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## Monkeypox: An emerging zoonotic pathogen

Masoumeh Beig, Mehrdad Mohammadi, Fatemeh Nafe Monfared, Somaieh Nasereslami

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### Abstract

Monkeypox virus (MPXV), which belongs to the orthopoxvirus genus, causes zoonotic viral disease. This review discusses the biology, epidemiology, and evolution of MPXV infection, particularly cellular, human, and viral factors, virus transmission dynamics, infection, and persistence in nature. This review also describes the role of recombination, gene loss, and gene gain in MPXV evolution and the role of signal transduction in MPXV infection and provides an overview of the current access to therapeutic options for the treatment and prevention of MPXV. Finally, this review highlighted gaps in knowledge and proposed future research endeavors to address the unresolved questions.

**Key Words:** Poxviridae; Orthopoxviruses; Monkeypox viruses; Epidemiology

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**Core Tip:** Since May 13, 2022, cases of monkeypox have been reported to the World Health Organization (WHO) from 12 Member States that are not endemic to the monkeypox virus across three WHO regions. This emergent pathogen is a significant concern worldwide after severe acute respiratory syndrome coronavirus 2 and requires epidemiological and other data on the virus. The objective of this review is to report comprehensive data on this virus.

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## INTRODUCTION

Monkeypox virus (MPXV) is one of the human orthopox viruses (OPVs), which consist of variola virus (VARV), cowpox virus (CPXV), and vaccinia virus (VACV)[1]. Monkeypox has similar clinical manifestations to smallpox, but has a milder rash and a lower fatality rate[2]. The aims of this review are to describe the current data on MPXV evolution, epidemiology, and infection-control mechanisms.

### **History of monkeypox virus**

When two smallpox-like illnesses appeared in monkey colonies housed for scientific study, the first cases of monkeypox were discovered in 1958[3]; therefore, the name monkeypox and the first human case of the virus were registered in 1970 in the Democratic Republic of the Congo[4]. Attempts to destroy the MPXV have since been documented in humans in other Central and West African countries [5].

### **Morphology, genome organization, and morphogenesis**

The morphology of MPXV virions has been shown to include brick- or ovoid-shaped particles[6]. Membrane links, a tightly packed core containing enzymes, transcription factors, a double-stranded DNA genome, and an outer membrane protecting the whole structure have been observed[7,8]. Although its whole life cycle occurs in the cytoplasm of infected cells, its genome contains linear double-stranded DNA (197 kb). The genome encodes all the proteins necessary for viral DNA replication, transcription, and virion assembly[6,9]. Cells infected with the poxvirus generate the intracellular mature virus and extracellular enveloped virus, two contagious viruses[10,11] (Figure 1).

## INFECTION BIOLOGY, DIAGNOSIS, AND TREATMENT

### **Animal models**

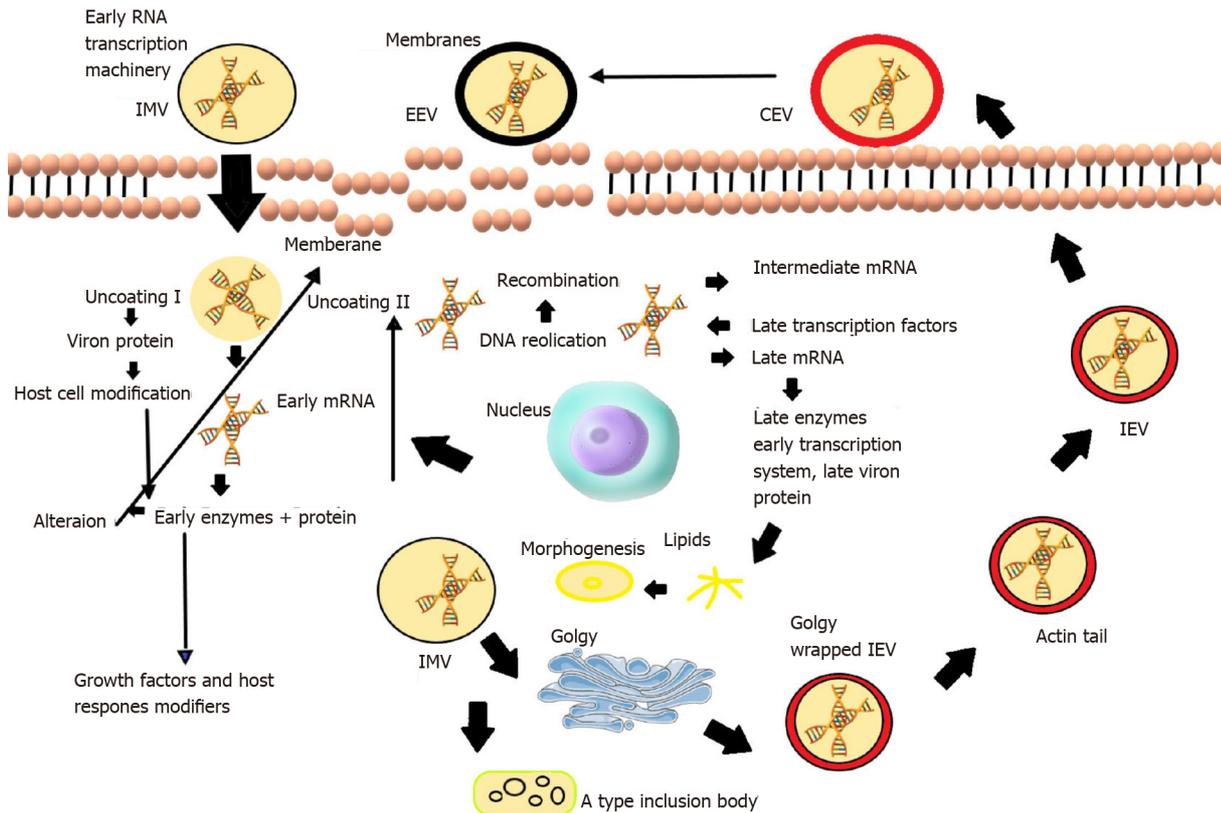
An animal model for studying ethnic illness uses a channel of contamination that matches the herbal transmission of the virus or displays development, morbidity, and death similar to those seen during ethnic infection[12,13]. The animal model also has to mirror human instances in at least one or more methods of transmission[14]. Additionally, the red patches on all MPXV-examined animals at the vaccination site showed a decrease in size compared to nearby animals, and beginning around 14 d after the challenge, a continual rise in body size across fully breathing animals in the vaccinated group[15, 16]. In a study that examined the sensitivity of 38 inbred strains of mice (32 classical inbred stresses and six wild strains), only three of the wild-derived strains (CAST/EiJ, PERA/EiJ, and MOLF/EiJ) were highly sensitive to MPXV, whereas all other inbred lines were strong after intranasal MPXV infection[2, 17].

### **Transmission**

Human-to-human and animal-to-human transmission are two potential MPXV transmission pathways [18]. Human-to-human transmission stability is correlated with droplet infection and interactions with body fluids, patient factors, and skin lesions in a contaminated individual[6,18]. The Congo Basin group is more virulent than the West African group and contributes more to interpersonal transport[19]. Direct contact and ingestion of the herbal viral host's food are the two routes by which transmission occurs from animals to humans[20,21]. Furthermore, zoonotic transmission can occur *via* direct touch, including blood, body fluids, and mucocutaneous lesions on a contaminated animal[22].

**Sexual transmission of MPXV:** MPXV outbreaks are not typical, as many patients are unrelated to travel to Central or West Africa and episodes of the virus in endemic areas. The MPXV is currently observed among men who have sex with men (MSM) in the United Kingdom. In the studies conducted, a high proportion of simultaneous sexually transmitted diseases and frequent anogenital symptoms were found, which indicates the possibility of transmission during close skin-to-skin or mucous contact during sexual activity[1,23,24].

**Transmission by MPXV-contaminated surfaces:** Although co-transmission between people and animals was identified as the primary method of infection dissemination in several investigations, transmission in patient care staff *via* surfaces contaminated with MPXV was seldom recorded. The



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**Figure 1 Cycle of monkeypox virus.** When the virion binds and fuses with the host cell membrane, the viral core is released in the cytoplasm. Enzymes, and then factors, initiate transcription. Most virions remain in the cytoplasm. Virus- and host-encoded proteins concerning cell surface-associated enveloped virions and cell surface-associated enveloped virions guard them to complement activation. IMV: Intracellular reduced virion; EEV: Extracellular enveloped virion; CEV: Cell surface-associated enveloped virion; IEV: Intracellular enveloped virion.

MPXV may also spread indirectly *via* contaminated objects. However, the environmental contamination of surfaces with MPXV is not well understood[25].

### Diagnostic methods

**Phenotypic approaches:** Phenotypic methods: According to the clinical diagnosis, in MPXV infection, a prodromal sickness usually accompanies it with a variety of symptoms over 3-5 d, including fever > 38.3°C, back pain, myalgia, headache, acute asthenia, pharyngitis, drenching sweats, malaise, and notably lymphadenopathy[6,26-28]. Vesiculopustular rashes begin on the face during 1-10 d of development, affecting 95% of patients[29], followed by the palms and soles (75%), oral mucosa (70%), genitalia (30%), and conjunctiva (20%). These skin lesions evolve from macules to papules, vesicles, pustules, and finally, scabs or crusts that fall[28]. Lesions in MPXV patients appear monomorphic, pea-sized, and complex, similar to smallpox[30]. The presence of lymphadenopathy in MPXV infection is one of the clinical markers that set it apart from smallpox, along with lesion appearance and limited centrifugal spread[31]. These skin manifestations compromise the skin eruption period of the disease, in which patients are contagious. Before that, patients are not able to transmit the virus. The natural history in patients without complications regularly lasts 2-4 wk[28]. Possible detection of MPXV based on clinical signs is essential to identify suspicious cases during surveillance. Nevertheless, the clinical case definition for MPXV based on unconfirmed studies has high sensitivity (93% to 98%) and low specificity (9% to 26%)[31,32]. Virus transmission occurs by direct bodily contact with pores and skin then skin lesions, along with sexual contact; or contact with contaminated materials, such as clothing, bedding and dishes, within 21 d before signs appear. Laboratory research does not validate the clinical definition, but an epidemiological link, including contact with a proven case does[28].

**Genetic methods:** It is recommended that genetic techniques, including polymerase chain reaction (PCR) or real-time PCR (RT-PCR), be performed in a biosafety level 3 facility[33].

Routine detection of MPXV DNA in clinical and veterinary specimens and cell cultures infected with MPXV is performed by RT-PCR targeting conserved regions of the outer coat protein (*B6R*) gene, I DNA polymerase E, the DNA-dependent RNA polymerase subunit 18 (*rpo18*), and the *F3L* genes[33,34]. Restriction fragment length polymorphism (RFLP) of genes or PCR-amplified gene fragments is also

used to detect MPXV DNA, but RFLP is time-consuming and requires viral culture[35]. Additionally, as RFLP of PCR products requires enzymatic digestion after gel electrophoresis, it may not be an appropriate method in a clinical setting where speed, sensitivity, and specificity are essential. Whole genome sequencing (NGS) is valuable in detecting MPXV and OPVs, but this technique is expensive, and downstream sequencing records processing requires extensive computing[36-38]. Therefore, NGS may not be a suitable detection method in resource-poor locations in sub-Saharan Africa. Although RT-PCR remains the optimal method for the identification of MPXV, this must be complemented by genome sequencing technology to provide information on the genome, which is essential for evidence-based epidemiology (Figure 2)[32].

**Immunological methods:** These methods include enzyme-linked immunosorbent (ELISA) and immunohistochemical assays to determine IgG and IgM antibodies and detect viral antigens[39]. Immunochemical analysis can distinguish poxvirus from herpes virus infection using polyclonal or monoclonal antibodies to all OPVs[11]. It has been shown that antibodies to the virus also have cellular responses and enhancements at the time of disease onset. Approximately 5 d and 8 d or more after the onset of the rash, IgM and IgG are formed in the serum, respectively[40]. Detection of IgM and IgG antibodies in unvaccinated individuals with a history of inflammation and severe illness may increase indirect MPXV discrimination. Despite this, these methods are not specific for MPXV detection and can detect other types of OPVs[32,41]. On the other hand, IgM can assess MPXV infection in people with a history of smallpox vaccination[42]. A positive IgM capture ELISA test indicates recent exposure to OPV (possibly MPXV in endemic areas) in vaccinated individuals.

Conversely, a positive IgG capture ELISA test indicates that a person has been exposed to OPV through vaccination or natural infection. Therefore, IgM and IgG in a sample are strong evidence of recent exposure to an OPV in previously vaccinated or naturally infected individuals. Thus, IgM in individuals vaccinated against smallpox in MPXV-endemic regions reflects recent exposure to MPXV [43,44].

**Electron microscopy:** MPXV under an electron microscope appears intracytoplasmic brick-shaped with lateral bodies and a central core measuring about 200–300 nm. Although this method is not a definitive diagnostic technique as OPV species cannot be differentiated morphologically, it provides a clue that the virus belongs to the Poxviridae family [45].

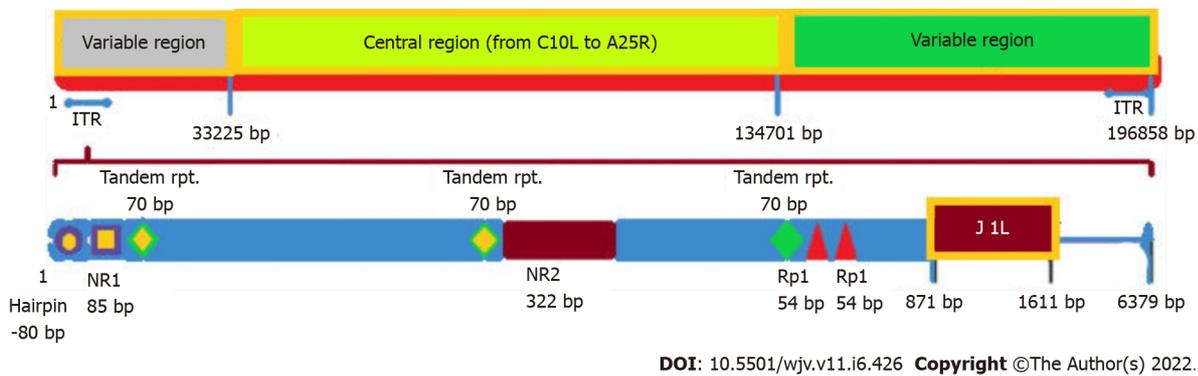
### **Virus-host interaction**

**Host and tissue tropism:** Members of the OPV family are thought to exhibit diverse spectra of host tropisms[46]. Although the reservoir host for MPXV has not been definitively identified, many mammalian species are naturally infected with MPXV[47]. Thus, it is believed that MPXV has a wide host range. Previously, after the challenge with Congo Basin MPXV, large amounts of viral DNA and viable virions died in a variety of animal tissues, suggesting broad tissue tropism. The immunohistochemical and histopathological tests by Falendysz *et al*[48] found that the MPXV antigen was identified in ovarian, brain, heart, kidney, liver, pancreatic, and lung tissues, and ovarian tissues were susceptible to MPXV[49].

**Host responses to the virus:** PXVs develop many strategies to escape the host's immune response to infection. Natural killer (NK) cells kill virus-infected cells by secreting cytokines that stimulate the activity of other cell types, such as T cells and dendritic cells[50]. MPXV infection can change lymphocyte numbers, NK cell changes in non-human primates (NHPs), lymphadenopathy, and lymphocyte consumption in MPXV-infected NHPs. Gavin *et al*[51] using prairie pooches showed a noteworthy increment in the number of all NK subsets (CD16- CD56-, CD16+, CD56+, and CD16+ CD56+) on the seventh day after vaccination. Moreover, the expression of chemokine receptors (CXCR3, CCR5, CCR6, and CCR7) on each NK cell subset suggest that, following the MPXV challenge, receptor expression was delayed or reduced[11,52]. Hammarlund *et al*[53] anticipated that MPXV has a safe avoidance component such as CPXV. The avoidance process utilized by MPXV ensures the viral store is resistant by repressing the activation of CD4+ and CD8+ T cells after interaction with MPXV-infected cells. Acknowledgment of MPXV-infected monocytes by antiviral CD4+ and CD8+ shows that MPXV does not activate the generation of cytokines (IFN- $\gamma$  or TNF- $\alpha$ ) by virus-specific T cells[52]. Antiviral T-cell responses are substantially increased following contamination with VARV alone. However, T-cell cytokine responses decreased by 95% after co-infection, including MPXV and VARV, and by 80% when low-dose MPXV was added (VARV: MPXV ratio was 10:1)[54].

### **Treatment**

**Vaccination:** The smallpox vaccine protects humans against smallpox. The smallpox vaccine incorporates a live vaccinia virus, and not a killed virus[55]. Vaccinated people must take precautions, as the vaccine can result in side effects[56]. Most humans have mild reactions such as flank pain, fever, and body aches[51]. However, some people may react differently, and some side effects can be life-threatening[57]. Although smallpox vaccination can shield humans from smallpox for approximately 3–5 years, its potential to protect humans then decreases, and for long-term protection, additional vaccinations may be needed[58]. Several reviews suggest that smallpox vaccination provides cross-protection



**Figure 2 Genomic structure of monkeypox virus.** The entire genome consists of over 196858 bp along the central genomic vicinity of 101476 bp. Both extreme variables (right is longer than left) include a 6379 bp inverted terminal repeat. ITR: Inverted terminal repeat.

against common OPV species and MPXV. Of humans vaccinated against smallpox, 85% did not develop MPXV infection[59]. The smallpox vaccine (ACAM2000™) was advocated by the Centers for Disease Control and Prevention (CDC)[60].

The attenuated vaccine, IMVAMUNE, is no longer available in MPXV areas[61]. A third-generation modified Ankara vaccine has been selected with the aid of the Food and Drug Administration (FDA) and the European Medicines Agency to prevent varicella or monkeypox in adults (age 18 years) with a high risk of VARV and MPXV infection[61,62]. Unlike the ACAM2000 vaccine, IMVAMUNE is no longer used in humans with immunodeficiency, such as immune disorders and atopic dermatitis. Neither ACAM2000 nor IMVAMUNE is used in specific populations[61,62]. Vaccination is also recommended for sexually high-risk individuals, including MSM, and those with a history of sexually transmitted diseases such as human immunodeficiency virus (HIV), syphilis, and gonorrhoea. However, there are no statistics on immunization, including smallpox vaccines JYNNEOS®/IMVANEX® that may confer protection against sexually transmitted MPXV[51,63].

**Antivirals:** There is no approved, safe remedy for MPXV infection. A 4-trifluoromethylphenol derivative and tecovirimat (ST-246 or TPOXX®), supported by the FDA, have been examined using animal models[64]. These agents have been shown to be beneficial in infected animals. According to a CDC report, clinical trials, including on tecovirimat, show that although the treatment is well tolerated and safe, there are inadequate statistics on its usefulness in treating monkeypox in humans[61,65,66]. Similarly, *in vitro* studies with cidofovir or brincidofovir (CMX001 or hexadecyloxypropyl-cidofovir) reduced viral DNA polymerase, and is an acyclic nucleoside phosphate conjugate of cidofovir[61,66,67]. However, brincidofovir has increased cytotoxicity and higher antiviral activity than cidofovir towards VARV, MPXV, VACV, and CPXV *in vitro*.

Brincidofovir has a high selectivity index and is 25-fold greater than cidofovir. Cidofovir is a nucleotide monophosphate analog. Another dynamic agent against poxviruses is NIOCH-14, a precursor of tecovirimat[66-68]. Although the activity of NIOCH-14 towards VARV, MPXV, and ECTV is similar to that of tecovirimat in *in vitro* studies, its production is less complicated than tecovirimat, and has been recognized as an essential antiviral in the future. Ribavirin and tiazofurin inhibited the activity of every OPV tested including VARV and MPXV[59,61,68,69]. Saquinavir, ritonavir, and nelfinavir are protease inhibitors, and efavirenz, stavudine, and zidovudine are reverse transcriptase inhibitors and have been used against OPVs. In addition, two adenosine analogs (C-ca3-Ado and C3-Npc A) have been shown to have protective activity against OPVs in viral replication assays, and these analogs are also inhibitors of S-adenosylhomocysteine hydrolase (SAH)[59,61,67,68]. These SAH hydrolase inhibitors have broad antiviral activity but had no detectable effect on CPXV *in vitro*. Using specific mechanisms, cidofovir and N-(2-hydroxypropyl) methacrylamide inhibited viral duplication in PXVs. However, adefovir and dipivoxil showed no sizeable activity against poxviruses.

Furthermore, adenosine oxide N1 had a considerable effect on OPV by inhibiting CPXV viral reproduction *in vitro* by blocking viral mRNA translation[52,68,70]. Although there is no optimal therapy, MPXV is managed only by supportive than evidential treatment, and is only suitable for symptomatic individuals[66,68]. Thus, environmentally friendly MPXV vaccination and antiviral agents are required to prevent transmission from asymptomatic people.

**Biocidal agents and disinfectants:** On June 5, 2022, a study was conducted to assess the published data regarding the antiviral effect of biocides and disinfectants against MPXV and orthopoxviruses. Vaccinia viruses must be rendered inactive by at least four log<sub>10</sub> using 70% ethanol (70%, 1 min), peracetic acid (0.2%, 10 min), and probiotic cleanser (1%-10%, one h) on contaminated surfaces. These tests also demonstrated the efficacy of glutaraldehyde (2%; 10 min), orthophthalaldehyde (0.55%, 5 min), iodine (0.04%-1%) and sodium hypochlorite (0.25%-2.5%; 1 min). Vaccinia virus was not affected by copper

levels (99.9%) but MPXV was at 3 min[71].

## CONCLUSION

As of May 2022, instances of MPXV have been recorded in nations where the infection is not endemic and are still being reported in several endemic nations. As a result, MPXV is no longer restricted to areas where it is endemic as, in recent years, visitors from Africa have brought MPXV to the United States, the United Kingdom, Israel, and Singapore. MPXV is a dangerous reemerging pathogen. MSM males in the United Kingdom have contracted MPXV *via* community transmission without directly interacting with travelers from endemic nations. In addition, a study reported admission to the Hospital for Infectious and Tropical Diseases in Romania of a 26-year-old HIV-positive male with high fever (up to 39 °C), chills, rectal pain, vesiculo-pustular rash, dysphagia, and skin lesions primarily in the anogenital area who had developed a mild form of the disease. This was the first MPXV case officially verified in Romania with suspicious epidemiological and clinical symptoms. Excellent knowledge on how to prevent and control MPXV infection, and improve contact tracing is required. This is particularly true in populations with high-risk characteristics. Public health officials and medical professionals should rule out MPXV in all patients who exhibit the typical rash and risky sexual behavior, especially those who have recently had sex with partners who visited countries where MPXV cases have been reported or partners who exhibit the same clinical symptoms even if they do not travel abroad[72]. As a result, it is essential to focus more on national and international research efforts for laboratory diagnosis, infection control, and treatment strategies. These strategies should also support sexual health and other specialized services in managing this condition. For MPXV outbreaks around the world, the Surveillance Outbreak Response Management Analysis System must be established and implemented.

## FOOTNOTES

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