirs; (4) avoiding contact with any objects that have come into contact with ill animals or people; and (5) avoiding contact with (5) Frontline personnel caring for Mpox virus-infected patients and other high-risk individuals who are expected to interact with infected individuals should wear the appropriate personal protective equipment (PPE) designed to protect against airborne infectious agents, such as N-95 masks, fully covered water-resistant gowns, and double-layered gloves.^{66,67}

Research has shown that smallpox vaccination offers a reliable defence against illnesses caused by Mpox and other orthopoxviruses⁶⁸. When administered early in the incubation phase, it can postpone the start of disease or minimise the severity of the illness.

However, patients who have impaired immune systems face the risk of suffering serious side effects^{69,70}. Mpox has become more prevalent since smallpox was eradicated in 1980 as a result of the end of immunisation initiatives to prevent viral illnesses. The attenuated strains used in the next-generation smallpox vaccines, including ACAM2000 (this is live vaccinia virus), LC16m8 (this one is attenuated vaccinia virus) and Modified Vaccinia Ankara (is also attenuated vaccinia virus) not only have a better safety profile than the first- and second-generation smallpox vaccines, but they also successfully stimulate the production of antibodies in atopic and immunocompromised patients⁷¹⁻⁷⁵. The live smallpox vaccination ACAM2000 has the benefit of being delivered in a single dosage. This medication is not recommended since it can proliferate in the cells of immunocompromised people. Furthermore, pregnant women and anyone with eczema or atopic dermatitis should not be vaccinated. A small number of vaccinated people may experience cardiac problems. Increased lesioning at the location of immunisation is normal and should be expected. The LC16m8 vaccine is an attenuated smallpox virus vaccine.

IMVAMUNE, a modified live attenuated vaccine, has fewer adverse effects and a safer profile than ACAM2000. Known as IMVAMUNE in the US and IMVANEX in Europe, it has limited replication ability, making it suitable for all adults, including those with weakened immune systems. Even after two doses of the live ACAM2000 vaccine, no skin lesions appear at the vaccination site⁷⁶.

Given the global impact of monkeypox virus (MPV), particularly in the context of the COVID -19 era, it is imperative that national and international research efforts be intensified. These efforts should focus on identifying virulence markers of the disease, understanding the factors that influence MPV evolution, which include both host and viral factors, investigating human behaviours that contribute to zoonotic spillover events, finding surrogates for asymptomatic infections, and examining the determinants of immunity for both the virus and the host. The goal of this research is to prevent MPV from filling the ecological gap left by the eradication of Variola major virus (VARV) and potentially evolving into a more dangerous pathogen. To achieve this, preventive epidemiological surveillance of MPV in endemic and high-risk areas, particularly in Nigeria, is essential. This surveillance should be conducted routinely and not only in response to outbreaks. Support the inclusion of routine and regular epidemiological surveillance of MPV in humans and animals in existing surveillance systems, such as the Surveillance Outbreak Response Management and Analysis System (SORMAS). This would allow early detection and effective management of monkeypox epidemics⁷⁷. The Sewer Coronavirus Alert Network (SCAN) was established in 2020 to test wastewater samples for COVID-19 virus presence. Since June 2022, it has expanded to include Monkeypox Virus (MPXV) detection in water

plants. Combining this network with phylogenomic analysis could provide insights into MPXV's spread and evolution. Although criticized for being reactive, SCAN has proven effective in anticipating virus spread, enabling frontline workers to control outbreaks and prevent a potential new Monkeypox pandemic.

15. Conclusion and future directions for Mpox research and control measures.

Indeed, the Mpox virus has long been a neglected zoonotic disease with the potential to spread and/or be used as a bioweapon. There are now no traditional guidelines for clinical care, medications, or vaccinations although the condition was originally diagnosed in 1958 and first recorded in a human in 1970⁷⁸. Limited research on the human immune response to Monkeypox virus (MPXV) infection remains unanswered. Understanding immune defenses and the potential for mucosal protection resulting from smallpox immunization or MPXV infection is essential. Investigating tissue-resident memory T cells and IgA in MPXV-related respiratory complications is essential. Factors like behavior, geography, diet, medical history, immunological factors, and genetics may also impact MPXV susceptibility and immunity. The virus can spread globally and has the potential to expand beyond Africa. Effective management strategies, such as ring-vaccination and pre-exposure vaccination, are needed to combat the outbreak. Increased surveillance efforts, vaccination availability, and antiviral medications can help mitigate the threat of MPXV. Also, it is a clear call to action for public health authorities to assume responsibility for informing specialists in the field of their results and being open and truthful with the general public. Recent outbreaks around the globe have highlighted the significance of rigorous and ongoing surveillance as well as the creation of novel preventative and therapeutic strategies. To stop rising transmission rates or virulence, appropriate and effective medicines as well as active monitoring activities are urgently needed⁷⁸. Considering At least in endemic regions and possibly globally, the Mpox virus is the most major orthopoxvirus in humans. It takes a lot of effort to prevent Mpox, which is no longer a rare condition. The human Mpox virus should be monitored and controlled using the insights learnt from the Covid-19 epidemic.

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