**SEMINAR: MONKEYPOX**

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**ABSTRACT**

Monkeypox is a zoonotic illness caused by the monkeypox virus (MPXV), an *Orthopoxvirus* in the same genus as variola, vaccinia and cowpox viruses. Since the detection of the first human case in the Democratic Republic of Congo (former Zaire) in 1970, the disease has caused sporadic infections and outbreaks, mainly restricted to some countries in West and Central Africa. In July 2022, the World Health Organization (WHO) declared monkeypox as a public health emergency of international concern on account of the unprecedented global spread of the disease outside previously endemic countries in Africa and the need for global solidarity to address the previously neglected disease. The 2022 outbreak has been primarily associated with close intimate contact, including sexual activity, and most cases have been diagnosed among men who have sex with men, which often present with novel epidemiological and clinical characteristics. In the 2022 outbreak, the incubation period ranges 7 to 10 days, most patients present with a systemic illness that includes fever and myalgia and a characteristic rash with papules that evolve to vesicles, pustules, and crusts in the genital, anal or oral regions and often involve the mucosa. Complications that require medical treatment, such as intravenous antiviral therapy, antibacterials, and pain control, occur in up to 40% of patients and include rectal pain, odynophagia, penile oedema, and skin and anorectal abscess. Most patients have a self-limited illness; between 1 to 13% require hospitalization, and the case fatality rate is below 0.1%. The diagnosis can be made through the demonstration of O*rthopoxvirus* DNA by polymerase chain reaction from lesions or body fluid swabs. Patients with severe manifestations and those at risk of severe disease (e.g., immunosuppressed people) may benefit from antiviral treatment (e.g., tecovirimat). The current strategy for postexposure prophylaxis or preexposure prophylaxis for those at high risk is vaccination with the nonreplicating modified vaccinia Ankara (MVA). Antiviral treatment and vaccines are not yet available in endemic countries in Africa.

## INTRODUCTION

Monkeypox is a zoonotic viral infection caused by the monkeypox virus (MPXV) that results in a rash similar to that of smallpox. However, person-to-person spread beyond immediate close contacts and case-fatality rate are significantly lower in monkeypox than in smallpox infection.

Monkeypox was first described in 1958 among monkeys shipped from Singapore to Denmark.[1] During the following decade, additional outbreaks were reported in captive monkeys in the United States, the Netherlands and France.[2] The first case of monkeypox infection in humans was reported in 1970 in the Democratic Republic of the Congo (DRC), formerly Zaire, in a 9-month-old boy who was the only member of his family without a smallpox vaccination. A prior vaccination against smallpox was estimated to be 85% effective in preventing monkeypox,[3] although the long-term efficacy of smallpox vaccination is unclear.[4]

After the first human case, sporadic outbreaks were reported in some countries in West and Central Africa, mainly among children in rural rainforest areas. Based on clinical presentation and genomic sequencing results, MPXV isolates were classified into two clades. Between 1981 and 2017, MPXV clade 1 caused several outbreaks in DRC, with high fatality rates (1-12%).[5–11] Most of these cases were not laboratory confirmed due to lack of local diagnostic infrastructure and because most patients lived in difficult-to-reach rural settings, as well as challenges associated with civil unrest and the existing health system. During this period, very few human monkeypox cases were reported in West Africa, but in 2017, Nigeria experienced a large outbreak with 122 confirmed cases of the MPXV clade 2.[12–14] The progressive rise of cases in DRC and the reemergence of monkeypox in Nigeria in 2017 were attributed to the discontinuation of smallpox vaccination in 1980, waning immunity, frequent use of bushmeat as a source of animal protein by the population, and the spreading urbanization with encroachment into forest and swamp areas.[15] Despite this concern, there was a global neglect of the African outbreaks and dearth of related research.

Monkeypox generated some international attention in 2003, when 71 human cases were reported in the United States.[16–18] Between 2003 and 2022, a few travel-related cases were reported outside endemic countries in Europe, America, and Asia.[19–28] However, from May 13th 2022, a global outbreak consisting of community spread of a new MPXV lineage, clade 2b, in newly affected countries worldwide led to the declaration of a public health emergency of international concern (PHEIC). By mid-October 2022, over 70,000 cases in more than a hundred countries had been reported, [29–31] and several clinical studies have suggested that the disease has novel epidemiological and clinical characteristics.[32–35]

In this seminar, we discuss the virology, epidemiology, clinical presentation, treatment, and prevention of monkeypox in light of the 2022 global outbreak.

## VIROLOGY

Monkeypox is an *Orthopoxvirus* (double-stranded DNA virus) in the same family as the variola virus (the causative agent of smallpox), vaccinia virus (the virus used in the smallpox vaccine), and cowpox virus. Electron microscopy of cells infected with MPXV shows a brick-like virion ranging from 200 to 250 nm, indistinguishable from the virions of variola or vaccinia viruses.

The monkeypox genome is large, with about 200 kilobase pairs, and encodes approximately 190 proteins to build viral particles and modulate numerous host processes. Two distinct clades of monkeypox that show ~0.5% genomic sequence difference had been historically identified in different geographic regions of Africa.[36] Clade 1, which has case fatality rates of 1%-12%,[5–11,37] is usually responsible for disease in Central Africa and the Congo basin whereas clade 2, which is less virulent, with case fatality rates below 0.1%, is found in West Africa.[36,38,39] The genomic differences between clade 1 and 2 viruses occur in regions which encode for important virulence genes[40] and likely explain the differences in clinical severity. For example, the gene encoding a complement control protein (CCP) that prevents initiation of the complement pathway is missing in clade 2 virus strains, and animal models of monkeypox using the clade 1 virus with a CCP deletion led to reduced morbidity and mortality in prairie dogs.[41]

A new lineage B.1 classified as clade 2b for its close relationship to clade 2, has been identified in the 2022 global outbreak.[42,43] Unlike RNA viruses, the double-stranded DNA of orthopoxviruses is very stable, and their DNA polymerase has a proofreading exonuclease activity, resulting in a low mutation rate (1-2 nucleotide changes per year). The new B.1 lineage has been associated with strains circulating in Nigeria during the 2017 outbreak;[12] however, there has been a divergence of up to 50 single nucleotide polymorphisms (especially APOBEC3-related mutations), which represents a mutation rate 6-to-12 times higher than previously estimated.[43] Since the APOBEC3 human protein serves as a cellular defense mechanism by introducing errors into the viral genome, mutations of this type are indicative of a large amount of human-to-human transmission. One key question is how these variations affect MPXV transmissibility, virulence, and human adaptation.

## EPIDEMIOLOGY

Within the decade following the first case in humans in 1970, 59 cases of human monkeypox were reported in West Africa and Central Africa;[5–7] with a mortality rate of 17% in children under 10 years old.[8,37] After smallpox eradication and subsequent discontinuation of routine smallpox immunization in 1980,[44] the World Health Organization (WHO) monitored human monkeypox cases with the concern that lower immunity levels to smallpox would increase population susceptibility to MPXV.[45] From 2000 to 2015, there were three spatiotemporal clusters indicative of outbreaks of suspected monkeypox cases in the DRC,[9] including 760 laboratory-confirmed cases between 2005 and 2007.[10] A 20-fold increase was reported between 1981-86 (0.72 per 10,000 population) and 2006–07 (14.42 per 10,000).

Similarly, an increase in monkeypox cases in Nigeria has been reported since 2017,[12] after nearly 40 years of no reported cases.

According to a 2022 WHO report, monkeypox was considered endemic in several African countries, including Benin, Cameroon, the Central African Republic, DRC, Gabon, Ghana (identified in animals only), Ivory Coast, Liberia, Nigeria, Sierra Leone, and South Sudan. Between January and May 2022, DRC was the country that reported the greatest number of suspected monkeypox cases with 1,284 cases and 58 deaths.[46] However, there is a considerable and regrettable gap between confirmed and suspected cases, which indicates an inadequate laboratory testing capacity.

Aside from the general increasing trend in endemic countries, several sporadic cases and outbreaks of monkeypox have been reported in non-endemic countries (e.g., UK, US, Israel, Singapore) after travel to or animal importation from endemic areas.[18–20,27,28] In 2003, 71 cases (35 laboratory-confirmed) of human infection in the United States were related to prairie dogs that had been in contact with African rodents.[16,17,21] Between 2018 and 2021, seven cases of monkeypox were diagnosed in the United Kingdom, four of them related to travel from endemic countries.[23,24] In July 2021, two returning travelers from Nigeria were diagnosed with monkeypox in Texas and Maryland, respectively.[25,26]

In the 2022 global outbreak, the first cases of monkeypox were reported to the WHO in May;[47] since then, the number of cases has continued to increase worldwide. Many early cases occurred in people who had attended an international pride event held on the Spanish island of Gran Canaria, which linked to transmission chains in several European countries.[48–51] However, by the end of May, locally acquired infections and community transmission became predominant in all affected countries.[48] On July 23, 2022, the WHO declared this outbreak of monkeypox a public health emergency of international concern.[52]

## PATHOGENESIS

Monkeypox viruses can enter the host via the respiratory or skin route (FIGURE 1).[53,54] The route of entry and the clade of MPXV may affect how the illness manifests.

In the respiratory tract, MPXV can infect airway epithelial cells, while in the skin the virus infects keratinocytes, fibroblasts and endothelial cells establishing productive and cytopathic infection.[55,56] Moreover, antigen presenting cells such as macrophages, dendritic cells, and in the skin, Langerhans cells, are infected abortively, allowing them to survive long enough to carry antigens to draining lymph nodes. Viral replication, gene expression, and virion assembly in the host cell cytoplasm results in mature virions (MV) with a single lipid membrane, followed by the release of extracellular virions (EV) with an additional envelope.[57] These are two antigenically distinct forms which contain 25 or 6 surface proteins, respectively.

MPXV spreads from the initial site of infection to draining lymph nodes by migration of antigen presenting cells and by direct viral access to lymphatic vessels. After initial replication in the lymph nodes resulting in a low-grade primary viraemia, MPXV may target other large organs, spleen and liver, where it amplifies and results in a second major viraemia wave that could then allow the virus to further spread to distant organs such as the lung, kidneys, intestines and skin.

In the nonhuman primate models of respiratory-acquired clade-1 MPXV, [58–60] the virus replicates in the respiratory epithelium during the incubation period (post-challenge day 4),[59] then it spreads to the regional lymph nodes and lymphoid organs, including the tonsils, spleen, liver, and colon, where it amplifies up to day 6. Finally, the virus is detected in the blood on day 8, with increasing concentration through day 10, along with widespread lesions in the skin and mucous membranes. Ulcerating lesions in the mouth and pharynx release large quantities of virus particles disseminated through large respiratory droplets.

The primate models of subcutaneous inoculation show viral replication only in the skin and lymphatic system, displaying mild, localized disease after clade 2 MPXV infection.[60] The respiratory, gastrointestinal and genito-urinary tracts may be affected after skin inoculation of clade 1 MPXV.[60] Data on skin inoculation in humans are limited to those obtained from vaccination with the vaccinia virus or variola virus (i.e., variolation), which resulted in locally-restricted lesions around the point of entry.[61] Similarly, during the 2022 outbreak, patients sexually acquiring the MPXV present with local anogenital lesions and some develop a limited number of distant lesions (face, limbs, and trunk), but extensive disseminated skin lesions are rare. [32–35]

Histopathologic analysis of skin lesions in the vesicular stage may reveal ballooning degeneration of keratinocytes, prominent spongiosis, dermal edema, and acute inflammation.[62] In the pustule stage, apoptotic keratinocyte debris are predominant, along with a few viable keratinocytes, and inflammatory cells. Viable keratinocytes may be multinucleated or exhibit cytopathic damage, such as eosinophilic inclusion bodies, prominent nucleoli, and "ground glass" chromatin. Immunochemistry shows the virus in the cytoplasm of all keratinocytes within the affected —but not the non-affected— epidermis. The lymphocytic infiltrate is predominantly T-cell with CD4 and CD-8 positive elements.[63]

MPXV infection stimulates humoral and cellular immune responses that restrict viral replication and induce prolonged immunity in recovering patients.[64] Humoral immune response consists of *Orthopoxvirus*-specific IgM, IgG antibodies against multiple antigen targets with long-term persistence of residual IgG memory B cells [65] that protect from reinfection or from developing severe disease.[4,66] After vaccinia virus vaccination, specific memory B cells may last for decades, though only 50% of individuals have protective levels (i.e, neutralizing antibodies titers greater than 1:32) after 20 years. It is likely that cross-protective immunity against monkeypox may similarly wane over time. Cellular immune response is characterized by a rapid expansion of activated effector CD4+ and CD8+ T-cells, followed by a decrease over time, which tends to normalize after 12-20 days from symptom onset.[64,67]

After vaccinia virus vaccination, memory T cells were found to persist for up to 50 years (half-life of 8-15 years) but does not necessarily provide robust protection against MPXV as shown in numerous cases MPXV breakthrough infection in people vaccinated in childhood. Effector CD4+ T cells play a role in enhancing recall and differentiation of B cells into antibody-secreting cells, while CD8+ T cells kill infected macrophages to prevent viral spread. Most patients show specific T-cells able to produce several Th1 inflammatory cytokines (IFN-γ, IL-1β, IL-6, IL-8, TNF and MCP-1). HIV-infected individuals with high CD4+ T cell counts (> 350) also demonstrate a similar poxvirus-specific T-cell response, [4] but there are limited data in those with low CD4 counts (<350). CD4 depletion appears to decrease protective antibodies and increase infection severity in the animal model, which is concerning.[68,69]

**TRANSMISSION**

Animal-to-human and human-to-human transmission can occur. Although the MPXV has been isolated from several rodents and non-primate animals in Africa (such as rope squirrels, tree squirrels, Gambian rats, dormice, and monkeys),[70] the exact animal reservoir of the virus is still unknown. It has been suggested that monkeys and humans are incidental hosts of the infection.[45]

Animal-to-human transmissions could occur from noninvasive exposures to infected animals (e.g., touching the animal, cleaning its cage, hunting or processing its meat) and also from a bite or scratch from an infected animal, with the former having a remarkable lower risk of transmission.[71,72] Genomic analyses have revealed a number of zoonotic spillovers into human populations, suggesting that MPXV may persist in wildlife reservoirs while occasionally infecting humans.[73]

Human-to-human transmission of MPXV can occur by respiratory secretions, direct contact, vertical transmission, percutaneous transmission, or indirect contact through fomites.

Respiratory transmission occurs when large respiratory droplets from the transmitter host deposit on the mucous membranes of the mouth and nose of the recipient host. Prolonged face-to-face contact, such as household contact, may be required for transmission to occur via this route.[26,32–35,51,74] Activities resulting in resuspension of dried material from lesions (eg, shaking contaminated linens) may also present a risk and should be avoided.

Direct contact with infectious sores or lesions on mucous membranes is the primary mode of transmission during the 2022 outbreak. MPXV can spread during activities that include close, intimate contact with an infected individual, and transmission may be facilitated by a breach in the recipient's skin or mucosa, such as microscopic abrasions that occur during sexual activity.

During the 2022 outbreak whilst MPXV DNA is nearly always found in skin samples, it is detected less frequently and at lower viral loads in other body compartments. For example, it can be detected in only 60-70% of anus and throat samples, 50%

of semen and 20% of blood and urine samples. [32,34,75,76] Moreover, the viral load in skin samples is higher by about two orders of magnitude compared to that in other body locations (viral load 10^6 vs 10^4 copies/ml; Ct values ~ 22 vs ~ 28).[75–78] In addition, evidence of replication-competent virus isolation has been reported more frequently from skin samples than from throat swabs, and semen samples.[76,78] Altogether these data support that intimate sexual contact is the main route of transmission, with the respiratory route playing a less important role.[32–35,51]

The period of infectiousness lasts from the onset of clinical manifestations until all skin lesions have scabbed over and re-epithelialization has occurred. Viral DNA is detectable by qPCR for a median time of 25 days in the skin, 16 days in the pharynx, 14 days in the rectum, 11 days in semen, and 5 days in blood; and it would take until day 41 for 90% of cases to have undetectable viral DNA levels in skin lesions.[78] Semen is unlikely to represent a major potential source of transmission during the course of disease or after complete recovery, as previously observed in other zoonotic viruses, because viral loads in semen are generally low and viral clearance is fast.[78]

Vertical transmission to the fetus can occur, sometimes leading to congenital monkeypox, although the risk at different stages of pregnancy has not been determined.[70] Among four pregnant women with monkeypox in the DRC, one had a healthy infant, two miscarried, and one had a fetal death with diffuse maculopapular skin lesions, consistent with vertical transmission.[79] At least, twelve pregnant women were infected during the 2022 outbreak, but vertical transmission was not observed in any case.[80] This difference may be partly related to the greater invasiveness of clade 1 compared to clade 2.

Percutaneous transmission has been reported after needlestick injuries from supplies used to collect cutaneous lesion samples.[81,82] The monkeypox lesions appeared at the site of the needlestick. The recommendation is against using sharp instruments to opening or aspirating monkeypox lesions and recapping used needles because of the risk for sharps injuries.

Environmental contamination of the household of monkeypox cases and patient care environment with MPXV DNA has been reported, including detection of replication-competent virus.[83–85] Investigations in respiratory isolation rooms of a hospital found viral DNA in rooms, bathrooms, anterooms, health care workers' PPEs, and non-touch surfaces (e.g., >1.5 meters from the bed).[86] It is unclear whether indirect contact with fomites is a frequent transmission pathway; however, the widespread surface contamination of the patient care environment calls for a systematic approach to surface cleaning, and appropriate use of PPEs by health-care workers.

Risks of transmission may vary in different settings, including households, congregated settings, health-care facilities and in the community. Prior to the 2022 outbreak, transmission occurred mainly within the household, and sustained human-to-human spread was rare. In one report from the DRC, the secondary attack rate in households was as high as 9%.[87] Additionally, monkeypox outbreaks

had been described in congregate living situations, such as prisons.[88] By contrast, household transmission has been rare during the 2022 outbreak, accounting for only 0.6%-to-3% of cases,[32,34] including several pediatric cases and one neonatal case who lived in a home with an infected adult.[89,90] Most infections in 2022 have been associated with community transmission with an estimated reproductive number (Ro) ranging from 1.40 to 1.80, which implies a potential for sustainable local transmission.[91] Healthcare-associated transmission of monkeypox had been reported in a dozen of cases in Africa,[92] and in one case outside endemic regions prior to May 2022.[23] The risk of transmission during the 2022 outbreak has been low, with only a small number of transmission events reported following exposure to fomites or needlestick injuries.[81,82,93,94]

Subclinical or asymptomatic monkeypox infection is considered to be rare. However, seroepidemiological studies in Africa[3,95] and retrospective PCR detection in male sexual health clinic attendees in France[96] and Belgium [97] suggest that some patients may have asymptomatic infection. The potential for transmission from an individual with an asymptomatic infection is uncertain.

**POPULATION AT RISK**

Historical risk factors for acquiring the infection in African countries include living in forested areas (specifically near sites habitable to squirrels), living in a home with monkeypox, male sex, and age <15 years.[10,11] During 2022 most patients diagnosed with monkeypox have been identified among men who have sex with men (98% of the patients in a report of 528 cases from 16 countries),[32] and many reported high-risk sexual behavior as a potential risk factor. Some of the patients have reported having multiple or anonymous sexual partners in the previous two weeks, attending 'sex-on-premises' venues (e.g., saunas or bathhouses) or 'group sex' sessions, and using recreational drugs during sex. Concomitant sexually transmitted infections have been reported in 16-to-29% of individuals tested in the published cohorts,[32–35] with gonorrhea, chlamydia, and syphilis being the most common infections. A substantial part (i.e., 33-to-42%) of patients infected with MPXV are on pre-exposure prophylaxis to prevent acquiring HIV (i.e. sexually active HIV-negative adults),[32,98] and a high percentage are people living with HIV (36-to-42%).[32–34] However, it is not yet known whether HIV infection affects a person's risk of acquiring monkeypox, and future studies shall determine the relative contribution of sexual behavior, access to sexual health care, and biologic risk.

Groups at higher risk for progressing to severe disease in African countries were children, pregnant women, and immunocompromised individuals, including people living with HIV. [22,79,99] However, among the few children, adolescents, and pregnant women infected during the 2022 outbreak, there have been no severe cases or adverse neonatal outcomes.[80,89] The risk of severe disease in persons with HIV is unclear. Reports from individuals hospitalized during the 2017-18 outbreak in Nigeria indicate that persons with advanced and uncontrolled HIV infection might be at higher risk for severe, extensive, or prolonged monkeypox disease following infection.[12] In contrast, studies from European countries, where most patients are receiving effective antiretroviral therapy (and had undetectable HIV RNA) have

noted no evident excess in complications, hospitalizations, or deaths among persons with HIV infection and monkeypox to date.[32,34,100] Two studies including 72,[34] and 241[32] people living with HIV found no differences in clinical features or clinical outcomes between those with or without HIV.[34] Another publication found that although patients with HIV were more likely to have a higher rash burden, there was no association between HIV status and severe illness.[100]

## CLINICAL PRESENTATION

The clinical presentation of monkeypox cases associated with the 2022 outbreak[32–35] differs from previous reports.[12–14,22] (TABLE 1) Prior to the 2022 outbreak, the mean incubation period of MPXV infection was 5-to-13 days (range from 4-to-21 days).[22,101] Persons with a history of an animal bite or scratch may have a shorter incubation period than those with only tactile exposures (9 vs 13 days, respectively).[72] During the 2022 outbreak, the mean incubation period generally spans from 7-to-10 days following exposure.[32,34,102,103] The shorter incubation period may be due to the direct viral inoculation through sexual transmission.

Monkeypox has historically caused systemic symptoms attributable to a viremic phase of illness that typically occurs before the skin rash, lasts 1-to-5 days, and includes fever, myalgias, sore throat, and generalized lymphadenopathy.[14] In the 2022 outbreak, systemic symptoms are common and may occur before the rash (prodromal stage) or shortly after the rash appears (early clinical stage), although rashes without systemic illness have been reported.[34,101] In this outbreak, generalized swelling of the lymph nodes has not been commonly seen, although regional lymphadenopathy is often associated with the lymph catchment area of skin lesions.[34]

The skin eruption of monkeypox usually lasts for 2-to-3 weeks and progresses through several stages in the following order: macules of 2-to-5 mm that evolve into papules, vesicles, and then pseudo-pustules (i.e., papules that resemble pustules but contain solid debris instead of fluid or pus).[35] Lesions are well-circumscribed, and often develop umbilication (a central depression on the top of the lesion). Between 7 and 14 days after the rash begins the lesions crust over, dry up and fall off. The lesions typically appear and evolve simultaneously on any given part of the body.[32–35] Some cases diagnosed in the 2022 outbreak present lesions at different stages simultaneously,[100] and not all lesions progress from one phase to another in order.[33,34]

The number of skin lesions in MPXV infection may vary from a few to one thousand. In endemic regions, between 20-42% of patients present with >100 lesions,[12,14,22] and immunocompromised individuals may present with >1000 lesions. In the 2022 outbreak, most patients present with 1-to-20 skin lesions and cases with more than 100 lesions have been extremely rare (0-4%).[32–35] Single lesions are reported at a rate of 10%-to-12% and represent a significant risk of misdiagnosis, particularly with syphilis chancres.[32–34]

The rash location during previous outbreaks usually involved all parts of the body, with the face, trunk and limbs being the most affected (Fig2- A1,B1).[12–14,22] Conversely, during the 2022 outbreak, lesions were mainly located on the anogenital and perioral areas (Fig2- C1,D1,E1,F1,H1).[32–35] Genital lesions may present as one or two solitary lesions (Fig2- E1) or multiple lesions that affect the penis, scrotum, and pubis. Genital lesions are commonly accompanied by surrounding edema, which may progress to severe swelling of the penile glans or foreskin so that the retracted foreskin cannot be returned to its normal position (i.e., paraphimosis, Fig2- C1). In the perianal region, lesions can involve the buttocks, the anal margin (Fig2- D1), or the anorectal mucosa resulting on proctitis (i.e., rectal pain, pain on defecation, tenesmus, serosanguineous discharge, or bleeding). In the perioral region, lesions of the oral mucosa or lips are ulcers or crusts (Fig2- F1); lesions on the tongue are usually circular, white, and centrally depressed (Fig2- H1); and tonsillar lesions are painful and cause difficulty swallowing. Among cases that started with the rash presenting in the genital and oral areas (possibly the site of inoculation), some presented with subsequent spread to the face and trunk after; in other cases, the face or extremities have not been involved at all; and a few cases presented with solitary primary lesions in the face or fingers alone (Fig2– G1). Occasionally, secondary bacterial infections may cause abscesses, and coalescing lesions in any region can lead to large plaques or ulcerations.

Data gathered during the 2022 outbreak suggest a relationship between the location of the lesions and the site of inoculation. For instance, MSM who engage in anal-receptive sex present with proctitis more frequently than MSM who do not engage in anal-receptive sex.[34] Similarly, individuals reporting oral-receptive sex are more likely to present with tonsillitis.

The differential diagnosis should consider several skin infections, poxviruses infections, and sexually transmitted infections.[104] Varicella (chickenpox, Fig 2- A2) is the most likely diagnostic consideration in a patient presenting with a vesicular rash. Lesions appear in successive crops, normally coexist with lesions in different stages of development, and have a fluid content. *Herpes Simplex Virus* is a differential diagnosis of monkeypox in cases of perioral vesicles and anal ulcers (Fig2- F2). Also, impetigo (Fig2- C2) caused by infection with group A *Streptococcus* should be considered because of presentation with vesicles and pustules, although the characteristic golden crust suggests impetigo. Other non-infectious skin conditions may show a similar clinical picture including erythema multiforme, pompholyx and blistering diseases, such as dermatitis herpetiformis (Fig2- B2) associated with gluten-sensitive enteropathy, and aphthous ulcers (Fig2- H2).

The differential diagnosis should consider other poxviruses, including *Molluscum contagiosum* (Fig2- D2), which presents as single or multiple small papules with central umbilication (anogenital location in adults may be a confounding sign in the diagnosis); the replication-competent smallpox vaccine (ACAM2000), which may cause local skin lesions; and *Tanapox virus*, another African poxvirus that causes a febrile prodrome and skin lesions lasting several weeks without sequelae.[105] Orf and

bovine stomatitis (also caused by parapoxviruses) can produce localized skin lesions similar to those of monkeypox that are difficult to distinguish clinically (Fig2-G2), but a previous contact with sheep/goats or dairy cows, respectively, facilitates the differential diagnosis of these conditions.

Several sexually transmitted infections may present with signs and symptoms that overlap with those of monkeypox, including ulcerated lesions in primary syphilis (Fig2- E2), or lymphogranuloma venereum. In patients with proctitis, lymphogranuloma venereum, chlamydia, gonorrhea, and syphilis should be considered. Additionally, throat features of monkeypox may be mistaken for bacterial tonsillitis or primary syphilis.

## COMPLICATIONS

Severe complications of monkeypox infection reported historically include bronchopneumonia, sepsis, ocular infection, and neurological manifestations. Ocular involvement may consist of conjunctivitis and lesions on the eyelids,[106] as well as keratitis that may lead to corneal scarring and blindness.[107,108] A systematic review of studies from 2003 to 2021 reported neurological clinical features such as encephalitis, seizures, and confusion in about 2% of monkeypox cases.[109] Radiographic imaging of some cases of encephalitis are consistent with acute demyelinating encephalomyelitis but polymerase chain reaction of cerebrospinal fluid was negative for poxvirus DNA.[110] Low mood and other mental health issues, including a case of suicide, were reported in Nigeria,[111] although it is unclear whether these conditions are caused by the neurological tropism of MPXV, or if they are rather the result of stigma and isolation. In the 2022 outbreak, some novel, albeit rare severe complications, have been identified, including a few sporadic cases of myocarditis,[32] epiglottitis,[32] peritonsillar abscess,[107] rectal wall perforation with associated abscess in patients with proctitis,[33] and hemophagocytic lymphohistiocytosis. Other less severe but more commonly reported complications during 2022 include rectal pain or pain on defecation in 14-to-36% of cases;[32–35] difficulty swallowing in 5-to-14% of cases; inflammation of the penis in 8-to-16% of patients; and secondary bacterial infection in 3-to-4%. Finally, some patients may present with a morbilliform rash of pink-to-red spots on the trunk, arms, and legs following the administration of certain antibiotics (e.g., ampicillin or amoxicillin).[34]

During the 2022 outbreak, the hospitalization rate was low (1%-to-13%) and primarily aimed at isolating the patient[32,112] or providing adequate pain management and treating secondary infections.[32,113] Some nasal, conjunctival, corneal and perianal lesions have also led to hospitalization. Most individuals have experienced a self-limited disease with symptoms lasting from two to four weeks.

The historically reported case fatality rate associated with monkeypox infection is heterogeneous and varies from 1-12% in cases infected with clade 1 in Central Africa (deaths occurred primarily within the second week of illness)[12–14] to <0.1% in most outbreaks caused by clade 2a.[22,39] One exception was an outbreak of clade 2a in Nigeria (2017-18), which resulted in a fatality rate of 3.6%, with several

deaths among HIV-positive immunosuppressed individuals.[12] During the 2022 outbreak, the case fatality rate has been below 0.1% (26 deaths have been reported out of 71,096 cases up to Oct 10, 2022);[30] a few of them were related to encephalitis, though details are still emerging.[31]

## DIAGNOSTIC INVESTIGATIONS

Monkeypox is diagnosed based on suspected epidemiological and clinical findings and confirmed by nucleic acid amplification testing (NAAT), such as real-time or conventional polymerase chain reaction (PCR).

The diagnosis should be suspected in patients who present with an unexplained acute rash, including mucosal lesions in the conjunctiva, mouth, penis, vagina, or anorectal area, in patients who present with proctitis or lymphadenopathy, and in patients with flu-like symptoms after high-risk exposure.[114] A probable case is defined as someone with a clinical suspicion and epidemiological risk factors for infection (e.g., close or intimate contact with a case of monkeypox, part of a social network or community experiencing monkeypox activity, recent travel to areas where large outbreaks of monkeypox have been reported).

Patients with suspected or probable monkeypox should be offered NAAT testing, either generic to orthopoxvirus (OPXV) or specific to monkeypoxvirus (MPXV, preferable). The recommended specimen type for laboratory confirmation of monkeypox is skin lesion material, including swabs of lesion surface and/or exudate, and lesion crusts. Swabbing of the lesion should be done vigorously to ensure adequate viral DNA is collected. Unlike herpes simplex lesions, which are usually filled with fluid, MPXV lesions can be filled with solid material (as shown on the histopathology), making it difficult to unroof the lesions.[62] If there are multiple lesions, a few of them can be sampled. MPXV DNA testing of a throat swab may serve for research or epidemiologic purposes but is generally not used in the clinical setting. A positive NAAT result has been found in some blood specimens, but the clinical significance of viraemia is not well stablished.[24,78]

West and Central Africa face diagnostic challenges associated with limited access to NAAT platforms and cold chain requirements for sample preservation, which hamper case confirmation in remote areas and, therefore, an understanding of monkeypox epidemiology. In rural health clinics or regional hospitals in low-income countries without access to high-precision PCR instruments, Loop-Mediated Isothermal Amplification (LAMP) diagnostic assays may be a viable alternative.[115] This approach has been proposed as a point-of-care diagnostic tool for several neglected tropical diseases,[116] and showed promising results in other emerging viruses, such as Chikungunya[117] or hantaviruses causing hemorrhagic fevers.[118] Another approach for determining the etiology of an outbreak of rash illness involves deploying a field analytical facility to conduct PCR assays from blotting papers soaked in pustular exudate collected in remote areas.[119]

Serologic testing for MPXV can be used to support a diagnosis of monkeypox, particularly if NAAT testing cannot be performed. IgM detection from recent acutely ill patients (4 to 56 days after rash onset) or IgG in paired serum samples, collected at least 21 days apart, with the first being collected during the first week of illness, can aid diagnosis. Patients with monkeypox have detectable levels of anti-orthopoxvirus.[120]

Skin tissue biopsies are additional clinical samples that can be considered for diagnostic testing, only if clinically indicated. The histologic features of monkeypox are very similar to those of smallpox, vaccinia, and cowpox, but are useful to differentiate from other infections such as herpes simplex virus, and varicella.

In patients with proctitis, proctoscopy may show evidence of mucosal inflammation or friability. However, distinguishing monkeypox from other sexually transmitted infections is difficult with a visual inspection solely. In many cases, routine proctoscopy is not possible due to the severe pain. If rectal wall perforation is suspected, rectal magnetic resonance imaging should be performed as part of the evaluation. Some patients with a sore throat and impaired swallowing present with ulcerative pharyngitis or tonsillitis. The presence of these symptoms with a negative result in the Strep A rapid test suggests monkeypox as a possible cause.

**TREATMENT**

The approach to the clinical management of monkeypox includes both general supportive care and use of antivirals with activity against the MPVX. Approximately half of patients during the 2022 outbreak have required pain relief medications (e.g., for oral or anogenital lesions). Additionally, for the treatment of proctitis, stool softeners and topical lidocaine have been used, while for the treatment of pruritus, warm baths and oral antihistamines may prove beneficial. Supportive care requiring catheterization may be warranted for those who have or are at risk for dehydration, those who require more intensive pain management and those experiencing severe disease or complications. In patients with extensive anogenital ulcers or abscesses, drainage, debridement, and wound management are required; antibiotics are prescribed for secondary bacterial infections.

The efficacy of any antiviral agent against monkeypox infection has not been evaluated in randomized or non-randomized trials. Three antivirals, tecovirimat (intravenous and oral),[121–123] cidofovir (intravenous and topical),[124] and brincidofovir (oral), are potential options for treating monkeypox. These antivirals, approved for the treatment of smallpox based on animal models and safety data in healthy individuals, are expected to be effective against monkeypox as well. [125,126]

The preferred agent is tecovirimat, which is recommended in selected patients with severe illness (e.g., infection of the eye, encephalitis, severe proctitis) or those at risk of developing severe illness (children under eight, patients with atopic dermatitis, pregnant women, nursing mothers, and immunocompromised patients).[127,128] Tecovirimat inhibits an orthopoxvirus protein that is essential for dissemination within an infected host. Human randomized controlled studies on the

efficacy of tecovirimat are lacking. In non-human primate models of monkeypox disease tecovirimat improves survival, [121] and case studies in humans show anecdotal improvement of symptoms and viral clearance.[24,128,129] The medication is well tolerated with most commonly reported side effects being headache, nausea, and abdominal pain, although the adverse effects profile was similar to placebo in a trial involving 360 healthy volunteers.[121] As of now, tecovirimat is available only under emergency authorization; clinical trials evaluating its efficacy in the treatment of human monkeypox are in progress.[130–132]

Cidofovir competitively inhibits the incorporation of deoxycytidine triphosphate (dCTP) into viral DNA by the viral DNA polymerase, disrupting chain elongation. Cidofovir has in vitro activity against monkeypox[133] and has been shown to be effective against lethal monkeypox challenge in animal models.[124] The most important safety concern of cidofovir is the dose-dependent nephrotoxicity, which can be reduced by co-administration with probenecid.[126,134] Cidofovir is contraindicated in persons with proteinuria (2+ or greater) or baseline serum creatinine greater than 1.5 mg/dL, and it is not recommended during pregnancy due to embryotoxicity found in rats and rabbits and the lack of adequate studies in pregnant women.[135] The oral analog of cidofovir, brincidofovir, may offer a better renal safety profile than cidofovir; however, three patients in the UK experienced derangement in liver function requiring discontinuation of the treatment.[24] In the EU, the use of cidofovir from monkeypox is not authorized, while in the US, the CDC holds an expanded access protocol. In the UK, only tecovirimat is recommended on the grounds of insufficient evidence on cidofovir.

**VACCINATION**

The smallpox vaccine has gone through three generations of medical technology but only the second and third generation vaccines are currently licensed: ACAM2000, a replication-competent smallpox vaccine, and IMVANEX (also known as JYNNEOS or IMVAMUNE), a live, nonreplicating vaccine .[136] These can be used in two situations: pre-exposure to prevent infection and disease among those at high risk, or post-exposure (ideally within four days of exposure) to ameliorate infection and disease.[137]

First-generation vaccines (e.g., Dryvax) consist of live unattenuated vaccinia virus. Its effectiveness in preventing monkeypox was demonstrated in a surveillance study of human-to-human transmission of monkeypox in Africa.[3] Vaccination was associated with a remarkable reduction in the secondary attack rate (7.5% vs 1.3%) among 2,278 household contacts enrolled in the study. First-generation vaccines were withdrawn since they were manufactured using crude methods that would make them ineligible for licensure today.

ACAM2000 is a second generation, replication-competent vaccine derived from a single clonal viral isolate from Dryvax that exhibited reduced neurovirulence in animal models.[138] Immunogenicity testing showed non-inferiority to Dryvax and clinical trials showed a similar safety profile.[139] Because ACAM2000 is infectious, it can cause serious side-effects (i.e., progressive vaccinia, encephalitis and eczema

vaccinatum) and it is contraindicated in immunocompromised patients, those with skin disorders, underlying heart disease, and pregnant women.[137]

IMVANEX is a third-generation vaccine based on the replication-deficient modified vaccinia ankara (MVA). In the non-human primate model the vaccine elicits robust humoral and cellular immune responses,[140] along with clinical protection against severe monkeypox diseases and death,[141] but clinical efficacy data against monkeypox in humans is lacking. In human volunteers, peak neutralizing antibody titers after two doses of an MVA vaccine are similar to those seen after single dose ACAM200, but with no serious adverse events noted in clinical trials.[142] Because of their attenuated phenotype, these vaccines have an improved safety profile and can be administered to immunocompromised individuals. Third generation MVA vaccines are administered subcutaneously in two doses four weeks apart. However, owing to shortages in vaccine supplies, several countries have authorized intradermal administration, which requires one-fifth of the volume of the subcutaneous route, in individuals older than 18 years.[143,144] Intradermal administration is supported by extrapolations from other infections where the intradermal route enhances immunogenicity and phase 2 studies showing equivalent antibody responses with both MVA administration routes.[145] To reach larger population, some countries have adopted single dose intradermal administration.

Vaccination programs in newly affected countries have focused primarily on MSM, who are at the highest risk of acquiring monkeypox. Globally, vaccines are being administered primarily in North America and Europe, whereas African countries remain without access to vaccines. Although global alliances, such as GAVI or The Global Fund, emphasize the importance of increasing vaccine coverage in African countries, so far, supplies have been cornered by wealthy countries.[146]

**DISEASE CONTROL**

In endemic countries, such as DRC and Nigeria, the national health authorities developed comprehensive disease control plans.[147,148] This included targeted epidemiological investigations in high-risk areas, improved laboratory-based surveillance capacity, laboratory diagnostics, the development of regional capacities to implement effective responses at the local level, and enhanced research activities.[147] However, the absence of access to vaccines[149] precludes these countries from stopping the transmission or developing a clear strategy for immunization once these vaccines become available. To be effective, vaccination may need to target risk groups in forested areas of West and Central Africa where zoonotic spillover traditionally causes outbreaks. Additionally, understanding how emerging sexual transmission affects local epidemics is crucial.

Current guidance for the 2022 outbreak control does not support mass vaccination of the entire population and relies on surveillance, contact tracing, and vaccination of high-risk groups to control the monkeypox outbreak.[150] Therefore, newly affected countries will require intervention prioritization in key populations. Although early associations with MSM may have been influenced by social, environmental, or biological factors, the epidemiology of this emerging infection may change over

time.[151] Of particular interest are congregated settings, such as prisons, dormitories and schools, as well as sexual networks of heterosexual individuals. Based on the above recommendations, US and Europe have started mobilizing their stockpiles of smallpox vaccines. Conversely, the corresponding strategic supplies of the WHO for smallpox outbreaks in Africa are not yet being distributed.[149] Despite the enormous difference in access to vaccines, the WHO has explicitly committed with a unified response to the 2022 monkeypox emergency, including the discontinuation of distinguishing between endemic and non-endemic countries.[29]

At this time, it is uncertain whether the outbreak can be contained in certain countries. However, even if some countries in Europe and North America can eliminate MPXV, other countries in Africa will remain affected, which will not only be inequitable but also a threat to future outbreaks worldwide. Therefore, wealthy nations must do more to control the spread of the virus in African human populations. Moreover, MPXV is unlikely to be eradicated due to its wide host range and elusive animal reservoir, in contrast to its close relative Variola, which primarily causes infections in humans.[152] The animal reservoir likely lies in Africa; therefore, a clear OneHealth framework effort must be directed toward that region. MPXV may also expand its endemic range through reverse zoonosis within newly infected countries. The establishment of a reservoir of MPXV in a wild animal population (e.g., rodents) in a previously non-endemic region would make control and eradication much more challenging.

## CONTROVERSIES AND UNCERTAINTIES

Many aspects of the epidemiology of human monkeypox, especially relating to the natural reservoirs, modes of transmission and predictors of clinical disease course remain largely unknown (PANEL 1). The knowledge gaps must be addressed through further research and intervention, including topics relevant to African nations related to behavior change regarding animal exposure, and address knowledge gaps, misconceptions and stigma associated with monkeypox.

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## CONTRIBUTORS

OM, DO, BT, CG, JJM, MM, and CO planned, wrote and revised the text. OM wrote the first draft and led the work.

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**PANEL 1: UNANSWERED RESEARCH QUESTIONS**

**1) Virology**

- Has the virus genetically changed with mutations conferring and advantage over other forms of the virus?
- Does the B.1.lineage result in milder clinical presentation than prior lineages?
- Are the different complications associated to specific strains and what is the of role genomic variation?

**2) Pathogenesis**

- Is the pauci-symptomatic or localized presentation seen in the 2022 outbreak compared to the disseminated presentation of typical monkeypox disease associated with a lower degree of immune protection following infection?

**3) Transmission**

- How much does the respiratory route contribute to the spread of monkeypox infection in the 2022 outbreak?
- What is the relative transmissibility by type of sexual exposure?
- What is the extent to which and time in which viable virus remains present in different body fluids? Could it lead to asymptomatic carriage of the virus and the possibility of onward transmission?
- Can MPXV be lodged in privileged sites (the eyeball, synovial fluid, spinal cord or the testicles) and pass the virus to others long time after recovering?
- What is the extent to which viable replication competent virus persists in the environment.
- What role and how much does asymptomatic infection contribute to spread of the infection?

**4) Population at risk**

- Does HIV infection affect a person's risk of acquiring monkeypox and why are people with HIV overly represented in published cohorts?
-What are the risk factors for severe disease?

**5) Clinical presentation and complications**

-What is the precise cause of death among patients who die of MPXV infection?

**6) Diagnostics**

- Can adapted point of care diagnostics such as antigen detection based rapid tests circumvent the lack of access to NAAT in West and Central Africa?

**7) Treatment**

- What is the efficacy and optimal timing and duration of antiviral therapy?

**8) Vaccines**

- What is the effectiveness, optimal number of doses, optimal administration route, and time of protection of MPXV third generation vaccines?
- Does having been vaccinated against smallpox decades ago protect against monkeypox today?

**9) Disease control**

-Will the outbreak remain limited to certain risk groups, or will it bridge to new target populations? What are the populations at highest risk of MPXV transmission other than MSM?
- Is there community transmission going undetected?
- Is it feasible for certain countries to stop transmission and eliminate MPXV?
- Upon elimination in a given country, how likely is it that new outbreaks will occur due to imported cases from countries without the ability to eliminate the disease?

**PANEL 2: SEARCH STRATEGY AND SELECTION CRITERIA**

Information for this seminar was obtained from peer-reviewed articles (retrieved from PubMed by searching with the terms "monkeypox" and "smallpox"), releases from reference institutions (e.g., U.S. Food and Drug Administration, World Health Organization, and Centers for Disease Control and Prevention), and general books of clinical practice. Only pieces published in English were included; no time restrictions were applied.

**FIGURE LEGENDS**

**FIGURE 1:** Proposed mechanism of the spread of monkeypox virus throughout the body and relation to the transmission route.

Legend: The clinical presentation of monkeypox may be influenced by the microorganism virulence factors, the host immunity, and the transmission route. Compared to previous outbreaks, MPXV in the 2022 outbreak is thought to spread through close contact or sexual contact, causing predominantly localized lesions instead of extensive disseminated lesions. It is possible that the localized nature of the disease results in lower levels of viremia and consequently less virus in respiratory excretions. As the respiratory route becomes less important, transmission continues to occur through direct contact via dermal inoculation, perpetuating the cycle of clinical presentation and transmission. Nevertheless, MPXV in prior outbreaks, mainly from DRC, is thought to be transmitted primarily through the respiratory tract followed by disseminated disease. Both the dermal inoculation and respiratory routes could contribute to animal-to-human transmission.

**FIGURE 2:** Monkeypox clinical presentation and differential diagnosis

Legend. Credits are to Dimie Ogoina (A1), Fernando Gruber (A2), Cristina Galván (B1, B2, C2, D2, G2, H2), Adrià Mendoza (C1), José Miguel Cabrera (D1, F1, H1), Irene Fuertes (E1, G1), Martí Vall-Mayans (E2), Rosa Taberner (F2).

[A] Discrete rash on the thorax caused by monkeypox (Nigeria) and varicella (Spain); [B] A generalized monkeypox rash (DRC) and a blistering rash caused by Dermatitis Herpetiformis (Spain); [C] Localized monkeypox lesions causing penile edema (Spain) and impetigo associated with scabies (Malawi); [D] Localized perianal rash caused by monkeypox and Molluscum Contagiosum (both in Spain); [E] Solitary monkeypox genital ulcer and primary syphilis chancre (both in Spain); [F] Lip lesion caused by monkeypox and Herpes Simplex (both in Spain); [G] Hand lesions caused by monkeypox and Orf virus infection (both in Spain); [H] Monkeypox lesions on the tongue and aphthous ulcer on the labial mucosa (both in Spain).

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