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Insights into poxviruses: virology and vaccines

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Due to the successful eradication of smallpox worldwide and the cessation of smallpox vaccination campaign in 1980, the human population seems to be more susceptible to poxvirus infection. In the last years, an increased detection of zoonotic orthopoxviruses (OPXVs) has also been observed. In particular, in the past 50 years, a high incidence of monkeypox virus (MPXV) disease (MPOX) in both Central and Western Africa was reported. MPXV is not as lethal as variola virus (VARV), the etiological agent of smallpox, but it represents a threat to public health. The global events of MPOX in May 2022, and the ongoing outbreaks in Central and Western Africa in August 2024, have prompted the World Health Organization (WHO) to declare MPXV a Public Health Emergency of International Concern. Preventive vaccination remains the most effective control against MPXV. Smallpox vaccines of the second and third generations have been suggested for high-risk groups, in spite of several limitations, such as some adverse events, reduced immunogenicity, and manufacturing issues. The emerging threat of MPXV highlights the urgent need for the development of vaccines that can effectively control MPOX and potentially prevent diseases caused by other related OPXVs. Our study aims at introducing basic information on the biology of poxviruses, and on factors that may contribute to the reemergence of zoonotic poxviruses. It also summarizes the evolution of vaccinia-based vaccines and strategies that may control and prevent future outbreaks.

KEYWORDS

MPXV, monkeypox virus, MPOX, monkeypox disease, OPVX, orthopoxvirus, smallpox vaccines, novel vaccines platforms

Introduction

Poxviruses comprise a large family of enveloped, double-stranded DNA viruses that infect a plethora of vertebrate and invertebrate species (International Committee, 2024). They are tissue-specific for epithelial cells, where they can develop cutaneous lesions (Obermeier et al., 2024). According to the last revision of the International Committee on Taxonomy of Viruses (ICTV) Master Species List, the Poxviridae family includes the Entomopoxvirinae and Chordopoxvirinae subfamilies, which are subdivided into 22 genera (International Committee, 2024). Viruses of the same genus share similar morphology, antigenic properties, and host range. Among poxviruses, orthopoxviruses

(OPXVs) are extensively studied due to their pathogen and zoonotic potential in humans. This genus includes the most notable species, variola virus (VARV), the etiological agent of smallpox, and vaccinia virus (VACV), the live vaccine vector for the smallpox vaccine, but other animal-infecting OPXVs, such as cowpox (CPXV), camelpox (CMLV), and monkeypox (MPXV) viruses can cause human zoonoses (Silva et al., 2020).

Smallpox is one of the most devastating viral diseases, with a fatality rate estimated to be up to 50% (Hanna and Baxby, 1913). As a result of a successful vaccination campaign, smallpox was globally eradicated in 1980, which represents a major milestone in public health (Fenner et al., 1988). Three main factors contributed to this success: i) the exclusive human reservoir; ii) the long-lasting immunological memory provided by vaccination; and iii) the absence of vaccine-resistant strains (Moss, 2011).

From the 1980s onward, a decline in herd immunity was observed, which has contributed to the resurgence of MPXV and the emergence of other OPXVs, particularly in groups of individuals who have never been exposed to these viruses in the past. Data from studies of humans vaccinated with the traditional vaccinia viruses were reported to offer 85% protection during MPXV outbreaks in Africa in the 1980s (Fine et al., 1988).

MPOX is a viral zoonotic smallpox-like disease illness, endemic to Central and Western Africa (Bunge et al., 2022). Phylogenetically, MPXV forms two major clades: the Central African clade (also known as the Congo Basin clade or Clade I, with Subclades Ia and Ib) and the Western African clade (Clade II, with Subclades IIa and IIb). The Central African clade has a fatality rate of 10% compared to 3.6% for the Western African clade (Djuicy et al., 2024). After the first human case, identified in 1970 in the Democratic Republic of Congo (DRC), several outbreaks of MPXV infection have been reported in Africa. In the last two decades, the landscape of MPOX has changed, as sporadic outbreaks in non-endemic countries were also observed with increased human-to-human transmission. In 2013, the Bokungu Health Zone reported a 600-fold increase in MPXV cases (Nolen et al., 2016), and in 2022–24, novel clusters of MPXV have emerged not only in endemic countries, but also in several non-endemic countries across Asia, America, and Europe. According to the latest data from the World Health Organization (WHO), as of November 30, 2024 and since January 2022, 117,663 confirmed cases have been reported across 127 countries, with 263 reported deaths (Organization, 2024a)[1]. These data demonstrate a global expansion of MPOX cases that prompted WHO to classify it as a Public Health Emergency of International Concern.

Vaccinations are one of the most effective preventive measures. WHO and the US Centers for Disease Control and Prevention (CDC) suggest the use of VACV-based vaccines of the second and third generations, such as ACAM 2000, MVA-BN (also known as IMVANEX/IMVAMUNE/JYNNEOS), and LC16m8 (Organization, 2022)[2]. However, despite their pivotal role in controlling the current outbreak in high-risk groups, they present several limitations, such as adverse events, reduced immunogenicity, and challenges related to unavailable doses. Likewise, the potential to evolve and the wide animal reservoir underscore the urgent need for the development of vaccines that may effectively control MPOX and potentially prevent diseases caused by other related OPXVs (Hazra et al., 2022).

This review discusses the biology of poxviruses, the factors that contribute to the reemergence of zoonotic poxviruses, current vaccines, and prospects strategies to control and prevent future outbreaks. It also describes *prime-boost* vaccinations, as a new methodology to enhance immunity against pathogenic OPXVs.

Poxvirus biology

The poxviridae family

The classification of poxviruses has been changed over time. Firstly, the viruses were classified based on their clinical features. Then they were classified in the same family as chickenpox (Varicella-Zoster virus) and syphilis (the spirochete *Treponema pallidum*). This classification was based on the morphology of virions or on cytoplasmic inclusion bodies such as Guarnieri bodies (Guarnieri, 1892), observed after infections with Variola and Vaccinia, or Marchal bodies (Downie, 1939; Marchal, 1930), identified after Ectromelia virus (ECTV) and Cowpox virus (CPXV) infections. Although the designation of cytoplasmic inclusion bodies was not precise

Abbreviations: APOBEC3, Apolipoprotein B mRNA-Editing Catalytic Polypeptide-like 3 Enzymes; dsDNA, double-stranded DNA; dsRNA, double-stranded RNA; CDC, Center for Disease Control and Prevention; CEF, chick embryo fibroblasts; CEV, Cell-Associated Enveloped Virus; CMLV, Camelpox Virus; CpG, Cytosine phosphate Guanine dinucleotide; CPXV, Cowpox Virus; ECTV, Ectromelia Virus; EMA, European Medicines Agency; EN-IND, Expanded Access Investigational New Drug; EVs, Extracellular Virions; FDA, Food and Drug Administration; HIV, Human Immunodeficiency Virus; ICTV, International Committee on Taxonomy of Viruses; ITRs, Inverted Terminal Repeats (ITRs); IV, Immature Virions; WVs, Wrapped Virions; MSM, Men who have Sex with Men; MPOX, Monkeypox disease; MPXV, Monkeypox Virus; MVA-BN, Modified Vaccinia Ankara - Bavarian Nordic; MVs, Mature Virions; NHPs, Non-Human Primates; NYCBH, New York City Board of Health; OPXV, Orthopoxvirus; ORFs, Open Reading Frames; PRRs, Pattern Recognition Receptors; RK, Rabbit Kidney cells; SAGE, Strategic Advisory Group of Experts on Immunization; SNPs, Single-Nucleotide Polymorphisms; TLR, Toll-Like Receptors; UTR, Untranslated Region; VACV, Vaccinia Virus; VARV, Variola Virus; WHO, World Health Organization.

1 https://worldhealthorg.shinyapps.io/mpx_global/

2 https://www.who.int/publications/i/item/WHO-MPX-Immunization

TABLE 1 Natural host of the poxvirus species.

Virus	Abbreviation	Viral genus	Natural host
Variola	VARV	Orthopox	restricted to humans
Vaccinia	VACV	Orthopox	broad host range natural host unknown
Cowpox	CPXV	Orthopox	broad host range natural host in rodents
Ectromelia	ECTV	Orthopox	restricted to mice natural host in rodents
Monkeypox	MPXV	Orthopox	natural reservoir in African rodents
Camelpox	CMLV	Orthpox	camels
Buffalopox	BPXV	Orthopox	restricted to water buffalo
Cantagalo and Aracatuba vaccinia	CTV and ATV	Orthopox	cattle and probably rodents
Akhmeta	AKMV	Orthopox	rodents
Borealpox	BRPV	Orthopox	red-backed voles and shrews natural host unknown
Orthopox Abatino	OPVA	Orthopox	captive macaques and cats natural host unknown
Molluscum contagiosum	MCV	Molluscipox	restricted to humans
Orf	ORFV	Parapox	sheep, goats, and humans
Paravaccinia	PV	Parapox	restricted to cattle
Bovine papular stomatitis	BPSV	Parapox	restricted to cattle
Deerpox	DPV	Parapox	various deer
Sealpox	SPV	Parapox	harbour and grey seals
Tanapox	TPV	Yatapox	monkeys and insects?

within the poxvirus family, Kato et al. refined the classification after histochemical reactions and morphological characteristics of inclusion bodies, introducing the A-type (acidophilic) and B-type (basophilic) classification (Kato et al., 1959). In 1953, at the Sixth International Congress for Microbiology (Fenner and Burnet, 1957), a poxvirus subcommittee was established, where Fenner and Burnet presented a review with the characteristics of the poxvirus family, following the modern classification system.

The poxvirus family can be divided into two subfamilies: the Entomopoxvirinae (poxviruses that infect insects) and the Chordopoxvirinae (poxviruses that infect vertebrates). Entomopoxvirinae and Chordopoxvirinae were divided into genera based on several studies of genomic DNA analyses and the immune response within the genera.

Target hosts

Poxviruses include 22 genera and numerous species, four genera (*Orthopoxvirus*, *Molluscipoxvirus*, *Parapoxvirus*, and *Yatapoxvirus*) of which have demonstrated pathogenicity in humans (Reynolds, et al., 2018). They have a different host-species restriction, so that VARV and Molluscipoxvirus are present only in humans, whereas species of the OPXV genus, as well as Parapoxviruses and Yatapoxviruses, are present in wild animals and can cause human zoonoses.

In general, zoonotic poxviruses have intermediate hosts, which are animals that live in close proximity to humans (i.e., cows, buffaloes, monkeys, camels, rodents, deer, seals), and not necessarily the natural host. Thus, the names of zoonotic poxvirus species do not always indicate the true natural reservoir host (Shchelkunova and Shchelkunov, 2022).

The natural host of various poxvirus species is illustrated in Table 1.

Poxvirus structure and genome

Compared to other viruses, poxviruses are endowed with an envelope and an asymmetrical shape (Hyun, 2022). By the electron microscope, they appear as brick-shaped and can reach dimensions from 200 to 400 nm (Knipe and Howley,

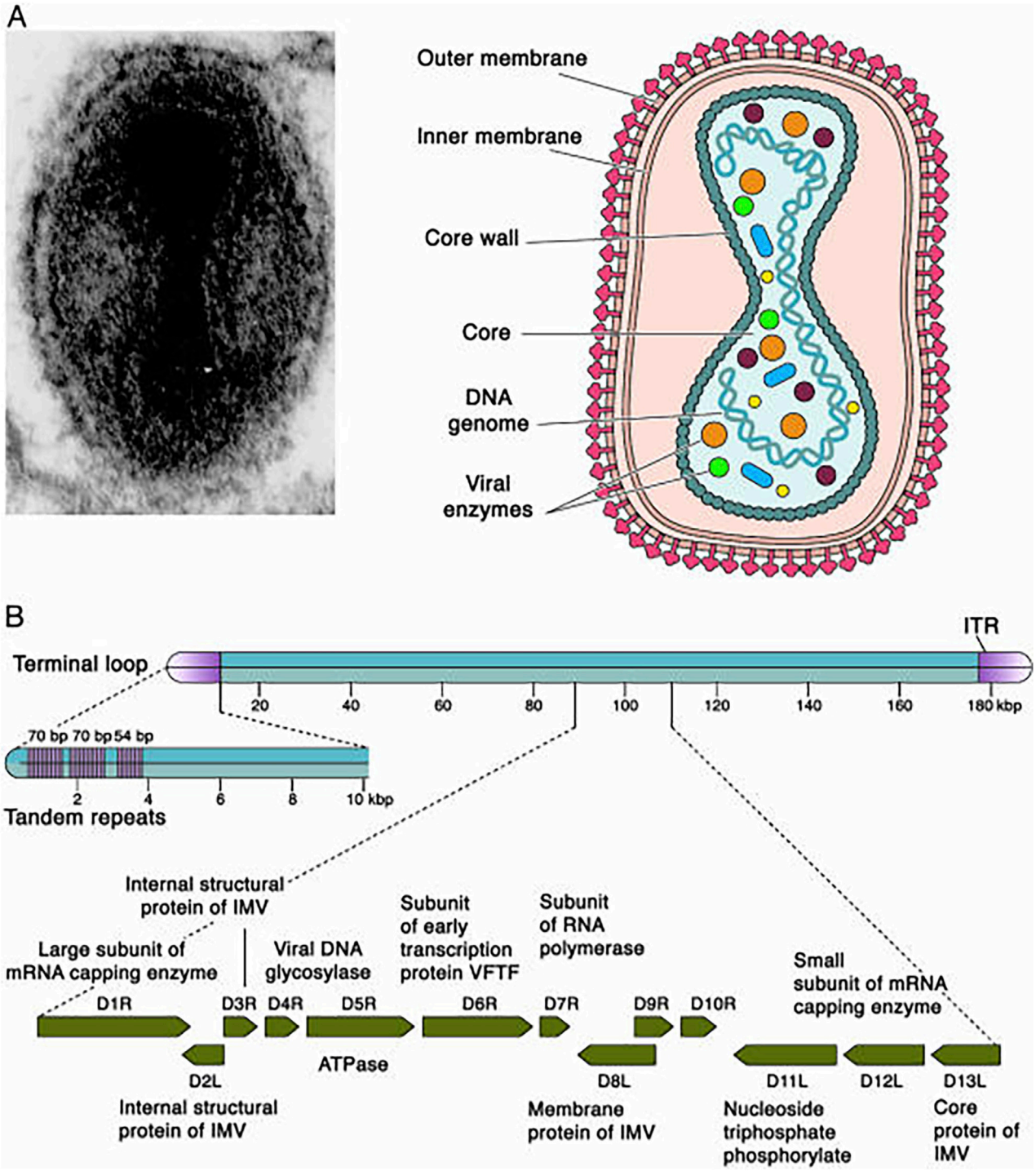

FIGURE 1
(A) A representative poxvirus by electron microscopy (left) and schematic structure (right); **(B)** Structural features of VARV genome. The complete linear double-stranded DNA genome is shown with some gene sequences encoding for specific proteins (Harrison et al., 2004).

2007). The four basic components of poxviruses are the core, lateral bodies, outer membrane, and the outer lipoprotein envelope that can be either single or double. The outer membrane and the envelope are important for the interactions with the host. The central core is enclosed by a dense layer of dumbbell-shaped structure known as the palisade

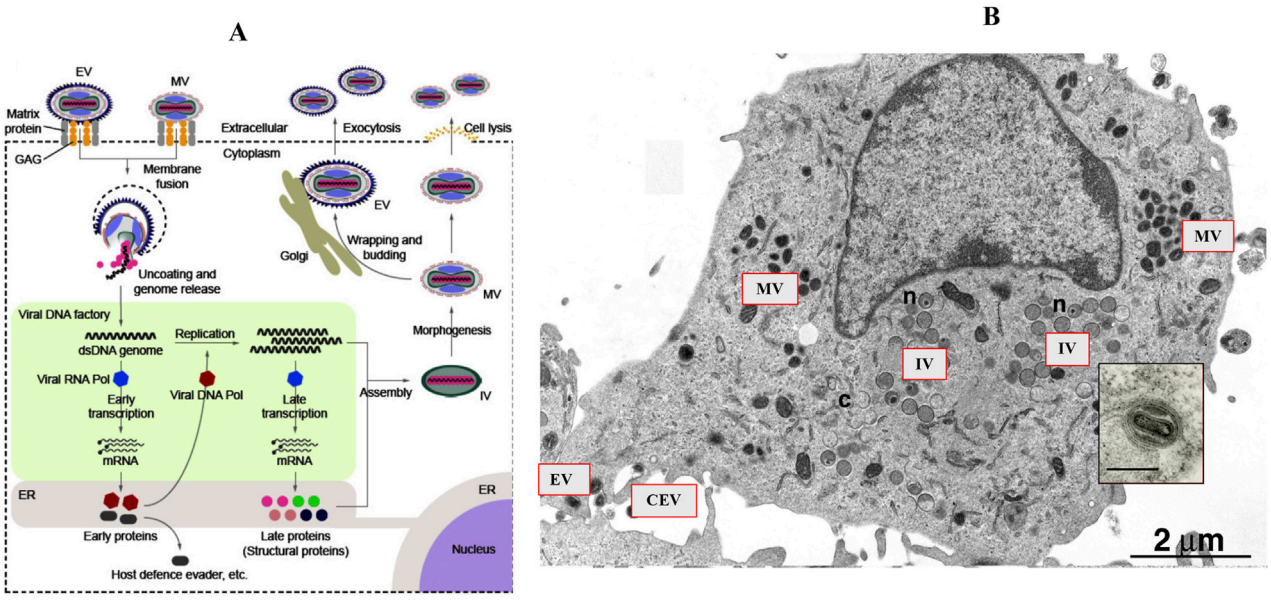

FIGURE 2
(A) The replication cycle of poxviruses. The attachment and membrane fusion of virions depend on interactions among viral surface proteins, extracellular GAGs, and matrix proteins of the host cell. After uncoating, the genomic DNA forms DNA factories, where transcription, replication, and translation occur, leading to the assembly of IV. Subsequently, morphogenesis occurs in the cytoplasm, and IV are shaped into MV. MV can enter the Golgi complex, where they are wrapped in an envelope and bud to form EV. EV are released from the cells through exocytosis, whereas MVs can be released through cell lysis (modified from Lee et al., 2023, https://creativecommons.org/licenses/by/4.0/); **(B)** Electron microscopy of a Vero cell infected by vaccinia virus, 48 h after infection. Inset, a vaccinia virus in penetration (bar, 250 nm). GAG glycosaminoglycan; EV enveloped virus; MV mature virus; IV immature virus; CEV cell-associated enveloped virus; c cytoplasm; n nucleoplasm.

layer, which contains the viral double-stranded DNA (dsDNA) and core fibrils. The internal structure, including the palisade layer, lateral bodies, and the central core, is surrounded by the outer membrane (Hyun, 2022).

The genome of the virion contains a noninfectious, linear, A + T-rich double-stranded DNA that varies from 130 kbp in parapoxviruses to more than 300 kbp in some avipoxviruses (Knipe and Howley, 2007), and codes for more than 100 non-overlapping *Open Reading Frames* (ORFs) (Yang et al., 2011). The genome of each poxvirus has a central collinear region flanked by dynamic regions, that vary in gene content among species, and terminal regions that contain *Inverted Terminal Repeats* (ITRs), which are identical, but oppositely oriented sequences at the two ends of the genome (Knipe and Howley, 2007). The ITRs include a *hairpin loop* that connects the two DNA strands, a short-conserved region (less than 100 bp), important for the resolution of the replicating concatemeric forms of DNA, *short tandem repeated sequences*, and several *open reading frames* (ORFs) (Merchlinsky and Moss B, 1989). The central region contains genes that perform housekeeping functions, like transcription, replication, and virion assembly. The terminal regions of poxviruses differ from one another and encode proteins implicated in host range infection and pathogenicity (Yeh et al., 2022), Figure 1.

Viral cell entry and pathogenesis

Unlike other DNA viruses, POXVs replicate only in the cytoplasm of infected cells. The mechanism of entry is described in three steps: attachment of the virus to the host cell membrane, hemifusion, and release of the viral core into the cellular cytoplasm.

The infectious particles of POXVs are the extracellular virions (EVs) and the mature virions (MVs) (Smith et al., 2002). These forms enter the host cells through different pathways. MVs enter the host cell membrane pH independently. In contrast, EVs, because of the additional membrane, require a more complex entry process. EVs enter host cells via endocytosis, where the acidic environment of the endosome removes the outer envelope. This uncoating process releases the viral core into the cytoplasm (Figure 2) (Schmidt et al., 2012).

Viral entry mechanisms

It is suggested that some important components of the cellular membrane might be involved in the initial binding of the poxvirus membrane to the host cells, such as glycosaminoglycans (including heparan sulfate and chondroitin), and laminin (Moss, 2012). On the other hand, there is evidence of important vaccinia virus proteins,

encompassing D8 (Matho et al., 2018), A27 (Chung et al., 1998), A34 (Blasco et al., 1993), A26 (Chiu et al., 2007), and H3 (Lin et al., 2000) that entail binding to the host cell membrane. Other transmembrane proteins, including L1 and A27, participate in hemifusion (Duncan and Smith, 1992).

Viral replication and gene expression

The viral core, released into the cytoplasm, contains enzymes responsible for initiating a series of events that lead to transcription (Rosales et al., 1994), DNA replication, and protein synthesis. Infectious poxvirus particles contain a transcriptional system including *early*, *intermediate*, and *late genes* (Knipe and Howley, 2007).

Following the entry into the cytoplasm, the DNA-dependent RNA polymerase, present in the viral core, activates the transcription of *early mRNA* that is translated into a variety of proteins, including factors necessary for the synthesis of viral DNA and the transcription of *intermediate genes*. With the disruption of the viral core, the expression of *early genes* ceases, whereas DNA replication progresses to form concatemeric molecules. The progeny of DNA activates the transcription of *intermediate genes* in *intermediate mRNA*, which are translated into enzymes and factors for the late gene expression. The final products of *late genes* include virion structural proteins, enzymes and early transcription factors. The formation of membrane structures and viral genome, that derived from the segregation of concatemeric DNA intermediate, are assembled and packaged in immature virions (IV) and then into the intracellular mature virions (MV), the first infectious form.

In the cytoplasm, MVs may behave in different ways: i) they remain free in cytoplasm; ii) they move to the cell surface for exocytosis through microvilli; iii) they acquire a second envelope from the trans-Golgi network to form wrapped virions (WVs), which bud from the host cell as extracellular enveloped virions (EVs) (Roberts and Smith, 2008).

Viral spread and immune evasion

MVs are more abundant than EVs and are thought to be responsible for the transmission between hosts, whereas EVs facilitate virus dissemination within an infected host (Schmidt et al., 2012).

The outer envelope of EVs presents specific transmembrane surface proteins, that include A33 (Roper et al., 1996), A34, A36 (Parkinson and Smith, 1994), A56 (Shida, 1986), F13 (Moss, 2011) and B5 (Yu et al., 2021), which are important for infectivity and cell-to-cell spread. In particular, A33, A36, B5, and A34 contribute to the formation of actin tails and microvilli, which are essential for viral dissemination among cells.

Once infected, the innate immune system activates different signaling pathways: (TLR)-dependent and (TLR)-independent toll-like receptors, various host pattern recognition receptors (PRRs), including DNA and RNA sensors, and inflammasome components to detect the virus. They are triggered by the expression of antiviral responses (Yu et al., 2021). Poxviruses have several immune evasion mechanisms, such as the synthesis of a variety of immunomodulatory proteins, that interfere with the antiviral defenses, triggered by the host pattern recognition receptors (PRRs).

Pathogenesis of poxviruses

Infections by poxviruses manifest a broad spectrum of pathogenicity. The infection can be either localized, self-limited in the skin, or systemic, and characterized by a generalized rash. The rash progresses through different stages such as macules, papules, vesicles, pustules and scabs (Obermeier et al., 2024).

The type of infection is dependent on the species of poxviruses, the route of entry, the genus/species of the susceptible animal and its immune status (Knipe and Howley, 2007). Although it is difficult to understand the natural pathogenesis due to the lack of data on the disease pathogenesis in its natural host, there is clinical evidence from animal models, such as ectromelia infection in mice and MPOX infection in non-human primates, that depicts the specifics of human pathogenesis (Knipe and Howley, 2007). Pathogenesis is divided into two phases: primary viremia and prodromal stage (Kumar et al., 2022). Primary viremia starts with the accumulation and replication of the virus in the primary inoculation site, including the nasopharyngeal, oropharyngeal, and the intradermal region. Then, the virus disseminates to all the lymph nodes and the secondary organs, where it subsequently replicates. When the virus ultimately reaches the skin and the tertiary organs, the prodromal stage (or secondary viremia) starts (Majie et al., 2023).

Poxviruses' transmission may occur through i) skin lesions (cuts, abrasions, or bites) of infected animals; ii) body fluids or respiratory droplets of infected individuals; iii) ingestion of contaminated food or water; iv) contaminated objects; or iv) cannibalism (Buller and Palumbo, 1991; Grant et al., 2020; Vivancos et al., 2022).

Among human poxvirus infections, only smallpox is not a zoonotic one. Zoonotic infections may spread as a primary transmission (from animal to human) or as a secondary transmission (from human to human). Transmission by infected animals to humans occurs through animal body secretions or animal bites. Transmission from human to human occurs mainly through the inhalation of large airborne respiratory droplets from infected persons, especially during prolonged face-to-face exposure or close contact. Transmission via contaminated objects (fomites) in household and healthcare settings or contact with infectious lesions from the rash or scab may also occur (Vivancos et al., 2022).

In recent MPOX outbreaks, it was observed that most confirmed cases were linked to unprotected intercourses and anogenital lesions. Although MPOX is not classified as a sexually

transmitted infection, evidence suggests that direct sexual contact is a primary route of acquisition.

Multiple studies demonstrated that up to 95% of reported cases are middle-aged males, particularly among men who have sex with men (MSM) (Bhat et al., 2023; Kumar et al., 2025). A disproportionate gender distribution of patients was also observed. However, it remains unclear whether the virus may infect sperm cells or reproduce in the genital canal (Kumar et al., 2025).

Although there are very limited data on MPOX infection during pregnancy, a few studies described fetal infection with MPXV in one out of four infected pregnant women had fetal death, of which two delivered miscarriages in their first trimester, and one delivered healthy baby (Mbala et al., 2017), thus, demonstrating the existence of vertical transmission of MPXV (Fahrni and Choudhary, 2022).

Resurgence of zoonotic poxviruses

Due to the successful eradication of smallpox worldwide, smallpox vaccination was interrupted in 1980. Since then, no other OPXV has widely circulated among humans. Young adults under the age of 44 and children have not been exposed to OPXVs, which has led to a number of increased cases of zoonotic OPXV infections (Simpson et al., 2020).

Although these viruses are sporadic and cause self-limited outbreaks, in 2020, epidemiological data, estimated by a mathematical model, envisaged a higher reproduction number of MPXV, when compared to previous data where this number was not reached (Grabenstein and Hacker, 2024).

MPXV is not as lethal as VARV, but causes significant morbidity and death (Meyer et al., 2002). Children and immunocompromised individuals, particularly those with human immunodeficiency virus (HIV) infection, have a higher incidence of fatality than older and healthy individuals (Bunge et al., 2022). Data from an active surveillance program, conducted in Zaire between 1980 and 1985, demonstrated that children aged from 0 to 4 years had a case fatality rate of up to 14,9% (Jezek et al., 1987). Likewise, in early 2024, most MPOX cases occurred in <15-year-old children with a case fatality risk of >10% (Grabenstein and Hacker, 2024).

Overview of MPOX

A pox-like disease was first identified in 1958 in non-human primates (NHPs), in a research center in Denmark that used their tissues and organs in cultured isolates of the poliomyelitis virus (von Magnus et al., 1959).

MPXV shares similarities in structure, clinical manifestation, and serological markers as OPXVs, particularly VARV and VACV. In 1970, the first case of human MPOX was reported in a 9-month-old baby, in the Democratic Republic of Congo (DRC) (Breman et al., 1980). MPOX was restricted to the tropical rainforest regions of Central and Western Africa.

MPXV in humans has an incubation period of 5–21 days (European Centre, 2022), then progresses to the prodromal and rash phases (McCollum and Damon, 2014). The prodromal phase includes atypical symptoms such as inguinal lymphadenopathy, fever, headache, and fatigue. After the fever onset, cutaneous lesions like rash start to develop initially on the face and subsequently spread to other regions of the body. Lesions (10–150) have been observed, which can persist for no more than 4 weeks. Infectivity of these lesions lasts until all the scabs desquamate.

Factors for the resurgence of MPOX

Along with the reduction in herd immunity, due to the cessation of the smallpox vaccination campaign, the global outbreak of MPXV in 2022 was influenced by other factors.

Novel transmission patterns

The most frequent human-to-human transmission of MPXV occurred in 99% of cases through the close contact among adult men, 94% of reported cases being due to male-to-male sexual contact or intimate contact 3 weeks before the symptoms of infection (Parums, 2024). These data suggested that sexual contact may be a novel route of transmission, even though MPOX was not considered a sexually transmitted disease before (Sadeghpour et al., 2021).

Viral evolution

Although the MPXV genome has a low mutation rate (Isidro et al., 2022), phylogenetic analysis indicates that the 2022 strain depicts an ongoing viral evolution from the 2017–2018 Nigeria outbreak. A means of 50 single-nucleotide polymorphisms (SNPs) has been identified, some of which may have been triggered by apolipoprotein B mRNA-editing catalytic polypeptide-like 3 (APOBEC3) enzymes. APOBEC3 belongs to the family of cytidine deaminases and it is an essential component of the innate immune system. These enzymes can introduce mutations into viral DNA through deaminase and deaminase-independent mechanisms. In some circumstances, APOBEC3 might not completely inactivate the viruses, and mutated variants can acquire mechanisms that escape from the immune system. The precise role of APOBEC3 in the introduction of these mutations and its contribution to the evolution of virus remains to be fully elucidated.

Ecology and geographic distribution of MPXV

Poxviruses, and in particular those of the OPXV genus, are known for a long-lasting stability in the environment (Forni et al., 2023).

Zoonotic transmission or spillover to humans might emerge from the alterations of the natural habitat of wild animals (Domán et al., 2022). MPXV spatial-temporal analyses indicate that environmental changes (deforestation and hydrological changes) determined the geographic pattern of MPXV reservoirs.

Migration of wild animals to the Congo Basin may have led to the development of the two major clades, likely influenced by the rainforest extension, and allowed the virus to adapt to different natural hosts. Despite the limited understanding of MPXV in wild mammals, the epidemiology of human cases suggests that some of the reservoir species dwell in the rainforest (Forni et al., 2023).

Some recent studies suggest that MPXV circulates in a wide range of wild mammals, particularly in rodents and in non-human primates (NHPs) (Kumar et al., 2022).

It is believed that the 2022 MPOX outbreak occurred as a result of a spillover from animals to humans (Happi et al., 2022; Riopelle et al., 2022). The spillover of MPXV was due to the wide geographical coverage of the MPXV hosts (Tu, 2015).

Thus, it is difficult to determine the natural host, because of the cross-reactivity with other OPXVs.

The climatic or ecological changes can result in multiple epizootics in rodent and NHP populations, followed by an increase in zoonotic transmission (Morgan et al., 2022).

Poxvirus-based vaccines

Evolution of poxvirus-based vaccines: from variolation to third-generation smallpox vaccines

Smallpox is the only human disease that has been eradicated worldwide, and this achievement is attributed to the use of vaccines (Fenner et al., 1988).

The evolution of poxvirus-based vaccines is based on the methods of vaccine preparation and on the selection of strains by their reactogenicity. Thus, it reflects both the progress in virus attenuation strategies and a better understanding of immune responses. The development of smallpox vaccines is divided into three generations.

Variolation and first-generation vaccines

The roots of smallpox vaccination were found in the variolation practices from the 10th to the 18th centuries to control disease, including intranasally insufflation or cutaneous inoculation of smallpox material scabs.

This precursor methodology led to the term of "vaccination" and to the development of the first smallpox vaccine, pioneered by Edward Jenner in 1798, that introduced a safer and more reliable alternative to variolation using cowpox material. This represented the initial use of poxviruses as vaccines.

In the subsequent years of the Jennerian vaccine, smallpox vaccines did not use CPXV, but other OPXVs, which for a long time have been known as "vaccine virus." The origins of VACV are unknown. VACV might have been the hybrid result between CPXV and VARV.

The first-generation smallpox vaccines were based on live, non-attenuated VACV strains and production systems. The most common VACV strains used worldwide include the New York City Board of Health (NYCBH) (North America and West Africa), Lister (UK), EM-63 (Russia and India) and TianTan (also known as Temple of Heaven, China), usually administered percutaneously, by scarification with a bifurcated needle, or with a jet injector (Moss, 2011).

There was a steady improvement in the methods of preparing vaccines. Early smallpox vaccine manufacturing introduced the "vaccine farms," that were rapidly adopted around the world and were considered a safe method at the time (Esparza et al., 2020). It consisted in the propagation and harvesting of VACV strains from the skin of live animals (e.g., calf, sheep, water buffalo). Based on WHO requirements, other production methods were applied such as lyophilization and dried frozen vaccine batches to provide a better efficacy and safety profile (Kmiec and Kirchhoff, 2022). This contributed significantly to the success of smallpox eradication.

Second-generation vaccines

Although the first-generation vaccines were used for the eradication of the smallpox, significant adverse events were reported, particularly in individuals with immune deficiency, such as eczema vaccinatum, progressive vaccinia, and post-vaccinal encephalitis (Fulginiti et al., 2003).

To reduce adverse events, a second-generation of live vaccines were designed, that were produced in tissue cultures such as rabbit kidney (RK) cells, chick embryo fibroblasts (CEF), human MRC-5 lung cells, and African green monkey kidney epithelial cells (Vero). There are two VACV strains used in the second-generation vaccines, such as Lister (used for the production of RIVM, Lister, and Elstree-Bavarian-Nordic vaccines) and NYCBH (used for the production of ACAM2000 and CJ-5030022 vaccines) (Monath et al., 2004).

In 2007, the Food and Drug Administration (FDA) approved the use of the ACAM2000 vaccine for over 18-year-old healthy people that were at high-risk for smallpox infection (Food and Drug Administration US, 2007). ACAM2000 is under surveillance of Expanded Access Investigational New Drug

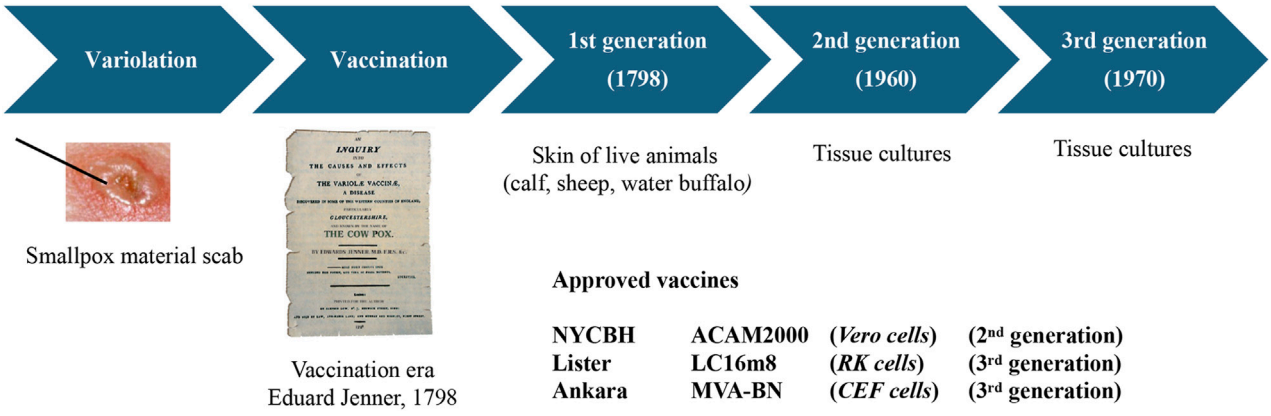

FIGURE 3
Development of the smallpox vaccines.

(EN-IND) application for MPOX prevention (Sehulster and Chinn, 2003).

Although they have a better safety profile compared to the first-generation vaccines, they still carry the risk of serious adverse effects. Given that ACAM-2000 is a live VACV-based vaccine, it is contraindicated in individuals with eczema, human immunodeficiency virus (HIV) infection, cardiovascular diseases, pregnant women, and other specific conditions (Frey et al., 2009). Clinical data have observed that ACAM2000 can cause myocarditis and/or pericarditis, with an average of 5.7 cases per 1,000 primary vaccinees. Attenuated vaccines were thus developed, sustaining immunogenicity and protective effectiveness (Jacobs et al., 2009).

Third-generation vaccines

In contrast with live VACV-based vaccines used in the first and second generation, the third-generation vaccines were developed through classical attenuation after multiple passages of VACV strains in specific tissue cultures. During this process, gene deletions and mutations were introduced into the virus genome, that resulted in the attenuation of the virus pathogenicity, which could not replicate in human cells anymore (Perdiguero et al., 2023). There are four VACV strains used in the third-generation vaccines, such as Ankara, Copenhagen, Lister, and NYCBH. Among these, two of them represent the most important vaccines, that include MVA-BN (Modified Vaccinia Ankara - Bavarian Nordic, that used the Ankara strain) and the LC16m8 (that used the Lister strain) (Paran and Sutter, 2009) both approved for smallpox and MPOX in high-risk groups.

According to clinical data, the third-generation vaccines, and in particular the MVA vaccine, are safer and suitable for a broader range of individuals, including immune-compromised subjects (Volz and Sutter, 2017).

Because the LC16m8 vaccine is partially replicating, it is contraindicated for immune-suppressed individuals or during pregnancy (Gubser et al., 2004).

The history of the generation of the different poxvirus-based vaccines is illustrated in Figure 3.

Current MPOX vaccines

Within the same genera, poxviruses share a similarity greater than 90% in the DNA genome. In particular, a 96.3% homology is revealed between MPXV and VACV (Gubser et al., 2004).

Multiple serologic studies demonstrated that production of antibodies induced by the smallpox vaccine could cross-neutralize other OPXV proteins, thus providing a cross-protection against MPOX (Poland et al., 2022).

Due to the reemergence of MPOX in non-endemic countries, on August 24th, 2022 the WHO's Strategic Advisory Group of Experts on Immunization (SAGE) revised and updated the strategy of the 2013 smallpox vaccination with the aim at using available smallpox vaccines such as ACAM 2000, MVA-BN, and LC16m8 (Organization, 2022)[2] for MPOX vaccination in subjects with high-risk of exposure to MPXV.

During the 2024 MPOX outbreak, SAGE recommended the use of MVA-BN and LC16m8 vaccines in endemic regions and for high-risk individuals in non-endemic settings (Organization, 2024b)[3].

3 https://www.who.int/publications/i/item/who-wer-9934-429-456

TABLE 2 The characteristics of smallpox vaccines.

Characteristics	ACAM2000	MVA-BN	LC16M8
VACV strain	New York City Board of Health (NYCBH)	Chorioallantoic Vaccinia Ankara (CVA)	Lister
In vitro cell culture	African Green monkey kidney epithelial cells (Vero)	Chick embryo fibroblasts (CEF)	Primary rabbit kidney cells (RK)
Vaccine type	Live replicating virus	Live-attenuated, non-replicating in mammals	Live-attenuated, minimally replicating virus
Virus concentration	US 1–5× 10^8 or 2.5–12.5 × 10^5 PFU[a]/ml	US, EU 0.5–3.95 × 10^8 IU[b]/0.5 mL	Japan ≥2.5–5× 10^7 PFU[a]/vial
Dose regimen	1 dose	2 doses (4-week intervals)	1 dose
Route of administration	Percutaneous, scarification with a bifurcated needle	Subcutaneous injection	Percutaneous inoculation (15 punctures)
High-risk population	Healthy adults exposed to OPXVs (laboratory or healthcare personnel, animal care or military personnel)	Healthy adults and under 12-year-old adolescent people; immunocompromised individuals, pregnant and breastfeeding women	Healthy adults and children

[a]Plaque-Forming Units.
[b]Infective Units.

Among these, MVA-BN, popularly known as JYNNEOS (called IMVAMUNE in Canada, and IMVANEX in Europe) is the only vaccine approved against MPOX. Recently, this vaccine was added to the prequalified list, and its use has been extended to individuals aged 12 years and older (Organization, 2024c)[4]. Moreover, LC16m8 vaccine is now in the list of the Emergency Use (Organization, 2024d)[5].

ACAM2000 has a complex safety profile, and its use for MPOX requires an additional informed consent (Sah et al., 2023). The characteristics of these three vaccines are summarized and illustrated in Table 2.

Geographic distribution of MPOX vaccines

As WHO has recently declared MPOX a Public Health Emergency, several organisations, such as the U.S. Center for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), European Medicines Agency (EMA), and Japanese KM Biologics, have adopted several mitigation approaches to control and prevent the current and future MPOX outbreak.

CDC recommends two doses of the JYNNEOS vaccine as the "best protection" against MPXV (Prevention US C.D.C, 2024). Meanwhile, Food and Drug Administration US (2024) expanded the approval of the ACAM2000 vaccine to individuals at high risk of MPOX infection. European Medicines Agency (2024) has recently recommended the vaccination of adolescents aged from 12 to 17 with IMVANEX. Although LC16, produced by Japanese KM Biologics is not yet internationally commercialized, a single dose of the vaccine has been approved domestically in Japan and in the DRC since June 2024 (Organization, 2024e)[5].

From September 2023 to November 2024, 1,137,000 doses of the MVA-BN vaccine were allocated to nine African countries by the Access and Allocation Mechanism, involving African CDC, Coalition for Epidemic Preparedness Innovations, Gavi, Vaccine Alliance, UNICEF and WHO (MPOX report, 2024)[6,7].

High vaccination rates lead to successful vaccination campaigns and effective control of infection. Factors that lead to successful vaccination campaigns are not only related to the development of safe and effective vaccines, but they had to ensure logistical issues and an equitable distribution (Gates, 2022).

A meta-analysis, including 61 studies with 263,857 participants from 87 countries, evaluated the prevalence of MPOX vaccine acceptance and uptake (Sulaiman et al., 2024). The overall global rate of intention to vaccinate against MPOX was 60.9%, and there was a substantial variation observed across the six WHO regions. The highest rate of intention to vaccinate was in the Western Pacific Region at 73.5% (95% CI, 63.0%–82.9%), whereas the African Region had the lowest rate at 41.9% (95% CI, 36.6%–47.4%).

Obstacles preventing the global distribution of MPOX vaccines include their high cost, low availability, and poor

4 https://www.who.int/news/item/13-09-2024-who-prequalifies-the-first-vaccine-against-mpox

5 https://www.who.int/news/item/19-11-2024-who-adds-lc16m8-mpox-vaccine-to-emergency-use-listing

6 https://cdn.who.int/media/docs/default-source/documents/emergencies/20241109_mpox_external-sitrep_-42.pdf.

7 https://www.who.int/news/item/15-03-2025-the-multi-partner-access-and-allocation-mechanism-allocates-238000-doses-of-mpox-vaccine-to-four-countries

accessibility. Logistical obstacles impede the global use of the MPOX vaccine since, in nonendemic regions, there is a limited availability and accessibility, whereas in higher-income countries stockpiles of millions of doses are available. Although in 2024 African countries accounted for more than 95% of all cases and deaths due to MPOX, few vaccines were available for prevention. Vaccine equity is critical to the fight against MPOX, and it necessitates collaboration among the different stakeholders (Shafaati et al., 2025).

Public knowledge, perceptions and attitudes towards the MPOX vaccine

Vaccines against MPOX may be administered to individuals at high risk as primary preventive vaccination before exposure or as post-exposure vaccination after contacts with an MPOX case (European Center D.P.C, 2025)[8]. A meta-analysis, including 61 studies with 263,857 participants from 87 countries reported a prevalence of MPOX vaccine uptake among people living with HIV (35.7%) compared to the general public (20.2%) and among the LGBTQI+ community (39.8%) (Sulaiman et al., 2024). The higher rates of acceptance and uptake observed among the LGBTQI+ community (73.6% and 39.8%) may indicate the group's higher risk perception, which plays a high role in vaccine acceptance. Among the healthcare workers, the prevalence of intention to be vaccinated against MPOX (51.9%) is comparable to the acceptance rate of the COVID-19 vaccine (55.9%–65.7%) (Sulaiman et al., 2024).

The effective control of the MPOX outbreak requires widespread acceptance and uptake of the vaccine, especially among high-risk groups such as the LGBTQI+ community and frontline healthcare workers. The outbreak of MPOX occurred at a time when global vaccine hesitancy was at an all-time high levels period (Bergen et al., 2023). Vaccine hesitancy, defined by WHO as "*a delay in the acceptance or refusal of vaccination despite the availability of vaccination services*", jeopardizes the success of vaccination programs (MacDonald and SAGE Working Group on Vaccine Hesitancy, 2015). Several studies have attempted to identify key determinants of intention and hesitancy to vaccinate against MPOX.

High-risk groups and people with poor vaccination histories should be targeted. People living with HIV, men who have sex with men (MSM), and people with pre-exposure prophylaxis may need a priority status for the MPOX vaccine. In a survey conducted in France, among MSM persons living with HIV or with pre-exposure prophylaxis, 52 out of 155 (33.6%) of the participants declared to be hesitant about MPOX vaccination (Zucman et al., 2022). Factors associated with the acceptance behavior were subjects with an increased number of sexual partners during the previous months, the fear about MPXV infection or the endorsement of mandatory COVID-19 vaccination in high-risk groups (Zucman et al., 2022). Participants declaring that the smallpox vaccine should be compulsory for people at risk were significantly associated with higher acceptance of MPXV vaccination ($p < 0.001$) (Zucman et al., 2022).

Another study conducted in Africa revealed that individuals who have never been vaccinated against other diseases were at a higher risk of MPOX vaccination hesitancy for themselves and for their children, despite the vaccine being available (Du et al., 2025).

A meta-analysis aiming at evaluating the rate of MPOX vaccine acceptance and uptake globally found that age, sex, level of education, and level of income are among the most reported sociodemographic determinants of MPOX vaccine acceptance (Sulaiman et al., 2024).

Therefore, public health intervention programs should consider these sociodemographic characteristics to design tailored strategies to address vaccine hesitancy and optimize the uptake rates. Modifiable behavioral factors associated with MPOX vaccine acceptance, such as concerns about the disease, the perception of risk, and knowledge about MPOX, should be considered to increase knowledge and awareness, especially among high-risk groups such as the LGBTQI+ community.

The source of information is another important factor to be considered when implementing strategies to combat misinformation. Studies have found that "infodemic", defined by WHO as "*too much information, including false or misleading information in digital and physical environments during a disease outbreak*" was strongly linked to vaccine hesitancy (Pierri et al., 2022). Interventions should aim at increasing the public's positive perception of MPOX vaccines and encourage them to get information from reliable sources like health institutions.

Engaging health professionals and institutions in media campaigns has been recommended as a potent method to face the problem (Shasha et al., 2022). It is important to address vaccine hesitancy through health education programs, especially in risk populations, to promote MPOX vaccine uptake. A meta-analysis involving 16 studies with 9,066 participants reported poor knowledge about MPOX vaccines in 53.4% of them (Tanashat et al., 2024).

Tailored long-term public messages targeting specific groups should be structured by health authorities. Healthcare professionals should increase their knowledge and awareness about MPOX vaccines. Trust in the safety and effectiveness of vaccines highly influences the decision to get vaccinated.

Clinical and case studies related to MPOX vaccines

Clinical development of ACAM 2000, MVA-BN, and LC16m8 was initiated and progressed as a measure against the potential use of smallpox as a biological weapon (Nalca

8 https://www.ecdc.europa.eu/en/mpox

and Zumbrun, 2010; Grabenstein and Hacker, 2024). At that time, MPOX was rare and unpredictable, making it difficult to conduct clinical trials.

In the last two decades, multiple studies have evaluated the safety of these vaccines in human volunteers through clinical trials. These studies were in line with the current clinical guidelines, with sample sizes ranging from several dozen to several hundred participants (Volz and Sutter, 2013; von Krempelhuber et al., 2010; Pittman et al., 2019; Morino et al., 2024).

A multicenter randomized controlled study testing ACAM2000 has concluded that this vaccine is effective and suitable as a booster dose for individuals previously vaccinated against smallpox (Food and Drug Administration US, 2022). LC16m8 has demonstrated potential in animal models, but further research is necessary to determine its long-lasting protection (Saijo et al., 2006; Hirani et al., 2023). In a study by Okumura et al. (2024) 1,006 adults were vaccinated with LC16m8, including those living with HIV (346 individuals), with an acceptance rate of 90.3% in the HIV group and 94.6% in the uninfected group. None of the participants developed MPOX, and vaccine's efficacy could not be measured. There is also an ongoing clinical trial conducted with LC16m8 in Colombia, but the results are not yet available (Tomotsugu et al., 2025). A total of twenty-two clinical trials established the strong efficacy and safety of the MVA-BN vaccine, as it ensured protection of individuals of various age groups from MPOX infection (Stittelaar et al., 2005; von Krempelhuber et al., 2010).

An analysis of adverse events, reported from the surveillance systems of America, Australia, Canada, and the Netherlands, revealed no unexpected adverse events following immunization related to MVA-BN vaccine (Muller et al., 2024; Duffy et al., 2022; van der Boom and van Hunsel, 2023).

JYNNEOS' safety was evaluated in more than 7,800 individuals (Organization, 2023)[9]. A case-control study estimated the vaccine's effectiveness as 36% after a single dose and 66% after two doses (Deputy et al., 2023). According to another study in the German population, a single dose of the MVA-BN vaccine's effectiveness was 84.1% in people without HIV compared to 34.9% in people living with HIV (PLHIV) (Hillus et al., 2025).

In a cohort of 849 healthcare workers from the DRC, who were vaccinated with two doses of MVA-BN, most of Congolese participants remained seropositive (Priyamvada et al., 2022) 2 years after the vaccination. A study by Berry et al. (2024) estimated that the vaccine's effectiveness remains >59% for up to 10 years, even after a single dose. MVA-BN is well tolerated in children, as none of the 87 English children (median age, 5 years old), who received one MVA-BN dose in a study conducted from 1 June to 30 November 2022, experienced serious adverse events nor developed MPOX disease after vaccination. Among survey respondents, 36% reported no symptoms, 40% reported only injection-site reactions, and 24% experienced systemic symptoms with or without local reactions (Ladhani et al., 2023). Furthermore, in October 2024, Bavarian Nordic announced a clinical trial to expand the vaccine's approval to children aged from 2 to 11 (Bavarian Nordic, 2024).

In a pivotal Phase 3 randomized clinical trial, MVA-BN showed a more favorable safety profile compared to ACAM 2000 (Pittman et al., 2019). Nevertheless, a single dose of MVA-BN was shown to induce low titers of VACV-specific and MPVX-specific neutralizing antibody titers compared to a single dose of ACAM 2000 (Mason et al., 2024). However, the vaccine's efficacy against MPOX infections for one dose of MVA-BN suggests high effectiveness.

A recent study suggests that smallpox vaccination does not provide full protection against MPOX during the ongoing outbreak (Clinical Trials, 2024), and ACAM2000 has been linked to significant adverse events, underscoring the need for the development of MPOX-specific vaccines (EPAR, 2024). The clinical studies related to MPOX vaccines are summarized and illustrated in Table 3.

The new generation of MPOX vaccines and prospects

In spite of the important role of smallpox vaccines of the second and third generation against MPXV infection, there are issues in vaccine supply and uncertainty about cross-protection to more virulent strains (Mucker et al., 2024).

Due to the potential accidental release of VARV or the use of other pathogenic OPXVs as biological weapons, a large number of new vaccine platforms, using novel recombinant DNA and mRNA-LNP vaccine technologies, have been developed in the last decades (Rappuoli et al., 2021). Unlike attenuated vaccines, that contain the complete pathogen, these platforms were designed to deliver only the specific pathogen antigens, and provided a similar humoral and cellular immunity as live-attenuated vaccines tested in both mice and NHPs (Fogg et al., 2004).

Despite the complexity of the VACV proteome, that encodes approximately 200 proteins, several proteins have been identified on the surfaces of the MV and EV infectious forms of OPXVs as vaccine targets for MPOX, including the MV proteins encoded by L1R and A27L genes and the EV proteins encoded by B5R and A33R genes (Hooper et al., 2003). These immunogens can be expressed by recombinant DNA, protein-based subunits, or mRNA-lipid nanoparticle (LNP) vaccines.

Several studies have been performed by a number of groups applying these vaccine platforms. Golden et al. prepared a genetic vaccine, by combining two MV (L1 and A27) and two EV (A33 and B5) VACV antigens (Golden et al., 2011). The serum of NHP or rabbits immunized with this DNA vaccine

9 https://www.who.int/groups/global-advisory-committee-on-vaccine-safety/topics/mpox#:~:text=In%20conclusion%20in%202022%2C%20after,profile%20reported%20in%20clinical%20trials.

TABLE 3 Clinical studies related to MPOX vaccines.

Author	Country	Trial number	Vaccine	Study design	Individuals enrolled	Study population	Findings summary
Pittman et al.	USA 2019	NCT01913353	MVA-BN ACAM2000	Open-label, randomized trial	443	Healthy individuals aged 18–42.	MVA-BN displayed favorable safety profile compared to ACAM 2000
FDA	USA 2022	NA	ACAM2000 Dryvax	Multi-center, randomized controlled trial	2684	Unvaccinated and vaccinated individuals against smallpox vaccine aged 18–84.	ACAM2000 is suitable as a booster dose for individuals previously vaccinated against smallpox
Priyamvada et al.	DRC 2022	NCT02977715	JYNNEOS	Clinical cohort	849	High risk individuals exposed to MPOX aged ≥18.	Most of Congolese individuals who received two doses of vaccine remained seropositive after 2 years
Deputy et al.	USA 2023	NA	JYNNEOS	Case-control study	1,146	High risk individuals exposed to MPOX aged ≥18.	The vaccine's effectiveness is 36% after a single dose and 66% after two doses
Ladhani et al.	UK 2023	NA	MVA-BN	Follow-up study	87	Children exposed to MPOX aged 0–16	No serious adverse events were reported
Okumura et al.	Japan 2024	JPRN-jRCT1031230137	LC16m8	Open label randomized trial	1,006	High risk individuals exposed to MPOX aged ≥18.	The effectiveness of LC16m8 in MPOX remains inconclusive, and vaccine's efficacy could not be measured
Bavarian Nordic	Denmark 2024	NCT06549530	MVA-BN	Ongoing clinical study	Ongoing	Healthy children aged 2–11	Data not yet available
Tomotsugu et al.	Colombia 2025	NCT06223919	LC16m8	Ongoing clinical study	Ongoing	High risk individuals exposed to MPOX.	Data not yet available
Hillus et al.	Germany 2025	NA	MVA-BN	Combined prospective and retrospective, multicenter, observational study	9,904	High risk individuals against MPOX infection aged ≥18	The MVA-BN vaccine's effectiveness by 14 days or later after a single dose was 84.1% in people without HIV compared to 34.9% in people with HIV.

NA, not available; USA, united states of america; UK, united kingdom; DRC, the democratic republic of congo; MVA-BN, Bavarian Nordic developed MVA under the brand name MVA-BN®.

could provide full protection in VACV intranasal murine challenge models. The cocktail of antibodies anti-MV and anti-EV could control the animal weight loss and the development of pox lesions and might replace vaccinia immune globulin used against the adverse effects of live VACV-based vaccines to treat OPXV diseases (Golden et al., 2011). More recently, Radaelli et al. evaluated in a murine model the preventive and protective activity of DNA recombinant genetic vaccines in combination with Fowlpox virus recombinants and/or purified recombinant proteins, all of them expressing the same antigens (VACV A33, B5, L1, and A27 proteins) using different *prime-boost* regimens, using either systemic and/or mucosal delivery (Radaelli et al., 2024). A single dose of any combined immunogen induced a very low antibody response. However, the double shot immunization *priming* with the DNA vaccine followed by two boosts with Fowlpox recombinants revealed high neutralizing antibody titers and recovery from challenge-induced weight loss in mice. This study provides important information on the putative efficacy of optimized vaccination protocols that may enhance immune responses.

Among these vaccine platforms, mRNA vaccines are considered a promising tool against MPOX (Monath et al., 2004). Mucker et al. demonstrated the efficacy of mRNA-1769 vaccine in an NHP model of lethal MPXV infection, that presented fewer MPXV lesions and a lower viral load in the blood than strain-572 MVA, although not identical to the licensed MVA-BN vaccine against MPOX (Mayer et al., 2024).

Up to now, there are two mRNA vaccines in a Phase I/II clinical trial, including BioNTech's BNT166a (NCT05988203) and Moderna's mRNA-1769 (NCT05995275) (Organization, 2024f)[10]. The results of these experiments may be highly

10 https://www.who.int/publications/m/item/mpox-vaccine-tracker—list-of-vaccine-candidates-in-research—development

relevant for Public Health to control current and future MPXV outbreaks.

However, these vaccine platforms have some limitations, such as lower immunogenicity, lower stability, a need for an advanced delivery system, and the inflammatory nature of mRNA vaccines. As mRNA vaccines require advanced infrastructures and ultra-low temperature storage, the large-scale production and efficient distribution may limit their accessibility in low-income countries.

Several advanced strategies are employed to address these limitations. Immunostimulatory sequences are inserted in DNA vaccines, such as cytosine-phosphate-guanine dinucleotide (CpG) and double-stranded RNA (dsRNA) motifs, that can significantly boost their immunogenicity by triggering a more robust and specific immune response (Wu et al., 2011). Modifying the 5′ and 3′ untranslated regions (UTR) and encapsulation of mRNA into liposomes, as well as the use of an electroporation delivery system for DNA vaccines, are being used to enhance the stability and cellular uptake (Tan et al., 2023; Cheng et al., 2023).

Several studies, evaluating the integration of DNA vaccines into the host genome in murine models, have reported a minimal frequency of integration. These studies indicate that the risk exists, but it is relatively low (Ledwith et al., 2000). However, in the future, those vaccines must be evaluated in various animal models, and not only in mice (Guarner et al., 2004; Aid et al., 2023; Falendysz et al., 2023; Li et al., 2024). One potential strategy in MPXV vaccine development is the combination of DNA vaccines with recombinant viruses to enhance the immune response, as described by Radaelli et al. (2024). It is also important to develop a vaccine that provides a robust immunity across the diverse MPXV clades, as seen in other vaccines such as Bimervax (PHH-1V), which targets the alpha and beta variants of SARS-CoV-2 (Leal et al., 2023; England et al., 2024).

These approaches, supported by advances in immunoinformatics for epitope optimization, may revolutionize MPOX-specific vaccine design (Bhattacharya et al., 2022).

Conclusion

The continuous evolution of poxviruses requires sustained research and the development of new monkeypox-specific vaccines to prevent potential future outbreaks.

The transmissibility landscape of the recent MPOX outbreaks has changed over the last two decades. Although MPOX is not classified as a sexually transmitted infection, evidence suggests that direct sexual contact may represent the primary route of acquisition.

Vaccines are one of the most effective preventive measures to rapidly respond to new poxvirus outbreaks. WHO and CDC suggest the use of smallpox vaccines of the second and third generations against MPXV infection, such as ACAM 2000, MVA-BN (also known as IMVANEX/IMVAMUNE/JYNNEOS), and LC16m8 (Organization, 2022)[2].

Studies have revealed that the MVA-BN vaccine has a better safety profile than ACAM 2000. Moreover, the MVA-BN vaccine's effectiveness remains >59% for up to 10 years, even after a single dose (Berry et al., 2024) whereas clinical studies with LC16m8 are limited.

Public perception is important, as vaccine hesitancy, fueled by misinformation and distrust, can jeopardize vaccination campaigns. Studies have reported high rates of MPOX vaccine acceptance and uptake among high-risk groups such as the LGBTQI+ community, healthcare workers, people living with HIV, men who have sex with men (MSM), and people with pre-exposure prophylaxis. Therefore, health authorities should develop and implement tailored public messages targeting specific groups.

Although health authorities such as FDA, CDC, and WHO have expanded MPOX vaccination recommendations to various target groups, high costs, low availability, and poor accessibility limit the equal geographic distribution of MPOX vaccines.

Additionally, recent studies indicate that these vaccines do not provide complete protection (Clinical Trials, 2024), and a lack of data on the efficacy against diverse strains of MPXV was also reported.

Given the limitations of the smallpox vaccine used against MPXV, this review emphasizes the urgent need to develop new MPOX-specific vaccine designs, applying the latest scientific strategies, such as electroporation delivery systems for DNA vaccines, encapsulation of mRNA vaccines in lipid nanoparticles (LNPs), and different prime-boost immunization protocols.

Two mRNA vaccines, BioNTech's BNT166a (NCT05988203) and Moderna's mRNA-1769 (NCT05995275), are currently in a Phase I/II clinical trial (Organization, 2024a)[10].

However, these vaccine platforms may represent limitations related to the manufacturing process and logistical factors. Large-scale production and efficient distribution of vaccines are essential, especially for mRNA vaccines that necessitate advanced infrastructure and ultra-low temperature storage, may limit their availability and accessibility in low-income countries.

Thus, it is important to ensure vaccine efficacy and safety, as well as efficient population immunization, vaccine accessibility, communication, and education efforts to raise awareness about their safety and effectiveness, to protect against future infectious disease outbreaks.

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AM and IM, conceptualization, writing and original draft preparation, review and editing, AR, CM, EH, and CZ,

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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