Received: 28 September 2022 | Revised: 29 November 2022 | Accepted: 14 December 2022

DOI: 10.1111/all.15633

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

REVIEW ARTICLE

A compilation answering 50 questions on monkeypox virus and the current monkeypox outbreak

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Abstract

The current monkeypox disease (MPX) outbreak constitutes a new threat and challenge for our society. With more than 55,000 confirmed cases in 103 countries, World Health Organization declared the ongoing MPX outbreak a Public Health Emergency of International Concern (PHEIC) on July 23, 2022. The current MPX outbreak is the largest, most widespread, and most serious since the diagnosis of the first case of MPX in 1970 in the Democratic Republic of the Congo (DRC), a country where MPX is an endemic disease. Throughout history, there have only been sporadic and self-limiting outbreaks of MPX outside Africa, with a total of 58 cases described from 2003 to 2021. This figure contrasts with the current outbreak of 2022, in which more than 55,000 cases have been confirmed in just 4 months. MPX is, in most cases, self-limiting; however, severe clinical manifestations and complications have been reported. Complications are usually related to the extent of virus exposure and patient health status, generally affecting children, pregnant women, and immunocompromised patients. The expansive nature of the current outbreak leaves many questions that the scientific community should investigate and answer in order to understand this phenomenon better and prevent new threats in the future. In this review, 50 questions regarding monkeypox virus (MPXV) and the current MPX outbreak were answered in order to provide the most updated scientific information and to explore the potential causes and consequences of this new health threat.

KEYWORDS
monkeypox, orthopoxvirus, outbreak, viral infection, zoonosis

1 | INTRODUCTION

We are currently facing the largest and most widespread outbreak of monkeypox (MPX) in history with more than 55,000 identified cases in 103 countries worldwide. The vast majority of cases (99.11%) have been reported in locations without a previous history of monkeypox virus (MPXV) exposure.[1] MPXV, a double-stranded DNA virus that belongs to the *Orthopoxvirus* genus and the *Poxviridae* family, was identified in 1958 by the virologist Preben von Magnus and colleagues while studying two smallpox-like disease outbreaks in cynomolgus monkeys.[2] MPX became a zoonotic disease when the first case in humans was reported in 1970 in a 9-month-old boy in Zaire (current Democratic Republic of the Congo—DRC). MPX became endemic in DRC and spread to other African countries, mainly in central and west Africa.[3]

The transmission of MPX in the current outbreak occurs in 95% of cases during sexual encounters, with a recent study finding that 98% of transmission cases involved men having sex with men (MSM).[4] Although MPX is a self-limited disease that usually lasts 2–4 weeks, severe symptoms and complications have been reported, including secondary infections, proctitis, tonsillitis, bronchopneumonia, sepsis, encephalitis, and infection of the cornea with ensuing loss of vision.[3,5] The prognosis of MPX depends on multiple factors such as initial health status, concurrent diseases, and comorbidities. In that sense, patients with a compromised immune system are at higher risk of severe MPX; moreover, the infection by MXPV may aggravate chronic conditions such as certain autoimmune or atopic diseases.[6]

Many causes could be responsible for the current unprecedented explosion of MPX cases, such as viral microevolution or the cessation of smallpox vaccination in 1980. However, human interventions like deforestation or climate change dramatically increase the possibilities of encountering pathogenic new species with a profound impact on the spread of zoonotic diseases. In fact, MPX is believed to have originated in the rainforests of western and central Africa, where over 500 square kilometers are being lost each year. It has been estimated that over 15,000 diseases will spread into a new species for the first time in the next 50 years as a result of rising global temperatures.[7]

This review aims to provide the most updated scientific information on MPXV and the current MPX outbreak answering 50 questions in relation to MPX virological characteristics, immunology, clinical signs and symptoms, epidemiology, diagnostics, and treatments. This review also explores the possible causes and consequences of the current MPX outbreak.

2 | MPX: VIROLOGICAL CHARACTERISTICS, CLINICAL SIGNS AND SYMPTOMS

2.1 | What is the taxonomy of monkeypox virus (MPXV)?

Monkeypox virus (MPXV) is classified in *Orthopoxvirus* genus, *Chordopoxvirinae* subfamily, *Poxviridae* family, *Chitovirales* order, *Pokkesviricetes* class, *Nucleocytoviricota* phylum, *Bamfordvirae* kingdom, *Varidnaviria* realm. The *Orthopoxvirus* genus includes 12 poxvirus species that infect humans and animals and consist of three zoonotic agents: cowpox virus, MPXV, and vaccinia virus.[8,9] MPXV is taxonomically related to other orthopoxviruses that are vaccinia, cowpox, variola virus (the agent of smallpox), molluscipoxvirus (Molluscum contagiosum virus), parapoxviruses (bovine papular stomatitis virus, grey sealpox virus, orf virus, pseudocowpox virus, and red deerpox virus), and yatapoxviruses (tanapox virus and yaba monkey tumor virus), which cause human infection, and some of them are zoonotic.[9,10]

2.2 | What are the phylogenetic characteristics of MPXV? What are the differential features of the MPXV isolates from the 2022 outbreak?

MPXV isolates are classified into 3 genetically distinct clades: (i) the clade I (formerly designated as clade 1), which is also known as "Central African" or "Congo Basin" clade and contains isolates from the Congo Basin, (ii) the clade IIa (formerly designated as clade 2) also known as "West African" clade and contains isolates from the countries in western Africa, and (iii) clade IIb (formerly designated as clade 3), which is descendant from clade IIa and includes the isolates of 2022 MPX outbreak.[9,11–13] The clade IIb consists of 4 lineages: A.1, A.2, A.1.1, and B.1 lineages. The MPXV isolates of the 2022 outbreak mainly belong to lineage B.1 and minorly belong to lineage A.2.[14,15] The lineage B.1 isolates include approximately 50 single-nucleotide polymorphisms (SNPs) compared with lineage A.1, which is the ancestor of lineage B.1 and contains isolates of 2018–2019 cases. Recently deposited 9 genome sequences from the USA, Thailand, and India, which are classified as lineage A.2, have 16 distinct genetic variations (9 nonsynonymous, 3 synonymous, 1 stop gained variation, and 3 amino acid deletion in the OPG174 gene) in comparison with other lineages. A study that compared the 2018 and 2022 MPXV genomes reported that 46 new consensus mutations, which include 24 nonsynonymous mutations, are detected in 2022 isolates, and these mutations are intensely located in 10 viral proteins: D2L-like, OPG023, OPG047, OPG071, OPG105, OPG109, A27L-like, OPG153, OPG188, and OPG210.[16] Another study that compared the MPXV genome sequences from the 2017 outbreak in Nigeria and the sequences from the 2022 outbreak showed that 55 SNPs were consistent in the 2022 isolates, and 25 of these SNPs were nonsynonymous leading to amino acid substitution in 20 viral proteins.[17] The mean nucleotide substitution rate of A.2 lineage is determined as 5.53×10^{-5} substitutions per base/year, and this indicates a more moderate rate in comparison with the substitution rate of B.1 lineage, which has 1.13×10^{-4} substitutions per base/year.[14,15] This indicates an accelerated mutation rate of lineage B.1 viruses that are dominant in Europe.

Recent studies indicate that most of the 2022 MPXV isolates have GA > AA mutations, which are indicative of the motif of apolipoprotein B mRNA editing catalytic polypeptide-like3 (APOBEC3) enzyme of the host. Clade 1 and the isolates prior to 2017 in clade 2 do not have any enrichment for GA > AA mutations, whereas the 2017–2022 isolates of clade 2 have enrichment of GA > AA mutations. The 2022 MPXV isolates have approximately 50 SNPs in comparison with 2018–2019 isolates, and it is found that 46 SNPs are GA > AA and TC > TT nucleotide replacements.[14,18] The viral genome editing maintained by APOBEC3 may play a role in the epidemiological characteristics of the 2022 MPX outbreak and may accelerate the transmission speed between humans. Another datum about the 2022 MPXV isolates is that the viral genome has a sequence of 30 T bases. However, the role or function of this sequence remains unclear.[19] The aforementioned genetic differences between the clades of MPXV might be responsible for their variable pathogenesis and transmission rates.

2.3 | When was MPXV identified for the first time? What is its main reservoir?

MPXV was identified in 1958 in Denmark, and the virus was isolated from the nonfatal cases of pox-like lesions in cynomolgus monkeys.[2] The reservoirs of MPXV are many mammals (domestic pig, African hedgehog, gray short-tailed opossum, Southern/common opossum, rope squirrel, and some other squirrel species, prairie dogs, woodchuck, dormouse, African brush-tailed porcupine, Gambian giant rat, Gambian rat, jerboa, etc.) and nonhuman primates (chimpanzee, rhesus macaques, orangutan, cynomolgus monkey, Asian monkeys, mona monkey, grivet, mangabeys, Western red colobus, etc). Although there are many reservoirs for MPXV, human transmission has only been proven from the orangutan, cynomolgus monkey, string squirrel, and prairie dog.[9,20–22] Human-to-animal transmissions have been reported in the 2022 MPX outbreak and seem to be epidemiologically significant.[9,23] However, further evidence on this transmission route should be demonstrated.[24]

2.4 | When was MPX disease described for the first time? In what regions of the world is MPX considered an endemic disease?

A 9-month-old boy with fever and pox-like rashes from the DRC in 1970 was the first described and confirmed human MPX case in medical history. Following the first human MPX case, several cases were reported from Liberia, Nigeria, and Sierra Leone in 1970 and

1971. MPX is endemic in some African countries, that is, DRC, Nigeria, Cameroon, Côte d'Ivoire, Liberia, Sierra Leone, Gabon, and the Central African Republic. However, DRC is the only highly endemic country with continuous reports of MPX cases during the past 50 years. The second country with the highest number of cases in past decades is Nigeria.[25]

2.5 | What are the modes of transmission of MPX? What is the transmission rate?

MPX shares the transmission modes of smallpox.[26] Prolonged cutaneous contact involving virion-rich lesion sites is the main transmission mode of MPXV.[27] MPXV is present in biological fluids such as blood, saliva, and sperm. However, contamination through body fluids and salivary droplets plays a minor role compared with direct cutaneous contact with maculopapular eruptive (MPE) lesions (Figure 1).[27,28]

MPXV transmission occurs in 95% of cases during sexual intercourse, with a recent study finding that 98% of transmission cases involved MSM (Figure 1).[4] These findings are corroborated by the preferential development of MPE in the oropharyngeal and anal regions. Up to now, the exact duration of virus persistence in vaginal and seminal fluids has not yet been established.[4,27] As opposed to other viral diseases, such as SARS-CoV-2 infection, people infected by the MPXV seem to become able to spread it only after the appearance of the first symptoms.[29]

Materno-fetal transmission of MPX has been established, resulting in miscarriage during the first trimester or stillbirth. In the latter case, diffuse cutaneous maculopapular lesions were present on the stillborn's head, trunk, and extremities.[30] Regarding the possible transmission of MPXV via maternal milk, low number of data concerning the presence of viral particles in maternal milk has been obtained; therefore, the transmission by this route remains unclear.[31,32] However, the Centers for Disease Control and Prevention (CDC) still recommends as a preventive measure not to breastfeed the baby from 3 to 4 weeks of age because cutaneous and mucosal transmission is still possible. It is important to note, however, that the presence of vaccinia virus, another virus of the orthopoxvirus family, has been demonstrated in cow's milk. Although these data in humans are still insufficient, they constitute an additional argument for the suspicion of the presence of this family of viruses in human maternal milk and the interest to study them further.[33]

Transmission from animals to humans has been suggested through animal bites, ingestion of infected animals, or contact with the blood of such animals (Figure 1).[3]

In the current outbreak, the World Health Organization (WHO) considers that the R0, that is, the average number of infectious disease cases arising from transmission from a single infected individual, is >1 in MSM and <1 in other populations. For example, in Spain, one of the most affected countries, the R0 for MPX is currently at 1.8 in MSM populations, to be compared with the figure of 2.88 observed during the peak of SARS-CoV-2 transmission.

2.6 | Can MPX be considered a sexually transmitted infection (STI)?

Sexually transmitted infections (STI) are defined by their transmission taking place during sexual intercourse, with syphilis, chlamydia,

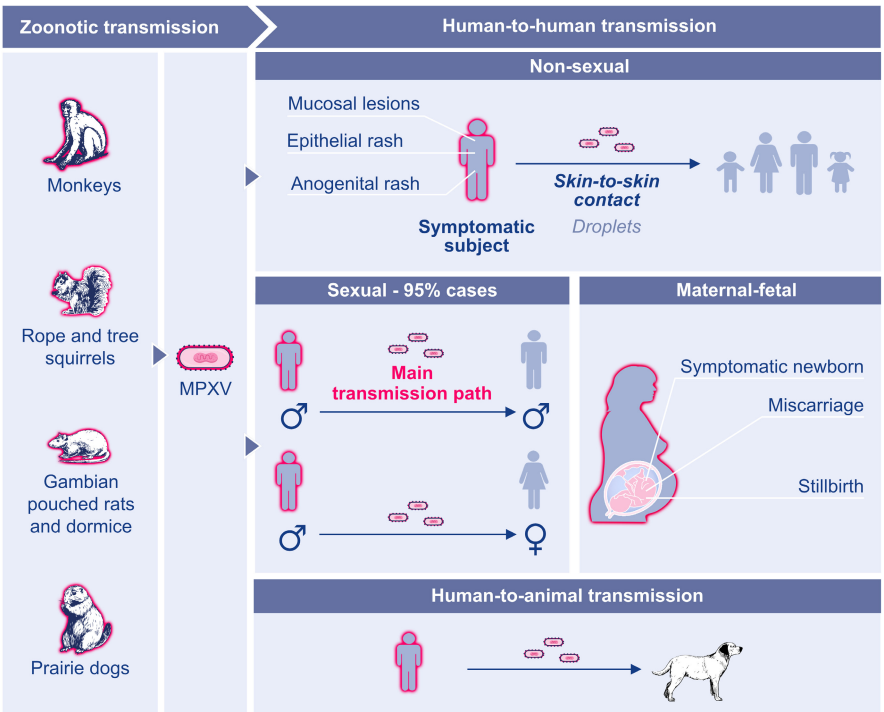


FIGURE 1 Modes of transmission of MXPV. The main mode of transmission is through direct and close skin contact with MPX lesions, in majority within the MSM (Men-Sex-Men) population. Zoonotic transmission and materno-fetal transmission are also documented. Transmission through blood and genital fluids has been suggested but is still under investigation. Human-to-animal transmission has been documented;[23] however, further evidence on this transmission route should be demonstrated.[24]

gonorrhoea, human immunodeficiency virus (HIV), and herpesvirus being typical examples. These pathogens are mainly transmitted through epithelial contact during anal, vaginal, or oral intercourse. Some STIs can be asymptomatic and thus hamper epidemiological surveillance, while others cause symptoms such as vaginal or urethral discharge, pain, or genital ulcers.[34]

Similar to the pathogens mentioned previously, MPX fulfils the criteria of STI. Indeed, a study conducted in 16 countries between April and June 2022 showed that 95% of MPX infections had been transmitted during sexual intercourse, with a prevalence of 98% of MSM intercourse.[4] Various skin symptoms were present, mainly genital and oral: cutaneous rash (95%), anogenital rash (73%), and mucosal lesions (41%). These symptoms relate to the mode of transmission of the disease, which takes place in most cases through prolonged epithelial and mucosal contact involving lesions of patients with the above-mentioned dermatological lesions.[4,27,35] The findings of this study were corroborated by those from other cohorts.[36,37]

Body fluids are involved to a lesser extent. Viral DNA is detected in sperm, saliva, and other fluids at least 16 days after the onset of symptoms and infectious virus was isolated from sperm in a reported case.[27,35,38] Nevertheless, it is not yet clear whether the virus could persist longer in other individuals and no precise duration has been established. Indeed, a more recent study detected the presence of the virus up to 19 days in semen after the first symptoms.[39] Another study, this time performed on crab-eating macaque archival tissue, detected the presence of the virus up to 37 days after exposure in the testicles.[40] Because of this uncertainty, the CDC recommends the use of condoms for 8 weeks after infection, but further research is needed to get a more precise knowledge of its persistence.

However, the amount of DNA measured in body fluids is lower than in skin lesions, as demonstrated by comparing cycle threshold (Ct) values.[38] Moreover, data on virion viability in body fluids are lacking, except for a study performed in monkeys and having concluded that a concentration of 10^4–10^5 copies/ml was required for infection.[41]

Some studies have tested for the presence of viral particles on inert surfaces by performing swabs followed by qPCR.[42,27,43] It has been shown that viral particles are present on various surfaces in the room of an infected person, such as door handles, light switches, and toilet seats. Specifically, the areas that have been in direct contact with the hands show the highest viral load.[42] It has also been reported the case of a transmission that the authors suspect to be linked to contaminated sheets. These observations are reinforced by the fact that it is documented that poxvirus, another virus of the same family as MPXV, can survive for a long time outside the body.[44] Furthermore, the presence of viral particles in fecal matter strengthens the possibility of contamination through poor hand hygiene and sewage.[45]

Taken together, these data suggest that, although MPX may behave as an STI, its main transmission mode is through skin contact. Indeed, among infected subjects, some had not engaged in sexual intercourse. The body fluids mode of transmission is also still under investigation, and so do the viability and the infectious dose of MPX.[4,38]

2.7 | Can the transmission of MPXV occur via the respiratory route? Can MPXV DNA be detected in saliva and oropharyngeal lesions?

In addition to skin and mucosal symptoms, as described previously, patients infected by MPXV may also develop flu-like symptoms. Multiple studies have reported lethargy, fever, myalgia, and lymphadenopathy reminiscent of smallpox infection, albeit to a lesser extent.[4,36,37,46] Cough has not been reported as an MPX symptom, but viral DNA in saliva and oropharyngeal lesions has been described.[38] In the context of the recent SARS-CoV-2 pandemic, these observations have fostered the hypothesis of a potential transmission through droplets or aerosols.

The actual contribution of this mode of transmission is still to be determined. CDC stated that the demonstration of salivary viral DNA is suggestive of potential transmission by droplets in indoor settings, particularly among members of the same household or hospital residents.[47]

On the contrary, previous studies do not support this hypothesis, suggesting that this transmission mode is marginal.[4,27,38] The main reasons are the low levels of viral DNA in body fluids, including saliva, and the lack of data on the viability of MPXV in saliva.[27,38,48] Moreover, the variations in the measured DNA levels as a function of sampling site (skin or saliva) and sampling time raise multiple questions regarding public health. Indeed, improved characterization of droplet transmission would lend basis to implementing preventive measures such as face mask recommendation, personal isolation, and the duration thereof, which could approach 1 month in this case.[27,28]

The prevalence of MPXV transmission through respiratory droplets is currently challenging to evaluate, even in subjects who became infected without sexual intercourse. Indeed, it is not yet established whether nonsexual contamination occurs through skin contact or through respiratory droplets.[4,28,38]

To summarize, MPXV transmission through respiratory droplets is possible and deserves more attention because it happens without direct interhuman contact. A better understanding of the respiratory transmission of MPXV or any virus is paramount for efficient strategies in the prevention of ongoing and future epidemics, and for combatting the current ones.

2.8 | What is the incubation period of MPX?

The incubation period of MPX, which corresponds to the interval between contact with the infected person and the time that the first symptoms appear, is usually 6 to 13 days but can be extended up to 21 days.[3] The duration of this period is known to depend on the transmission route, being longer for noninvasive exposure compared with invasive exposures. Therefore, for intact skin contact with MPXV, the incubation

period is around 13 days, whereas, for MPXV contact with broken skin or mucous membranes, the typical incubation period is 9 days.[49]

2.9 | What are the symptoms and complications of MPX?

Classically, WHO distinguishes 2 phases in the disease of MPXV infection: an initial invasive period, lasting up to 5 days, manifested as fever, headache, lymphadenopathy, myalgia, asthenia, and malaise; followed by a secondary skin eruption phase, occurring 1 to 3 days after onset of fever.[3] However, in the current 2022 outbreak, many cases are not presenting this typical picture, being the skin rash the primary symptom at onset and appearing before the fever and other constitutional symptoms. Even in some cases, the prodromal symptoms of the typical initial phase are absent.[50]

Generally, MPX is a self-limited disease usually lasting between 2 and 4 weeks. However, more severe complications have been reported, including secondary infections, proctitis, tonsillitis, bronchopneumonia, sepsis, encephalitis, bacterial cellulitis, and infection of the cornea with ensuing loss of vision.[3,5] The complications are usually related to the extent of virus exposure and patient health status, generally affecting children, pregnant women, and immunocompromised patients.[3,5,51,52]

2.10 | What are the characteristics of the skin lesions induced by MPXV?

The typical rash is manifested as macules and papules of 0.5 to 1 cm in diameter, with progressive evolution into a vesicular and pustular rash, often with umbilication by day 5–7th (Figure 2A).[53] During a period of 2 to 4 weeks, the rash resolves with scabbing and desquamation.[3] Classically, all skin lesions are the same size and are often in the same development phase. However, in the current 2022 outbreak, atypical lesions at different stages of development (asynchronous) have been described.[3]

The rash usually begins on the face and trunk and later spreads centrifugally to extremities, palms, and soles, as well as oropharyngeal (Figure 2B), genitalia, conjunctivae mucous membranes, and cornea. However, in the current 2022 outbreak, patients with isolated lesions in the genital (Figure 2C) or perineal/perianal area without skin involvement in other sites have been reported.[50,54]

3 | MPXV—IMMUNE SYSTEM INTERACTION

3.1 | What is currently known about the immune response to MPXV?

There are very few studies about the immune response to MPXV in infected individuals. The studies about MPX cases usually contain only clinical and epidemiological data, not humoral or cellular immune response against MPXV or any related parameters. Some studies suggest that antibodies and memory B-cells, but not CD4+ and CD8+ T-cell responses, play a canonical role for recovery and long-term protection from smallpox and MPX.[55,56] A study including 37 confirmed MPX patients has investigated IgG and IgM responses regarding clinical signs and previous smallpox vaccination status of the patients.[57] IgG and IgM development profiles of smallpox-vaccinated and -unvaccinated patients with MPX are reported to be similar. IgG response appeared on day 1 after rash onset in vaccinated patients, while IgG response was detected on day 2 in unvaccinated patients. However, in both vaccinated and unvaccinated patients, IgM response was detected on day 2 after rash onset, with more rapid onset of IgM in unvaccinated patients. IgM titers were detectable until day 77 and day 126 in vaccinated and unvaccinated MPX patients, respectively. However, detectable IgG was observed until day 147 in vaccinated and day 139 in unvaccinated MPX patients from a total of 168 days analyzed.[57]

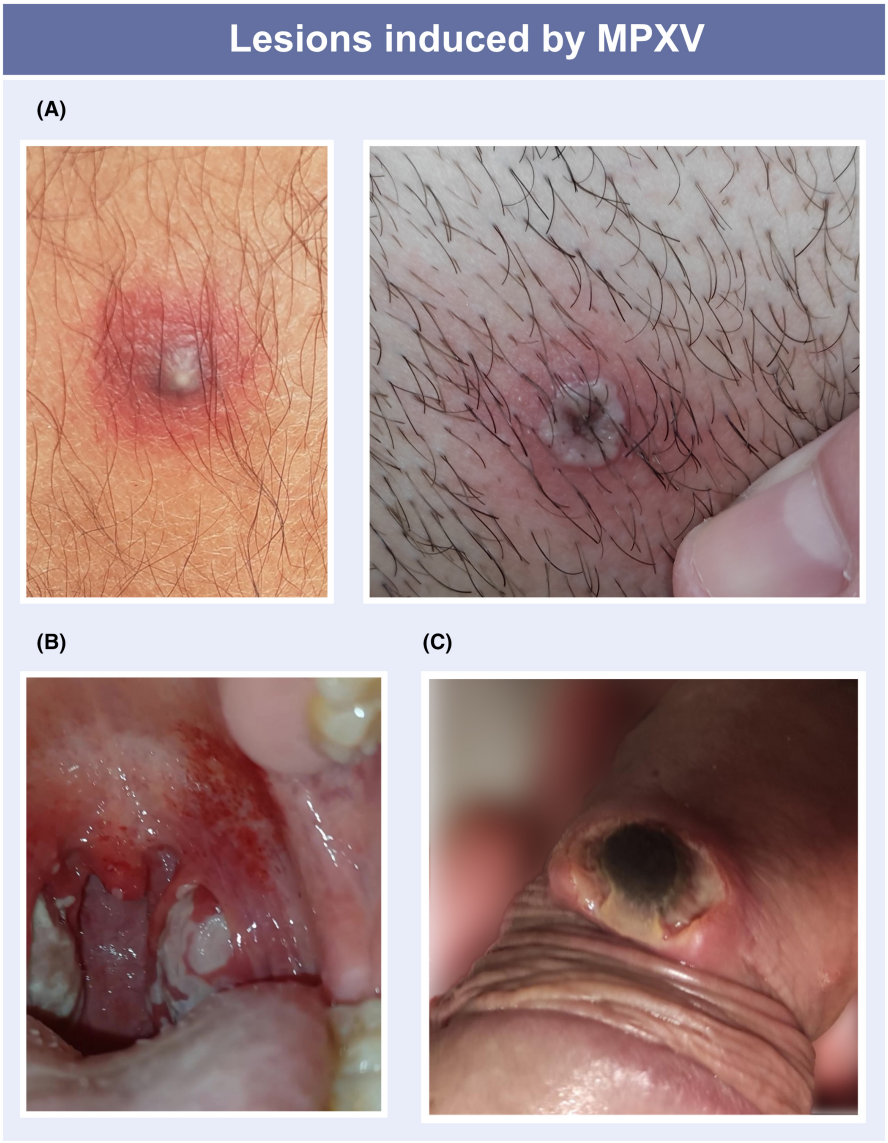
3.2 | What are the immune mechanisms involved in poxvirus infections?

Poxvirus-encoded virokines act intracellularly or extracellularly interfering with the response to infection within the cell, including the induction of an antiviral state, oxidative burst, and apoptotic pathways, and the immune system through downregulation of immune recognition molecules, such as major histocompatibility complex (MHC) class I and CD4 as an immune evasion.[58] Unlike cowpox virus (CPV), MPXV did not interfere with MHC I and II expression or intracellular transport of MHC molecules. Antiviral CD4+ and CD8+ T cells recognize vaccinia virus-infected monocytes but not MPXV. MPXV is able to abrogate T-cell responses against other viruses and anti-CD3 stimulation.[59] Variola virus B17 and MPXV-B16 proteins inhibit type I IFN-induced signaling.[60] Another study showed that the F3L gene of MPXV suppresses the immune type I interferon system by preventing the phosphorylation of PKR and eIF2alpha (Table 1).[61]

CD94, a molecule preferentially expressed by NK cells, is essential for the resistance of C57BL/6 mice to mousepox.[62] Rhesus macaques infected with MPXV 2.5×10^6 PFU Zaire 79 strain showed increased NK cell frequency and absolute numbers in the peripheral blood and lymphoid tissues. However, MPXV infection caused a loss in NK cell functions, including cytotoxicity capacity and secretion of IFN-γ and TNF-α. It may explain the death of MPXV-infected nonhuman primates (Table 1).[63]

Another study showed that a moribund cynomolgus monkey exhibited neutropenia, excessive inflammatory responses and high-load viremia after experimental infection with the Liberia strain of MPXV and concomitant bacterial sepsis.[64] Serum samples from mild ($n = 4$), moderate ($n = 5$), severe ($n = 5$), and serious ($n = 5$) patients with confirmed MPX were evaluated for cytokine profiling. All cases had elevations in serum concentrations of IL1β,

FIGURE 2 Lesions induced by MPXV.



IL-1RA, IL-2R, IL-4, IL-5, IL-6, IL-8, IL-13, IL-15, IL-17, CCL-2, CCL-3, CCL-4, and CCL-5 but only increased IL-2R, IL-10 concentration, and granulocyte macrophage-colony stimulating factor were significantly higher in serious cases in comparison with patients in other severity groups.[65] It was speculated that diazepam and alprazolam may augment the severity of poxvirus infections such as cowpox or MPX.[66]

HeLa cells were infected with three orthopoxviruses (MPXV, vaccinia virus, and cowpox virus) following 6 h, and differences in the cellular response were analyzed by using microarray analysis. It was found that a majority of 96% of the assayed cellular transcripts remained unchanged. Out of 30,484 individual genes and transcripts in MPXV-infected cells, 321 showed expression changes larger than twofold. While 219 out of 321 transcripts (68.2%) were upregulated, 102 out of 321 transcripts were downregulated. Infection-induced changes in the gene expression profiles of cowpox virus, MPXV, and vaccinia virus-infected cells were observed to be largely different. It was found that the most significant changes are histone mRNAs, leukocyte migration, and Toll-like receptor signaling transcripts. Findings might reflect an inadequate subversion of the hosts' antiviral response for orthopoxviruses (Table 1).[67]

4 | MPX OUTBREAK OUTSIDE ENDEMIC REGIONS

4.1 | When did MPX become a zoonotic disease?

MPX has been defined as a zoonotic disease (animal-to-human transmission) since 1970 when, as previously mentioned, the first case in humans was reported in a 9-month-old boy admitted to the Basankusu Hospital in Zaire (currently, DRC).[25,68] MPX became endemic in DRC and spread to other African countries, mainly in central and west Africa. In particular, human cases of MPX have been reported in 11 African countries so far: Benin, Cameroon, the Central

TABLE 1 Immune mechanisms involved in MPXV infection

Target immune mechanism or component	Description	References
Type I IFN signaling	MPXV-B16 proteins inhibit type I IFN-induced signaling	Fernández de Marco et al[60]
Type I IFN signaling	F3L gene of MPXV suppresses the immune type I interferon system by preventing the phosphorylation of PKR and eIF2alpha	Arndt et al[61]
NK cell frequency and function	MPXV infection in Rhesus macaques causes an increase in NK cell frequency but a loss in their functions, including cytotoxicity capacity and secretion of IFN-γ and TNF-α	Song et al[63]
MHC I and II expression	Unlike other poxviruses such as cowpox virus, MPXV does not interfere with MHC I and II expression or intracellular transport of MHC molecules	Hammarlund et al[59]
T-cell responses	MPXV prevents T-cell receptor (TcR)-mediated T-cell activation	Hammarlund et al[59]
Cytokine profile	MPXV induces a higher increase in IL-2R, IL-10 concentration and granulocyte macrophage-colony stimulating factor in serious cases in comparison with patients in other groups with less severity	Johnston et al[65]
Gene expression profile	Infection-induced changes in the gene expression profiles of cowpox virus, MPXV, and vaccinia virus-infected cells are largely different. Most significant changes are histone mRNAs, leukocyte migration, and Toll-like receptor signaling transcripts	Bourquain et al[67]

African Republic, DRC, Gabon, Cote d'Ivoire, Liberia, Nigeria, DRC, Sierra Leone, and South Sudan.[3]

4.2 | Is the current outbreak outside endemic regions the first that has occurred in history?

Since its first identification in humans in 1970, MPX has been reported throughout the above-mentioned African regions where MPX is considered endemic. However, outbreaks outside these endemic regions have occurred in the past two decades. The first MPX outbreak outside of Africa was reported in the United States of America (USA) in 2003 (Figure 3).[69] Overall, before the current 2022 outbreak, the cases outside Africa were reported in the USA (49 cases: 47 in the initial outbreak in 2003 and 2 cases in 2021), the United Kingdom (UK) (7 cases in 2018, 2019, and 2021), Israel (1 case in 2018), and Singapore (1 case in 2019), which represent a total of 58 cases in 18 years (Figure 3).[22,68,70]

4.3 | What are the characteristics of previous outbreaks?

The first MPX outbreak reported outside of Africa occurred in May and June 2003 among residents of the midwestern USA (Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin) (Figure 3).[69,70] This outbreak was linked to contact with infected pet prairie dogs (*Cynomys* species). These pets had been housed at the facilities of an Illinois animal vendor with infected rodents (including Gambian pouched rats and dormice) that had been imported into the USA from Ghana.[70] It has been shown that certain activities associated with animals were more likely to cause MPX infection,[71] specifically touching a sick animal or its bedding, receiving a scratch or bite that caused a skin lesion, and cleaning the cage.

The subsequent outbreaks outside of Africa were described in individuals who traveled from African countries where MPX is endemic or cases of human infection due to contact with pets infected by rodents imported from Africa (Figure 3). Of note, MPX infection was not attributed to person-to-person contact. After these sporadic cases, the largest international outbreak of MPX started in 2022.

4.4 | How different is the current 2022 outbreak compared with previous ones?

The main characteristic that distinguishes the 2022 outbreak from the previous ones is that it is the most widespread and largest outbreak reported in countries beyond Africa.[72] From the previous 58 cases detected outside Africa from 2003 to 2021, in 2022, the number of affected patients increased to more than 55,000.[1,22] The cases of the current outbreak are increasing fast, and their evolution can be checked on CDC world map website.[1]

In previous outbreaks, infection was detected following direct contact with infected animals or after travels to areas affected by MPX, such as the African continent.[73] In 2022, with few exceptions, the cases have been detected after human-to-human sexual contact, no travel-related and without connection with exotic pets.[15]

During the 2022 outbreak, the vast majority of affected patients are young male adults in their 30s (approximately 85% of the cases between 20 and 40 years old),[74] with less impact on those age ranges where the population had received smallpox vaccination.[25] During the first outbreak reported in 1970, patients' age was 4–5 years, progressively increasing up to 10 years old from 2001–2009 and to 21 years old until 2019.[74] Even if some cases of vaccinated people have also been reported,[74] the cessation of vaccination programs has been pointed out as a cause of the largest and international spreading of the current outbreak.[22] Related to the number of

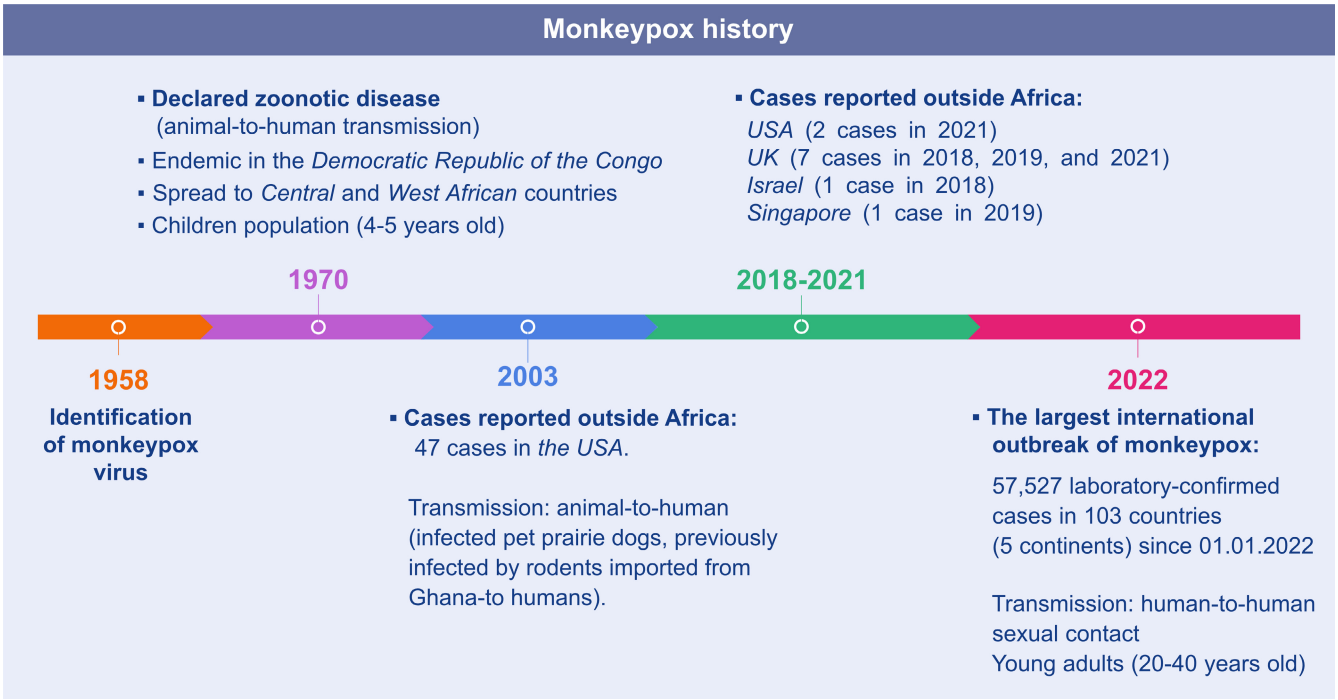


FIGURE 3 MPX history. Timeline detailing relevant dates in the history of MPX, from the identification of MPXV in 1958 to the current 2022 outbreak.

deaths caused by MPX, during the previous outbreaks in Africa, the mortality ranged from 1 to 10%, affecting more children and not vaccinated population.[74] With the impact on patients and international regions, the authorities enhanced epidemiology surveillance, considered preventive measures such as quarantine, and began vaccination programs previously discontinued in the 80s.[22]

4.5 | How many cases of MPX have been described so far in the current 2022 outbreak? What are the most affected countries?

On the date this text is written, there have been 57,527 laboratory-confirmed cases in 103 countries since May 1, 2022 (Figure 4).[1] Most cases (57,016; 99.11%) have been reported in locations without a previous history of MPXV exposure. Although MPX has historically been associated with central and West African countries, the current 2022 outbreak has affected five continents (Africa, America, Asia, Europe, and Oceania). The most affected countries worldwide have been the USA ($n = 21,893$), Spain ($n = 6749$), Brazil ($n = 5726$), France ($n = 3785$), Germany ($n = 3530$), and the UK ($n = 3484$). The remaining countries have presented less than 2000 cases (Figure 4).[1]

Eighteen deaths have been reported to date due to MPX, affecting 10 countries. Ten of them have occurred in 3 historically affected countries (Central African Republic, Ghana, and Nigeria), but 8 of them have happened in other locations without previously reported cases (Spain, Brazil, India, Ecuador, Cuba, Peru, and Belgium). Ghana and Nigeria have been the most affected, with 4 deaths reported.[1] A previously compromised immune system is associated with the most severe disease evolution in patients with MPX.[1] In previous outbreaks, sepsis and bronchopneumonia were the main death causes.[52] During the outbreak of 2022, there have been reports of deaths caused by encephalitis in Spain and India, as well as respiratory failure, acute renal failure, and final septic shock in Peru.[75]

All the data presented is subject to change.[1]

4.6 | How has WHO classified the 2022 outbreak?

The current 2022 outbreak was confirmed in May 2022 by WHO when 12 member states confirmed more than 90 cases of MPX. As previously commented, these confirmed cases did not have travel links to endemic countries.[76,77] Initially, during the meeting of the International Health Regulations (IHR) in June 2022, the committee agreed that the current MPX outbreak did not constitute a Public Health Emergency of International Concern (PHEIC). However, they warned that the situation should be closely monitored.[78] Indeed, in just a month, the confirmed cases rose from 3000 in 47 countries to 16,000 in 75 different countries.[79] Due to this increase, on July 23, 2022, the IHR decided by consensus that the multicountry 2022 MPX outbreak constituted a PHEIC.[79–81]

4.7 | What is the case fatality ratio of MPX in endemic regions versus in the 2022 outbreak?

According to WHO statements, the case fatality ratio (CFR) of MPX has historically ranged from 0 to 11% in the general population,

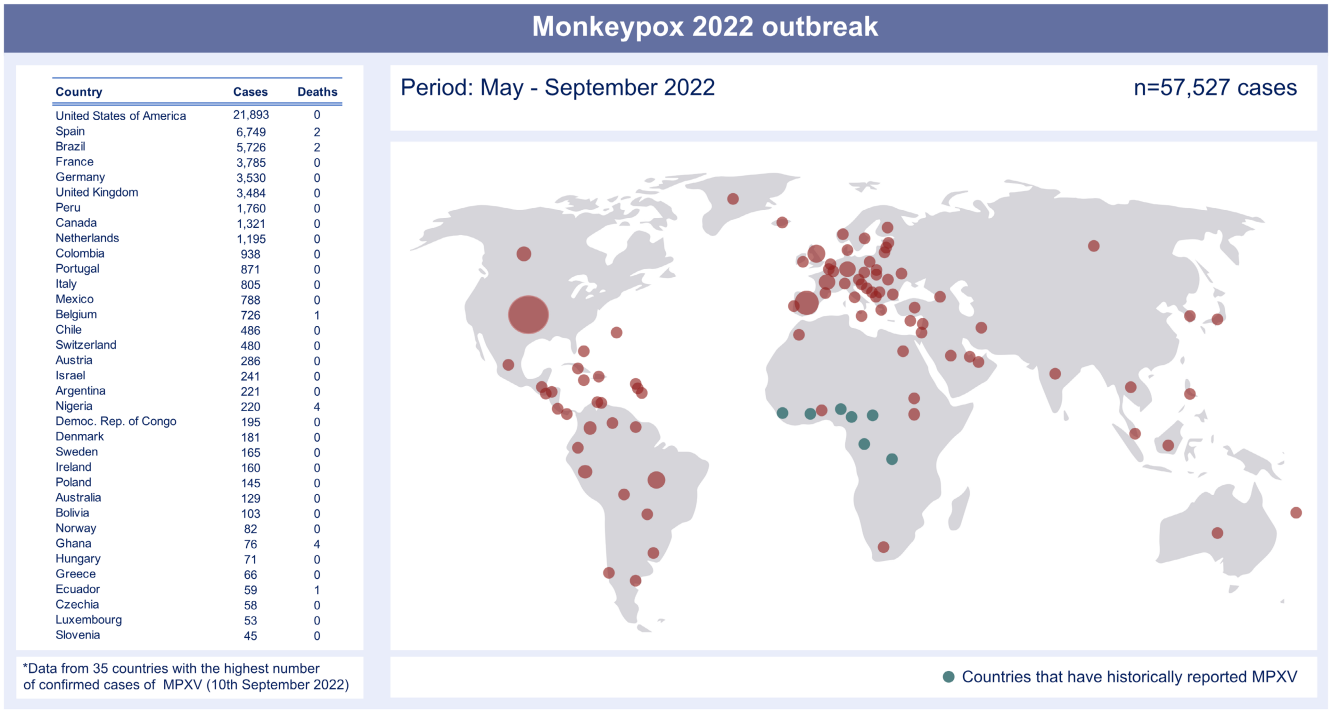


FIGURE 4 Number of confirmed cases of MPX worldwide in the 2022 outbreak (from May to September 2022). Biorender software was used to create the map under an academic license.

TABLE 2 MPX CFR reported in the scientific literature.[25]

Reference	Monkeypox CFR (Case Fatality Ratio)	Time and Localization of study
Breman et al[68]	17%	1970–1980; 5 Central and West African countries
Jezek et al[147]	10%	1981–1986; Zaire
Jezek and Fenner[148]	9.8%	1981–1986; DRC
Mwanbal et al[149]	3.3%	1996–1997; Kasaï Oriental, Zaire
Hutin et al[150]	3.7%	1996-February 1997; DRC
Meyer et al[151]	33%	February — August 2001; province of Equateur in DRC
Di Giulio et al[152]	17% (1970–1979), 10% (1981–1986), 1.5% (1996–1997), 0% (2003)	Rainforest areas of central and western Africa (including Cameroon, the Central African Republic, Gabon, Cote d'Ivoire, Liberia, Nigeria, and Sierra Leone) and DRC
Nolen et al[153]	9.6%	Second half of 2013; Bokungu Health Zone of the DRC

while in recent times, it has been around 3–6% (Tables 2 and 3).[3] The currently available literature reports a different death rate of MPX in endemic regions, such as Africa, depending on the viral clade. As previously mentioned, in those regions two different clades of the virus are reported: the Central African clade (including the Congo Basin), which is more virulent, and the West African clade.[82]

A recent review about MPX reports that, across all countries, the calculated pooled estimate CFR was 8.7%. Instead, separating data by clade, the CFR for the Central African clade (10.6%, 95% CI: 8.4–13.3%) was significantly higher than that of the West African clade (3.6%, 95% CI: 1.7–6.8%). Furthermore, it is reported that, in the past, MPX death rate has been higher among children; in particular, during the African outbreaks from the 1970s to the 1990s, 100% of deaths (47/47) were in children younger than 10 years of age; instead, over the last two decades (2000–2019), only 37.5% (6/16) of deaths occurred in children <10 years old.[25]

In estimating the MPX death and mortality rate, it is essential to consider that percentages may be affected by the availability of advanced medical care.[82] According to the data published by WHO on 22 August 2022, MPX CFR all over the world in the current outbreak

TABLE 3 MPX CFR reported to WHO, by WHO Region, from January 1, 2022, to August 22, 17:00 CEST.[83]

Region	CFR
Africa	1.73% (7/404)
America	0.01% (2/20438)
Eastern Mediterranean	0.00% (0/35)
Europa	0.01% (2/20652)
South-East Asia	7.14% (1/14)
Western Pacific	0.00% (0/121)
96 Member States/territories across all 6 WHO regions	0.03% (12/41664)

is estimated to be 0.03%.[83] At the time of revision of this paper (November 2022), the MPX CFR worldwide in the current outbreak is 0.04%.

5 | CAUSES AND CONSEQUENCES OF THE CURRENT MPX OUTBREAK

5.1 | Is MPX outbreak related to viral microevolution?

To rapidly get the first insights on phylogenetic placement and evolutionary trends of the 2022 outbreak causing MPX, the MPXV genome sequence was publicly released on 20 May 2022 by Portugal, as well as on additional sequences released in the National Center for Biotechnology Information (NCBI) before May 27, 2022, with 15 sequences in total (most of them from Portugal). In summary, genomic and phylogenomic data to date insights into the evolutionary trajectory of the 2022 MPXV outbreak strain and shed light on potential mechanisms and targets of human adaptation. In a recent publication, scientists showed that accelerated evolution of this human MPXV, potentially driven by the APOBEC3 action, suggested that viral genome sequencing might provide sufficient resolution to track the transmission dynamics and outbreak spread, which seemed to be challenging for a presumably slow-evolving double-stranded DNA virus.[14] Together with the adopted strategy of real-time data sharing, microevolution studies may help guide novel outbreak control measures and subsequent research directions.

5.2 | Is the current MPX outbreak an overlapping pandemic with SARS-CoV-2?

It is important to consider the recent spread of MPX in light of the ongoing COVID-19 pandemic, and the potential for coinfection between SARS-CoV-2 and the MPXV. This may result in changes related to infectivity patterns, severity, management, or response to vaccination in one or both diseases. That could also negatively impact the efficiency of diagnostic tests used in both diseases. In addition, the interaction between both viruses could facilitate the emergence of a new variant of concern (VOC) of SARS-CoV-2 with features that could further impact the current pandemic management strategies, such as increased capability for immune evasion or escape, and burden the health care system as a whole. It is too soon to understand whether the current MPX outbreak is an independent phenomenon, or whether it has been exacerbated by the COVID-19 pandemic. This uncertainty calls for precautious actions to be taken by healthcare authorities to contain such outbreaks as cases mount and interaction with other infectious agents, not least SARS-CoV-2, leads to the emergence of variants of increased pathogenicity.[84] In addition, it has to be considered that the public awareness due to COVID-19 and substantially decreased international travels may have been a limiting factor for the further spread of the MPXV.

5.3 | Is the cessation of smallpox vaccination in 1980 a possible cause of the 2022 MPX outbreak?

MPXV is closely related to smallpox virus. In that respect, although smallpox infection vaccines are believed to offer protection against MPX, in theory, it is not an easy task to contain, limit the spread, or treat newly emergent MPX cases. That is mainly due to the stoppage of smallpox vaccination programs during the past 40 years and the shortage of effective vaccines because they are only available in limited stockpiles. The increasing number of unimmunized population and the waning immunity of immunized individuals due to the cessation of smallpox vaccination in 1980 could have had a significant impact and could have been one of the facilitators of the current MPX outbreak.

Despite the presence of treatments and vaccines that offer hope for limiting the transmission and progression of MPX outbreaks, such countermeasures are not readily available in the meantime.[84]

5.4 | Are deforestation and climate change possibly related to the 2022 MPX outbreak?

Most of the world's species are yet to be uncovered: it is estimated that 8.7 million species of animals, plants, and fungi live in the world, and only 1.2 million species have been scientifically described.[85] Many of these species dwell in unexplored areas, such as tropical forests and may harbor viruses and bacteria with the potential to cause severe human diseases.[86] Human interventions like deforestation increase the possibility of encountering these pathogenic new species. COVID-19 and Ebola are both diseases, which come from a reservoir of wild animals. In both cases, humans moving into forests or consuming wild animals are thought to have been responsible for the disease crossing the species barrier. Similarly, MPX is believed to have originated in the rainforests of western and central Africa, where over 500 square kilometers are being lost each year.[87]

Deforestation also contributes to climate change, which profoundly impacts the spread of zoonotic diseases. Rising temperatures cause animals to change their habitat, making them more likely to enter areas of human habitation.[88] A recent study estimated that over 15,000 diseases will spread into a new species for the first time in the next 50 years due to rising global temperatures.[7]

5.5 | Can demographic changes possibly contribute to the 2022 MPX outbreak?

Population growth is a key driver of deforestation, habitat loss, and climate change—three challenges that amplify the threat of zoonotic disease outbreaks. It took thousands of years for human numbers to reach 1 billion (in 1758) and just 264 years to reach 8 billion (in 2022). Although the growth rate is slowing, human population growth could continue into the next century, depending on fertility rates in the next few decades. The United Nations medium-fertility projection shows the global population increasing to 8.5 billion in 2030 and 9.7 billion in 2050, eventually peaking at 10.4 billion in the 2080s.

Socioeconomic factors such as poverty (with its far-reaching consequences on feeding and housing habits) and chaotic urbanization are also favoring zoonotic outbreaks.[89] Hunting, handling, preparing, and consuming bushmeat were consistently implicated in developing clinical MPX.

5.6 | Has an improved surveillance and reporting system contributed to uncovering increased MPX cases?

Until present, the outbreaks of MPX have consistently occurred in populations living in rural areas, in small villages (<1000 people) adjacent to or within humid evergreen tropical forests—at the so-called human–animal interface.

There has been an increase in the number of individual outbreak reports over time since 1970. A total of 35 individual outbreaks have been reported outside the DRC, 20 of which have occurred since 2010. Intensified surveillance was carried out in DRC between 1970–1979, 1981–1986, and 2005–2007, specifically accounting for increased reports during these times. Passive surveillance has been implemented since 2000 when MPX started to be reported to Integrated Disease Surveillance and Response (ISDR). Available ISDR data show a progressively increasing annual case burden since 2001.[90]

Accessible and affordable point-of-care rapid diagnostic testing would allow reliable discrimination between MPX and other viral infections for surveillance and outbreak reporting purposes. In addition, the increase in the population of susceptible individuals since the cessation of the smallpox vaccination program has to be factored in.[91]

5.7 | Are we currently facing an increase in zoonotic diseases? What are the potential consequences of the current MPX outbreak?

The Anthropocene has in its store several challenges, including the increased risk of pandemics with novel viral, fungal, or bacterial strains. As agriculture expands with habitat destruction, human populations grow, and international trade of plants and animals' booms, there is an increased chance of encountering new zoonotic diseases.

Combatting zoonotic diseases is not a simple process, as it requires a concerted effort from all communities across the globe. Reducing land use (e.g., agriculture, mining, and deforestation) and biodiversity loss (through, e.g., protected areas) will decrease the chance of encountering new pathogenetic species. Continuous monitoring of human, live stocks, and wild animal populations for signs of a new zoonotic disease facilitates timely public health interventions to prevent an outbreak from becoming a pandemic. The One Health Zoonotic Disease Prioritization process brings together representatives from human, animal, and environmental health sectors, as well as other relevant partners, to prioritize zoonotic diseases of greatest concern for multisectoral, One Health collaboration in a country, region, or other areas.

Six out of every 10 infectious diseases in humans are zoonotic, which makes it crucial that each nation strengthen its capabilities to prevent and respond by using the One Health approach. One Health is an approach that recognizes the connection between people, animals, plants, and their shared environment and calls for experts in human, animal, and environmental health to work together to achieve the best health outcomes for all.[92]

6 | DIAGNOSIS OF MPX

6.1 | What are the criteria used in the diagnosis and differential diagnosis of MPX?

Criteria used in the diagnosis of MPX are the occurrence of (1) typical mucosal and/or skin lesions combined with (2) systemic symptoms and/or (3) potential contact to an MPX-infected individual in the last few weeks.[4] Primary differential diagnostic evaluations of oropharyngeal or anogenital ulcerations suspected to be caused by MPX are STI,[93] such as HIV (antigen/antibody testing of blood for HIV has to be done), disseminated gonococcus infection (culture or nucleic acid amplification for *Neisseria gonorrhoeae*), primary or secondary syphilis (blood testing for antibodies, analysis of sore from a lesion for *Treponema pallidum*, nucleic acid amplification), chancroid (identification of *H. ducreyi* by nucleic acid amplification), lymphogranuloma venereum (nucleic acid amplification of specimen for *Chlamydia trachomatis*), and granuloma inguinale also called donovanosis (visualization of Donovan bodies on tissue crush or biopsy). Most importantly, even in patients with confirmed MPX infection, coinfection with other STI has to be evaluated if there are risk factors for STIs (Table 4). In that respect, testing for other pathogens

is important not only for the differential diagnosis but also for the possibility of coinfections.

Differential diagnostic evaluations in patients with skin lesions, which present rashes and/or vesicular-papular and macular eruptions,[94] include skin infections caused by varicella-zoster virus (VZV) such as chickenpox, which are from their clinical appearance quite similar to MPX lesions of the skin. Chickenpox can be distinguished from MPX by the higher number of lesions (usually more than 5–10 lesions in patients with chickenpox). The spreading of the VZV-caused lesions over the whole body, including the hairy scalp, contrasts with the spreading of the MPX lesions in a defined area limited to a particular body site in a high number of MPX patients and the less frequent involvement of palms and soles. Furthermore, there is a lack of concomitant anogenital or oropharyngeal ulcerations in VZV-infected individuals. Another VZV-induced virus (re-)infection, which has to be considered is Zoster, characterized by vesicular-papular lesions limited to a dermatome or presenting as a rash of vesicular-papular lesions in the case of disseminated Zoster, which might occur in immunosuppressed patients. Both forms go along with malaise and skin pain. Final differentiation of all conditions has to be done by nucleic acid amplification of VZV versus MPX from skin swabs. Other differential diagnostic evaluations in patients with oropharyngeal lesions are herpes simplex virus (HSV) infections such as primary HSV infection of the oral region called gingivostomatitis herpetica, which occurs rarely in adults but more frequently in immunosuppressed or very young children with fever as a prodromal symptom. Gingivostomatitis herpetica is initially characterized by clustered vesicles of the oral mucosa, which develop into painful ulcerations with a typical red margin. In addition, in individuals with genital and anogenital lesions, HSV infection of the genital region or HSV recidivans in loco, which goes along with (recurring) painful burning blisters and ulcers and can be caused by HSV1 or HSV2, has to be evaluated as a differential diagnosis. Eczema herpeticum, a widespread HSV infection with umbilicated, sometimes superinfected vesicles, accompanied by malaise, fever, and swollen lymph nodes in particular in individuals with atopic dermatitis has to be differentiated from MPX by nucleic acid amplification of the disease-causing virus as well (Table 5).

Hand-mouth and foot disease, which starts with flat discolored spots of palms and soles and goes along with thin blisters of the palmar and plantar areas, oral mucosa and perioral region represents another differential diagnosis to MPX infection. Hand-mouth and foot disease is caused by Enteroviruses, most often Coxsackie A16 or Enterovirus 71, and is characterized by mild symptoms. It is very common in children but rare in adults. Eczema molluscatum, a viral infection caused by molluscum virus, which affects atopic individuals, in particular patients with atopic eczema, is characterized by skin-colored, glassy, umbilicated papules on eczematous skin and represents another differential diagnosis to MPX infection. In contrast to MPX infection, papules vary in size, and no general symptoms such as fever, malaise, or pain accompany the skin lesions (Table 5).

TABLE 4 Sexually transmitted diseases and their diagnostic evaluation to be considered in the differential diagnosis of MPX and in the evaluation of coinfections.

Sexually transmitted disease	Diagnostic evaluation
HIV	Antigen/antibody testing of blood for HIV
Gonococcal infection	Culture or nucleid acid amplification of *Neisseria gonorrhoeae*
Primary or secondary syphilis	Blood testing for antibodies, analysis of sore from lesions for *Treponema pallidum*, nucleic acid amplification
Chancroid	Nucleic acid amplification for *H. ducreyi*
Lymphogranuloma venereum	Nucleic acid amplification for *Chlamydia trachomatis*
Granuloma inguinale	Visualization of Donovan bodies on tissue crush or biopsy
HSV genitalis	Nucleic acid amplification for HSV1 or HSV2

6.2 | What differential diagnostic evaluations are used to differentiate MPX from smallpox?

Most challenging is the differential diagnostic evaluation of MPX infection from other pox diseases such as smallpox disease (Variola major and minor), which is also caused by a virus of the *Poxviridae* family and results in quite similar symptoms as MPX. Smallpox-infected individuals are highly contagious, and smallpox infections go along with severe courses in about 30% of patients affected by Variola major and mild courses in patients affected by Variola minor. One important differential diagnostic feature which could help to distinguish MPX from smallpox disease is lymphadenopathy, that is, large swollen lymph nodes observable in patients with MPX infection only but not in patients with smallpox disease and the fact that smallpox disease has been eradicated already in 1977; therefore, there have not been new cases since then. Final differentiation between both Orthopoxviruses could be done with the help of polymerase chain reaction (PCR), electron microscopy, or viral culture of swabs.

Cowpox virus is another member of the *Orthopoxvirus* genus and can be transmitted from infected cows, cats, or other animals to humans and causes Cowpox.[95] Cowpox is a mild, self-limited disease characterized by fever, swollen lymph nodes, and painful blisters and ulcerations of the hands and other body regions, which got in contact with the infected animal.[96] Cowpox infections are much more limited to the body area of transmission and occur nowadays only very rarely. They can be distinguished from MPX infections by analysis skin swabs as well (Table 5).

6.3 | What laboratory diagnostic tests are used in the diagnosis of MPX?

The preferred laboratory diagnostic test for MPX is real-time or conventional PCR with high sensitivity and accuracy. Protocols of PCRs for the diagnosis of MPX have been published in the last decades.

TABLE 5 Differential diagnostic evaluations in patients with skin lesions who present rashes and/or vesicular-papular and macular eruptions.

Disease	Differences with MPX	Diagnosis
Chickenpox	Higher number of lesions (>5–10), spreading of lesions over the whole body including the hairy scalp, less often palms and soles affected, missing concomitant anogenital or oropharyngeal ulcerations	Nucleic acid amplification of VZV
Zoster	Vesicular-papular lesions limited to one or more dermatomes, unilateral, malaise, and skin pain	Nucleic acid amplification of VZV
Gingivostomatitis herpetica	Initially clustered vesicles of the oral mucosa, which develop into painful ulcerations with a typical red margin. Young children, immunosuppressed adults most often affected. It goes along with prodromal symptoms such as fever	Nucleic acid amplification of HSV
HSV recidivans in loco	Recurring painful burning blisters and ulcers	Nucleic acid amplification of HSV
Eczema herpeticum	Widespread umbilicated, superinfected vesicles accompanied by malaise, fever, and swollen lymph nodes. Individuals with atopic dermatitis most frequently affected	Nucleic acid amplification of HSV
Hand-mouth and foot disease	Flat discolored spots of palms and soles, blisters of the palmar and plantar area, oral mucosa and perioral region. Most frequently in children, rarely in adults	Coxsackie Virus A16, Enterovirus Virus 71
Eczema molluscatum	Skin-colored glassy umbilicated papules on normal or eczematous skin, papules vary in size, no general symptoms such as fever, malaise or pain accompany skin lesions	Poxvirus
Smallpox disease	Usually no lymphadenopathy	Orthopoxvirus
Cowpox	Fever, swollen lymph nodes, painful blisters, and ulcerations of the hands and other body sites which got in contact with infected animals	Orthopoxvirus

In that sense, during the first outbreak outside Africa in 2003, the first RT-PCR protocol to detect MPXV from rash samples was developed.[97] Afterwards, additional protocols were established, some of them able to distinguish Central African and West African clades.[98] Most of the previously real-time PCR protocols based on MPXV-specific primers from clade I or IIa seem to be accurate for the detection of MPXV in the current 2022 outbreak.[99] However, WHO, in a report of May 2022, advised that the specific PCR protocol to be used as a diagnostic test should be verified by trained personnel beforehand.[100] Currently, PCR kits for MPXV detection are being rapidly and continuously developed.

So far, laboratory tests based on the detection of antigens and antibodies do not provide accurate diagnostic confirmation of MPX since Orthopoxviruses present a relevant serological cross-reactivity. Moreover, previous or recent immunization with vaccines based on vaccinia can also elicit false positives. For these reasons, tests for serology and antigen detection are not recommended for the diagnosis of MPX.[3]

6.4 | What samples are suitable for the diagnostic tests?

Samples from skin lesions are the most optimal for PCR testing in the diagnosis of MPX. Samples should be taken with swabs from vesicles, pustules (from fluid or roof), or dry crusts, collected in different tubes.[3] It is advised to collect samples from at least three skin lesions at different locations.[101] Biopsies might also be considered. Besides samples from skin lesions, swabs from the oropharynx might also be collected. In this case, a negative PCR result with this type of sample should be taken with precaution and further testing of skin lesions would be necessary.

Blood samples are considered unsuitable for PCR in the diagnostic testing of MPX since viremia is limited in time concerning sample collection after the onset of symptoms.[3] In addition to samples for diagnostic testing, other specimens at different body locations might be collected for research purposes. In that context, studies have shown that MXPV can be detected in saliva, semen, urine, and feces.[38]

6.5 | What procedures and measures are followed for obtaining and handling the samples?

Sample collection and laboratory testing of samples from suspected or probable cases of MPX should be performed by qualified personnel in facilities with Biosafety Level 2 (BSL-2). All samples from suspected MPX cases should be handled as potentially infectious, and a risk-based assessment should be performed to establish specific measures to avoid laboratory transmission. In that sense, standard operating procedures should be followed and specific training on adequate use of personal protective equipment (PPE) and sample handling should be provided. Vaccination of personnel in charge of sample collection, storage and testing is encouraged.

Once collected, samples from skin lesions should be placed in a sterile and dry tube and should be stored in dark at a cold temperature (2–8°C or −20°C for short term and −20°C or lower temperature for long term). Although with optimal storage conditions the DNA of MPXV is stable in lesion skin samples for long periods of time,[102] it is

recommended to perform the diagnostic testing within the following hours of the specimen collection.[100]

6.6 | What are the exclusion criteria in the diagnosis of MPX?

According to CDC, cases that might be excluded as suspected, probable, or confirmed cases of MPX are those with an alternative diagnosis that can completely explain the disease. However, as MPX rash can be mistaken with other diseases such as syphilis or herpes and coinfection of MPXV and other infectious agents is possible, MPXV testing should be considered in subjects with a suspicious rash even if they have a positive diagnostic test for another infectious agent. Another exclusion criterion is based on individuals with symptoms compatible with MPX who do not develop a rash in the first 5 days of the disease onset. Additionally, the lack of a positive PCR detecting MPX or Orthopoxvirus using high-quality samples may also be considered as another exclusion criterion in the diagnosis of MPX.[103,104]

7 | TREATMENT AND PREVENTION OF MPX

7.1 | What are the current treatments available to treat MPX?

It is currently unknown what is the best way to cure MPX. In order to facilitate the best information, U.S. Food and Drug Administration (FDA) has established a dedicated MPX website.[105] Antivirals are promising treatment options.[106] There are three antivirals used for the treatment of MPX, namely cidofovir, tecovirimat, and brincidofovir (described in detail in the following section). Also, safety is just scarcely studied. In the UK, tecovirimat showed no adverse effects but brincidofovir did, yet for clear conclusions power was limited.[36] Single-patient reports have been described.

7.2 | What is the role of supportive care in the treatment of MPX?

Most cases of MPX have been treated symptomatically in the 2022 outbreak.[107] Solely in patients with severe disease, immunocompromised patients, children younger than 8 years, and pregnant individuals, antiviral treatment should be considered. Thus, this is relevant for a rather small percentage of cases.

7.3 | What antivirals are effective to treat MPX?

As mentioned, there are now three antivirals used for the treatment of MPX, namely tecovirimat, brincidofovir, and cidofovir. Tecovirimat prevents the cellular transmission of the virus by inhibiting the functions of a major envelope protein. As an antiviral blocking VP37 protein detected on the surface of Orthopoxviruses, it was previously tested in prairie dogs, administered orally, and showed more effectivity than cidofovir.[20] Tecovirimat is now the first-line therapy due to oral availability and is currently licensed for the treatment of MPX (European Medicines Agency—EMA), approved for smallpox by the FDA in 2018 and also available in Europe. The CDC holds an Expanded Access–Investigational New Drug (EA-IND) protocol that allows its use in non-variola Orthopoxviruses such as MPXV.[107] It is effective with a good safety profile and it was approved "under extraordinary conditions." The most common side effects are headache and nausea.[108]

As second-line therapy recommended in Europe,[106] brincidofovir is active against dsDNA viruses.[109] Brincidofovir was approved by the FDA for the treatment of smallpox in 2021, and, as with tecovirimat, the CDC holds an EA-IND that allows its use in MPX. Efficacy had been demonstrated in animals.

Cidofovir blocks viral replication by selectively inhibiting viral DNA polymerases. Cidofovir, which has demonstrated promising results for herpes virus and CMV, represents a nucleotide analog given intranasally and showed good results in Africa. Yet, because of its disadvantages, cidofovir now represents only the third-line treatment. It needs to be injected, has high renal and hematological toxicity, and has a potential carcinogenic, teratogenic, and reprotoxic effect.[106]

7.4 | What are the criteria for the prescription of antivirals to treat MPX?

The prognosis for MPX infection depends on multiple factors, such as previous vaccination status, initial health status, concurrent diseases, and comorbidities. The majority of patients will experience self-limiting infections.[106,110] Therefore, antiviral treatment, if available, should be considered in patients with severe infection or at risk for severe infection, in conjunction with a pediatric infectious diseases specialist. Those might include patients with severe disease (e.g., conditions requiring hospitalization), people who are at risk for severe disease such as immunocompromised patients, children (specifically <8 years of age), pregnant women, and people with skin diseases and/or aberrant local infection.[106,110] Variola immune globulin (VIG) can be considered for prophylactic use in an exposed person with severe T-cell immunodeficiency for which smallpox vaccination following exposure to MPXV is contraindicated and in pregnant women with severe disease. As previously mentioned, as of fall 2022, tecovirimat is EMA-approved in Europe since 2022, but not available in every country. In the USA, it is approved by the FDA and available through a compassionate use program.[111,112]

7.5 | What are the recommendations for pregnant women exposed to MPXV?

There are currently no evidence-based recommendations for pregnant women, but they are at higher risk of acquiring severe disease.

There are scarce case reports of pregnant women describing vertical transmission with a poor fetal outcome.[113,114] Therefore, postexposure prophylaxis might prevent a poor fetal outcome. WHO recommends the MVA-BN nonreplicating vaccine as postexposure prophylaxis, but the benefits should outweigh the risks. About 300 women have been studied after vaccination without severe adverse events.[115]

In PCR-confirmed pregnant women, tecovirimat is the first-line antiviral recommended by the CDC for women with severe illness. Animal studies have not shown embryotoxic and teratogenic effects till now. Cidofovir and brincidofovir should not be used in the first trimester of pregnancy. VIG is probably safe in pregnancy, like the other specific immunoglobulins.[114]

Serial ultrasound might elucidate signs of vertical transmission in the fetus. In case of infections in the last weeks of pregnancy, Caesarian section might be chosen as a delivery strategy in women with active lesions to prevent the risk of fulminant disease in the neonate. If vertical transmission is likely, for example, in a case where mothers became ill just before or after (vaginal) delivery, neonatal VIG should be considered.[114]

7.6 | What preventive measurements should be taken by subjects in close contact with infected patients?

Close contact for MPX is defined as anyone who has been exposed to an infected person during the infection period, that is, from the time symptoms start until all symptoms have resolved, which means complete healing of the rash with the formation of a fresh layer of skin.[116,117] A high-risk exposure with a probable or confirmed case of MPX involves direct contact with body fluids or lesion material, prolonged face-to-face contact without appropriate PPE (e.g., FF2 respirators), or fomites (e.g., contaminated linens).[22,117,118] Currently, asymptomatic contacts are not considered infectious; therefore, quarantine is not obligatory.[119] Interestingly, a recent study has found a prevalence of 6% of asymptomatic cases among individuals with confirmed MPX.[120]

Subjects in close contact should be advised to undergo active surveillance for MPX-like symptoms (skin rash/lesions, chills, headache, fever, sore throat, fatigue, and lymphadenopathy) for 21 days following the last exposure. This requires monitoring of temperature once or twice a day. In order not to mask early signs of MPX infection, the contacts should avoid taking antipyretics, such as acetaminophen, ibuprofen, and acetylsalicylic acid.[119,121]

Exposed health professionals can still work, yet they should not take care of patients at high risk for severe MPX disease (e.g., pregnant women, neonates, and immunocompromised patients).[117,121]

For the general population who has been exposed to an infected person during the infection period, hand hygiene, respiratory etiquette, and safe sex behaviors (e.g., using condoms and reducing the number of sexual partners) are recommended during the self-monitoring period.[117,119] If the symptoms occur, the subject should immediately self-isolate and contact their local healthcare provider. Before an in-person appointment, the healthcare staff should be informed about the possible exposure to MPX virus and the developed symptoms.

7.7 | Is there a vaccine against MPX?

The vaccine approved in Europe for the prevention of MPX is MVA-BN (called Imvanex® in the EU, Jynneos® in the USA and Imvamune® in Canada). It contains an attenuated form of the vaccinia virus called "modified vaccinia virus Ankara" (MVA), which is related to the smallpox virus. This vaccine received a marketing authorization valid throughout the EU on July 31, 2013, with the purpose of being used to protect against smallpox in accordance with official recommendations. In 2019, MVA-BN was approved for the prevention of smallpox and MPX in the USA. On July 22, 2022, EMA authorized under "exceptional circumstances" Imvanex® for the prevention of MPX.[122,123]

In the USA, on August 9, 2022, FDA issued an emergency use authorization (EUA) for MVA-BN vaccine (named Jynneos® in this country)[124,125] (Table 6). According to vaccine "summary of product characteristics," the standard regimen is a subcutaneous (SC) route of administration with an injection volume of 0.5 ml. Following the MPX outbreak as a Public Health Emergency, another regimen, that is, intradermal administration (ID) of an injection volume of 0.1 ml, was authorized on August 9, 2022, to increase the number of available Jynneos® vaccine doses by up to fivefold. In fact, according to the results of a clinical study published in Vaccine in 2015, the lower intradermal dose was immunologically noninferior to the standard subcutaneous dose.[126]

On September 28, 2022, the CDC posted the first effectiveness data, suggesting how well the vaccine is performing in the real-world setting. These data consider confirmed and probable MPX cases with illness onset between July 31 and September 3, and they make it possible to state that unvaccinated people had 14 times the risk of monkeypox disease compared with people who were vaccinated. These provisional data will be updated periodically.[127]

ACAM2000® is another vaccine licensed for smallpox by the FDA in 2015 (Table 6). It is a replication-competent Vaccinia virus vaccine derived from a plaque-purified clone of the same New York City Board of Health strain that was used to manufacture Dryvax vaccine, one of the vaccines used in the eradication of smallpox. Until 2019, ACAM2000® was the only Orthopoxvirus vaccine licensed in the USA and it was used for pre-exposure prophylaxis against Orthopoxvirus infection among persons at risk for such exposures.[128] It has been made available for the prevention of MPX under an Expanded Access–Investigational New Drug application (EA-IND).[124]

TABLE 6 MPX vaccines currently available.

Vaccine	Vaccination schedule	Smallpox license	MPX license	Target population	Special notes
MVA-BN Bavarian Nordic (JYNNEOS®, USA; IMVANEX®, EU; IMVAMUNE®, CANADA) Third generation	Two doses at intervals of 28 days	EU (Imvanex®) authorized under exceptional circumstances (2013); Canada (Imvamune®): full authorization (2013); USA (Jynneos®): full authorization (2019)	USA (Jynneos®) full authorization (2019) Canada (Imvamune®): full authorization (2019); EU (Imvanex®): authorized under exceptional circumstances (2022)	Adults over the age of 18	No data in pregnant or feeding. Persons with atopic dermatitis may experience more intense local skin reactions (such as redness, swelling and itching) and other general symptoms (such as headache, muscle pain, feeling sick or tired), as well as a flare-up or worsening of their skin condition.
ACAM2000® KM Biologics Second generation	Single dose	Multiple countries	USA for PEPV (postexposure preventive vaccination)	Adults aged 18–64 years of age	Warnings and precautions for certain population groups (e.g., in PLWH, children and in particular infants <12 months of age, or pregnant women, where it can cause fetal harm) apply. ACAM2000 live vaccinia virus may be transmitted from a lactating mother to her infant causing complications in the infant from inadvertent inoculation. Live vaccinia virus contained in ACAM2000® could be transmitted -> warning to infants, pregnant, people who have problems with their immune system. Persons with atopic dermatitis may experience more intense local skin reactions (such as redness, swelling and itching) and other general symptoms (such as headache, muscle pain, feeling sick or tired), as well as a flare-up or worsening of their skin condition.
LC16 (Japan) Third generation	Single dose	Japan since 1975	Japan since August 2022	Infants and children (all ages) as well as adults	Contraindicated in any person who is immunosuppressed or has atopic dermatitis, or during pregnancy.

7.8 | What are the indications of the vaccines for the prevention of MPX?

The "summary of product characteristics" of MVA-BN vaccine reports in the section "indications and usage" that the vaccine is indicated for the prevention of smallpox and monkeypox disease in adults 18 years of age and older determined to be at high risk for smallpox or monkeypox infection.

Currently, WHO does not recommend mass vaccination for MPX based on data on the evolution of the epidemic. Vaccine stocks are limited so far, so vaccination is offered in relation to the degree of the risk of contagion. In fact, different countries have made different decisions regarding population groups for which vaccination should be reserved.

In August 2022, when planning MPX vaccination for target population groups, the WHO European Region stated the need to consider MPX vaccination as a pre-exposure prophylaxis in people at risk of exposure regardless of gender or sexual identity. In addition, communities at high risk of exposure or with high-risk behaviors, such as MSM with multiple sexual partners, occupational sex workers, health workers at high risk of exposure, health workers administering smallpox vaccines competent for replication, and laboratory personnel working with orthopoxviruses or performing diagnostic tests for MPX are considered as priority target of MPX vaccination.[129]

In an update published in October 2022 by the WHO, experts considered the epidemiological impact of the infection to identify the priority target categories: In particular, although all individuals can be infected regardless of sex, age, or lifestyle habits, there is a prevalence of cases of infections among MSM with multiple sexual partners. The WHO recommended to identify a heterogeneous working group for the drafting of indications on MPX vaccination, composed not only by health professionals (such as doctors, researchers, and health authorities) but also by sexual health providers, LGBTQ+ groups and sex workers to ensure a proposal and acceptance of vaccination based on equity.[130]

In the current epidemiological situation of monkeypox outbreaks outside of endemic countries, close contacts of a Monkeypox case should receive MPX vaccination. A close contact is a person who has been exposed to someone who is a confirmed or probable case of monkeypox, beginning from when symptoms first appeared to when all scabs have fallen off, under the following circumstances: face-to-face exposure (including health workers without appropriate PPE), direct physical contact (including health workers without appropriate PPE), including sexual contact or contact with contaminated materials such as clothing or bedding (including health workers without appropriate PPE).[131]

In addition to pre-exposure prophylaxis, the MPX vaccine can be administered as postexposure prophylaxis, that is, to vaccinate individuals who have had contact with a confirmed case of MPX.[122] During periods of national supply constraints, some national health authorities recommended the possibility of administering MPX vaccination as postexposure prophylaxis in immunosuppressed individuals and other risk groups: In this case, the MPX vaccine should be administered as soon as possible, ideally within 4 days after risk exposure. The administration between 4 and 14 days may still be effective in decreasing symptoms, although may not prevent the disease. Early administration is recommended as it will result in a more effective protective response.[132] With regard to this specific use, as postexposure prophylaxis, studies are currently underway to evaluate the efficacy of the MVA-BN vaccine in the current context.

7.9 | What are the contraindications of the vaccines for the prevention of MPX?

In the "contraindications and warnings" section of the MVA-BN vaccine, it is stated that the vaccine is contraindicated in case of hypersensitivity to the active substance or any of the excipients, or trace residues. The other excipients of the vaccine are as follows: trometamol, sodium chloride, and water for injections, as well as possible residues in trace amounts of chicken proteins, benzonase, gentamicin, and ciprofloxacin. The administration should always take place in the presence of health personnel able to manage timely treatment in case of anaphylaxis. It is also stated in the "summary of product characteristics" that it is preferable to intersperse the administration of a dose of MVA-BN vaccine and the anti-SARS-CoV-2 vaccine for 28 days. There are no contraindications regarding the administration of the vaccine in immunocompromised individuals, being constituted by a nonreplicating virus. However, it must be specified that immunocompromised individuals may have a reduced antibody response. MVA-BN can be administered to people with HIV infection or atopic dermatitis, although the latter have developed more local and general symptoms in clinical trials after vaccination.[133]

ACAM2000®, which is a replication-competent Vaccinia virus vaccine, has more contraindications than MVA-BN. In that sense, ACAM2000® is contraindicated in subjects with immunosuppressant illnesses such as lymphoma, leukemia, or AIDS. It should not be administrated to subjects with allergy to the active substance or the excipients contained in the vaccine. ACAM2000® is also contraindicated in subjects with hypertension, coronary artery disease, cardiomyopathy, or diabetes mellitus.[134] Individuals with atopic dermatitis may experience more intense local skin reactions (such as redness, swelling, and itching) and other general symptoms (such as headache, muscle pain, feeling sick or tired), as well as a flare-up or worsening of their skin condition.

7.10 | Can smallpox vaccines protect against MPXV?

The world health authorities agree that smallpox vaccines protect against MPX virus. CDC stated that smallpox and MPX vaccines are effective at protecting people against MPX when given before exposure to the virus and that postexposure vaccination may help prevent the disease or make it less severe.[135]

Historical data report that smallpox vaccination was approximately 85% protective against MPXV (Table 7), while the review of the literature currently available shows that unvaccinated individuals accounted for about 80–96% of MPX cases. In fact, MPXV is closely related to the virus that causes smallpox.[25] Over the years, epidemiological studies revealed that approximately 90% of confirmed human MPX cases had not been infected with other poxviruses, and most cases were born after the end of the smallpox virus eradication program, very likely having not been vaccinated with smallpox vaccine.[118] In 2018, Kalthan et al. published the results of an investigation carried out during an MPX epidemic that involved five villages in the Central African Republic and reported that the < 10 years and 21–30 years age groups were the most affected and that young age and the absence of smallpox vaccination were associated with severe presentations in 87.5% of cases.[136]

TABLE 7 Smallpox vaccination protection against MPX in scientific literature.

Reference	Smallpox vaccine protection against MPX	Time and Localization of study
Fine et al[154]	85%	1980–1984, Zaire
Jezek et al[147]	13% (43/338) patients had visible vaccination scar(s)[a]	1981–1986, Zaire
Nasir et al[155]	85%	Review data of Nigeria
Gong et al[156]	85%	Review

[a]The study reports the smallpox vaccination rate among human MPX cases.

Data supporting the efficacy of smallpox vaccination in protecting against MPX derive from investigations carried out in recent years on MPX epidemics that have occurred in countries where the virus is endemic. In 1980, WHO reported that the overall attack rate of contacts without smallpox vaccination scar (7.2%) was significantly different from those with vaccination scar (0.9%).[137] Moreover, in support of this thesis, in 2010, Rimoin et al. reported a dramatic increase in average annual incidence of MPX in DRC, which was hypothesized to be due to a lack of vaccination as only 24% of the local population and 4% of the MPX patients had prior vaccination. Those data suggested that vaccination >25 years earlier could have protected individuals against an Orthopoxvirus infection and, furthermore, that lack of vaccination in these populations could have contributed to a higher incidence of infection.[91]

We underline that at the present state of knowledge it is not possible to accurately estimate the efficacy of smallpox vaccination in protecting against MPX due to the scarcity of studies in the literature.

7.11 | What is the protective immunity duration of the vaccines?

Antibody and T-cell responses could be maintained for up to 75 years after smallpox vaccination. As commented in the preceding section, previously smallpox-vaccinated people are estimated to be 85% protected against MPX because of cross-protection occurring across Orthopoxviruses.[138] However, it was reported that systemic Orthopoxvirus infection is not entirely protected by remote (30 years prior) smallpox vaccination.[139] The duration of immunity is unknown for current FDA-approved vaccinia virus

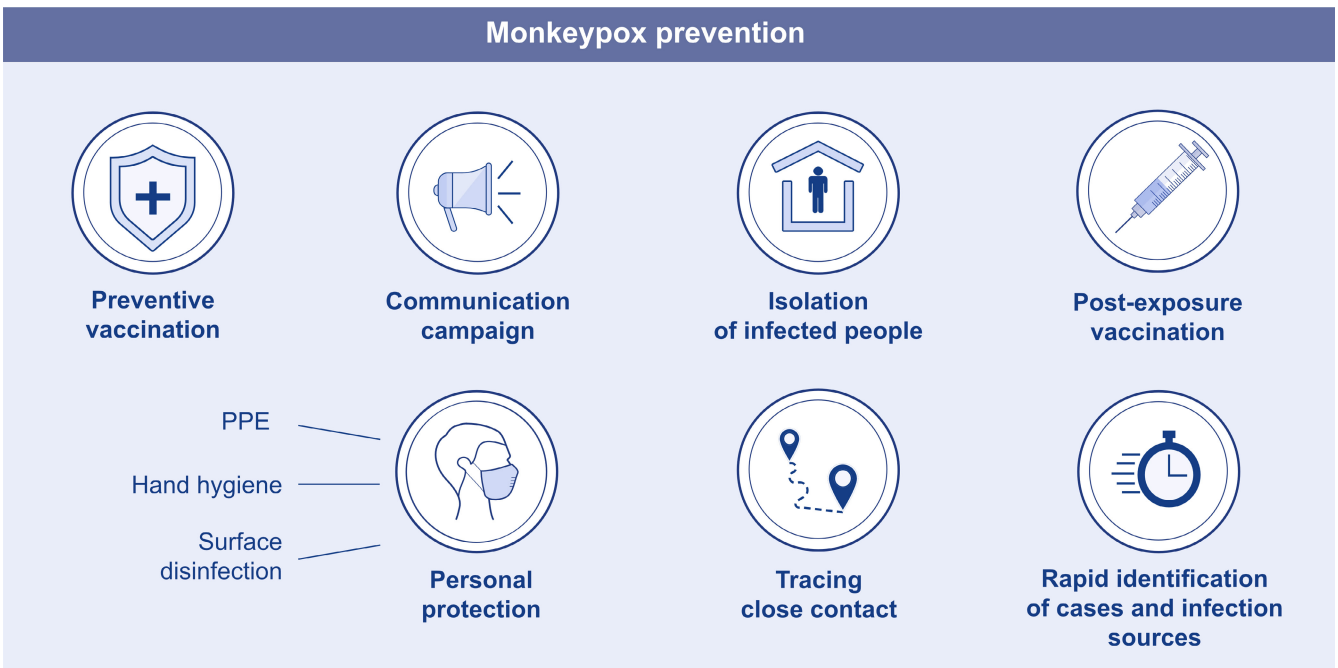


FIGURE 5 Preventive measures for the control of MPX.

vaccines, namely ACAM2000® and MVA-BN (also named Jynneos® or Imvanex®).[124,125] Even though there is a lot of cross-reactivity amongst orthopoxviruses, MPXV has more antibody epitopes.[140] A study showed in *Macaca fascicularis* vaccinated with the smallpox vaccines (LC16m8 and Lister) and challenged with monkeypox viruses Zr-599 strain that a single vaccination with LC16m8 provides long-term immunity against monkeypox viruses.[141] More than 50,000 students in Japan received the LC16m8 vaccine, with little side effects being recorded.[142]

7.12 | What measures are currently being applied by the different countries for the prevention of MPX?

Different countries have implemented several control measures to prevent the spread of MPX (Figure 5). The rapid identification of cases and possible sources of infection and the tracking of close contacts with infected people are essential measures to reduce the spread of MPX.[143] Most of the preventive measures have been implemented in this way including measures related to the isolation of infected people, good hand hygiene, personal protective equipment use, or surface disinfection.[144,145] At present, WHO is developing guidance for authorities with technical recommendations to help countries with surveillance, laboratory work, clinical care, and infection prevention. Complementarily, WHO, states, and agencies are designing campaigns to prevent MPX human transmission, mainly orientated to high-risk groups but also to a broader general population.

Following WHO recommendations regarding vaccination,[146] mass vaccination is not recommended, and preventive vaccination is only recommended for people at high risk of exposure. The postexposure preventive vaccination is only recommended for close contact with confirmed cases.[146]

8 | CONCLUSION

Since MPX became a zoonotic disease in 1970, the current 2022 outbreak is the most widespread and largest ever experienced outside endemic areas with more than 55,000 confirmed cases in just 4 months. The figure contrasts with previous sporadic outbreaks in which 58 cases were confirmed over 18 years. The new threats that human beings are experiencing in recent years in the form of pandemics and outbreaks are placing the consequences of human interventions, such as deforestation or climate change, in the spotlight.

Although MPX is not a severe disease having a CFR of 3–6%, the current explosion of cases has prompted health authorities to take measures in order to contain the outbreak in an efficient and sustained manner. With a R0 of 1.8 in certain populations such as MSM in some of the most affected countries, the current MPX outbreak presents unique characteristics. In that sense, the efficient responses to the current threats and the preventive measures for those that may occur in the future require a strong support from society and institutions to scientific research and innovative progress in medicine.

ACKNOWLEDGEMENTS

Grant from research program Talento Investigador of the Community of Madrid (Regional Ministry of Science, Universities, and Innovation, Madrid, Spain. Grant number: 2019-T1/BIO-12690). Grant from Instituto de Salud Carlos III (ISCIII) through the project PI20/00351 (Spanish Ministry of Science and Innovation) (Co-funded by the European Union).

CONFLICT OF INTEREST

MA has received research grants from Swiss National Science Foundation, Bern; research grants from the Stanford University; Leading House for the Latin American Region, Seed Money Grant. She is in the Scientific Advisory Board member of Stanford University-Sean Parker Asthma Allergy Center, CA; Advisory Board member of LEO Foundation Skin Immunology Research Center, Kopenhagen; and Scientific Co-Chair of World Allergy Congress (WAC) Istanbul, 2022. CAA has received research grants from the Swiss National Science Foundation, European Union (EU CURE, EU Syn-Air-G), Novartis Research Institutes, (Basel, Switzerland), Stanford University (Redwood City, Calif), and SciBase (Stockholm, Sweden); is the Co-Chair for EAACI Guidelines on Environmental Science in Allergic diseases and Asthma; is on the Advisory Boards of Sanofi/Regeneron, Stanford University-Sean Parker Asthma Allergy Center, Novartis (Basel), GlaxoSmithKline (London, Zurich), Bristol-Myers Squibb (London, New York) and SciBase (Stockholm); and is the Editor-in-Chief of Allergy. BS has received funding from DFG (German research foundation, BMBF and EU, and consulting fees from GSK, Sanofi and Novartis. The other authors have no conflict of interest within the scope of the submitted work.

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How to cite this article: Cabanillas B, Murdaca G, Guemari A, et al. A compilation answering 50 questions on monkeypox virus and the current monkeypox outbreak. *Allergy*. 2023;78:639-662. doi:10.1111/all.15633