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REVIEW

Monkeypox Virus Infections in Humans

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SUMMARY Human monkeypox is a viral zoonosis endemic to West and Central Africa that has recently generated increased interest and concern on a global scale as an emerging infectious disease threat in the midst of the slowly relenting COVID-2019 disease pandemic. The hallmark of infection is the development of a flu-like prodrome followed by the appearance of a smallpox-like exanthem. Precipitous person-to-person transmission of the virus among residents of 100

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countries where it is nonendemic has motivated the immediate and widespread implementation of public health countermeasures. In this review, we discuss the origins and virology of monkeypox virus, its link with smallpox eradication, its record of causing outbreaks of human disease in regions where it is endemic in wildlife, its association with outbreaks in areas where it is nonendemic, the clinical manifestations of disease, laboratory diagnostic methods, case management, public health interventions, and future directions.

KEYWORDS *Monkeypox virus*, monkeypox, *Orthopoxvirus*, virology, outbreak, public health, immunization, tecovirimat

INTRODUCTION

Monkeypox is a viral zoonosis of humans that had remained a neglected tropical disease in sub-Saharan Africa for over half a century until an initial cluster of infections involving dozens of United Kingdom residents was recognized and reported to local health authorities in May 2022, according to the World Health Organization (https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385). The emergence of this disease has been characterized by rapid international spread with a growing number of cases in new geographic areas, a preponderance of disease among members of a distinct social group, and a somewhat atypical clinical presentation, collectively being shaped in part by increased international travel, human behavior, and viral pathogenicity. In the regions of West and Central Africa where it is endemic, an increase in human case counts since the year 2005 has been fueled by climate change, deforestation, warfare, human migration, and decreased herd immunity from remote smallpox vaccination (1–3). In this review, we discuss the taxonomy and phylogeny of *Monkeypox virus*, its association with the smallpox vaccination campaign, its history of causing outbreaks of human disease in regions where it is endemic in wildlife, its association with outbreaks in areas where it is nonendemic, the clinical manifestations of disease, laboratory diagnostic methods, case management, public health interventions, and future directions.

TAXONOMY AND PHYLOGENY

Monkeypox virus is a member of a closely related group of large (220 to 450 nm by 140 to 260 nm), brick-shaped or ovoid, double-stranded DNA viruses in the *Orthopoxvirus* genus within the *Poxviridae* family, which includes *Akhemeta virus*, *Alaskapox virus*, *Camelpox virus*, *Cowpox virus*, *Variola virus* (the cause of smallpox), and *Vaccinia virus* (believed to be a hybrid of *Cowpox virus* and *Variola virus*) (4–8). *Variola virus* is the only member of this group that has no animal reservoir. Orthopoxviruses of animals that have not been associated with zoonotic transmission include *Abatino macacapox virus*, *Ectromelia virus* (the cause of mousepox), *Raccoonpox virus*, *Skunkpox virus*, *Taterapox virus* (the cause of gerbilpox), and *Volepox virus* (4).

The nomenclature of *Monkeypox virus* is currently in a state of flux. *Monkeypox virus* has historically been subclassified into two distinct genetic lineages or clades, namely, Central African and West African, the former being associated with an increased burden and severity of human disease in countries where it is endemic (9). To avoid socio-geographic discrimination, the World Health Organization (https://www.who.int/news/item/12-08-2022-monkeypox--experts-give-virus-variants-new-names) has renamed these clades using Roman numerals, with clades I and II representing the former Central African and West African clades, respectively, and recently a classification system has been proposed based on the PANGOLIN approach developed for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) sequences (10). Phylogenetic analyses of human *Monkeypox virus* isolates have tracked viral evolution over time (10–14). Host APOBEC3 cytidine deaminases, which convert cytosine to uracil in exogenous DNA, are antiviral immune factors produced by mammals and are believed to contribute to the emergence of new *Monkeypox virus* sublineages through incidental generation of nonsynonymous and nonsense single-nucleotide polymorphisms during viral replication (10, 12–16). Based on these observations, clade II has been subdivided into two clades, IIa and

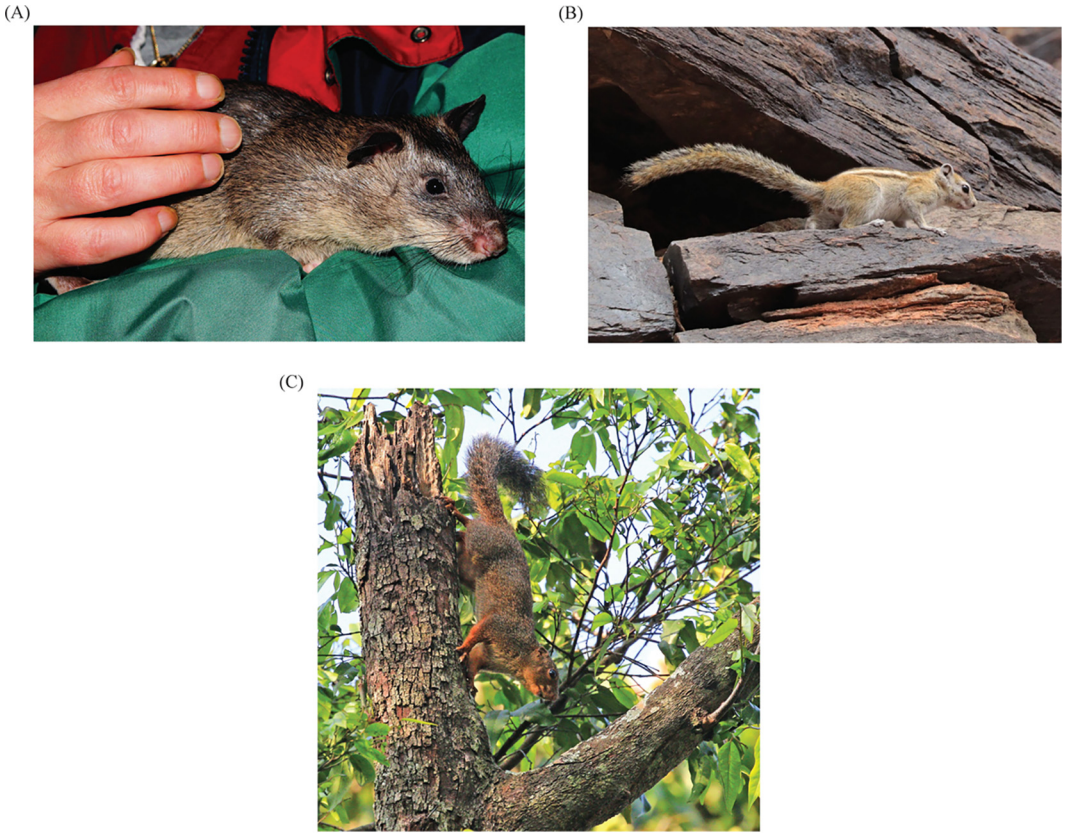

FIG 1 (A) Gambian giant rat (*Cricetomys gambianus*). (Reproduced from https://commons.wikimedia.org/wiki/File:Cricetomys_gambianus_Gambian_giant_Rat-1914404.jpg, by K. Paulick, available under the Creative Commons CC0 1.0 Universal Public Domain Dedication.) (B) Congo rope squirrel (*Funisciurus congicus*). (Reproduced from https://commons.wikimedia.org/wiki/File:Congo_rope_squirrel_%28Funisciurus_congicus%29.jpg, by C. J. Sharp, licensed under the Creative Commons Attribution-Share Alike 4.0 International license.) (C) Red-legged sun squirrel (*Heliosciurus rufobrachium*). (Reproduced from https://commons.wikimedia.org/wiki/File:Red-legged_sun_squirrel_%28Heliosciurus_rufobrachium%29_2.jpg, by C. J. Sharp, licensed under the Creative Commons Attribution-Share Alike 4.0 International license.)

IIb, with the latter being representative of strains associated with the 2022 human monkeypox virus outbreak in countries where it is not endemic (10).

DISCOVERY AND ENDEMICITY

Monkeypox virus was discovered in the summer and fall of 1958 as the cause of two outbreaks of a nonlethal smallpox-like skin disease of captive cynomolgus monkeys that were being used for polio vaccine manufacturing and research at the Statens Serum Institut in Copenhagen, Denmark (17). Outbreaks of monkeypox infection involving primates at research institutions in the Netherlands, United States, and France were subsequently reported in the late 1950s and 1960s (18–20). *Monkeypox virus* has historically been endemic only in the rainforests of Central and West Africa. According to the World Health Organization (https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385) and several published reports, countries in the African subcontinent where zoonotic transmission of *Monkeypox virus* has been confirmed include Cameroon, Central African Republic, Democratic Republic of the Congo, Gabon, Ivory Coast, Liberia, Nigeria, Republic of the Congo, Sierra Leone, and South Sudan, while only imported cases have been reported in Benin (1, 21–27). Although *Monkeypox virus* has been isolated from animals in Ghana, no known locally transmitted human cases have been reported from that country (28).

The name monkeypox is a misnomer, since arboreal African rodents, including Gambian pouched rats (*Cervicectomys gambianus*), rope squirrels (*Funisciurus* spp.), and red-legged sun squirrels (*Heliosciurus rufobrachium*) serve as the natural reservoir of the virus, while monkeys and other primates are believed to be accidental hosts (Fig. 1A, B, and

C) (21, 29–32). The environmental milieux is believed to play a role in the endemicity of *Monkeypox virus* in the African subcontinent. Ellis and colleagues reported that annual rainfall, seasonal temperatures, soil characteristics, and vegetation patterns are associated with the prevalence of *Monkeypox virus* infection (33). Molecular analysis of a large collection of preserved terrestrial *Funisciurus* spp. skin specimens housed at the American Natural History Museum in New York City, NY, USA and the Royal Museum for Central Africa in Flemish Brabant, Belgium, demonstrated that *Monkeypox virus* was circulating among reservoir animals in the late 19th century, and so human infections likely occurred prior to the discovery of the virus in 1958 (18, 34).

BREACHING THE ANIMAL-TO-HUMAN HOST SPECIES BARRIER

The first known case of human monkeypox infection was reported in September 1970 and involved a 9-month-old boy from the Democratic Republic of the Congo (DRC) who presented with a nonfatal smallpox-like illness characterized by the development of a centrifugal pox-like rash that resolved 2 weeks later (35). This child was never vaccinated against smallpox, although all other members of his family and virtually all residents of the village where he resided had previously been vaccinated, and there were no secondary cases of infection (35). *Monkeypox virus* was recovered in cell culture from the child's skin scabs that were sent to the World Health Organization (WHO) Smallpox Reference Centre (36). Unfortunately, the boy developed measles 2 months later and succumbed to that illness (35).

Shortly after the description of the first case, virologically and/or serologically confirmed cases of nonlethal human monkeypox infection were reported in West Africa (37). From October 1970 to May 1971, four Liberian children under the age of 10 years (three of whom were playmates who handled monkey carcasses), a 4-year-old Nigerian girl with no known animal exposure, and a previously immunized 24-year-old male from Sierra Leone who handled a deceased monkey had developed monkeypox infection (37, 38). Three of the children experienced a severe clinical course but eventually recovered (37, 38). All six of the pediatric cases were associated with a negative smallpox vaccination history, and all seven cases occurred in remote tropical rainforest villages where monkeys (which, at the time, were mistakenly believed to be the natural reservoir of the virus) were a regular part of the diet (37, 38). There were 24 susceptible (unvaccinated) household contacts, although none appeared to have developed the disease (37). However, one additional human case of monkeypox infection in this cluster that came to light during the WHO's 24th World Health Assembly in 1971 (https://apps.who.int/iris/bitstream/handle/10665/85833/Official_record193_eng.pdf?sequence=1&isAllowed=y) was reported from Nigeria and arose in the mother of one of the affected children (38).

FIVE DECADES OF HUMAN MONKEYPOX INFECTIONS IN AFRICA

Following the gradual cessation of the global smallpox vaccination campaign between the early 1970s and early 1980s and the eventual eradication of smallpox by 1977, case counts of human monkeypox infection in Africa steadily increased (39–43). From 1970 to 1979, a total of 47 cases of human monkeypox infection were reported in Central and Western Africa, including 38 in the DRC, four in Liberia, three in Nigeria, one in Sierra Leone, and one in the Ivory Coast (42). Three adults and one 8-year-old child from this cluster were vaccinated against smallpox (42). The age of affected individuals ranged from 7 months to 40 years, with a median age of 4 years, and over 80% of the cases occurred in children less than 10 years of age (42). There were eight (17%) attributable deaths due to monkeypox, all of which occurred in unvaccinated children between the ages of 7 months and 7 years, and all but one of the seven adults experienced mild to moderate disease (42). From 1980 to 1981, an additional 12 cases were reported in the DRC and other Central and West African countries, including Cameroon (42, 43).

In 1982, active monkeypox surveillance activities began in the DRC, leading to an even greater increase in case detection (41–43). During this time period, 386 cases occurred in the DRC, while only 18 were reported in other countries where the disease

TABLE 1 Distinguishing features of clade I and clade II monkeypox virus variants[a]

Characteristic	Clade I	Clade II
Endemicity	Cameroon, Central African Republic, Congo, DRC, Gabon, South Sudan	Benin, Cameroon, Ivory Coast, Liberia, Nigeria, and Sierra Leone
Severity	Usually moderate to severe	Usually mild to moderate
Household transmission rate	7.5–12.3%	0–3.3%
Mortality	10.6%	1–6%

[a]From references 1, 21–27, and 42.

is endemic, with the vast majority occurring in young children (40, 44). This age distribution implies that exposure to the virus was common, such that people in this region encountered it early in life. Monkeypox infections may, in some cases, be subclinical. Population-based seroprevalence studies conducted in the DRC, Ivory Coast, and Sierra Leone revealed that 15.4% of unvaccinated children less than 15 years of age had *Orthopoxvirus*-specific antibodies, with one-third of these individuals having no recollection of symptoms or evidence of a smallpox vaccine mark (40).

The onset of the AIDS epidemic in Africa in the early to mid-1980s prompted the WHO to gradually redirect its public health resources, leading to the eventual termination of the DRC monkeypox surveillance program in 1986 (40). During the following decade, confirmed case numbers of human monkeypox infection declined, with no cases being reported to WHO beyond 1992, until a cluster of 344 cases among a predominantly unvaccinated cohort in the DRC from 1996 to 1997 was described (31, 40). Outbreaks in the DRC have been commonplace since then, with over 1,000 cases per annum being reported since 2005 (1, 41, 44, 45). In contrast, case reports of human monkeypox infection from countries other than the DRC were infrequent between 1970 and 2016 (1). Nigeria experienced a complete hiatus of cases over a 39-year stretch until 2017, at which point a large country-wide outbreak of over 120 laboratory-confirmed or suspected infections involving clade II was recorded (1, 46–48). While increasing numbers of cases can reflect increasing surveillance efforts, and it is certainly true that cases and outbreaks have occurred in the absence of adequate surveillance to detect them, the increasing numbers of cases being reported from the African subcontinent do not appear to be solely a consequence of increase surveillance activities, and it is plausible that shifting patterns of land use and population density, among other factors including declining population immunity, are providing opportunities for more zoonotic transmission and larger resulting outbreaks (1, 27, 49). The distinguishing features of clade I and II monkeypox virus infections are summarized in Table 1, and the geographic distribution of monkeypox virus in Africa is shown in Fig. 2.

MECHANISM OF ZOONOTIC AND HUMAN-TO-HUMAN TRANSMISSION

Transmission of *Monkeypox virus* occurs through close physical contact with animals or humans, their body fluids, via contaminated droplet particles from respiratory secretions, or infected skin lesions, and indirectly by way of fomites (21, 27, 30, 47, 50–52). Unlike smallpox, airborne transmission of *Monkeypox virus* to humans has not been clearly demonstrated, with no expert consensus to date (53, 54). However, the UK Health Security Agency (https://www.gov.uk/guidance/high-consequence-infectious-diseases-hcid) considers *Monkeypox virus* clades I and IIa to be potentially airborne, while researchers at the UK National Health Service (NHS) have demonstrated evidence of aerosolization with clade IIb virus (55). Airborne transmission is theoretically possible, since infective *Monkeypox virus* aerosols can be artificially generated under controlled laboratory settings and by virtue of the recent observation that aerosolized virus is capable of causing the classical signs of monkeypox illness in research primates (56, 57). Percutaneous exposure, including animal bites, and consumption of animal meat are common modes of transmission in endemic areas (21, 47, 51, 52, 58). In humans, the rate of secondary infections among unvaccinated close contacts ranges from 7.5% to 12.3% within affected households and 3% for others (21, 42). Tertiary (infection acquired from a contact of a secondary case) and quaternary (infection acquired from a tertiary case) cases have been described among close contacts but rarely occur (59, 60). Among vaccinated contacts, the overall secondary attack rate is

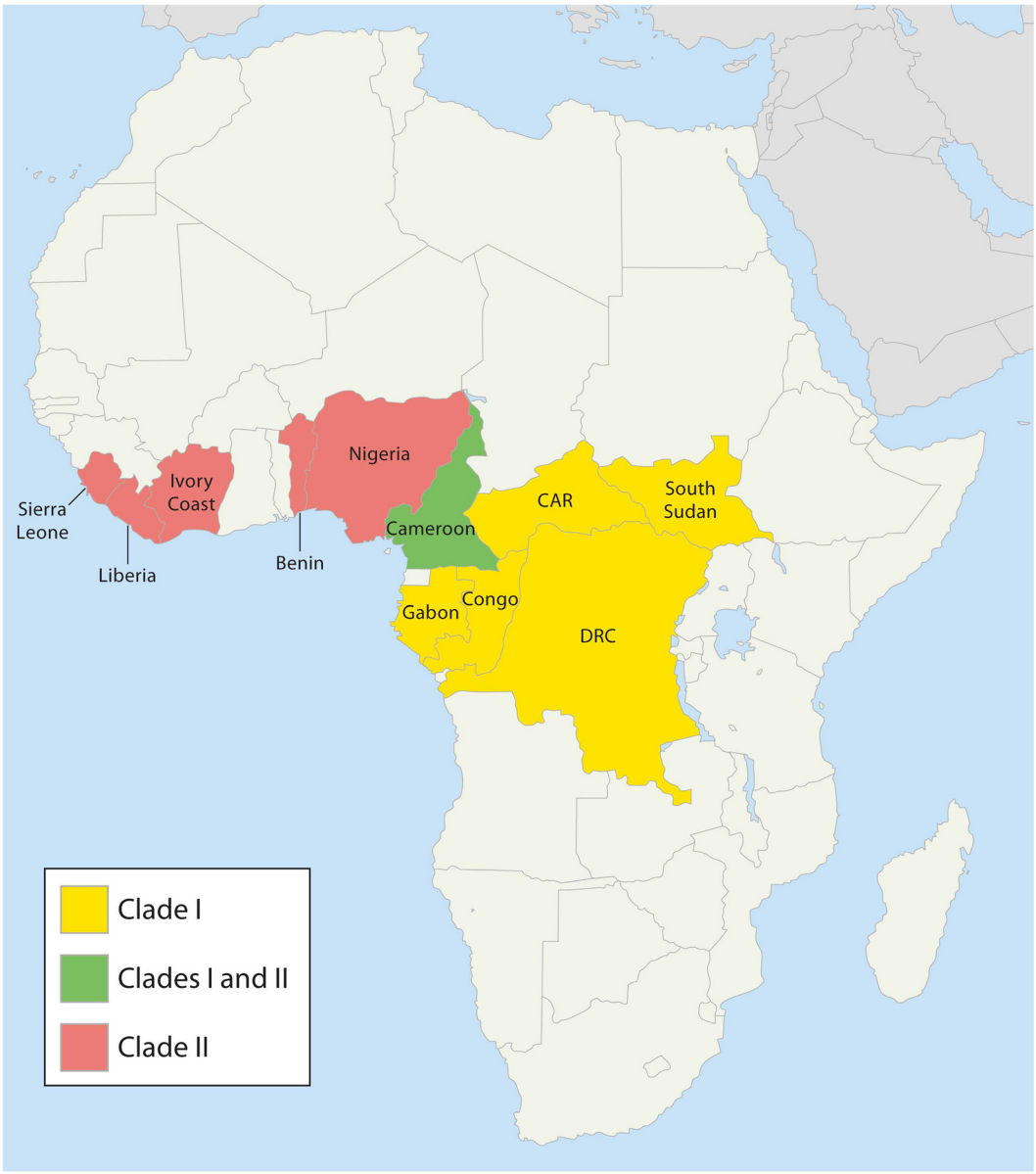

FIG 2 Clade-based geographic distribution of locally acquired or imported human monkeypox virus infections in African countries (yellow, clade I, formerly known as the Central African clade; red, clade II, formerly known as the West African clade; green, clades I and II). Only imported cases have been reported in Benin. In Ghana, no human cases have been reported, although clade II is endemic among terrestrial rodents in that country. CAR, Central African Republic; DRC, Democratic Republic of the Congo. (Adapted from https://commons.wikimedia.org/wiki/File:Africa_location_map_without_rivers.svg, by Eric Gaba, available under the terms of the GNU Free Documentation license, version 1.2.)

less than 1% (59). In contrast, the median secondary infection rate among presumably nonimmune household contacts was approximately 50% during a 2013 outbreak in the Bokungu region of the DRC (45). In the latter setting, secondary transmission occurred most commonly among individuals who shared a bed and among children who played with a case patient (45). Primary infections, in contrast, have almost always been associated with handling an infected animal rather than from incidental environmental exposure (45). More recently, the detection of *Monkeypox virus* DNA in semen among infected persons in countries where it is nonendemic, coupled with the subsequent recovery of culturable virus from seminal fluid, has sparked a high level of interest and debate over the role of sexual activity in person-to-person transmission of the disease (61–65). This hypothesis of sexual transmission is supported by European and American reports of severe proctitis following exposure to monkeypox virus among men who have sex with other men (MSM),

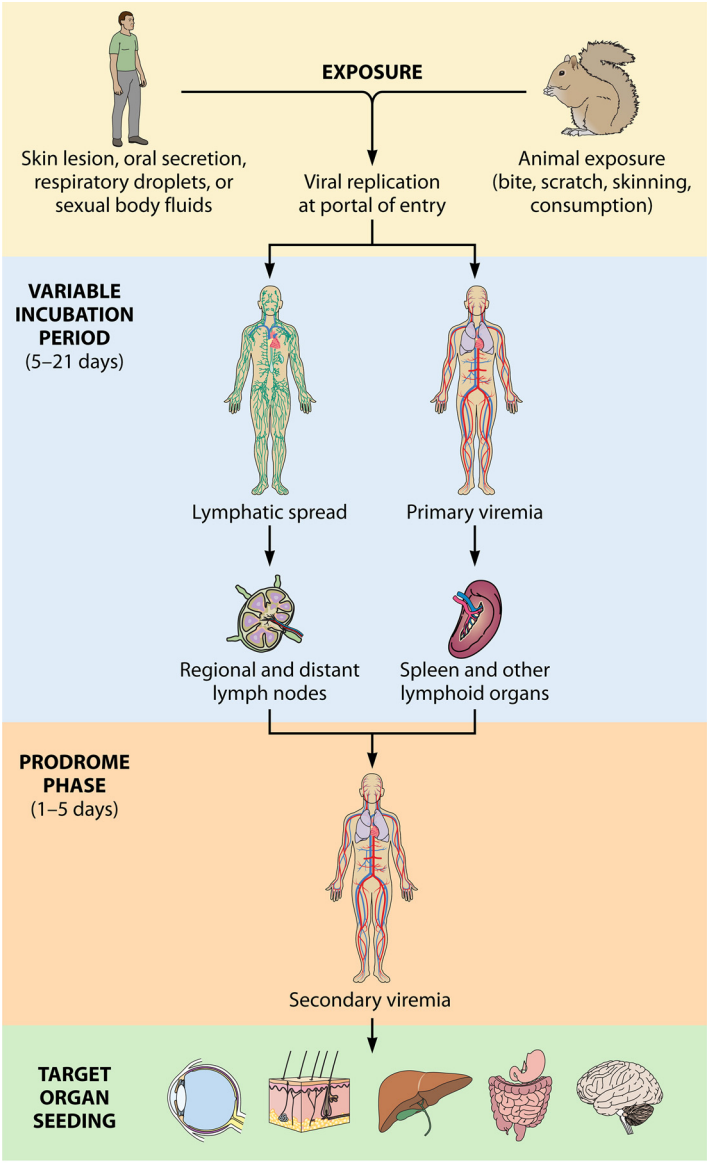

FIG 3 Pathogenesis of monkeypox virus (38, 42, 45, 72–74, 76, 77).

almost invariably in the setting of unprotected anal-receptive intercourse, and by Nigerian reports of human monkeypox infection among male heterosexuals with high-risk sexual behavior (61, 64, 66–71).

PATHOPHYSIOLOGY AND VIRULENCE

Our understanding of the pathophysiology of human monkeypox infection has relied on information from human modeling studies, outbreak investigations, and the findings of challenge experiments conducted in nonhuman primates and prairie dogs (38, 72–75). The incubation period ranges from 5 to 21 days (typically 7 to 14 days), after which the clinical manifestations of the disease become apparent in the vast majority of individuals (Fig. 3) (38, 42, 45, 75). Inoculation of *Monkeypox virus* into the skin, soft tissues, upper respiratory tract mucosa, or lungs is met with a robust local innate immune response evolving over a period of hours to days and typically involves recruitment of macrophages, fibroblasts, and polymorphonuclear leukocytes (38, 72, 76, 77). Despite these host defense mechanisms, the virus may evade containment (78, 79). The next phase involves viral dissemination via the lymphatic system to the

regional lymph nodes and an initial viremic phase with seeding of the splenic and tonsillar tissues (38). A secondary viremic phase follows, with seeding of the integument and viscera (including liver, intestines, kidneys, ovaries, testicles, and/or brain) (38). Corneal scarring may develop secondary to conjunctival or eyelid involvement or possibly by autoinoculation after contact with infectious skin lesions (57, 78, 80–82). Compared to clade II (formerly known as the West African clade), infection with clade I (formerly known as the Central African clade) is associated with a greater number of skin lesions, a higher probability of gastrointestinal tract involvement typified by granuloma formation (stomach, small intestine, colon, pancreas, peritoneal membrane), a longer duration of illness, more severe symptoms, and a 10-fold-greater plasma viral load (72).

The histopathologic and clinical features of human monkeypox infection are almost indistinguishable from those of smallpox, with the exception of a more severe clinical course and the absence of lymphadenopathy in the latter (57, 83). Furthermore, smallpox is usually transmitted by aerosols, which has yet to be demonstrated for monkeypox in natural settings. Transmission by way of an animal bite or scratch may introduce the virus into the bloodstream, resulting in a shorter incubation period and the possible absence of prodromal symptoms (57). Clade I has been shown to possess multiple virulence genes that are not found in clade II, the most important of which appears to be the monkeypox inhibitor of complement enzymes (MOPICE) gene, which is similar to its analog in *Variola virus*, the smallpox inhibitor of complement enzymes (SPICE) gene (41, 84–86). The MOPICE gene has been shown to encode a protein that interferes with the early phases of the host complement cascade (84). Clade I has other important virulence factors, including those that promote cellular apoptosis (85, 87, 88). There is also evidence of earlier viremia and more disseminated disease with clade I infections (73).

CLINICAL PRESENTATION OF MONKEYPOX INFECTION IN HUMANS

The clinical manifestations of human monkeypox infection exhibit many similarities to those of smallpox but are typically much milder in nature. Unlike monkeypox, smallpox is an eradicated disease, has no animal reservoir, and does not involve the lymphoreticular system (2, 47, 51, 52, 77, 89). The variable incubation period of monkeypox infection is followed by a 1- to 5-day prodrome that may include fever, chills, drenching sweats, headache, fatigue, backache, malaise, and enlarged, tender lymph nodes in the neck, axillary, and/or inguinal regions (2, 47, 51, 52, 77, 84, 89–91). Exposed individuals may also develop sore throat (from acute tonsillitis), productive or nonproductive cough, and an enanthem involving the mucous membranes of the oral, conjunctival, and/or genital regions (47, 77, 89, 91, 92). The postprodromal period is heralded by the onset of pathognomonic smallpox-like skin lesions, with or without internal organ involvement. Serious complications may arise as a result of primary viral inoculation, secondary seeding of target organs via the bloodstream, or bacterial superinfection and may include pneumonia, dehydration from gastrointestinal fluid and electrolyte losses, cellulitis with or without abscesses, conjunctivitis, blepharitis, keratitis with visual loss secondary to corneal ulceration, septicemia, and encephalitis (93). These sequelae may occur in up to 43% of unvaccinated individuals, compared with up to 9% of vaccinated individuals following infection with clade I (93).

Integumentary System

Skin involvement is the most commonly observed physical sign of human monkeypox infection. A classical polymorphic centrifugal rash is typically seen 2 to 3 days after the onset of fever and manifests initially as small (subcentimeter) macules that evolve over time in synchronous fashion to become papules, followed by vesicles, and then pustules, with each of the first three stages lasting for 1 to 2 days and the pustular stage lasting for 5 to 7 days, according to the U.S. Centers for Disease Control and Prevention (CDC; https://www.cdc.gov/poxvirus/monkeypox/clinicians/clinical-recognition.html) and other researchers (Fig. 4A and B) (2, 38, 89–92, 94–97). Like smallpox, the rash initially involves the face and trunk and

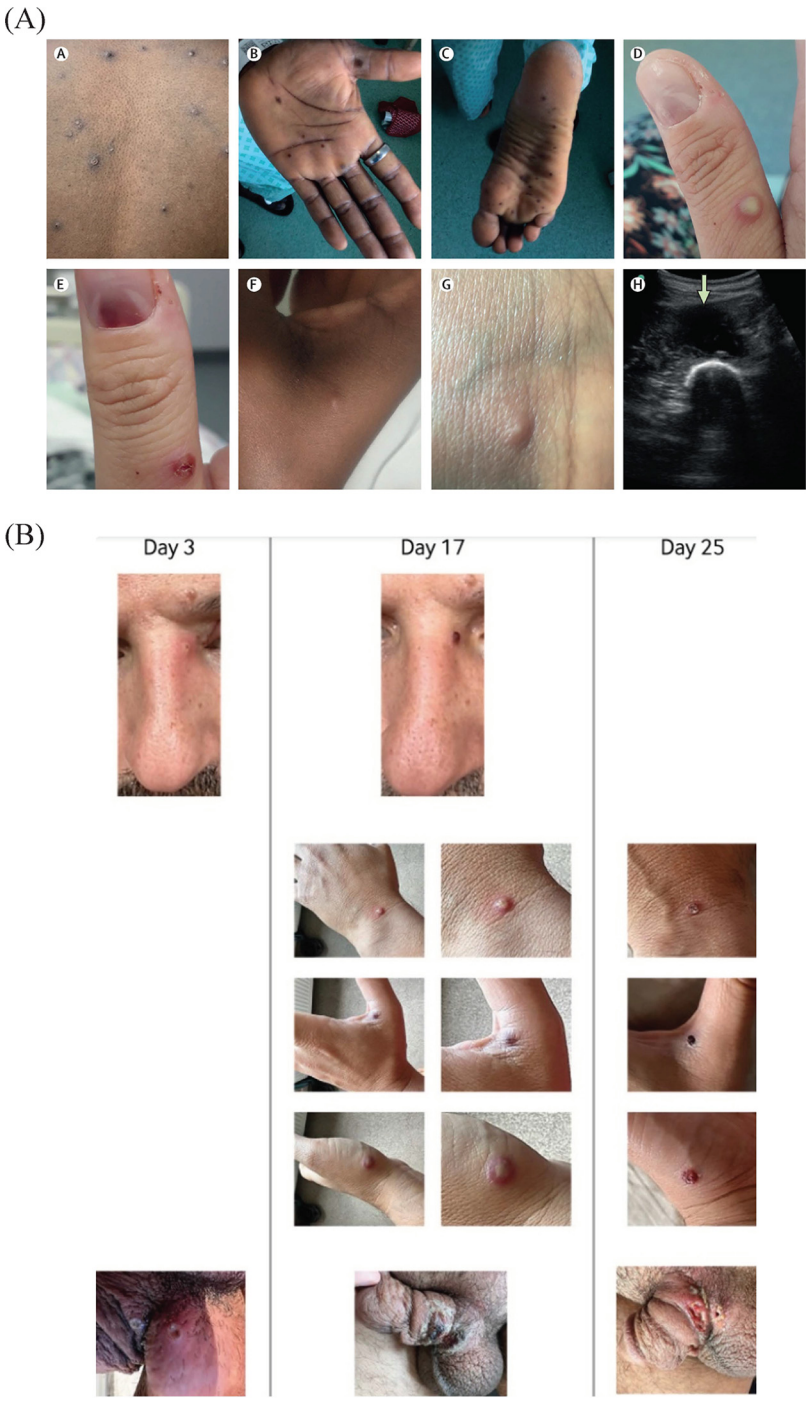

FIG 4 (A) Exanthem of monkeypox virus infection in humans. Vesicles and pustules (A, D, and H), macules (B and C), subungual lesions (F and G), and an ultrasonographic image of an abscess are shown. (The images are reproduced from reference 155, which was published under a Creative Commons BY-NC-ND 4.0 license.) (B) Nonsynchronous onset and progression of monkeypox skin lesions on the face, hands, and genitals (112). (The images are reproduced from https://commons.wikimedia.org/wiki/File:Monkeypox_lesion_progression.jpg, licensed under the Creative Commons Attribution 4.0 International license.)

is generally followed by involvement of the palmar and plantar surfaces, with resolution by umbilication, crusting, and scarring over the ensuing 1 to 2 weeks, as reported by the CDC (https://www.cdc.gov/poxvirus/monkeypox/clinicians/clinical-recognition.html) and other investigators (2, 38, 89–92, 94–97). Genital and/or anorectal involvement is considered a hallmark of monkeypox skin infection in countries where it is nonendemic, particularly among men who have sex with other men, although this presentation is not uncommon in other

patient populations, exposure scenarios, or jurisdictions (42, 48, 61, 62, 64–71, 98–102). During the early 1980s, approximately 33.3% and 19.2% of unvaccinated persons in the DRC with a known animal or human source of infection, respectively, had evidence of a genital mucous membrane involvement (98). In 2022, researchers in Spain found that among 185 human monkeypox cases enrolled in an observational study through a country-wide network of dermatology practices, 99% were MSM, 76% had genital, groin, or pubic lesions, and 42% were coinfected with HIV (99). Other concurrent studies conducted in Spain, France, and the United States found relatively comparable patient demographics, sexual practices, comorbidities, and frequencies of genital and/or anal skin involvement (100–102). Monkeypox skin lesions are typically monomorphic and discrete but may be semiconfluent or confluent (52, 59, 77, 89–91, 93, 96, 97). The skin disease is categorized as mild if there are $\leq$25 lesions, moderate if there are between 25 and 99 lesions, severe if there are between 100 and 250 lesions, and serious if there are $>$250 lesions (11, 42, 103). Lesions range in size from 0.5 cm to 1 cm in diameter and are typically larger and greater in number among unvaccinated patients compared to those with prior smallpox vaccination (91). Secondary bacterial infections of the skin, including carbuncles and cellulitis, represent the most common postviral sequelae of human monkeypox infection; they tend to disproportionately affect unvaccinated individuals or those with immunosuppression and may lead to permanent skin disfiguration, alopecia, or changes in skin pigmentation (77, 91, 93, 104–107).

Pulmonary System

Upper respiratory tract signs and symptoms of monkeypox virus infection may include pharyngitis, oral ulcers, and tonsillitis, with the latter occurring in up to 50% of cases (91). Lower respiratory tract involvement following monkeypox infection is a rarely acknowledged late manifestation of the disease in animals and humans (50, 108). Cynomolgus monkeys challenged with lethal doses of aerosolized monkeypox virus developed fatal necrotizing bronchopneumonia several days following exposure (50, 108). Nonhuman primate models have demonstrated that the clinical course of monkeypox-associated pulmonary infection is mediated by the interplay of the host immune response and the expression of viral genes that promote lung tissue destruction (109). Following experimental intrabronchial monkeypox virus challenge, lobar consolidation and/or ground-glass opacities typical of community-acquired pneumonia may be evident on chest radiography (110). Perhaps the earliest documented human case of monkeypox-associated lower respiratory tract infection occurred in a 2-year-old unvaccinated child from the DRC (111). This patient initially presented to hospital with a generalized monkeypox virus rash that was complicated by the onset of respiratory signs and symptoms consistent with pneumonia. Despite receiving empirical antibiotic therapy, the child developed febrile seizures and eventually died (111). In a case series of 282 patients with monkeypox in the DRC during the early 1980s, pneumonia and/or respiratory distress were diagnosed in 11.6% of unvaccinated individuals (with a mortality rate of 66%), compared to only 3.1% of those with previous smallpox vaccination (93). Secondary bacterial infection of the lung has also been reported in human monkeypox cases, albeit infrequently, and occurring later in the course of illness (50, 77, 112).

Central Nervous System

Headache is the most commonly documented neurologic manifestation of monkeypox infection and is typically experienced through the prodromal phase of the illness (46, 69, 89, 91–93, 113–116). During the postprodromal period, serious neurologic sequelae, including encephalitis, meningoencephalitis, and seizures, may be observed but are rarely encountered (99, 107, 111, 114, 117). In a series of 388 human monkeypox cases in the DRC during the early 1980s, only one individual (a 3-year-old unvaccinated female child) developed encephalitis and ultimately succumbed to her illness (98). In contrast, 9% (3/40) of hospitalized human monkeypox cases in Nigeria developed encephalitic seizures, with two attributable deaths (one in a 28-day-old female neonate and the other in an HIV-positive adult man) (107). Central nervous system complications have also occurred in immunocompetent children and adults. A previously healthy 6-year-old girl from Indiana, USA, who acquired monkeypox following

exposure to an imported pet developed encephalitis less than 1 week following the onset of prodromal symptoms but eventually survived with no residual neurologic sequelae (117). In this pediatric case, initial cerebrospinal fluid (CSF) analysis demonstrated a neutrophilic pleocytosis which became predominantly lymphocytic on repeat lumbar puncture performed several days later (117). Orthopoxvirus-specific IgM was detected in CSF, and magnetic resonance imaging demonstrated involvement of brain parenchyma (117). In 2022, public health authorities in Spain reported two cases of fatal encephalitis in previously healthy adult males with no epidemiologic link to other cases; *Monkeypox virus* DNA and *Orthopoxvirus* genus-specific IgM were detected in CSF samples obtained from both individuals (https://cdn.who.int/media/docs/default-source/blue-print/isabel-jado_case-control-studies_who-monkeypox-vaccine-research_2aug2022.pdf?sfvrsn=d81df2d0_3). The detection of *Monkeypox virus* in brain tissues harvested from infected animals suggests that neurologic signs and symptoms of monkeypox infection are mediated by viral neurotropism and arise secondary to invasion of the central nervous system through hematogenous spread or directly via the olfactory system (118).

Ophthalmic System

Eye pathology is a relatively infrequent but potentially sight-threatening complication of human monkeypox infection. Ocular manifestations may include conjunctivitis, blepharitis, blepharoconjunctivitis, subconjunctival nodules, keratitis, and corneal ulcers (81, 82, 89, 91, 93, 98, 107, 113, 119–123). Among 295 unvaccinated persons with monkeypox in the DRC between 1981 and 1986, 20.3% and 16.4% with an animal or human source of infection, respectively, had evidence of conjunctivitis, while 4% overall had evidence of keratitis or corneal ulceration (91). Three decades later, a study in the DRC found that 23% of human monkeypox cases had evidence of conjunctivitis, with eye involvement occurring primarily among children and in those with more debilitating prodromal symptoms (81). Conjunctivitis and photophobia were reported in 0.2% and 0.1%, respectively, of over 14,000 human monkeypox cases in Europe, according to the European Centre for Disease Control (ECDC) and WHO (https://monkeypoxreport.ecdc.europa.eu/). Permanent loss of vision, which usually follows the development of keratitis or corneal ulceration, has primarily been described among children in the DRC (77, 81, 91, 99).

Gastrointestinal System

Nausea, vomiting, and other gastrointestinal symptoms may occur with varying frequency during the course of monkeypox illness (89, 91, 92). Among cases of human monkeypox infection reported in the DRC during the early 1980s, 7.5% of unvaccinated persons experienced vomiting, watery diarrhea, dehydration, or malnutrition, while gastrointestinal symptoms were notably absent in vaccinated individuals (91). In a case series of American children and young adults who developed monkeypox infection following exposure to exotic pets, one-third experienced nausea or vomiting, with a slightly higher incidence in children (89). However, nausea and vomiting were reported in only 0.8% of human monkeypox cases in Europe, according to aggregate surveillance data released by ECDC and WHO (https://monkeypoxreport.ecdc.europa.eu/). In contrast, these symptoms were experienced by 9.2% of Americans with human monkeypox during the 2022 outbreak in countries where it is nonendemic (102). Diarrhea has been reported to occur in approximately 5% of human monkeypox cases (89). The presence of gastrointestinal symptoms during human monkeypox infection may be associated with a greater risk of prolonged hospitalization (89).

Hepatic transaminitis is common, occurring in approximately half of human monkeypox infections, with the median alanine aminotransferase and aspartate aminotransferase levels being twice the upper limit of normal (89). Gross involvement of the liver and other intraabdominal organs is an infrequent but potentially serious complication of monkeypox infection in humans and animals (72, 124–127). Immunohistochemical analysis of liver specimens harvested from macaques challenged with lethal doses of monkeypox virus revealed the presence of intracellular pox virus inclusions (124). Furthermore, monkey challenge studies

have demonstrated evidence of granulomatous changes in the stomach, intestines, and peritoneal membrane following subcutaneous or intranasal infection with the clade I variant of monkeypox virus but not with clade II (72). On the other hand, researchers employing a prairie dog infection model demonstrated that clade II monkeypox virus could be found in liver samples following intraperitoneal inoculation but not after intranasal challenge (125). A bioluminescent study of prairie dogs challenged with monkeypox virus demonstrated the presence of active viral replication in intestinal and hepatic tissues (126). Monkeypox virus was cultured from hepatic and splenic tissue obtained at autopsy from a 9-month-old girl in Gabon who died within 48 h of presenting to hospital with severe prodromal symptoms and hepatosplenomegaly (127).

Genitourinary System

Genitourinary tract manifestations of human monkeypox infection are common among MSM in regions where monkeypox is not nonendemic, and the symptomatology may be the primary reason for their seeking medical care (61, 66–69, 99–102, 112). Lesions on the external genitalia may be monomorphic, evolving together in a synchronous fashion from macules to the final pustule stage, although up to one-third of human monkeypox infections in a British case series had evidence of a polymorphic genital rash with lesions at different stages of progression (112). Individuals with exanthemata of the external genitalia often have concomitant oral and genital enanthema, penile edema, tender regional lymphadenopathy, and other sexually transmitted infections (66, 67, 69, 101, 112). Fever occurs in up to two-thirds of individuals with genital skin lesions, while other prodromal symptoms are less common (69, 100–102, 112). In one case series, dysuria was reported in 5% of patients with genital involvement (101).

Monkeypox virus has been detected in testicular, ovarian, and/or uterine tissue of macaques following subcutaneous, intranasal, or aerosolized exposure, and culture-confirmed gonadal involvement has also been observed in a prairie dog model (108, 124, 128). Although detection of monkeypox virus in human semen supports a sexual transmission hypothesis, it is unclear to what degree the reproductive organs are involved or if fertility is impaired (61, 129).

Pregnancy

Monkeypox infection in pregnant women has infrequently been reported but may be associated with congenital infection, stillbirth, miscarriage, preterm labor, or delivery of a healthy infant (91, 130–132). Although cases of monkeypox infection in pregnant women have been sparsely reported, this patient population is believed to be at increased risk of morbidity and mortality compared to nonpregnant adults based on the natural history of illness following smallpox infection (133, 134). Although vertical transmission to the developing fetus is believed to occur, studies in humans are limited to date (91, 131, 135, 136). In Europe, a pregnancy registry has recently been established to collate health outcomes information on women with human monkeypox infection (134).

Immunocompromised Hosts

Persons who are immunosuppressed represent a potentially high-risk group for infection with monkeypox virus and the development of serious sequelae. In countries where monkeypox virus is nonendemic, the vast majority of human monkeypox infections have occurred in MSM, with HIV infection being the most common comorbidity (61, 63, 66–69, 99–102, 112, 137, 138). Studies conducted in France, Germany, Spain, and the United States found that MSM with well-controlled HIV had clinical presentations and outcomes of monkeypox infection that were comparable to those in MSM without HIV (66, 67, 69, 99–102, 112, 137, 138). In contrast, African patients with HIV and low CD4 cell counts appeared to have a more protracted clinical course with a longer duration of illness, a heavier burden of skin lesions, bacterial superinfection of the skin, and genital ulcers (107). According to the UK NHS (https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/26782/inf16401.pdf), there have not been any reported cases of monkeypox infection in solid organ transplantation at the time of writing, although the potential for donor-to-recipient transmission exists (139).

FIG 5 Prairie dog. (Reproduced from https://commons.wikimedia.org/wiki/File:Prairie_Dog_%2819014508183%29.jpg, by W. Warby, licensed under the Creative Commons Attribution 2.0 Generic license.)

HUMAN MONKEYPOX INFECTION AMONG AMERICAN EXOTIC PET OWNERS

The first known cluster of human monkeypox infections outside of the African subcontinent occurred on U.S. soil in May 2003, wherein a multistate outbreak involving dozens of individuals between the ages of 1 and 51 years was attributed to close contact with infected prairie dogs acquired as pets from an animal distributor, with the ultimate source of infection being imported Gambian rats which transmitted the infection to the prairie dogs at an American pet distribution facility (Fig. 5) (140–146). Cases were initially described in the state of Wisconsin, followed by reports from Illinois, Indiana, Kansas, Missouri, and Ohio (92, 117, 140–145, 147, 148). The first reported case in this outbreak occurred in a 3-year-old girl from Wisconsin who was hospitalized for cellulitis after being bitten on the hand by a pet prairie dog on 13 May 2003 (52, 148). She presented with a prodrome of high fever, malaise, and tender cervical lymphadenopathy followed shortly afterwards by the development of a diffuse rash consistent with monkeypox (52, 148). Both of her parents experienced a similar illness, and the diagnosis of monkeypox infection was confirmed by viral culture and electron microscopy of a skin sample obtained from the mother (148). The child fully recovered after a

2-week hospital stay, although both of the family's pet prairie dogs eventually died of monkeypox (52). In Indiana, one family cluster involved a 6-year-old girl and her parents, who became ill after purchasing two infected prairie dogs from a flea market (117). These family members had the classical febrile prodrome and pathognomonic rash of monkeypox (117). Unfortunately, the child became severely ill and required intensive care unit admission for mechanical ventilation secondary to worsening somnolence (117). She was found to have radiologically confirmed encephalitis; however, this young patient survived and achieved a full neurologic recovery after several weeks of close medical follow-up (117).

There were no reported deaths during this multistate outbreak although, in total, three children experienced severe illness and about one-quarter of affected individuals required hospitalization for supportive care or isolation (140–144). Reynolds and colleagues found that individuals who became ill following a prairie dog bite or scratch had a shorter incubation period, more pronounced clinical symptoms, and a higher likelihood of requiring hospitalization than did individuals who were infected through noninvasive exposures such as respiratory droplets or skin contact (115). Overall, there were 47 confirmed or probable cases of human monkeypox infection during this outbreak, all being caused by clade II viruses, with no clear evidence of person-to-person transmission (115). On 11 June 2003, the CDC announced a temporary ban on the importation of six species of African rodents believed to be natural reservoirs of monkeypox and also on the sale and distribution of prairie dogs (149, 150). However, the ban was eventually revised in 2008 to permit interstate movement of prairie dogs (151).

TRAVEL-RELATED HUMAN MONKEYPOX IN NON-AFRICAN COUNTRIES, 2018 TO 2021

From 2018 to 2021, imported cases of human monkeypox infection caused by clade II were reported in several non-African countries, including the United Kingdom, Israel, Singapore, and the United States (51, 152–160). Two epidemiologically unrelated cases were diagnosed in the United Kingdom in September 2018, the first (case 1) being a 32-year-old male visitor from Nigeria and the other (case 2) being a 36-year-old male British resident who had returned from a trip to Nigeria (51, 152, 153). Case 2 purportedly consumed bushmeat and was in close proximity to a potential human case of monkeypox at a large family event during his trip (152, 153). Cases 1 and 2 presented with a febrile prodrome, lymphadenopathy, and eventually a rash involving the face, palms, and groin (152). Monkeypox virus DNA was isolated from the skin lesions of both cases (152). Indirect respiratory transmission of monkeypox virus from case 2 to a 40-year-old British female health care worker (case 3) wearing adequate personal protective gear was confirmed in late September 2018 and was believed to be related to handling contaminated bed sheets (51). Case 3 presented to their primary care provider with a classical clinical picture of monkeypox but had received postexposure smallpox vaccination within 7 days of exposure to case 2 (51). After public health authorities became aware of case 3, over 100 other health care workers were also offered postexposure prophylaxis (51). Cases 1 to 3 also required hospitalization and received antiviral therapy with brincidofovir, and all recovered from their illness (51, 155).

In December 2019, another case of monkeypox (case 4) was reported in a middle-aged male returning from Nigeria, and this individual presented with a brief coryzal prodrome followed by the development of a rash that was similar in nature and distribution to cases 1 to 3 (154, 155). In May 2021, UK public health authorities were alerted to a mild case of monkeypox infection among one member of a family that had recently traveled to Nigeria (154). This individual (case 5) was a middle-aged male who transmitted the infection to two other family members, including his 18-month-old female child (case 6) and his female spouse (case 7), and all three cases fully recovered from their illness (154, 155). The latter case was treated with the antiviral tecovirimat for 2 weeks (155). Only cases 1 to 4 had a febrile prodrome, while cases 1 to 5 also had a genital rash (155). None of these additional cases had previously been immunized with the smallpox vaccine (155).

In October 2018, a young adult male from Israel was diagnosed with monkeypox after returning from a Nigerian trip (156). He initially presented with a penile rash and viral prodrome approximately 12 days after handling rodent carcasses (156). His clinical presentation included bilateral tender inguinal lymphadenopathy, moderate thrombocytopenia, and hepatic transaminitis (156). This case was hospitalized for 24 h but had an otherwise-uneventful course (156).

In May 2019, a 38-year-old male Singaporean national was hospitalized with monkeypox after attending a wedding in Nigeria, where he consumed cooked bushmeat (157, 158). He presented with a putative clinical prodrome, lymphadenopathy, and a rash which involved the face, torso, palms, soles, and genitalia, and his clinical condition eventually improved with conservative measures (157, 158).

In July 2021, a nonelderly man who had recently attended a large social function in Nigeria was diagnosed with severe monkeypox infection after presenting to an emergency department in Dallas, TX, with a viral prodrome followed by the development of a diffuse centrifugal pox-like rash (159). The source of the exposure remained unknown, and there were no epidemiologic links to other imported Nigerian cases (159). He received antiviral treatment with tecovirimat and remained in hospital for over a month before being released (159). In the same year, a 28-year-old man from Maryland who had recently visited family members in Nigeria developed the classical prodrome, exanthem, and enanthem of monkeypox and was believed to have secondarily acquired the infection from a Nigerian case (160). Within 24 h of hospital admission, his clinical condition improved (160).

GLOBAL MONKEYPOX OUTBREAK IN COUNTRIES WHERE IT IS NONENDEMIC, 2022

On 7 May 2022, public health authorities in the United Kingdom and the WHO were informed of an imported case of human monkeypox infection in a traveler returning from Nigeria (https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON381; https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385). Contact tracing of potentially exposed individuals (health care workers, persons on the same flight from Nigeria, community members) failed to identify any additional epidemiologically linked cases, according to the WHO. However, by 25 May 2022, a total of 86 confirmed cases were reported in the United Kingdom, and none other than the first case had reportedly traveled to Africa (161). Within a span of a few weeks, case counts ballooned in the United Kingdom and elsewhere, as reported by the WHO (https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON392, https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON393).

By 5 October 2022, there were over 68,000 laboratory-confirmed cases of human monkeypox infection in 100 countries where it is nonendemic, according to the CDC (https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html). Outside of the United Kingdom, at the time of writing, cases had been reported in 38 other European countries as well as in Canada, the United States, Argentina, Aruba, Australia, Bahamas, Bahrain, Barbados, Benin, Bermuda, Bolivia, Brazil, China, Chile, Columbia, Costa Rica, Cuba, Curacao, Cyprus, Dominican Republic, Ecuador, Egypt, El Salvador, Georgia, Greenland, Guadeloupe, Guatemala, Guyana, Honduras, Hong Kong, Jamaica, India, Indonesia, Iran, Israel, Japan, Jordan, Lebanon, Martinique, Mexico, Morocco, New Caledonia, Panama, Paraguay, Peru, Philippines, Qatar, New Zealand, Russia, Saudi Arabia, Singapore, South Africa, South Korea, Sudan, Taiwan, Thailand, Turkey, the United Arab Emirates, Uruguay, Venezuela, and Vietnam (https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html). Cases during this epidemic have predominantly occurred among MSM (61, 64–69, 99–102, 112, 129, 137, 138, 162–172). Outbreaks in Spain, Germany, and other Western countries have been linked to Pride festivals and saunas in several locales (69, 164–166, 169, 171, 172). In recent samples, an apparent shift has occurred in the epidemiology, with a higher proportion of cases occurring among women and men who do not report having sex with men, according to the CDC (https://www.cdc.gov/poxvirus/monkeypox/cases-data/technical-report/report-2.html). This may represent new transmission in these populations, or improved ascertainment.

Public health experts around the world initially believed that physical contact with an infected person was the primary mode of monkeypox virus community transmission within countries where it is nonendemic. However, researchers from Italy recently reported the detection of monkeypox viral DNA in semen samples obtained from four young men in their 30s (two with HIV and two receiving preexposure HIV prophylaxis) who reported unprotected sexual intercourse with other men while attending a large Pride festival in Gran Canaria from 5 to 15 May 2022 (67). All but one of the cases presented with the usual viral prodrome, two had inguinal lymphadenopathy, and all had pruritic pox-like lesions in the genital or anal area (67). *Monkeypox virus* DNA was also detected in semen samples collected from the two index cases in Germany; both were men who had sex with men, with one of these individuals being an HIV-positive sex worker (61). In a Spanish case series, half of affected patients were found to have monkeypox DNA in semen (129). Furthermore, in a large multinational case series of human monkeypox infection in 16 countries where it is nonendemic, the majority of individuals who underwent seminal fluid analysis were also found to have *Monkeypox virus* DNA in their semen, and almost one-third of those who underwent testing for other sexually transmitted diseases had microbiologic evidence of coinfection (69). However, it is not yet clear whether the presence of monkeypox viral DNA in sexual body fluids represents an infectious risk, although these observations have prompted the World Health Organization and other public health agencies to reconsider the possibility of person-to-person spread via the sexual route, according to Reuters (https://www.reuters.com/business/healthcare-pharmaceuticals/who-looks-into-reports-monkeypox-virus-semen-2022-06-15). More recently, Heskin and coworkers found that the timing and pattern of symptom onset in relation to sexual intercourse and the opportunities for close contact that are afforded by sexual encounters suggest that monkeypox is capable of sexual transmission (62).

A recent review of the epidemiology of the current monkeypox outbreak in countries where it is nonendemic revealed that the preponderance of cases reported in the peer-reviewed literature had an atypical clinical presentation, characterized by an anogenital localization of the rash with relative sparing of the face and hands, as well as the presence of inguinal lymphadenopathy (68). These observations are consistent with those of other investigators (61, 66, 67, 69, 129, 164–167, 172, 173). Bragazzi and coworkers noted that approximately one-quarter of infected patients had a viral prodrome and only 11% had cervical lymphadenopathy (68). Infected patients in countries where monkeypox virus is nonendemic often reported experiencing rectal pain, bleeding, and tenesmus, believed to be secondary to the development of localized pox-like lesions (69, 99–101, 112, 129, 137, 138, 164, 165, 172, 173). Risk factors for human monkeypox infection in countries of nonendemicity include male gender, young age, sex with other men, unprotected anal intercourse, HIV positivity, and a history of sexually transmitted infections (68). There are also reports of a number of asymptomatic infections, although it is presently not known whether infectiousness is dependent on the presence of clinical signs or symptoms of disease (174).

CLINICAL SEVERITY OF HUMAN MONKEYPOX INFECTION

Monkeypox infections in humans are usually mild, although severe illness and death may occur. The two known clades of monkeypox virus, namely, clade I (found in Cameroon, Central African Republic, Congo, DRC, Gabon, and South Sudan), and clade II (found in Benin, Cameroon, Ivory Coast, Liberia, Nigeria, and Sierra Leone) have biologic differences that may affect the clinical course of the disease (1, 9, 27, 73, 175). Attributable mortality rates of 10.6% and 3.6% for infections caused by clades I and II, respectively, have been reported in regions of endemicity (27). However, it is plausible that milder cases of monkeypox infection are underascertained, and no deaths had occurred in any of the cases reported outside of Africa prior to the 2022 outbreak, including the 2003 exotic pet-related outbreak in the United States and the pre-2022 sporadic cases in the United Kingdom, Israel, Singapore, and the United States (3, 41, 144, 152–160). Among the more than 68,000 laboratory-confirmed cases of human

monkeypox infection in 100 countries of nonendemicity between 7 May 2022 and 5 October 2022, there were only 13 attributable deaths in nine jurisdictions (with Spain reporting three deaths, the United States and Brazil each reporting two deaths, and Belgium, Cuba, Czechia, Ecuador, India, and Sudan each reporting single deaths), according to the World Health Organization (https://www.paho.org/en/documents/weekly-situation-report-monkeypox-multi-country-outbreak-response-region-americas-9), Brazilian government (https://agenciabrasil.ebc.com.br/en/saude/noticia/2022-07/first-death-linked-monkeypox-confirmed-brazil), European CDC (https://www.ecdc.europa.eu/en/news-events/monkeypox-situation-update), Spanish Health Officials (https://cdn.who.int/media/docs/default-source/blue-print/isabel-jado_case-control-studies_who-monkeypox-vaccine-research_2aug2022.pdf?sfvrsn=d81df2d0_3), and the U.S. CDC (https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html), with all infections being caused by the "milder" clade II. The two monkeypox-associated deaths in the United States occurred in individuals with profound immunosuppression and were reported by public health officials in Texas (https://dshs.texas.gov/news-alerts/texas-confirms-first-death-of-a-person-with-monkeypox/#:~:text=The%20Texas%20Department%20of%20State,monkeypox%20played%20in%20the%20death) and California (http://publichealth.lacounty.gov/phcommon/public/media/mediapubhpdetail.cfm?prid=4058). In contrast, the two fatalities reported by Spanish health officials (https://cdn.who.int/media/docs/default-source/blue-print/isabel-jado_case-control-studies_who-monkeypox-vaccine-research_2aug2022.pdf?sfvrsn=d81df2d0_3) occurred in previously healthy MSM. Details regarding deaths occurring in other countries are not publicly available.

Using a prairie dog animal model, Hutson and coworkers demonstrated that the median lethal dose of clade I was 100-fold less than that of clade II (58). Elevated cytokine levels have been found to correlate directly with the number of skin lesions and are thus believed to be predictive of disease severity (176). Other risk factors for severe disease include young age, lack of previous smallpox vaccination, gastrointestinal symptoms, and the presence of an enanthem in the oral cavity (89, 177). Respective mortality rates of 1% and 11% for vaccinated and unvaccinated individuals with human monkeypox have been reported (1, 94). Given that the number of skin lesions is typically greater in human monkeypox infections caused by clade I compared to clade II, complications such as blindness, bacterial cellulitis, and septicemia are expected to occur more often in the former. This theory is also compatible with the observation that human-to-human transmission is more common with clade I infections (9).

LABORATORY DIAGNOSIS OF HUMAN MONKEYPOX INFECTION

The laboratory diagnosis of monkeypox infection in humans is crucial given the difficulty in differentiating the typical skin manifestations from those caused by other pox viruses and from the varicella zoster virus (VZV) (Table 2). Nonspecific laboratory abnormalities commonly seen in patients with monkeypox infection include peripheral leukocytosis, thrombocytopenia, hepatic transaminitis, hypoalbuminemia, and low blood urea nitrogen (89). Skin lesions are the diagnostic specimens of choice and are preferably collected during the vesicular or pustular rash stages (5, 6). Skin scrapings, fluid, crusted lesions, and biopsy tissue should be collected and placed in sterile containers and sent to a microbiology laboratory, where a broad array of methodologies can be used to identify and differentiate *Monkeypox virus* from other viral pathogens (5, 6).

Direct Detection Methods

Nonmolecular diagnostic modalities, including electron microscopy and antigen detection, were commonly performed before the advent of genomic methods (5, 6, 83, 89, 178, 179). Electron microscopy can differentiate orthopoxviruses from viruses in other genera, although it cannot provide species-level identification and is not readily available as a diagnostic tool in clinical laboratories (5, 6, 179). Similarly, antigen detection methods can be used to reveal the presence of hemagglutinin, which differentiates orthopoxviruses (hemagglutinin positive) from non-orthopoxviruses (hemagglutinin negative), although species-level identification is not possible and the assay is generally not available in most

TABLE 2 Differential diagnosis of skin lesions in human monkeypox infections[a]

Genus	Species	Endemicity	Exanthem
Orthopoxvirus	*Variola virus*	Eradicated	Centrifugal rash on face, palms, and soles
	Monkeypox virus	Central and West Africa (rodents)	Centrifugal rash on face, palms, and soles
	Vaccinia virus	Worldwide (smallpox vaccines)	Local (injection site) or generalized
	Cowpox virus	Europe and Asia (cattle, wild rodents)	Fingers; other sites by autoinoculation
	Camelpox virus	Middle East, Africa, Asia (camels)	Head, neck, limbs, genitalia
	Alaskapox virus	Alaska, USA (rodents)	Extremities
Parapoxvirus	*Orf virus*	Worldwide (sheep, goats)	Painful lesions on finger, hand and arms, face
	Pseudocowpox virus (milker's nodule)	Worldwide (cattle)	Painful lesions on hands, face
	Bovine papular stomatitis virus	Worldwide (cattle)	Painful lesions on hands, arm
	Sealpox virus	Ocean coastlines of Northern hemisphere (seals)	Painful lesions on hands
Yatapoxvirus	*Tanapox virus*	Equatorial Africa (wildlife)	Nodular lesions on extremities
	Yaba-like disease virus and *Yaba monkey tumor virus*	Equatorial Africa (wildlife)	Palms, extremities, face (occupational)
Molluscipoxvirus	*Molluscum contagiousum virus*	Worldwide (Humans)	Trunk, limbs (except palms and soles), face
Varicellovirus	*Varicella zoster virus*	Worldwide (Humans)	Centripetal rash on trunk, occasionally extremities and face

[a]From references 1, 6, 7, and 298–302.

clinical laboratories (6). However, a rapid *Orthopoxvirus* lateral flow antigen detection assay, Orthopox BioThreat Alert (Tetracore, Rockville, MD, USA), is commercially available and may be a useful point-of-care diagnostic tool (180). Viral culture is labor-intensive and is no longer performed for routine diagnostic purposes.

Molecular Diagnostics

Nucleic acid detection methods are considered the gold standard for the laboratory diagnosis of monkeypox virus infection and include conventional and real-time PCR, loop-mediated isothermal amplification, and next-generation DNA sequencing (5, 6, 181–189). Multiplex real-time PCR assays have been developed that provide sensitive and specific species-level identification to differentiate *Monkeypox virus* from *Variola virus*, *Cowpox virus*, and *Vaccinia virus* and to provide clade-specific identification for differentiation of clade II from clade I *Monkeypox virus* infections (182, 184, 185, 187, 188). The U.S. Centers for Disease Control and Prevention (https://www.cdc.gov/poxvirus/monkeypox/pdf/pcr-diagnostic-protocol-508.pdf) has recently published their real-time PCR procedure for the detection of Monkeypox virus DNA in skin lesions. Other clinical specimens, including nasopharyngeal swabs, throat swabs, cerebrospinal fluid, and urine, can be analyzed for *Monkeypox virus* DNA, although some assays are for research use only, given the lack of specimen-specific test validation, according to the World Health Organization (https://apps.who.int/iris/rest/bitstreams/1425052/retrieve). The availability of the complete genomic sequence of the *Monkeypox virus* responsible for the current outbreak in countries of nonendemicity (https://www.ncbi.nlm.nih.gov/nuccore/ON563414?utm_source=Blog&utm_medium=referral&utm_campaign=Monkeypox-genome&utm_term=ON563414&utm_content=20220526link1) serves as an important milestone, one which will help pave the way for the development of more robust molecular diagnostic assays.

Serology

Serodiagnosis of monkeypox infection can be considered when clinical material such as skin samples are not available for testing (5, 6). These methods include enzyme-linked immunosorbent assays (ELISAs), indirect immunofluorescence assays, hemagglutination inhibition tests, plaque reduction virus neutralization tests, radioimmunoassays (RIAs), radioimmunoassay adsorption (RIAA), and Western blotting (5, 6, 190–195). Humoral immunity following human monkeypox infection involves production of antibodies directed against *Monkeypox virus*-specific and *Orthopoxvirus* genus-specific epitopes (196, 197). Although

TABLE 3 CDC case definitions for monkeypox virus infection in humans[a]

Confirmed	Probable	Possible
Monkeypox virus DNA detection or culture growth	Detection of *Orthopoxvirus* DNA, viral particles (EM, IHC), or IgM (days 28–56 after rash onset), and no other *Orthopoxvirus* exposure	New monkeypox-like rash, high clinical suspicion, ≥1 epidemiologic risk factor(s) (travel, exotic animal contact, contact with infected case, and/or intimate contact with individual from group with high incidence of disease)

[a]From https://www.cdc.gov/poxvirus/monkeypox/clinicians/case-definition.html#:~:text=case%20report%20form.-,Confirmed%20Case,culture%20from%20a%20clinical%20specimen. EM, electron microscopy; IHC, immunohistochemistry.

immunologic cross-reactivity with other orthopoxviruses may be seen as a limitation, antibody detection methods may be helpful in ruling out infection or in supporting a presumptive diagnosis of monkeypox infection in the proper clinical context (196, 197). IgM-ELISA and IgG-ELISA (acute and convalescent) are the most practical serologic methods available for the diagnosis of monkeypox infection. IgM antibodies are usually detectable by day 5 after rash onset, compared to day 8 after rash onset for IgG (192). Reported sensitivities for IgM and IgG detection are 94.8% and 100%, respectively, while specificities are 94.5% and 88.5%, respectively (192).

Histopathologic findings of monkeypox infection mimic those of smallpox, VZV, and herpes simplex virus (178). However, immunohistochemistry using monkeypox-specific antibodies can be employed for laboratory confirmation (178).

CASE DEFINITION OF HUMAN MONKEYPOX INFECTION

According to the U.S. CDC (https://www.cdc.gov/poxvirus/monkeypox/clinicians/case-definition.html#:~:text=case%20report%20form.-,Confirmed%20Case,culture%20from%20a%20clinical%20specimen), a "confirmed case" of human monkeypox is one where *Monkeypox virus* is isolated in culture or *Monkeypox virus* DNA is detected by PCR or next-generation sequencing from a clinical specimen. A "probable case" is defined as one where there is genus-level detection of *Orthopoxvirus* DNA or identification of *Orthopoxvirus* particles on electron microscopy or immunohistochemistry of a clinical specimen, or demonstration of detectable levels of Orthopoxvirus-specific serum IgM from 28 to 56 days after rash onset, with no suspicion of other *Orthopoxvirus* exposure (including replicating *Vaccinia virus* in second-generation smallpox vaccines). A "suspect case" is one where an individual presents with a new exanthem compatible with monkeypox, there is a high degree of clinical suspicion of monkeypox infection, and there is at least one epidemiologic criterion, which may include travel to a country where monkeypox virus is endemic, contact with a living or dead exotic African animal (or its commercial by-products), contact with individuals with suspected, probable, or confirmed monkeypox infection, and/or intimate contact with individuals belonging to groups experiencing high attack rates of monkeypox. These case definitions are summarized in Table 3.

CROSS-PROTECTION FROM SMALLPOX VACCINATION

The global eradication of smallpox is one of the greatest achievements in modern medicine and was accomplished by an intensive vaccination campaign between the mid-1960s and mid-1970s under the auspices of the World Health Organization (198–203). Endemic smallpox had been eradicated from North America in the 1950s and eventually from the rest of the world in the 1970s, with the last reported naturally acquired case occurring in 1977 (198–203). In the early 1970s, routine smallpox vaccination of children in the United States, Canada, United Kingdom, and other European countries had thus ceased (198, 204). Longitudinal studies have shown that lifelong immunity following smallpox vaccination in otherwise-healthy individuals is the norm and confers a cross-protective efficacy of 85% against monkeypox infection (205). These observations are also consistent with evidence of serologically confirmed subclinical monkeypox infection in asymptomatic individuals who had received a smallpox vaccine up to 30 years prior to direct or indirect (fomite) exposure to infected prairie dogs during the 2003 U.S. monkeypox outbreak (206).

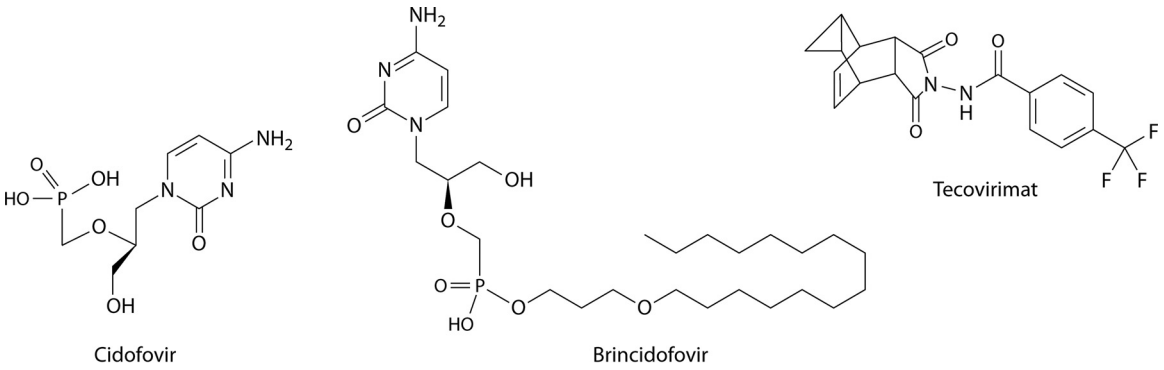

FIG 6 Chemical structures of cidofovir, brincidofovir, and tecovirimat. (Reproduced from https://commons.wikimedia.org/wiki/File:Cidofovir.svg, by Artur [username Ijfa-ag] [cidofovir], and from https://commons.wikimedia.org/wiki/File:Brincidofovir.svg [brincidofovir] and https://commons.wikimedia.org/wiki/File:Tecovirimat.svg [tecovirimat], by Ed [username Edgar181], all available under the Creative Commons CC0 1.0 Universal Public Domain Dedication.)

TREATMENT OF HUMAN MONKEYPOX INFECTION

The mainstay of therapy for human monkeypox infection involves nonpharmacologic supportive measures, although antiviral agents with activity against *Monkeypox virus* based on *in vitro* cell culture data, animal challenge studies, and phase I human trials are available for clinical use and should be considered for individuals with more severe manifestations of the disease or for postexposure prophylaxis in high-risk situations (207–248). Novel drugs, as well as those that are approved for other indications, have been investigated as potential therapeutic agents in this regard (Fig. 6).

Cidofovir

Cidofovir [(*s*)-1(3-hydroxy-2-phosphonylmethoxypropyl)cytosine] (HPMPC), a nucleotide analog of cytosine monophosphate that was approved by the U.S. Food and Drug Administration (FDA) in 1996 for the treatment of cytomegalovirus retinitis in patients with HIV, demonstrates potent activity against other herpesviruses and orthopoxviruses (207–210). This agent inhibits viral replication by binding to poxvirus DNA polymerase (207–212). Cidofovir preexposure prophylaxis has been shown to protect cynomolgus monkeys against intravenous infection with monkeypox virus, while cidofovir treatment of infected monkeys results in lower peripheral blood viral loads and skin lesion counts if administered within 48 h of inoculation (207, 213). Cidofovir treatment within 24 h of infection appears to be more effective than postexposure smallpox vaccination in preventing death of primates after lethal intratracheal challenge with monkeypox virus (214, 215). However, concurrent administration of cidofovir with smallpox vaccine may result in an attenuated humoral immune response following monkeypox virus challenge (214). Combination therapy with cidofovir and the chemotherapeutic agent mitoxantrone has been shown to have modest synergistic activity against monkeypox virus, based on a mouse model (216). Overall, these observations imply that for cidofovir therapy to be effective against human monkeypox infection, treatment may need to be initiated prior to the onset of the skin rash, which would require early diagnostic consideration and detection of viral DNA in peripheral blood samples during the prodrome phase. The standard adult human treatment dose of cidofovir (administered intravenously) is 5 mg/kg weekly for 2 weeks followed by 5 mg/kg biweekly (208). To mitigate the risk of nephrotoxicity, cidofovir must be coadministered with oral probenecid, which prevents the intracellular uptake of the drug by proximal renal tubular epithelial cells and requires intravenous prehydration with 1 to 2 liters of normal saline with or without additional fluid hydration post-cidofovir administration (207). The adult dose of probenecid is 2 g given 3 h prior to cidofovir administration, 1 g given 2 h postinfusion, and 1 g given 8 h post infusion (207). Cidofovir is contraindicated in individuals with a creatinine clearance of $<$55 mL/min, a baseline creatinine of $\geq$1.5 mg/dL, and significant proteinuria (207). Furthermore, the

safety and effectiveness of cidofovir in children and in pregnant or lactating women are unknown (207). Since cidofovir demonstrates embryotoxicity in animal models, the CDC (https://www.cdc.gov/poxvirus/monkeypox/clinicians/pregnancy.html) and other experts recommend avoiding this agent for the treatment of monkeypox infection in pregnant women, particularly during the first trimester, unless the disease is life-threatening (135, 250, 251).

Brincidofovir

Brincidofovir (hexadecyoxypropyl-cidofovir, also known as HDP-cidofovir or CMX001), is an orally bioavailable and less nephrotoxic lipid analog of cidofovir with demonstrated effectiveness in the treatment of orthopoxvirus infections, including Monkeypox virus, based on animal models (207, 208, 217–219). Brincidofovir is converted to cidofovir intracellulary, although it is not a substrate of the organic anion transporter in proximal renal tubular epithelial cells (220). Brincidofovir demonstrates greater *in vitro* potency than cidofovir by achieving higher intracellular concentrations of the active form of the drug, cidofovir diphosphate (220). Experience with brincidofovir for the treatment of human monkeypox infection has been limited. In 2018, three cases of human monkeypox infection in the United Kingdom (two imported, one nosocomial) received 1 or 2 weekly doses of brincidofovir, although treatment was discontinued prematurely in all three individuals due to hepatotoxicity (155). While brincidofovir does not have regulatory approval for the treatment of monkeypox in humans, the recommended dose is 200 mg weekly for three consecutive weeks, based on the results of phase I studies in healthy human volunteers and animal models of *Orthopoxvirus* infection (221). Of concern is the recent documentation of cidofovir-resistant strains of *Monkeypox virus*, which likely infers cross-resistance to brincidofovir, although the prevalence of such resistance remains unclear (222). The safety and effectiveness of brincidofovir for the treatment of monkeypox infection in pregnant or lactating women and in children are unknown (207). Akin to cidofovir, evidence of drug-induced embryotoxicity has been demonstrated in rat and rabbit models (250). Therefore, the CDC has recommended against the use of brincidofovir in pregnant women (https://www.cdc.gov/poxvirus/monkeypox/clinicians/treatment.html), and the existing FDA approval for the use of brincidofovir in the treatment of smallpox in pediatric patients does not extend to monkeypox infection in this patient population (https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/214460s000,214461s000lbl.pdf).

Tecovirimat

One of the most promising antivirals for the treatment of human monkeypox infection is tecovirimat {ST-246, or TPOXX; 4-trifluoromethyl-*N*-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2-(1*H*)-yl)carboxamide}, a small molecule that was discovered and codeveloped by SIGA Technologies (New York, USA) and the U.S. Government following high-throughput screening studies for drugs with activity against *Variola virus* (223–225). Tecovirimat was initially approved by the FDA (https://www.fda.gov/news-events/press-announcements/fda-approves-first-drug-indication-treatment-smallpox) in July 2018 for the management of biowarfare-related human smallpox infection in adults and children (226, 227). This approval was granted under the U.S. FDA Animal Rule for drugs (21 CFR 314.600–650), which involves extrapolation of efficacy based on the results of animal challenge studies when human drug trials would be deemed unethical given the nature of the disease being investigated (228, 229). A new intravenous formulation of tecovirimat manufactured by SIGA Human BioArmor was approved by the FDA on 19 May 2022 (https://investor.siga.com/news-releases/news-release-details/siga-receives-approval-fda-intravenous-iv-formulation-tpoxxr), based on animal data, and an expanded access protocol for intravenous tecovirimat administration for U.S. Department of Defense-affiliated personnel who are otherwise not eligible to receive oral tecovirimat has recently been implemented (https://clinicaltrials.gov/ct2/show/NCT05380752) with cosponsorship by SIGA Technologies (230). SIGA Human BioArmor recently announced Health Canada (https://investor.siga.com/news-releases/news-release-details/siga-announces-health-canada-regulatory

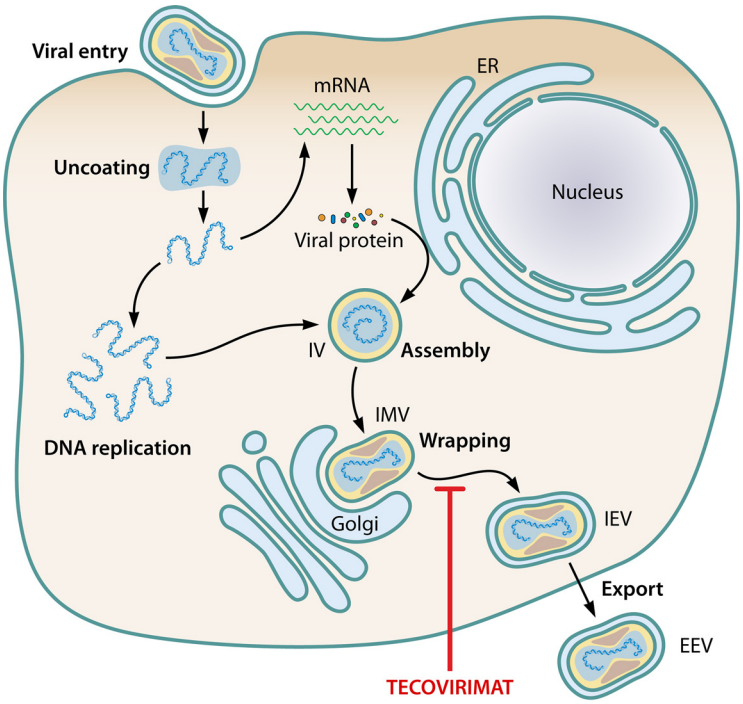

FIG 7 Mechanism of action of tecovirimat in a human cell infected with monkeypox virus (223, 225, 231–234, 249). IV, immature virus; IMV, intracellular mature virus; IEV, intracellular enveloped virus; EEV, extracellular enveloped virus; ER, endoplasmic reticulum.

-approval-oral-tpoxxr) and European Medicines Agency (EMA) (https://investor.siga.com/news-releases/news-release-details/siga-technologies-receives-approval-european-medicines-agency) approval of oral tecovirimat for the treatment of human smallpox infection, and the EMA approval also encompasses the treatment of monkeypox and other *Orthopoxvirus* infections in humans.

Tecovirimat inhibits VP37, a conserved *Orthopoxvirus* protein encoded by the *F13L* gene, which mediates Golgi-derived lipid "envelopization" (wrapping) and egress of intracellular *Orthopoxvirus* particles from infected host cells, a required step for viral dissemination (Fig. 7) (223, 225, 231–234, 249).

Nonhuman primate models have demonstrated tecovirimat's effectiveness and safety when used against *Variola virus* (235). In monkey challenge studies using lethal doses of aerosolized *Monkeypox virus*, tecovirimat administration conferred complete protection against infection when administered within 3 to 5 days of challenge and was also shown to improve survival when administered up to 8 to 10 days following exposure (236, 237). The effectiveness of tecovirimat may have been most convincingly demonstrated in a recent study using a prairie dog model, whereby animals were intranasally challenged with *Monkeypox virus* and subsequently administered a nondrug vehicle (control) or a 2-week course of tecovirimat beginning on days 0 or 3 of inoculation or after the onset of the pox rash (238). The investigators found that early treatment (postexposure prophylaxis) conferred almost complete protection against symptomatic disease (238). Furthermore, if treatment was initiated after the onset of rash, the animals survived, although manifestations of the disease were not prevented (238). Using a nonhuman primate model, Berhanu and coworkers found that postexposure treatment with tecovirimat alone or in conjunction with a second-generation smallpox vaccine (ACAM2000) prevented severe disease and death compared to those treated with vaccine alone, which did not confer any protection (239). Tecovirimat was found to be 83% protective if administered 4 to 5 days after exposure, compared to a protection rate of 50% if treatment was delayed until day 6 postexposure (239). In a mouse model, the combination of tecovirimat and brincidofovir demonstrated

synergy in reducing mortality from *Orthopoxvirus* infection, with no excess toxicity, suggesting that combination therapy using these two antivirals could be considered for human infections (240). However, coadministration of tecovirimat with a second-generation smallpox vaccine (ACAM2000) to primates exposed to lethal doses of *Monkeypox virus* resulted in similar outcomes at the cost of reduced vaccine-mediated immunogenicity compared to controls receiving only the vaccine (241). In contrast, tecovirimat protected T-cell-deficient knockout mice from lethal monkeypox virus challenge if drug was administered on the day of exposure (242).

Animal models have been instrumental in establishing a safe and effective dosing regimen of tecovirimat for the treatment of human *Monkeypox virus* infections. In animal challenge studies, the intravenous route of exposure for *Variola virus* (and, accordingly, for *Monkeypox virus*) bypasses the normal viral particle entry pathway through the respiratory tract, oral mucosa, or skin, thereby initiating what would normally be the second viremic phase following natural infection, and treatment initiation following such exposure would, thus, correspond to treatment initiation just prior to or during the prodromal or early rash phases in putative infections (224). Studies in animals have revealed that the drug is well tolerated and is not associated with any major adverse events (233). Pharmacokinetic and pharmacodynamic studies in cynomolgus monkeys inoculated intravenously with monkeypox virus have shown that a tecovirimat dose of either 400 mg or 800 mg daily for 14 days should prove to be safe and effective for prophylaxis or therapy of human monkeypox infections (243). Further research in nonhuman primates as well as phase I safety data in healthy human volunteers have led to the recommendation that in adults weighing at least 40 kg, the ideal dose of oral tecovirimat for the treatment of human monkeypox infection is 600 mg twice daily for 14 days (225, 244–248). Although resistance to tecovirimat has not emerged during therapy, *in vitro* cell culture studies have shown that mutations in the *F13L* gene decrease the half-maximal effective concentration of the drug by up to 95-fold (233). The standard adult dose of intravenous tecovirimat is 200 mg every 12 h for up to 14 days, according to the FDA (https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/214518s000lbl.pdf). Despite the lack of human safety data on the use of tecovirimat in pregnant and lactating individuals, studies in mice have demonstrated minimal distribution of the drug to placental tissue and breastmilk (225). Moreover, mouse and rabbit models have failed to show any drug-related adverse fetal effects or impact on maternal fertility (225, 237). Since pregnant women are at potentially increased risk of severe illness from monkeypox infection, the CDC (https://www.cdc.gov/poxvirus/monkeypox/pdf/tecovirimat-ind-protocol-cdc-irb.pdf) has endorsed the use of tecovirimat in this patient population under an investigational new drug application if an individualized clinical assessment deems the potential therapeutic benefits to outweigh any unknown pregnancy-associated risks. A liquid chromatographic assay using mass spectrometry has recently been developed for therapeutic drug monitoring of tecovirimat blood levels, although it has not yet received regulatory approval for routine diagnostic use (252). Table 4 provides a synopsis of the currently available antivirals with activity against *Monkeypox virus*.

Future Candidate Antivirals

Drug screening studies of adamantane molecules have identified newer-generation preclinical compounds capable of inhibiting *Orthopoxvirus* replication *in vitro* by binding to VP37, the same protein that is targeted by tecovirimat (253). These agents have not yet been studied in animal models and are likely years away from being candidates for human studies.

PRE- AND POSTEXPOSURE PROPHYLAXIS WITH SMALLPOX VACCINE

Given the close immunologic relationship of viruses belonging to the *Orthopoxvirus* genus, preexposure or postexposure prophylaxis with smallpox vaccines may prevent or reduce the burden of human monkeypox infection, according to the CDC (https://www.cdc.gov/poxvirus/monkeypox/clinicians/smallpox-vaccine.html). Ample stockpiles of live

TABLE 4 Currently available antiviral agents for the treatment of human monkeypox virus infection[a]

Drug	Mechanism of action	Indication	Dosage	Common adverse event(s)
Tecovirimat	Inhibits viral protein VP37, which mediates Golgi-derived lipid "envelopization" and exocytosis of intracellular orthopoxvirus particles	Treatment of smallpox in adults and children weighing ≥13 kg	600 mg orally twice daily for 14 days, or 200 mg i.v. every 12 h for 14 days	Headache, nausea
Cidofovir	Inhibits viral DNA polymerase	Not approved for treatment of orthopoxvirus infections	5 mg/kg i.v. wkly for 2 wks, followed by 5 mg/kg i.v. biweekly until symptom resolution	Nephrotoxicity, leukopenia, thrombocytopenia
Brincidofovir	Inhibits viral DNA polymerase	Not approved for treatment of orthopoxvirus infections	200 mg orally, wkly, for 3 consecutive wks	Gastrointestinal upset

[a]From references 207–212, 217–221, 223–229, 231–234, 237, and 244–248. Administered as intravenous (i.v.) dosing regimen of tecovirimat, based on recommendations from the FDA (https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/214518s000lbl.pdf).

attenuated vaccines that are effective against smallpox and monkeypox are maintained in the United States, Canada, and Europe, based on reports from the CDC (https://www.cdc.gov/smallpox/bioterrorism-response-planning/public-health/vaccination-strategies.html#:~:text=The%20Strategic%20National%20Stockpile%20(SNS),state%20or%20territorial%20health%20department), Health Canada (https://www.canada.ca/en/public-health/services/publications/healthy-living/canadian-immunization-guide-part-4-active-vaccines/page-21-smallpox-vaccine.html), EMA (https://www.ema.europa.eu/en/medicines/human/EPAR/imvanex), and other researchers (203, 254–257). These vaccines are derived from the closely related *Vaccinia virus* and appear to provide durable protection against all orthopoxviruses (256, 258). The immune response following smallpox vaccination or *Monkeypox virus* infection is a composite of humoral and cell-mediated functions, being mediated primarily by neutralizing antibody-producing B cells as well as $CD4^+$ and $CD8^+$ T lymphocytes (259–264).

First-Generation Vaccines

When the global smallpox eradication campaign was in full gear during the 1960s and 1970s, a wide repertoire of vaccines manufactured from biologically unique *Vaccinia virus* strains were being administered in various jurisdictions around the world (255, 256, 265, 266). In the United States and Canada, a lyophilized calf lymph-derived live attenuated vaccine using the New York City Board of Health strain of *Vaccinia virus* was predominantly used and was marketed under the tradename Dryvax (255, 256, 265, 266). A similar first-generation vaccine produced from the Lister strain of *Vaccinia virus* was used in Europe, Africa, and Asia (265). Serious adverse events following administration of these vaccines were reported in up to 2% of recipients and occurred more commonly in immunocompromised individuals and those with eczematous skin disorders (255, 256, 259). Such reactions included skin necrosis at the vaccine injection site and complications related to dissemination of the *Vaccinia virus* vaccine strain, including widespread infection of the skin, myocarditis, encephalitis, and/or death (255, 256, 259, 267). These vaccines have been replaced by newer-generation agents with a much-improved safety profile, although they were successfully used for pre- or postexposure prophylaxis of 30 contacts during the 2003 U.S. prairie dog-related human monkeypox outbreak (144). Although some countries maintain stocks of these first-generation vaccines for use in the event of an emergency, the United States replaced their national Dryvax stockpile with second-generation vaccines in 2008 (257).

Second-Generation Vaccines

At the turn of the 21st century's first decade, second-generation replication-competent attenuated *Vaccinia virus* vaccines were developed for biodefense purposes as successors to older-generation vaccines (203, 255–257, 268). Compared to first-generation products such as Dryvax, these newer vaccines were found to be equally safe and

immunogenic (269, 270). The FDA approved ACAM2000 in 2007 as a second-generation smallpox vaccine to replace the national stockpile of Dryvax, while Elstree-BN replaced the first-generation Lister strain vaccines being used in Europe (257, 268). In monkeypox virus challenge experiments involving nonhuman primates, a single dose of ACAM2000 administered 28 days prior to exposure provided complete protection from severe illness or death, with complete eradication of transmissible virus from the pharynx (271). Similarly, ACAM2000 administration 2 months prior to lethal monkeypox virus challenge in nonhuman primates resulted in complete protection against death, mitigation of any appreciable signs or symptoms of infection, elimination of pharyngeal viral shedding, and the development of neutralizing antibodies with titers comparable to those generated by Dryvax (272).

Third-Generation Vaccines

The unfavorable safety profiles of second-generation vaccines eventually paved the way for the development of a new generation of candidate vaccines using advanced cell culture techniques (255, 266). Modified *Vaccinia virus* Ankara (MVA) is the prototypical replication-deficient strain used in third-generation smallpox vaccines in North America and Europe (203, 255, 265). A *Monkeypox virus* challenge model in macaques demonstrated that prevaccination with a single dose of MVA averted death and provided almost complete protection from clinical illness (273). However, in a comparable study, a single dose of an MVA vaccine (IMVAMUNE) was inferior to a two-dose regimen (days 56 and 28 preexposure) or to a single dose of ACAM2000 in nonhuman primates exposed to aerosolized *Monkeypox virus* (271). The study authors argued that two doses of MVA vaccine, rather than a single injection, should be administered for preexposure prophylaxis in clinical practice (271).

Phase I clinical studies in human volunteers have found these third-generation vaccines to be safe and immunogenic, with minimal local or systemic adverse events and correspondingly high titers of neutralizing antibody (274, 275). In one study, all participants who had previously been immunized with a first-generation smallpox vaccine were found to have detectable antibodies following IMVAMUNE administration, irrespective of their baseline antibody levels at study entry (275). In a phase II trial of adult humans with HIV and a prior diagnosis of an AIDS-defining illness, administration of two (0 and 4 weeks) or three (0 and 4 weeks followed by a booster at 12 weeks) doses of MVA vaccine generated high titers of neutralizing antibodies with minimal adverse events (276).

Postexposure prophylaxis studies of third-generation smallpox vaccines demonstrated complete protection against death when administered within 24 h of a low-inoculum exposure, compared to incomplete protection if administered at day 3 postexposure or in the setting of a very heavy inoculum (275). In contrast, ACAM2000 was equally effective at days 1 and 3 of vaccination in the low-dose exposure setting (277). According to the CDC (https://www.cdc.gov/poxvirus/monkeypox/clinicians/smallpox-vaccine.html), MVA-based vaccines are incapable of replicating in human and other vertebrate cells, making them safe for use in immunocompromised persons and pregnant women, although establishing an international smallpox vaccine registry for pregnant women has been recommended to assess safety and efficacy in this patient population (134, 278).

The Advisory Committee on Immunization Practices (ACIP) in the United States recommends preexposure prophylaxis with third-generation vaccines (generic name MVA-BN, the tradename of JYNNEOS in the United States, IMVAMUNE in Canada, and IMVANEX in Europe) for laboratory workers involved in monkeypox diagnostic testing, as well as for health care workers who administer smallpox vaccine or who are involved in the care of patients with monkeypox virus infection (279). This replaces the previous ACIP recommendation in 2006, which involved the use of ACAM2000 (280). The National Advisory Committee on Immunization Practices in Canada (https://www.canada.ca/content/dam/phac-aspc/documents/services/immunization/national-advisory-committee-on-immunization-naci/guidance-imvamune-monkeypox/guidance-imvamune-monkeypox-en.pdf)

TABLE 5 Currently available vaccinia virus vaccines[a]

Product (trade name)	ACAM2000	JYNNEOS, IMVANEX, and IMVAMUNE
Formulation	Second-generation cell culture-based replication-competent attenuated vaccinia virus	Third-generation live, replication-deficient modified *Vaccinia* Ankara (MVA-BN)
Indication	Preexposure prophylaxis against smallpox and monkeypox	Preexposure prophylaxis against smallpox and monkeypox
Contraindications	Immunocompromise; eczema, infancy, pregnancy, breastfeeding, cardiac disease; allergy to vaccine component	Allergy to vaccine component
Dosing regimen and administration	One dose given subcutaneously using a bifurcated needle	Two doses given subcutaneously at days 0 and 28
Boosters	Every 3 yrs	Every 2 yrs
Efficacy	Limited data, although potentially comparable to that of Dryvax	Limited data, but potentially lower than that of ACAM2000 or Dryvax

[a]From references 251, 255–257, 259, 260, 263–275, 279, and 281.

recommends an exposure risk assessment and, if eligible, two doses of IMVAMUNE administered at least 28 days apart for monkeypox preexposure prophylaxis. A recent study showed that a 28-day vaccination interval resulted in significantly greater geometric mean antibody titers compared to a 7-day interval, although the clinical significance of these findings is unknown (281). The currently available vaccinia virus vaccines are summarized in Table 5.

PREVENTION AND CONTROL OF HUMAN MONKEYPOX INFECTION

For individuals with suspected or confirmed monkeypox infection, a surgical mask should be worn to prevent droplet transmission from respiratory secretions, dressings should be used to cover all skin lesions until they have crusted over, personal use items (e.g., towels, kitchen utensils) ought not be shared, cleaning (e.g., using dilute bleach solution or ethyl alcohol) of frequently touched environmental surfaces should be employed, contaminated clothing should be laundered in a hot wash cycle, and contact with household members and nonhousehold people should be avoided until the illness has resolved, according to the CDC (https://www.cdc.gov/poxvirus/monkeypox/clinicians/infection-control-home.html#:~:text=Avoid%20contact%20with%20unaffected%20individuals,shared%20with%20other%20household%20members), Government of Canada (https://www.canada.ca/en/public-health/services/diseases/monkeypox/health-professionals/interim-guidance-infection-prevention-control-healthcare-settings.html), and ECDC (https://www.ecdc.europa.eu/sites/default/files/documents/Monkeypox-infection-prevention-and-control-guidance.pdf). Healthcare workers involved in the care of patients with monkeypox should don gloves and gowns, ensure adequate respiratory protection using N-95 masks and face shields, and maintain excellent hand hygiene. Hospitalized patients with confirmed or suspected Monkeypox should be isolated under airborne, droplet, and contact precautions until further information is available to guide infection prevention and control efforts. Based on an average incubation period of 7 to 8 days for confirmed cases during the current multinational monkeypox outbreak, the CDC (https://www.cdc.gov/poxvirus/monkeypox/clinicians/monitoring.html) and ECDC recommend that close contacts of infected individuals should be observed for a minimum of 3 weeks following the last known exposure (282, 283). Domestic pets may be at risk of acquiring monkeypox from humans, as evidenced by a recent report of transmission to a dog from its household owners (284). The CDC (https://www.cdc.gov/poxvirus/monkeypox/prevention/pets-in-homes.html) has, therefore, recommended that persons with monkeypox infection avoid contact with domestic pets or other animals.

CONCERNS ABOUT A WIDESPREAD GLOBAL MONKEYPOX OUTBREAK

The emergence of infectious diseases such as monkeypox in nonendemic areas has created a great deal of anxiety in light of our frightening experience with COVID-19. Although much is not known about monkeypox infection in humans (which, like

Ebola, had remained a neglected tropical disease for years until the recent outbreak drew the belated attention of the developed world), it is instructive to compare the trajectory of cases during the current outbreak coupled with decades of repeated spillover outbreaks in Africa. While it is not clear at present whether the current outbreak will be contained, we will certainly experience repeated and potentially large introductions that are linked to travel, as demonstrated by recent events. The basic reproduction number (R0) is used by epidemiologists to estimate the risks and size of an outbreak and its resulting dynamics (where R0 = mean number of secondary infections transmitted from a single case in the context of a nonimmune population). Based on historical data at a time when approximately 85% of the population was immune to smallpox, R0 had been estimated to be 0.6 to 1.0 for clade I and lower for clade II (27, 47, 75, 258). In contrast, the R0 for measles is estimated to range from 11 to 18 (285). A Monte Carlo stochastic model constructed by researchers in the 1980s suggested that sustained human-to-human transmission of *Monkeypox virus* in a nonimmune population would be highly unlikely (286). Nevertheless, growing case counts arising from improved testing clearly indicate that the effective reproductive number has been above 1 for some time, as would be required for a sustained outbreak, and more recent mathematical modeling studies have shown that *Monkeypox virus* has the potential for sustained human-to-human transmission as population immunity declines following the cessation of smallpox vaccination (75, 287). McMullen and colleagues highlighted the importance of comprehensively addressing all potential factors involved in predicting viral transmissibility within a population (288). Furthermore, researchers from the United Kingdom have reported that R0 for monkeypox greatly exceeds 1 among networks of MSM compared to R0 levels below 1 in non-MSM sexual networks (289). Assuming a secondary attack rate (SAR) of 5% (which is comparable to rates reported for clade II in the African subcontinent), R0 approaches 10 in MSM networks where the average number of sexual partners exceeds 20 over a 3-week period of infectivity and may be higher under other modeling assumptions (289). Given the number of cases reported in the United Kingdom during the month of May 2022, the outbreak potential of the virus is indisputable, although the impact of non-MSM populations appears far smaller in this regard (289). Further investigations and enhanced surveillance will be required to define the risk to the population in general and the extent to which enhanced awareness and vaccination will be capable of controlling transmission. Public health researchers have resorted to the analysis of wastewater samples for Monkeypox virus and other emerging infectious diseases as an effective tool for disease surveillance (290, 291). Data from the New York City Department of Health (https://www1.nyc.gov/site/doh/data/health-tools/monkeypox.page#surveillance) and the UK Health Security Agency (https://www.gov.uk/government/news/uk-monkeypox-case-numbers-begin-to-plateau) indicated that the incidence of human Monkeypox virus cases peaked in early August 2022 and are now on a downward trajectory in these jurisdictions, although it remains unclear if decreased case numbers are secondary to the protective effect of vaccines and/or behavioral changes. Even if the current outbreak is contained, we can expect further introductions, and we should not lose focus of the importance of controlling monkeypox in Africa. To ensure that the global community is adequately prepared to deal with this emerging infectious disease challenge in the present and immediate future, WHO declared on 23 July 2022 that the current monkeypox outbreak is a global health emergency (https://www.who.int/europe/news/item/23-07-2022-who-director-general-declares-the-ongoing-monkeypox-outbreak-a-public-health-event-of-international-concern). Genomic analyses have been instrumental in informing outbreak investigation efforts and in enhancing our understanding of the evolution, transmissibility, and pathogenesis of Monkeypox virus in Africa and nonendemic countries (11, 292–294). Sequencing data have revealed that the 2022 Monkeypox virus outbreak in Europe and the Americas originated from clade IIb, with further evolution into distinct intracontinental sublineages over time (293, 294). Researchers from the University of Edinburgh (https://virological.org/t/initial-observations-about

-putative-apobec3-deaminase-editing-driving-short-term-evolution-of-mpxv-since-2017/830) used a molecular clock based specifically on changes characteristic of APOBEC3 editing to demonstrate that there has been sustained human-to-human transmission of *Monkeypox virus* for several years. Real-time molecular evolutionary analyses will be essential for optimizing ongoing surveillance of Monkeypox and other viruses of medical importance.

CONCLUDING REMARKS

Monkeypox infection in humans represents another unprecedented global health threat, one that is associated with significant morbidity albeit with a low mortality. Active surveillance, enhanced diagnostic capacity, availability of newer-generation vaccines for pre- and postexposure prophylaxis, introduction of a potent antiviral with activity against *Monkeypox virus*, and widespread patient education campaigns have created some degree of optimism for containing the current outbreak in nonendemic regions. Like COVID-19, our experience with monkeypox is an example of how emerging infectious diseases can only be tackled through international public health collaborative efforts and incorporating interventions that target high-risk populations, and renaming the virus to avoid stigmatization will be essential in this regard (295–297).

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