# A REVIEW OF GENOME ORGANIZATION, EVOLUTION, TRANSMISSION, CIRCULATION, AND CLINICAL MANIFESTATION OF MONKEYPOX VIRUS

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**ABSTRACT**

Mpox is an illness caused by the monkeypox virus (MPXV, genus Orthopoxvirus), which infects animals and humans. Genetically, there are two MPXV clades: The Central (1) and West (2), with two reported subclades for each. MPXV can be transmitted between animals, from animals to humans, and humans to humans. Since May 2022, a multi-country outbreak of mpox has been registered in non-endemic regions. After a decrease in the number of confirmed cases in 2023, a re-emerging spread of mpox clade I in Africa and various EU/EEA countries has been registered since mid-2024, and into 2025. According to available genomic data, nonsense or frameshift mutations of MPXV resulting in loss of protein-coding genes and noncoding genes or regulatory regions observed in endemic regions of Central Africa have been associated with human-to-human transmission of the virus. Urbanization caused by population growth in West Africa may increase the risk of human MPXV infection. The infection spread, especially among the countries of the European continent, has led to increased research on mpox prevention and therapy, with data being continuously updated. Monitoring of potential animal reservoirs and exploring new transmission routes are important. Over time, the MPXV has evolved by accumulating genome mutations, contributing to its adaptability and easier human-to-human transmission.

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## 1. Classification

Mpox is an illness caused by the monkeypox virus (MPXV), which infects animals and humans. MPXV is a member of the genus *Orthopoxvirus*, subfamily *Chordopoxvirinae*, family *Poxviridae* [1]. The genus *Orthopoxvirus* has 13 identified representatives. Among them, important human pathogens include variola, vaccinia, cowpox and rabbitpox viruses. MPXV was first isolated in monkeys in 1958 at the Primate Research Institute in Denmark [2]. In 1970, an outbreak study in the Democratic Republic of Congo (DRC) proved its importance in human pathology [3]. Since then, MPXV has been considered endemic to DRC and was detected in other 11 African countries [1,4]. Based on laboratory data, various small mammals are a reservoir of MPXV, animals infect the human population, whereby the chain of human to human transmission is relatively short in case of close contact. [5, 6] Genetically, there are two MPXV clades: The Central African (Congo Basin - DRC, Republic of Congo, Gabon, Cameroon and Central African Republic) and the West African (Nigeria, Benin, Côte d'Ivoire, Liberia, and Sierra Leone) and [7-9].

Central Africa's viruses are more virulent than those from West Africa [10, 11]. Studies show that infections caused by the Central African clade tend to be more severe, with a higher fatality rate (10%), in contrast to the West African strain, which has a fatality rate of 4% [12, 13]. These variations in virulence are linked to differences in genome structure, including deletions in gene regions and fragmentation of genes within open reading frames [14]. The genetic similarity between the two clades is at most ~95%, while the homology of viruses within the clade is on the order of 99% [9]. In addition to geographic distribution, they differ in clinical pattern, severity, and transmission [15-17]. The study of Happi et al.

[18], discussed a change in the nomenclature of the MPXV in three clades and separation of isolates from the Congo Basin in clade 1 and those from West Africa in clades 2 and 3. Clades 2 and 3 thus defined are less virulent and less likely to infect humans, unlike clade 1. This explains the zero reported deaths in the 2003 outbreak in the USA [19], where 90% of reported cases were from the Congo Basin [20].

**2. Structure**

Morphologically, MPXV has an oval or rectangular brick shape visible by electron microscopy, a characteristic of poxviruses. It measures 200 × 250 nm and has membranous surface tubules or filaments, a double-stranded core disc with linear double-stranded DNA (dsDNA), and a lipoprotein envelope [14]. The immature virion has spherical shapes, whereas the mature virion can be seen on negative staining in two forms: smaller ink (M) or larger capsular (C).

**3. Genomic organization and evolution**

MPXV's dsDNA genome is approximately 197 kb long and encodes around 180 proteins, which posed a significant challenge during de novo assembly of the entire genome [21, 22].

The MPXV genome is linear and features covalently closed ends, meaning it lacks free 3' and 5' ends. Each end of the genome contains 10 kb of inverted terminal repeats (ITRs) [22]. The genes are compactly arranged, with few intergenic regions longer than 100 bp. The central region of the genome contains genes crucial for transcription, replication, and the assembly of the virus, while the terminal, which varies between different poxviruses, encodes proteins that influence clinical symptoms and the virus's host range. The first fully sequenced MPXV genome from the 2022 outbreak (isolated as MPXV_U.S._2022_MA001) was deposited in the GenBank with accession ID ON563414 [118] on May 30, 2022 [23]. According to Oxford Nanopore sequencing, the genome of MPXV spans 197,205 base pairs of linear ds DNA.

The reported rate of poxviruses mutations per replication cycle is $10^{-5}$ to $10^{-6}$. Genome analysis of the 2022 U.S. MPXV outbreak indicates a notable accumulation of mutations as opposed to MPXV isolates from previous years. These mutations primarily occur at the 5' GA-to-AA sites within Apolipoprotein B mRNA Editing Catalytic Polypeptide-like 3 (APOBEC3), a protein with cytosine deaminase activity, and these G-to-A mutations are typically for MPXV 2 clade [23, 24]. The APOBEC3 proteins, known for their activity on single-stranded DNA, are studied in RNA viruses [24]. Their role in DNA viruses, however, has also been demonstrated [25-27]. APOBEC3 proteins play a crucial part in the innate immune defense of vertebrates by inhibiting virus replication through their cytosine uracil deaminase activity [24, 28].

In a study by Jones et al. [29], 47 MPXV genomes from Berlin, Germany, collected between May 20 and July 4, 2022, were analyzed. several nonsynonymous amino acid changes compared to the previous outbreak were revealed. Notably, the original 5' gene was duplicated in sequences isolated from two lesions one of the same patient. Additionally, four genes near the 3' end of the genome were either disrupted or completely deleted due to an 856-nucleotide translocation between the genome's endpoints. Such genomic rearrangements in orthopoxviruses could confer host-specific advantages, potentially enabling better adaptation and facilitating virus transmission between humans during the current mpox epidemic [29].

The greatest genome variability in orthopoxviruses is observed in the two inverted terminal repeat (ITR) regions at the genomeends, where immune escape factors predominantly reside and play a role in virulence [30, 31]. Mutations in these regions are believed to be the key mechanism for the rapid adaptability of orthopoxviruses following host switching [32, 33]. MPXV genomes from both West and Central Africa show mutation in the ITR regions [29, 34]. The evolution of MPXV genome stimulates its virulence, and ability to evade immune responses.

**4. Viral replicative cycle**

Poxviruses are obligate cellular parasites and their replication is realized only in the cytoplasm. MPXV has aerosol or intradermal transmission, can penetrate damaged skin or mucous membranes, where initial replication occurs and spreads to local lymph nodes [35]. In aerosol infection, the virus enters the respiratory tract. It has been established

that MPXV can enter the body through sexual contact [36, 37]. In addition, direct contact with materials contaminated with the virus, such as clothing, utensils, and furniture, is a prerequisite [36, 37].

Viral penetration occurs through micropinocytosis, viral endocytosis, and fusion with the cell membrane, enabling entry via various routes, including airborne and contact-based pathways. Upon inoculation, MPXV replication triggers generalised infection with organ involvement via the bloodstream. The virus replication occurs entirely in the cytoplasm of the host cell, under the control of two antigenically distinct virion forms: mature (MV) and enveloped (EV). Following the transcription and translation of MPXV mRNA, intracellular mature virions (IMVs) containing viral DNA are produced. IMVs, encapsulated in a membrane derived from the Golgi apparatus, form intracellular enveloped virions (IEVs). These IEVs then fuse with the host cell membrane, producing cell-associated virions (CEVs), which are subsequently released into extracellular spaces as extracellular enveloped virions (EEVs) [38]. Similar to other viruses, the members of *Orthopoxvirus* genus have evolved various mechanisms to avoid host defences, which facilitates their entry. and Orthopoxviruses may impair the pattern recognition receptors (PRRs) expressed by innate immune cells. PRRs include Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-1-like receptors (RLRs), C-type lectin receptors (CLRs) etc. responsible for recognizing molecules of infectious agents and released from damaged cells [39]. Once PRRs bind to microbial ligand [40]. , activation of inflammation-related transcription factors such as nuclear factor kappa B (NF-κB), interferon regulatory factors (IRFs), and activator protein-1 (AP-1) follows

Signal transduction of TLRs involves several types of intracellular adaptor proteins, such as MyD88, MAL, TRAM, TRIF, and SARM, which are crucial for triggering I immune responses [39, 41]. Any disturbance in those adaptor proteins can lead to an inadequate and incapacitated immunological response against viral infections. At this stage, orthopoxviruses contain genes encoding proteins that can interact with and disrupt the the physiological functions of adaptor proteins , followed by inhibition of transcription factors associated with inflammation, i.e. NF-κB [40]. Furthermore, MPXV and other members of Orthopoxviridae can affect cellular apoptosis, particularly the function of enzymes caspase-1, caspase-8, and caspase-9 [40, 42]. MPXV genome encodes a protein that mimics the activity of B-cell lymphoma-2 (Bcl-2) proteins, which are known to play an important role in the regulation of apoptosis [40, 43] and NF-κB activation [40, 44, 45]. Orthopoxviruses can also affect the immune response against viral agents by inhibiting the production of interferon regulatory factors (IFRs), thereby blocking the synthesis of interferon [40].

Orthopoxviruses and MPXV possess many genes encoding proteins that can disrupt different stages of host's inflammatory cascadecomprising cytokine and chemokine production, complement system activity, ubiquitin-proteasome pathway activity, and several other targets [40, 46].

Once able to evade the host immune system, MPXV can attack many systems of the host. Mpox and smallpox can have similar clinical manifestations. A specific symptom for mpox which is not detected in smallpox cases is lymphadenopathy (most often in submandibular, cervical, or inguinal region) [36, 47].

An important role in the development of lymphadenopathy plays the vaccinia complement control protein (VCP) [40], which consists of four short consensus repeats (SCRs). VCP can bind to some complement components (C3b, C4b and other), thus disrupting th esubsequent complement cascades [40]. Ultimately, VCP suppresses the inflammatory response [48, 49].

unchanged similar structure of the VCP protein has been found in other orthopoxviruses (variola, vaccinia, cowpox viruses). In contrast, , the VCP structure of MPXV is either shortened (clade 1) or deleted (clade 2) [40].

**5. Epidemiology and transmission**

Mpox primarily spreads in Africa regions, with MPXV transmission occurring through several routes: between small mammals, from animals to humans and within the human population [50]. The most common mode of animal-to-human transmission is through direct contact with an infected animal or its fluids [51]. Initially, human infections were linked to animal exposure, but in regions with high

rodent infestations and where hunting or preparing meat from wild animals is common, deciphering the source of infection is difficult [52]. Human-to-human transmission was observed in Nigeria and other parts of West Africa [53]. Close living conditions or shared use of utensils are potential sources of contamination, which could increase transmission risk within households. The first reported case of human mpox in the U.S. occurred in 2003, when the outbreak was linked to infected prairie dogs. Despite this, there was still a risk of human-to-human transmission [54]. In September 2018, MPXV was transmitted to a healthcare worker in the UK through contaminated bed linens [55]. Viral penetration can also occur through close contact or by respiratory secretions from an infected individual [52]. Vivancos et al. [56] reported an ongoing MPXV outbreak in the UK since May 2022, noting that all previously documented cases in the country were either imported or involved healthcare workers in contact with international cases [55]. In Israel and Singapore, cases of mpox were reported, with epidemiological data indicating the importation of the infection associated with travel to Nigeria [57]. Regarding the current mpox epidemic in Europe, there are no initial epidemiological links to sites in West and Central Africa, raising the possibility of long-term undetected transmission in the region [58]. The incidence of mpox was reported to range from 0.64 per 100,000 in 2001 to 50 per 10,000 in 2016 [59]. The death ratio ranged from 1 to 10%, with clade 1 being more virulent and deadly compared to clade 2. A major proportion of deaths due to MPXV are concentrated in Africa. After the year 2000 a significant demographic manifestation of mpox and increase in deaths primarily among children under 10 years and young adults was documented [59].

The reported epidemic from May 2022 to December 31, 2024, covered 122 countries, with only 7 being part of the previously endemic countries, and the confirmed mpox cases were 102,000 [60]. Globally, the cases of mpox peaked in August 2022. Althoughsignificantly fewer cases are reported presently, the transmission of clade 2b MPXV continues globally. Confirmed cases in the European Union/European Economic Area (EU/EEA) countries, including Bulgaria, by 12 February 2025 are 23 882 (reported from 29 EU/EEA countries) [61]. The current epidemic in non-endemic for the virus countries, is spreading mainly among men who have sex with men (MSM) and multiple partners. However, there is a potential for spread to other population groups. Clinical manifestations of mpox reported in the EU/EEA are mild to moderate. The severity may be higher in young children, pregnant women, and immunocompromised individuals. Case isolation, contact tracing, and rapid laboratory diagnosis form the core of the current mpox epidemic control strategy in most EU/EEA countries. Collaboration between public health and clinicians, especially sexual health professionals, is critical to identify as many close contacts as possible are. Early diagnosis, isolation, and effective contact tracing are key to epidemic control [3]. Based on the available data, ECDC assesses the likelihood of further spread of mpox in EU/EEA countries and globally in the coming months as medium-high, resulting in a moderate risk for the general population. The inability to rapidly contain the epidemic situation determines the WHO's change of strategy and designation of the disease. Mpox infection was defined as one of 'international concern and public health urgency' in 2022, after which it was specified again as 'moderate' in 2023 and since August 2024 mpox outbreak and rapid spread of a new virus strain in DRC, clade 1b, is defined as a public health emergency of international concern.

The current risk of further spread of MPXV among persons with multiple sexual partners in linked groups (including some MSM groups) is defined as high.

Transmission to healthcare workers from mpox patients (e.g., face-to-face contact for prolonged periods, contact with open lesions without gloves, intubation, or other invasive medical procedures), including nosocomial spread, is possible given the risk of transmission of other orthopoxviruses. In parallel, thousands of clinically compatible mpox cases are being reported in Africa, where the access to laboratory diagnostics remains limited. Measures have been developed for rapid detection and prevention of the virus. Currently, the primary alert is from the DRC, where the epidemic spread of mpox genetic lineage 1 has been observed. In

2024, DRC reported over 40,000 cases, over 9,000 confirmed cases, and 40 deaths. Burundi reported over 3 000 confirmed cases, and Uganda reported over 1 500 confirmed cases. Mpox clade 1 cases were reported also in Rwanda, Kenya, Zambia, and Zimbabwe. Outside of the African continent, only travel-associated cases due to MPXV clade 1 and/or sporadic cases with epidemiological links to travel-associated cases have been reported. No wider community transmission and no deaths have been reported due to MPXV clade 1 in any country outside of Africa [62].

The CDC has investigated recent MPXV outbreaks to assess possible transmission routes, such as hugging, kissing, and sexual contact (oral, anal, and vaginal). These modes of transmission may be connected to genetic mutations that enhance virus's ability to spread between individuals [63-65].

The 2022 epidemic indicates a divergence from the original two MPXV clades, especially regarding human-to-human transmission. This divergence is marked by the branching of clade 2, which is now referred to as clade 3 or "human MPXV" (hMPXV). The most notable difference between clades 1, 2, and 3 appears to be in the terminal inverted terminal repeats (ITR), which harbor genes responsible for encoding host response modifier (HRM) proteins. One of these is the mpox analogue of the poxviral inhibitor of complement enzymes (PICEs) or MOPICE protein, which was once thought to be a differential virulence factor between clade 1 and 2, with the absence of MOPICE in clade 2 contributing to its lower pathogenicity [9]. However, a solid study in rhesus macaques showed the opposite. Eliminating MOPICE increased replication in vivo and attenuated the adaptive immune system response [66]. The virulence determinants distinguishing the two clades seem more likely to be influenced by numerous genetic factors within the vast MPXV genome, including the open reading frames of D10L, B10R, B14R, and B19R [4].

The mpox outbreak in 2022 suggests that the MPXV virulence correlates with the genetic variability in the virus genome. All reported MPXV isolates are phylogenetically related to clade 2, and the estimated mortality rate is below 1% [67].

**6. Clinical manifestation of MPOX, pathogenesis and immune response**

Mpox has an incubation period ranging from 5 to 21 days, with an average of 6 to 13 days. The illness typically begins with symptoms such as fever, muscle aches, fatigue, and headache. Within three days of symptoms onset , a maculopapular rash develops, spreading centrifugally from the primary infection site to other areas of the body. In cases of disseminated rash, the palms and soles are affected. The skin lesions progress over about 12 days, evolving from macules to papules, then to vesicles and pustules before fading. Secondary bacterial infections may occur as a result of itching and subsequent skin damage. Lesions may also appear on the oral or ocular mucosa (enanthema). Lymphadenopathy is commonly observed in many patients before and during the rash phase. It is important to note that clinical manifestations in travel-related cases are generally mild, often with only a few lesions. The appearance of the rash marks the beginning of the infectious period, which typically lasts between two and four weeks [68, 69].

In most infected individuals, the symptoms are mild to moderate. However, clinical complications can include encephalitis, secondary bacterial skin infections, conjunctivitis, keratitis, pneumonia and dehydration. There is limited information on how mpox affects immunocompromised patients. In a 2017 outbreak in Nigeria among individuals co-infected with HIV, the disease was more severe, with more extensive skin lesions and genital ulcers compared to those who were HIV-negative.

**7. Laboratory diagnosis of mpox**

Laboratory confirmation of MPXV infection is based on a molecular assay detecting a unique viral DNA sequence in appropriate clinical material. PCR analysis can be used alone or in conjunction with sequencing. Molecular protocols to detect OPXV, particularly MPXV, some of which include differentiation of the Congo Basin and West African clade have been quickly validated. Some protocols involve two steps where the first PCR reaction detects OPXV but does not identify the species. The assay can then be followed by a second step, which is PCR-based or uses sequencing to detect MPXV species precisely [70].

Electron microscopy can be used as a laboratory approach to evaluate a sample for the presence of a potential poxvirus, but the high technical skills and facilities required, and the advent of modern molecular assays , has precluded this method from routine poxvirus diagnostic.

Virus isolation in Vero cell cultures is a relatively simple approach but is not recommended as a routine diagnostic procedure and should only be performed in laboratories with specially trained laboratory workers and BSL Class III biosecurity facilities.

A wide range of infectious agents cause skin rashes with similar clinical course to MPXV, making a diagnosis based on clinical presentation alone challenging, especially in cases with atypical presentation. Therefore, it is important to consider other potential causes of discrete skin lesions or disseminated rash. Examples of etiologic agents causing similar-appearing skin lesions at various stages of development include herpes simplex virus 1 and 2 (HSV 1 and 2), varicella zoster virus (VZV), molluscum contagiosum virus, enteroviruses, measles, scabies, Treponema pallidum (syphilis), bacterial skin infection, drug allergies, and parapoxviruses (causing orf and related conditions) [71].

In case case of clinical suspicion for mpox in human, skin lesions serve as a source material for etiological identification of the causative agent. In those clinical specimens, the demonstration of the virus occurs with the greatest frequency. In addition, naso- and oropharyngeal swabs can be examined in the first days of clinical manifestation (days 1-4). It is advisable to process at least two lesions similar in type from two different anatomical regions [70]. The material from those is placed in one container. When the lesions are different in type, the different specimens are not mixed and are placed in separate containers. Many studies, have detected viral DNA with varying frequency in a range of other clinical materials (saliva, ejaculate, urine, and faeces).

**8. Therapy and prevention of mpox infection**

Studies have shown that the smallpox vaccine offers cross-protection against poxviruses in 85% of those immunized. Although post-vaccinal immunity decreases over time, it is believed that the smallpox vaccine still provides some level of protection for adults over the age of 50. Following the official declaration of smallpox eradication by the WHO in 1980, vaccination ceased in many countries. In Bulgaria, the last individuals to receive the smallpox vaccine were those born between 1976 and 1978, and vaccination was not administered to all children. A scarification scar on the right arm, typically located in the deltoid muscle area, is considered a reliable indicator of smallpox vaccination [71].

Given the epidemic situation that has developed and the large number of mpox cases in non-endemic countries, the effects of various antiviral therapeutics and medications are beginning to be studied. A smallpox vaccine, ImvanexTM (Modified Vaccinia Ankara), is available in Europe and has been authorized for use by the European Medicines Agency under "emergency circumstances" [72]. A new mpox-specific treatment with Tecovirimat, an inhibitor of the VP37 assembly protein, was approved by the European Medical Association (EMA) in 2022, and a newer vaccine based on the Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN) platform was introduced in 2019 for prevention of the viral disease [72].

**9. State of research of the problem**

Although smallpox was eradicated over 40 years ago, infection with another Orthopoxvirus, the monkeypox virus, can produce a clinical presentation similar to that of smallpox [73]. Early theories suggested that MPXV was an extremely rare virus, not easily transmitted, and confined to the rainforest regions of Africa [74]. The ongoing MPXV epidemic in 2022, which spans over 100 countries worldwide, belies this claim.

In the 1980s, some authors put forward the opinion that t variola virus could be easily derived from the monkeypox virus [75], but that was not confirmed [76] and was even refuted subsequently [77]. Although sufficient differences between the MPXV and variola genomes have been demonstrated to rule out simple interconversion, the possibility remains that MPXV is a more ancient ancestor of the variola virus. Ultimately, sequence analysis of the two agents indicated the presence in MPXV DNA of only a 1,065-bp homolog that is part of the open reading frame of the variola genome but with many accumulated

deletions. This is an irrefutable evidence that MPXV is not a variola virus precursor but a distinct orthopoxviral agent that strengthens confidence in smallpox eradication's long-term success [73].

The cessation of variola vaccination has likely contributed to the rise in mpox cases, as the unvaccinated population serves as a key factor in the ongoing incidence increase . [78].

The largest mpox outbreak in West Africa's history began in Nigeria in September 2017 [79]. For the first 11 and a half months, no cases were exported; however, between September 2 and 23, 2018, three unrelated individuals infected with MPXV left Nigeria and traveled to two different countries [80]. Seven months later, a person of Nigerian nationality fell ill with mpox in Singapore [81]. These cases marked the first documented instances of MPXV being carried from the African continent by a human host. Meanwhile, several mpox outbreaks were also reported in laboratory and zoo animals, with no identified source of infection [82]. In 2003, the United States experienced an mpox outbreak, traced back to the shipment of rodents from West Africa [83]. The index case of the current mpox outbreak in Europe was confirmed in a UK resident on May 6, 2022, and was linked to a travelling to Nigeria. Sequencing of the first isolate from a patient in Portugal, with clinical material collected on 4 May 2022, suggested that the MPXV isolates were homologous to those imported into the UK in 2018-2019 and ere genetically related to the Nigerian MPXV strain belonging to the West African MPXV clade [84]. Other available MPXV sequences from patient isolates from the USA and Belgium also showed a closer genetic relationship with West African MPXV isolates and those from the UK in 2018 and 2019 [85]. The earliest date of symptoms onset in patients has been reported as April 3, 2022. Most reported cases were aged between 31 and 40 years (42%), of whom were male (99.5%). Among cases with known HIV status 43% were HIV positive. The majority of patients presented with rash (95.2%) and systemic symptoms such as fever, fatigue, mylagias, vomiting, diarrhoea, chills, sore throat or headache (64%). Just over 10.2% were hospitalized. Healthcare workers (over 40) were reported sick, and investigations are ongoing to determine whether the infection was due to occupational exposure. The highest proportion of viral DNA was detected in skin lesions (46.7%), followed by oropharyngeal swabs (19.1%) and rectal swabs (15.4%) [86]. The presence of viral DNA is not an evidence of the detection of viable virus and infectious potential of the clinical material concerned. However, it is subject to thorough analysis as it may be a reservoir for virus shedding and contamination of household products, especially when it comes to contaminated faecal water. The basis of the disease and transmission is puzzling due to the unusually high incidence of person-to-person transmission, with studies focusing on possible genetic modifications of the virus.

According to available genomic data, nonsense or frameshift mutations of MPXV resulting in loss of protein-coding genes and noncoding genes or regulatory regions observed in endemic regions of Central Africa have been associated with human-to-human transmission of the virus. The 2022 MPXV outbreak affecting multiple countries will likely have a single origin, with early signs of microevolution in the clusters of the outbreak. Urbanization caused by population growth in West Africa, especially in Nigeria, may increase the risk of human MPXV infection. The infection spread, especially among the countries of the European continent, has led to increased research on mpox prevention and therapy, with data being continuously updated. Monitoring potential animal reservoirs (e.g., rodents) and exploring new transmission routes are important. Over time, the MPXV evolved by accumulated genome mutations, contributing to its adaptability and easier human-to-human transmission. Studies suggest that variation in gene copy number may be a crucial factor in modulating the fitness of the virus [10].

In line with what has been discovered so far, the WHO Advisory Committee on Smallpox Virus Research at its twenty-fifth meeting (Geneva, 25 and 26 October 2023) reviewed reports from the two WHO Collaborating Centres on Smallpox Virus Conservation, taking into account the global implications of the COVID-19 pandemic and the global mpox epidemic since 2022. Variola virus research strategies need to be modified and updated about the threat of a global weakening of immunity to the virus, the spread of

immunosuppressive conditions, and the ongoing evolution of orthopoxviruses leading to adaptation to more efficient human-to-human transmission, as is the case with MPXV. The Advisory Committee encourages efforts towards a rapid MPXV diagnosis and sequencing of all virus isolates. Regarding research on antiviral therapeutic agents approved for use against MPXV, cowpox and vaccinia virus (Vaccinia Virus) infection are the antiviral agents Tecovirimat (approved in Europe) and NIOCH-14 (approved in the Russian Federation). Research on therapeutics with complex activity against orthopoxviruses, including variola virus and MPXV, is encouraged.

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