

Monkeypox: Virology, Pathophysiology, Clinical Characteristics, Epidemiology, Vaccines, Diagnosis, and Treatments

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ABSTRACT -- The World Health Organization, has declared the recent multiregional outbreak of monkeypox, a global public health emergency. Monkeypox is a zoonotic viral infection endemic to the west and central Africa. It belongs to the Poxviridae family, the Chordopoxvirinae subfamily, and the Orthopoxvirus genus. The Poxviridae family generally consists of complex, large, enveloped, and linear double-stranded DNA viruses. The initial clinical symptoms of monkeypox are often fever, severe headache, lymphadenopathy, myalgia, and fatigue. The skin lesions typically erupt within 1–3 days of the onset of fever. The rash tends to be more localized on the face and extremities than on the trunk. Monkeypox is often a self-limiting infection, and symptoms last from 2 to 4 weeks. It is isolated from various species, but the exact natural host is uncertain. Monkeypox is transmitted by close contact with infected humans or animals. Currently, no specific medication is available for monkeypox, and the existing therapeutics are the anti-viral agents approved for smallpox infection, including tecovirimat, cidofovir, and brincidofovir. Additionally, the U.S. Food and Drug Administration has approved Vaccinia Immune Globulin Intravenous for treating vaccination complications. It is diagnosed by PCR. There are currently two vaccines licensed by the U.S. Food and Drug Administration. According to the WHO guidance, the first-generation smallpox vaccines held in national reserves of some countries are not recommended as they do not meet the current safety and manufacturing standards. The interim guidance indicates that new and safer (second- and third generation) vaccines for smallpox, may be beneficial for monkeypox prevention, including JYNNEOS, which has been approved for the prevention of monkeypox. Human monkeypox was first reported in 1970. Since then, it has caused several outbreaks, mainly in central and west Africa. The first monkeypox outbreak outside of Africa occurred in the United States in 2003, linked to contact with infected pet prairie dogs. More recently (2018-2021), monkeypox cases have been reported in travelers from Nigeria to the United Kingdom, Israel, Singapore, and the US. Since May 2022, multiple monkeypox cases have been confirmed in several non-endemic countries, raising the concern of an emerging global pandemic. This review is an updated overview of our current state of knowledge regarding monkeypox virology, pathophysiology, clinical characteristics, epidemiology, vaccines, diagnosis, and treatment options.

INTRODUCTION

The Poxviridae family consists of nucleocytoplasmic viruses with large double-stranded deoxyribonucleic acid (DNA) measuring 200-400 nm. They can be detected by light and electron microscopy. The

Poxvirus family is a large group of viruses that infect a wide range of animals, including mammals, birds, insects, and reptiles. Humans are the primary reservoir and host of Variola and Molluscum Contagiosum viruses. The Poxviridae family is divided into two subfamilies: Entomopoxvirinae

ABBREVIATION. FDA: Food and Drug Administration; DNA: Double-Stranded Deoxyribonucleic Acid; RT-PCR: Reverse Transcription Polymerase Chain Reaction; WHO: World Health Organization; CDC: the U.S. Centers for Disease Control and Prevention; ITR: Inverted Terminal Repetition; RNAseq: The high-throughput deep RNA sequencing; ORFs: Open Reading Frames; IC: Intermediate Compartment; ER: Endoplasmic Reticulum; TGN: Trans-Golgi Network; IFN: Interferon; PAMPs: Pathogen Associated Molecular Patterns; PRRs: Pattern Recognition Receptors; NF-Kb: Nuclear factor kappa-light-chain-enhancer of activated B cells; API: Activator Protein 1; MyD88: Myeloid Differentiation Primary Response Gene 88; TRIF: Toll/interleukin-1 Receptor Domain-Containing Adapter-inducing Interferon- β ; CTLs: Cytotoxic T Lymphocytes; NK: natural killer cell; TNF: Tumor Necrosis Factor; IL-1 β : Interleukin-1 β ; MHC: Major Histocompatibility Complex; ACIP: Advisory Committee on Immunization Practices; MVA: Modified Vaccinia Ankara; RFLP: Restriction Fragment Length Polymorphism; ELISA: Enzyme-Linked Immunosorbent Assay; VIGIV: Vaccinia Immune Globulin Intravenous; AIDS: Acquired Immune Deficiency Syndrome; HAN: Health Alert Network; LRN: Laboratory Response Network; SNS: Strategic National Stockpile; EA-IND: Expanded Access Investigational New Drug; PPEs: Personal Protective Equipment

(insect-infecting viruses, with four genera and 31 species) and Chordopoxvirinae (vertebrate-infecting viruses, with 18 genera and 52 species). Monkeypox belongs to the Poxviridae family, the Chordopoxvirinae subfamily, and the Orthopoxvirus genus (1-5). Figure 1 depicts the major genera in the Chordopoxvirinae subfamily. Some, such as Orf and Molluscum Contagiosum virus, produce a localized, self-limited infection by inoculation to the skin or localized cell proliferation. Others, such as Variola, cause a fulminant systemic disease (6). Zoonotic Orthopoxvirus outbreaks have repeatedly occurred worldwide (7). Table 1 summarizes the zoonotic members of the Poxviridae family, transmissible to humans.

Although the exact natural reservoir of the monkeypox virus is uncertain, various potential hosts have been identified. Susceptible species to monkeypox virus include rope squirrels, tree squirrels, Gambian pouched rats, dormice, sooty mangabey, and non-human primates (8-10). Monkeypox is a zoonotic viral disease and one of humans' four pathogenic Orthopoxvirus species. Variola virus (the causative agent of smallpox), Cowpox virus, and Vaccinia virus are the other pathogenic species causing human disease (8). Monkeypox transmission occurs through direct contact with infected humans and animals. Contact with saliva and body fluids, feces, lesions, lesion exudate or crust material, respiratory droplets, and contaminated items are possible ways of human-human transmission. Contact with infected animals and consumption of infected meat are possible ways of animal-human transmission (2, 8, 9, 11). Monkeypox virus incubation time is about 4-21 days

(9, 12, 13). The clinical manifestations of monkeypox are very similar to the ones of smallpox. The initial clinical symptoms include fever, severe headache, lymphadenopathy (lymph node enlargement), myalgia, and asthenia. The timing of lymphadenopathy is the main distinguishing difference between monkeypox and smallpox; in monkeypox, lymph node enlargement occurs early and typically at the onset of fever. Skin lesions often appear within 1–3 days of fever onset, and their numbers vary from a few to several thousand. Patients frequently develop pleiomorphic skin lesions on the face, palm, soles, or near the genitals or anus. However, the rashes may also be on other areas like the chest, mouth, conjunctivae, and cornea. The infection can last up to 4 weeks (2, 9, 14, 15). Reverse Transcription Polymerase Chain Reaction (RT-PCR) is the method of choice for routine diagnosis of monkeypox virus and other orthopoxviruses (8, 16).

During the summer and fall of 1958, two outbreaks of a non-fatal pox-like disease were observed in cynomolgus monkeys in a laboratory in Denmark; the name monkeypox originated from this initial discovery (17). monkeypox was first reported in August 1970 in Bokenda (a remote village in the Equatorial province of the Democratic Republic of the Congo, called Zaire) in a 9-month-old child who was admitted to Basankusu Hospital. Initially, the patient was suspected of having smallpox, but after further sample evaluations, the monkeypox virus was isolated and confirmed (18-20).

Since the 1970s, monkeypox virus has caused several outbreaks, mainly in central and west Africa. The outbreak in the US in 2003 was the first monkeypox outbreak reported outside of Africa (21). There was not any report of monkeypox for several years until the monkeypox outbreaks occurred in Nigeria (2017-2021) and Cameroon (2018) (22-24). Moreover, since 2018, monkeypox cases have been reported in 109 locations, including 102 non-endemic countries and territories such as the United Kingdom, Israel, Singapore, and the United States, mainly from travelers infected in Nigeria (25-30).

In fact, between 2010-2021, the number of monkeypox cases had increased at least 10-fold compared to the 1970s. Moreover, the patients in the 1970s were primarily young children (the median age of 4 years old) but recently confirmed patients are predominantly young adults (the median age of 21 years old) (31, 32). It can be due to the cessation of smallpox vaccinations, which could provide some cross-protection against monkeypox (32). Previously

documented data have indicated that the smallpox vaccine (Vaccinia virus) was approximately 85% protective against monkeypox virus (33, 34).

The 2022 outbreak of monkeypox has a wide geographical spread. Several countries in both endemic and non-endemic regions have reported increased cases of monkeypox. More than 1,500 cases were reported in 43 countries by June 10, 2022, including Europe and North America. The World Health Organization (WHO) has warned about this formidable infectious disease challenge in 2022 (15, 35). In the United States, the Centers for Disease Control and Prevention (CDC) has been tracking an outbreak of monkey pox reported in several countries, including the US. Fifty-six thousand twenty-six confirmed cases have been reported globally until September 7, 2022. Fifty-five thousand five hundred fifteen of these cases were reported from 102 non-endemic locations, which had not historically reported monkeypox. Five hundred eleven cases were reported from 7 countries, which had historically reported monkeypox. Reports from CDC on September 7, 2022, indicate 21,274 total confirmed monkeypox/Orthopoxvirus cases in the US. (30, 36).

There are many knowledge gaps in the plan for managing monkeypox, including emergence, epidemiology, prevention, and treatment of this disease. This review aims to provide updated information about monkeypox virus for designing better prevention and treatment.

METHODS

Search strategy and criteria

Two databases, PubMed and ScienceDirect, were searched to identify the latest published information about monkeypox. The keywords used in this review were: (Monkeypox), (Monkeypox) AND (Molecular virology), (Monkeypox) AND (Pathophysiology), (Monkeypox) AND (Signs and symptoms), (Monkeypox) AND (Epidemiology), (Monkeypox) AND (Vaccine), (Monkeypox) AND (Diagnosis), (Monkeypox) AND (Treatment).

The criteria for inclusion were the texts written in English and the date of publication between 1/1/1970 and 8/3/2022. Non-relevant articles, experimental laboratory or animal model studies, animal serology studies, book and book chapters, meta-analysis, articles pertaining to non-specific anti-viral medications, author correspondences, letters to the editor, projection models, articles regarding co-infection studies with monkey pox and

other viruses, and reports regarding regulation and health workers were excluded. Missing publications were identified by going through the reference lists of the selected articles. During the eligibility step and in the screening process, articles without a full text, abstracts with insufficient information, or broad topic articles on Poxviruses that lacked focus on monkey pox were excluded. The study selection and flow diagram are depicted in Figure 2.

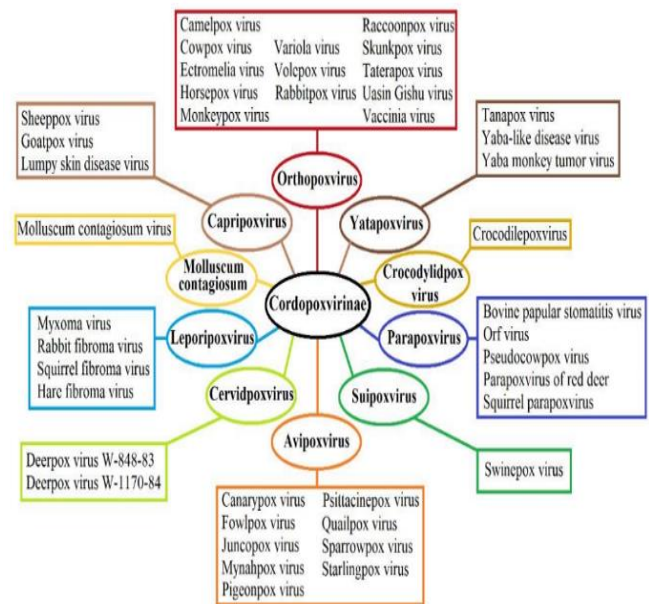


Figure 1. Major genera in the Chordopoxvirinae subfamily

Data extraction

After three steps of assessment for titles, abstracts, and full texts, the full text of each selected article was retrieved for detailed analysis. Data were extracted using a checklist, including authors' names, publication years, incubation, transmissions, reservoir, epidemiology, human clinical manifestations, diagnostic methods, complications, treatments, and prevention. From the search to the final data extraction, all processes were followed independently by two research experts (MS And SMMM). Probable discrepancies were resolved by the principal investigator (HGK).

Data Analysis

We used the content analysis method to analyze the data qualitatively. Content analysis is an objective, rule-guided method to make replicable and valid inferences. This method can analyze the characteristics of visual, verbal, and written documents.

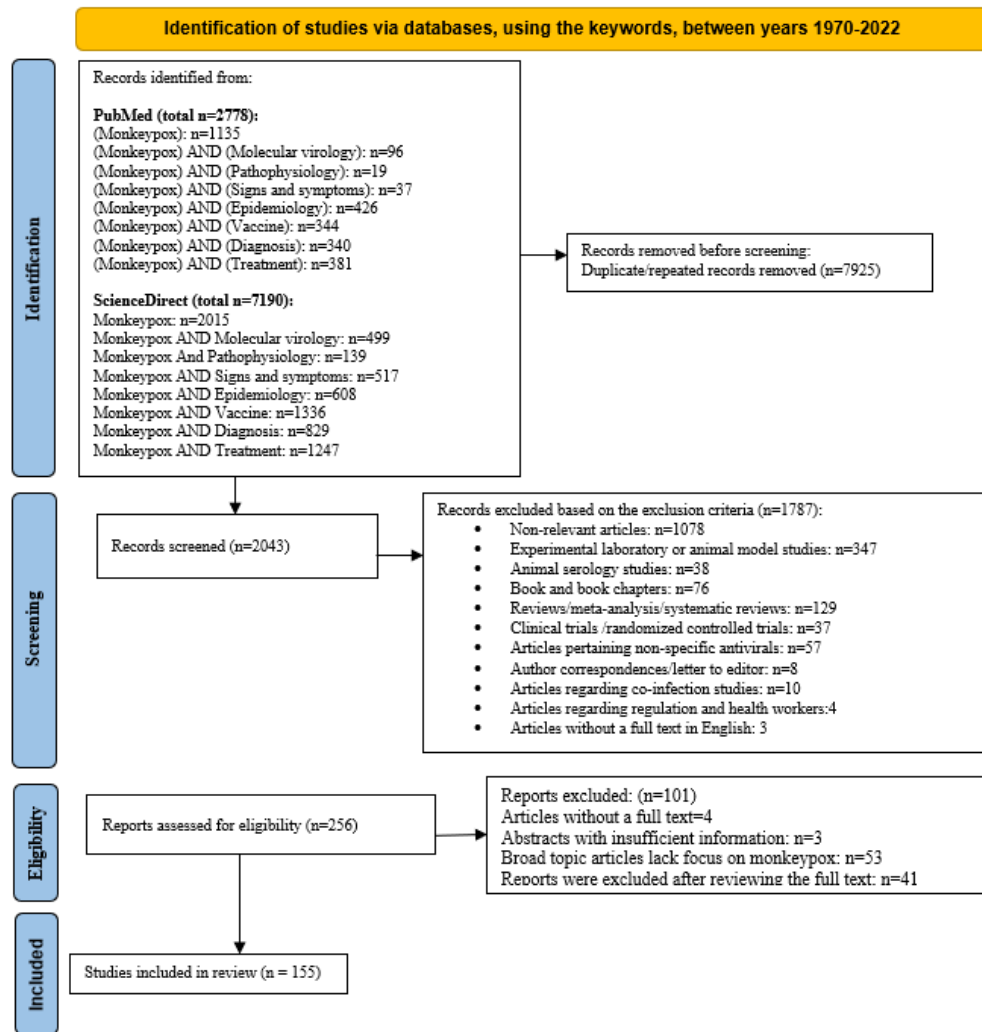


Figure 2. Study selection and flow diagram

RESULTS

Molecular virology

The Poxviridae family consists of complex, large, enveloped, and linear double-stranded DNA viruses. The Poxvirus family is a large group of viruses that infect a wide range of animals. The variola virus and Molluscum Contagiosum virus are human-specific. The Poxviridae family has two subfamilies: Chordopoxvirinae, which causes infection in vertebrates, and Entomopoxvirinae, which infect insects. The subfamily Chordopoxvirinae is subdivided into eighteen genera, including the Orthopoxvirus genus. Orthopoxviruses are large viruses (size range: 140–450 nm) with generally brick or oval-shaped forms (1, 9). Monkey pox belongs to the Orthopoxvirus genus and is one of the four human pathogenic Orthopoxvirus species. Variola virus, the causative agent of smallpox,

Cowpox virus, and Vaccinia virus are the other human pathogenic Orthopoxviruses (1, 84). Immunological cross-reactivity and cross-protection are common among Orthopoxviruses; infection with any member of the genus confers some protection against other members of the same genus (85).

Viral genome

Linear, double-stranded DNA genomes of Poxvirus range from 128 to 365 kbp, which are connected at both termini by an identical oppositely oriented sequence called an inverted terminal repetition (ITR) and form a continuous polynucleotide chain (5, 86).

The gene pool of Poxviruses is sufficient to replicate in host cell cytoplasm by virus-encoded proteins, enabling them to encode essential proteins, including RNA and DNA polymerases and mRNA biogenesis factors (87). The Poxvirus genes could be

classified into two major categories: highly conserved genes involved in cell entry, gene expression, DNA replication, and virion assembly, and less conserved genes that participate in specific host interactions (88) (Table 2).

The high-throughput deep RNA sequencing (RNAseq) studies have identified 118 Open Reading Frames (ORFs), which are expressed before viral DNA replication and 93 expressed after DNA replication. Close intervals and extensive overlap between ORFs make it challenging to distinguish intermediate and late genes (89).

Entrance and replication

The intracellular replication of the Vaccinia virus cycle has been well studied among Poxviruses. Poxviruses replicate entirely within the cytoplasm, which differs from most DNA viruses. The prototype Poxvirus genome has approximately 200 ORFs transcribed at the early, intermediate, and late stages of infection (89, 90). The viral transcriptosome (previously packed in the virus particles) provides immediate expression when the core enters the cytoplasm. The viral transcriptosome comprises transcription factors and enzymes necessary for the transcription of early genes. The multi-subunit RNA polymerase encoded by the virus makes an early protein that provides the replication of DNA. The replicated DNA provides a template for further replication and DNA synthesis and transcribes the intermediate and late genes (5, 88-90).

Some promoters have intermediate and late genes. The intermediate genes encode DNA binding/packaging elements, core-associated proteins, and late transcription factors. The late genes encode many morphogenesis and mature virion membrane proteins, including those involved in the virus entry and early transcription factors (89, 91).

After the virus's attachment, uncoating, and core entrance, the early viral gene starts to encode. It leads to the viral gene expression and remodeling of the endoplasmic reticulum, which establishes the viral factories (88, 92). These factories are sites for intermediate and late stages of transcription and translation (93); viral particle formation, transcription, and viral mRNA translation occur in these sites (69, 70, 76). Poxvirus DNA synthesis can be detected within two hours after infection in cell cytoplasm as virus factories are built from a single virion. The number of factories varies based on the viral disease (93, 94).

Additionally, before the genome is replicated, the early genes encode the nonstructural proteins, which

are essential for genome replication. The late genes are expressed following genome replication and encode structural proteins (89, 94). After late gene expression, virus assembly begins, and crescent membranes appear within the factories. Although a piece of direct evidence is lacking, the intermediate compartment (IC) between the endoplasmic reticulum (ER) and Golgi apparatus are considered possible sources of crescent membranes. Viral membranes typically form as open sheets, which are not connected to cellular organelles (93, 95, 96). These membranes enlarge to form spherical immature virions, which eventually condense into dense brick-shaped mature virions with a single external membrane. Depending on the poxvirus genus, some mature virions may obtain an additional outer membrane from the trans-Golgi network (TGN), endosomal cisternae, or plasma to form wrapped virions. Wrapped virions are subject to exocytosis as extracellular enveloped virions (93, 95, 97). Figure 3 depicts the entrance and replication cycle of the Poxvirus.

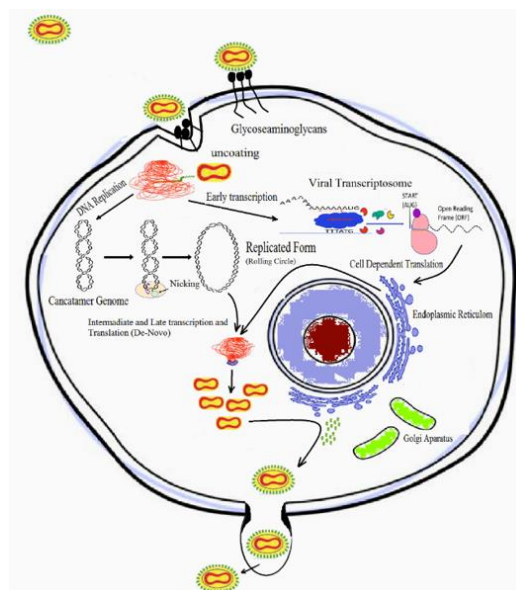


Figure 3. Viral entry into cells mediated through interactions between viral ligands glycosaminoglycans as cell surface receptors (chondroitin sulfate or heparan sulfate). Fusion and uncoating occur in a low pH-dependent manner, and the core enters the cytoplasm.

In the cell cytoplasm, the virus releases the prepackaged viral factors and enzymes necessary for providing early expression (viral transcriptosome) and DNA replication. The Poxvirus translation depends

Table 1. Summary description of zoonotic members of the Poxviridae family, which may cause infectious illness in humans

Name	Genus, name of the disease	Incubation	Transmission	Reservoir	Epidemiology	Clinical manifestations in animals	Clinical manifestations in humans	Complications	Diagnostic modalities	Treatment	Prevention
Variola virus (HS) (37, 38)	Orthopoxvirus, smallpox	7-17 days	Direct and prolonged face-to-face contact, through respiratory (coughed or sneezed) droplets, scabs and body fluids, contaminated clothing or bedding	Human	The incidence is highest during winter and early spring	No signs or symptoms	Severe headache, backache, fever ($\geq 101^{\circ}\text{F}$), enanthema over the tongue, mouth and oropharynx, maculopapular rash, pustules, pneumonia, cough.	Blindness due to viral keratitis, arthritis, and encephalitis. Death due to toxemia, associated with immune complexes, and Hypotension.	RT-PCR, antigen or nucleic acid detection, Electron-microscopy, culture on chorioallantois, Serology.	Antivirals: Tecovirimat, cidofovir, brincidofovir.	Smallpox vaccines: ACAM2000 [®] and JYNNEOS are FDA licensed. Aventis Pasteur Smallpox Vaccine (APSV) is an investigational vaccine that may be used in a smallpox emergency under the appropriate regulatory mechanism.
Molluscum contagiosum virus subtype 1 (HS) (39, 40)	Molluscipoxvirus, Molluscum Contagiosum (MC)	2-6 weeks	Direct contact with infected skin such as sexual, non-sexual, or by autoinoculation, contaminated fomites like bath sponges or towels. Vertical transmission is possible	Human, chimpanzees	Predominantly in children between 2-5 years old. In adults, sexually transmitted or during contact sports. Immunocompromised patients are at higher risk of infection	No signs or symptoms	Firm rounded papules from 2 to 5 mm, pink or skin-colored, eczematous plaques around one or more lesions.	Bacterial super-infection, molluscum dermatitis, conjunctivitis	Dermoscopy, Reflectance confocal microscopy, histopathological assay	Mechanical (cryotherapy, curettage, pulse dye laser Therapy), Chemical (Cantharidin, Potassium hydroxide, etc.), immunomodulatory (Imiquimod, Cimetidine, Diphencyprone), and anti-virals (cidofovir).	No FDA-approved vaccines are available.
Bovine papular stomatitis virus (41-44)	Prapaxvirus, Bovine papular stomatitis	2-4 days	By contaminated devices or organs of the infected animal	Calves, humans.	Primarily observed in spring and early summer. Morbidity rates may reach 100 percent.	Papules on the muzzle, lips, hard palate, and oral mucosa of calves. Viruses are shed in secretions of the respiratory and alimentary tracts (including saliva).	Nodules and pustules on the hands and sometimes on the face.	Not reported	Clinical and histopathological evaluations and findings, electron microscopy, PCR. Basophilic and eosinophilic intracytoplasmic inclusions may be investigated microscopically.	There is no treatment yet. Recurrent disease is possible because immunity is not long-lasting.	A live heterologous vaccine (based on attenuated Orf virus) has been designed, but its efficacy is still under investigation.

Table 1 continues...

Pseudocowpox virus (44-48)	Prapaxvirus, milker's nodule	5 to 15 days	By direct and indirect contact. Indirect contacts include the calf suckling of multiple cows, flies, and contaminated milking equipment and facilities.	Humans, cows. Also, it can be a coincidental infection in herds with bovine herpes mammillitis or Cowpox.	It is a usual problem in the autumn and spring in dairy cows, especially when minor trauma to the teat epithelium happens. Sometimes it appears as an endemic disease.	Papulovesicular and pustular on the udder and skin of dairy cows.	Localized lesions on the hands, fever, regional lymphadenopathy, and erythema multiforme.	Bullous pemphigoid-like eruption, erythema multiforme.	Real-time PCR, clinical signs, histopathology, and electron microscopy.	Symptomatic treatment, curettage and Cauterization.	There is no FDA-approved vaccine available. Treating the cows' mastitis, using gloves, soap, water, and disinfectants before and after milking or handling the suspected animals.
Cowpox virus (49-53)	Orthopoxvirus, Cowpox	7 to 12 days	Contact with infected animals, primarily domestic cats (sometimes predators of wild rodents)	Humans, wild rodents, such as bank voles and wood mice	Lack of smallpox vaccination (abandoned since 1977) may render the population more vulnerable to the cowpox virus. Since keeping wild and exotic animal pets has become popular, wild and domestic animal hosts are expanding. Worldwide spread. It is economically significant, particularly in sheep farming. Sheep may fail to grow properly. Endemic diseases in the west and central Africa. The first monkeypox outbreak reported outside of Africa was in the US in 2003. Since May 2022, multiple monkeypox cases have been confirmed in several non-endemic	Multiple nodular skin lesions with firm crusts. Erosive erythematous lesions on the hand, face, and trunk.	Erosive lesions on the hands, face, or Trunk (mostly a single lesion).	Ulcer necrosis.	Real-time PCR, Serological tests.	Cidofovir, antibiotics, Surgery (in case of necrosis)	Cross-protection with smallpox vaccination. Use disposable gloves and disinfectants for suspected animals.
Orf virus (6, 54)	Prapaxvirus, Orf disease	2 to 3 days	Infected lesions in animals or fomites, including feeding troughs, fences, barn doors, and shears.	Goats, sheep, humans.		Vesiculopapular eruption on the gums, lips, nose, or groin	1 to 4 papules on the hands, Maculopapular rashes, target lesions, acute weeping, nodules.	Erythema multiforme and vesicular eruptions. Secondary bacterial infection.	Cell culture, complement fixation, fluorescent antibody, biopsy, electron Microscopy.	No specific treatment is available. Antibiotics in case of super-infection	A live vaccine is available for animals
Monkeypox virus (6, 8, 9, 14, 55-64)	Orthopoxvirus, Monkeypox	4-21 days	Close contact with infected animals and humans. Contact with saliva, body fluids, feces, lesions, lesion exudate or crust material, respiratory droplets, and contaminated items.	Rope squirrels, tree squirrels, Gambian pouched rats, dormice, sooty mangabey, and non-human primates.		Asymptomatic, lethargy, inappetence, nasal discharge, respiratory distress, diarrhea, skin lesions.	Fever, severe headache, lymphadenopathy, myalgia, asthenia, and skin lesions.	Conjunctivitis, secondary bacterial super-infection, pulmonary distress, pneumonia, encephalitis, alopecia.	RT-PCR, Electron Microscopy, Immunohistochemistry, tissue culture, serum samples for antibody detection, Restriction Length Fragment Polymorphism (RFLP), and whole-genome sequencing.	Tecovirimat, cidofovir, brincidofovir, Vaccinia Immune Globulin Intravenous (VIGIV).	FDA licensed smallpox vaccines: ACAM2000® and JYNNEOS.

Table 1 continues...

Rabbitpox virus (65-70)	Orthopoxvirus, Rabbitpox	5 to 7 days	Close contact with the infected animals.	Rabbits	countries, raising the concern of an emerging global pandemic. (No human-to-human transmission and animal-to-human transmission have been reported)	Lymphadenitis, popular lesions of the skin and mucous membranes, keratitis or ophthalmia, and orchitis.	Not reported.	Corneal ulceration followed by perforation, iridocyclitis or glaucoma, Laryngitis (all reported in rabbits).	RT-PCR	ISATIN β -thiosemicarbazone, rutilantin A, brincidofovir.	There is no vaccine for Rabbitpox virus disease. But it has been used as a model for producing smallpox vaccines.
Horsepox virus (7, 71, 72)	Orthopoxvirus, Horsepox	Ten days (Acute) to 40 days (subacute disease).	Close contact with infected animals and lesions (animal-to-human and animal-to-animal).	Originally horses, and then Cattles.	It was reported frequently during the 19th and early 20th centuries. Then after, it became progressively rare. The last case was seen in Mongolia. (No human-to-human transmission was reported). Infection with the Vaccinia virus does not occur except in laboratory workers. It is a safe virus because it does not cause severe disease in immunocompetent humans or animals. Its spread is worldwide. New pathogenic strains in Brazil and Colombia.	Skin lesions on the muzzle, external and internal lips, and external nares.	Not reported.	Not reported.	RT-PCR, histopathology, and electron microscopy	Local antiseptic (iodo-polyvinylpyrrolidone)	There is not any vaccine for Horsepox virus disease. It has been used as a model for producing smallpox vaccines.
Vaccinia virus (6, 7, 73-78)	Orthopoxvirus	3 to 5 days	Close contact with infected animals and lesions in cow's teats (animal-to-human and human-to-human)	Cattle, rodents, humans.	It is a safe virus because it does not cause severe disease in immunocompetent humans or animals. Its spread is worldwide. New pathogenic strains in Brazil and Colombia.	Vesicular and pustular lesions, mainly in the udder, teats, and around the nose and mouth.	One skin lesion called "Jennerian vesicle," lymphadenopathy, fever, and malaise.	Eczema vaccinatum, encephalopathy, Secondary bacterial infection.	Real-time PCR, electron microscopy.	Lipid esters of cidofovir, silver nanoparticles, and an ointment containing 1.7 mg/g of the interferon inducer poly (ICLC) (a complex of polyriboinosinic-polyribocytidylic acid with poly-L-lysine and carboxymethyl-cellulose).	Vaccinia virus is a vaccine for the prevention of smallpox. Also, it is a potential treatment for cancer gene therapy.
Yaba monkey tumor virus (79-82)	Yatapoxvirus	Unknown	Bite from an arthropod vector (mosquito), infection in monkeys and humans.	Unknown	It was first reported in rhesus monkeys housed in outdoor pens at Yaba near Lagos in West Africa.	Skin lesions	Subcutaneous tumors, composed of histiocytes, actively divide and become spindle-shaped.	Lymphadenopathy, scar.	Real-time PCR, Intracytoplasmic inclusion Bodies, TEM, virus detection by immunohistochemistry	Not reported	Not reported

Table 1 continues...

Tanapox virus (6, 79-83)	Yatapoxvirus	Unknown	Bite from an arthropod vector (mosquito), infection in monkeys and humans.	Unknown	It was first recognized in September 1957, when an outbreak occurred among Wapakomo school children in Ngau and Kenya. The virus was isolated and identified after a second outbreak in 1962 in Africa (Tana River).	Skin lesions	Short febrile illness followed by the appearance and development of individual, hard, raised nodules that regress after 3-4 weeks.	Lymphadenopathy, scar.	Real-time PCR, electron microscopy, histopathological analysis, and nucleic acid testing	No effective treatment	No vaccination
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Table 2. Major proteins of the poxvirus replication complex

	Protein Name	Function	Expression Status
Attachment	A26	Binds laminin; associated. with A27	Late expression
	A27	Binds heparan; associated with A17	Intermediate Expression
	D8	Binds chondroitin	Intermediate Expression
	H3	Binds heparan	Intermediate Expression
	A16	Component of Entry and Fusion Complex	Intermediate Expression
	A21	Component of Entry and Fusion Complex	Late expression
	A28	Component of Entry and Fusion Complex and Binds to H2	Late expression
Entry Proteins	F9	Associated with Entry and Fusion Complex	Late expression
	G3	Component of Entry and Fusion Complex AND Binds to L5	Late expression
	G9	Component of Entry and Fusion Complex	Late expression
	H2	Component of Entry and Fusion Complex AND Binds to A28	Late expression
	I2	Component of Entry and Fusion Complex	Late expression
	J5	Component of Entry and Fusion Complex	Late expression
	L1	Associated with Entry and Fusion Complex	Late expression
	L5	Component of Entry and Fusion Complex and Binds to G3	Late expression
	O3	Component of Entry and Fusion Complex	Intermediate Expression
	E9L	DNA polymerase	Early Expression
Replication Protein	D5R	Helicase-primase	Early Expression
	D4R	Uracil DNA glycosylase	Early Expression
	A20R	Processivity factor	Early Expression
	B1R	Protein kinase	Early Expression
	I3L	Single-stranded DNA-binding protein	Early Expression

Table 2 continues...

			A50R	DNA ligase	Early Expression
			G5R	FEN1-like nuclease	Early Expression
DNA processing			A22R	Holliday junction resolvase	Intermediate Expression
			H6R	Topoisomerase	Late Expression
DNA packaging			A32L	ATPase	Intermediate Expression
			I1L	Telomere-binding protein 1	Intermediate Expression
			J2R	Thymidine kinase	Early Expression
Precursor metabolism			A48R	Thymidylate kinase	Early Expression
			F4L, I4L	Ribonucleotide reductase	Early Expression
			F2L	dUTPase	Early Expression
			CRM B,C,D,E,MT2,vCD30	Viral TNF Receptor	
		viroreceptors	IFN α,β,γ receptor homologue	Viral IFN Receptor	Early Expression
			IL1B receptor homolog	Viral IL1B Receptor	
			GPCR Homologue	Viral GPCR Receptor	
			SERP1, SPI3	Viral serpins (Extracellular)	
			Semaphorin Homologue	Viral Serpins (Intracellular)	
			Cytokine Homologue	Viral IL10	
			MC148R	Viral Chemokine	
	Viromimicry	virokines	vEGF,vVEGF	Viral Epidermal Growth Factor	Early Expression
			GP38, GIF	Viral Cytokine Binding Proteins	
			VCP, IMP	Viral Complement Binding Proteins	
			CBP1,2	Viral Chemokine Binding Proteins	
			C12L (vIL18-bp)	Viral IL18 Binding Proteins	
			B15R (vIL18-bp)	Viral IL-1 β -binding protein	
			CrmA SP1,2	Viral serpine (Intracellular)	
			M-T2, MT-4, M-T5	Viral antiapoptotic protein	
			MC066L	Viral antioxidant protein	
		virotransduction	vFLIPs, vTLP	Viral signal Transduction Inhibitor	Early Expression
			3 β -H3D	Viral steroid synthesis	
			E3L	Viral ds-RNA binding protein	
			K3L (v-eIF-2 α)	Viral Initiation Factor 2	

on host ribosomes to translate their mRNAs and uses the Endoplasmic Reticulum (ER) membrane during its replication and assembly. Along with Poxvirus replication (rolling circle) and generating the replicating form, intermediate genes are transcribed and translated. Subsequently, they lead to the expression of early and late genes and prepare them for packaging. Membrane structures will appear, and concatemers of viral genomes along with early transcribed factors are assembled into nude virions. The immature virions are transformed into mature virions by fusing into the outermost lipoprotein layer. Mature virions move along cellular microtubules. Some mature virions may obtain an additional outer membrane to form wrapped virions. Wrapped virions are subject to exocytosis as extracellular enveloped virions and are released by actin polymerization.

Studying DNA synthesis suggested that it begins near the genome termini by forming nicking in the extrahelical bases of the terminal hairpins. The DNA replication complexes interact with the various replicating factors and yield a large functional enzyme complex (5, 98). Several essential proteins are involved in the replication complex formation, such as the E9L protein, a DNA polymerase with both DNA polymerase and proofreading activity, and A20R, which seems to be a central molecule in the replication complex (99, 100). The critical proteins of the Poxvirus replication complex are summarized in Table 2.

The entry of enveloped viruses depends on membrane fusion. Electron microscopic images showed that interaction with glycosaminoglycans and laminins at the cell surface supports the virus entrance. Additionally, low pH (<6) in acidified endosomes could enhance the entry of virus particles. The orchestras of conserved proteins in Poxviruses play a crucial role in the attachment and fusion of Poxviruses as a part of their entry mechanisms (101, 102) (Table 2).

PATHOPHYSIOLOGY

Clinical Pathology

Due to a low number of published studies, the knowledge of the systemic pathology of human monkeypox is currently limited. Vesiculopustular rash, fever, pruritus, headache, myalgia, and lymphadenopathy are common symptoms of human monkeypox. The rashes could be developed in all body parts, especially the face, which is the most affected area (103).

The skin lesions of human monkeypox are the most prominent feature of the infection. The skin lesions are pleiomorphic and include papules, vesicles, pustules, umbilicated pustules, ulcerating, pseudo-pustular, and scabs (104-106). The lesions are indistinguishable from ordinary smallpox. In the microscopical examination, all the features of variola virus infection, including epidermal hyperplasia, ballooning degeneration of keratinocytes with intracytoplasmic inclusions, intraepidermal vesicles and pustules, and crust formation, can be observed. Dermal changes are similar to smallpox and include oedema and infiltration with lymphocytes, macrophages, and to a lesser extent, neutrophils, and eosinophils (105, 107). Hematology serum biochemistry and data from reported cases indicate that patients may develop hypoalbuminemia (mild to moderate), leukocytosis, thrombocytopenia, and asthenia (105, 106, 108).

The innate and acquired immune response in Poxvirus infection

The innate immune response provides a faster but less specific immune response. The first line of host defense includes anti-viral proteins, including type I interferons (IFNs) (109), proinflammatory cytokines (110), chemokines (111), and other anti-viral proteins. Pathogen-associated molecular patterns (PAMPs), which recognize viral DNA, RNA, envelope, or core proteins through a diverse recognition system named pattern recognition receptors (PRRs), are other measures of the innate immune response against Poxviruses (112, 113). The host activating sensor mechanism based on the pattern recognition system includes Double-Stranded-RNA-Activated Sensors, Dual RNA/DNA Sensors, DNA-Activated Sensors, and Toll-Like Receptor-Mediated Poxvirus recognition. These effector molecules mediate or orchestrate the adaptive immune response to contain Poxvirus infection at various stages (112).

The activation of proinflammatory genes depends on the activation of the transcription factors, including the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and activator protein 1 (AP1) (114, 115). Moreover, myeloid differentiation primary response gene 88 (MyD88) (116) and Toll/interleukin-1 receptor domain-containing adapter-inducing IFN- β (TRIF) are the pathways that share common downstream molecules (117).

Poxviruses encode diverse classes of immunomodulatory proteins to inhibit apoptosis, the activity of cytotoxic T lymphocytes (CTLs), and natural killer (NK) cells. These immunomodulatory proteins also inhibit the production of IFNs, chemokines, inflammatory cytokines, complements, and antibodies. For example, orthopoxviruses such as Cowpox virus and Vaccinia virus encode multiple homologs for tumor necrosis factor (TNF) and interleukin-1 β (IL-1 β) receptors (89, 112, 118). The acquired immune system in Poxvirus infection down-regulates the expression of the class I Major Histocompatibility Complex (MHC) receptors (119). For instance, the Molluscum Contagiosum virus encodes an MHC I homolog, MC080R, potentially acting as a ligand for inhibition of the NK cell receptors (112). Additionally, proteins with sequence similarity to MHC I domains are coded by genomes of Cowpox (120), Sheeppox (121), and Yaba-like disease (122) viruses. These homologs class I MHC receptors are endogenous viral antigens to evade the virus from circulating CD8 CTLs (112).

Cellular immunity against Poxvirus includes cytolytic immune cells, especially NK cells and CTLs, which are critical for the rapid identification and clearance of virus-infected cells (116). Monkeypox virus evades anti-viral CD4+ and CD8+ T cells by suppressing cognate T cell activation through MHC Class II downregulation (123). It has been observed that monkeypox virus infection in rhesus macaques induces massive expansion of NK cells. Subsequently, the migrating capacity of NK cells to tissues at early stages is reduced, and the cytotoxicity function and cytokine secretion are vastly compromised (124).

CLINICAL MANIFESTATION

Signs and symptoms

Monkeypox symptoms usually start within three weeks of exposure to the monkeypox virus, typically lasting for 2-4 weeks. Most of the human monkeypox's clinical characteristics are similar to the smallpox infection. Often the infected individual develops flu-like symptoms. Fever, headache, muscle aches, backache, and fatigue are the typical initial symptoms. After the onset of fever and lymphadenopathy, rashes appear 1-3 days later. The infection can be transmitted from when symptoms start until the rash has healed. The significant distinguishing difference between monkeypox and smallpox is the timing of the lymph node enlargement that often occurs early at the onset of

fever. Fever often subsides on the day of or up to 3 days after rash onset. The lesions are more localized on the face (95% of cases), palms and soles (75% of cases), oral mucous membrane, extremities, genitalia, conjunctivae, and cornea. In severe cases, lesions can affect large skin sections and slough them off. The rash will go through several stages before healing, including papules, vesicles, pustules, umbilicated pustules, ulcerating lesions, and scabs. The infection can last up to 4 weeks until the lesion peels off. A range of complications may occur after monkeypox infection, including secondary bacterial infections, respiratory symptoms (e.g., sore throat, nasal congestion, or cough), gastrointestinal complications, dehydration, sepsis, encephalitis, and corneal infection that may result in loss of vision. (15, 125-128).

EPIDEMIOLOGY

Monkeypox in humans was diagnosed for the first time in 1970 in the Democratic Republic of the Congo. It has spread to Africa's west and central regions in recent years, while its prevalence outside Africa has dramatically increased (129). The median age of monkeypox infection in Africa has increased from 4 and 5 years old in the 1970s and 1980s to 10 and 21 years old in the 2000s and 2010s. Outside of Africa, it is more common in men. The overall case fatality rate was 8.7% which significantly differs by clades (Central African: 10.6%, West African: 3.6%) (22). The epidemiologic studies of monkeypox are summarized in Table 3.

VACCINATION

There are currently two vaccines licensed by the FDA for preventing monkeypox infection: ACAM2000 (Sanofi Pasteur Biologics Co) and JYNNEOS (Bavarian Nordic A/S) (138). According to the WHO guidance (Vaccines and immunization for monkeypox: interim guidance, 24 August 2022), the first-generation vaccines held in the national reserves of some countries from the smallpox eradication programme (concluded in 1980) do not meet current safety and manufacturing standards and therefore are not recommended. The guidance recommends the use of the newer and safer (second- and third-generation) smallpox vaccines such as the JYNNEOS (also known as MVA-BN), that have been approved for the prevention of monkeypox. The interim guidance reiterates that the supply of newer

Table 3. Epidemiologic studies of monkeypox

Country, Year	Descriptive	Analytical
The Democratic Republic of Congo, 2021 (130)	Conducted on confirmed laboratory patients with monkeypox (n=1057); most common ways of transmission were: 1) contact with animals (such as mice and squirrels) or bites by domestic and wild animals (36.9%), 2) exposure to infected humans (33.3%) which was mostly intra-family transmission (90.5%) and through friends (6.4%). The incubation time was three weeks. In most cases, the disease symptoms began with a fever, then rashes appeared. Most rashes were observed in the face (98%) and the trunk (97.3%). Other common symptoms were fatigue (86.3%), lymphadenopathy (84.7%), shivering (83%), headache (54.8%), muscle pain (75.2%) and dysphagia (71.3%). 99.4% of cases had a fever before the rash. The interval from fever to rash was about two days (1-34 days).	The average annual incidence of the disease was 14.1 per 100,000. Students showed the highest reported incidence (31.7%). Males, especially between 5-9 and 10-19 years old, had a higher infection rate. Vaccination in the upper age group caused a threefold difference in the disease occurrence compared to the lower age unvaccinated group.
The Democratic Republic of Congo, 2010 (131)	Not Reported	760 patients were included. The incidence rate was 5.53 per 10,000 per year. The infection rate (per 10,000 per year) in vaccinated and unvaccinated individuals groups was 0.76 and 7.35, respectively. The highest infection rate was among girls aged 0-4 years (7.95 per 10,000 per year) and boys aged 10-14 (9.35 per 10,000 per year). Several factors include living in forest areas, male gender (6.99 per 10,000 per year vs. 4.12 in females), age under 15, and vaccination history affected the infection rate.
Sudan, 2010 (132)	19 patients under 32 years old were studied. 79% of patients were under 20 years of age. 52% of the patients were women. The incubation period was 14 days. In most patients, the symptoms were fever, lymphadenopathy, sore throat, joint pain, and rash. The median time interval between the exposure and the onset of skin rash was five days (1-6 days). Eight of the patients were hospitalized. Fourteen patients had been infected through contact with an infected human, and three were possibly infected by contact with infected animals. The most common symptoms were fever (n=16), lymphadenopathy (n=15), and rash (n=19). Skin rashes were mostly scattered throughout the body. In three cases, rashes were observed in the arms and feet.	Not Reported
USA, 2010 (133)	30 confirmed patients mostly over 18 years old were studied. The incubation time was 21 days. 80% of the patients had no history of vaccination. The disease was transmitted from animals to humans by directly touching the infected dogs (46.7%) or other infected animals (76.7%). Reported symptoms were skin rashes (100%), fever (93.3%), lymphadenopathy (56.7%), cough (56.7%), and conjunctivitis(13.3%) .	Not Reported
Nigeria, 2020 (134)	Conducted on 40 hospitalized patients with monkeypox. 77.5% of patients were male, and 67.5% were under 35. Nine patients also had HIV co-infection. The average duration of the disease was three weeks (in some cases lasted more than 28 days). In 57.1% of HIV-positive and 12.5% of HIV-negative patients, the disease duration was more than 28 days. In 65.7% of cases, the onset of symptoms started with rash and 34.3% with fever. Rashes were observed in all patients. Other symptoms included Fever (90%), lymphadenopathy (87.5%), genital ulcer (62.5%), somatalgia (62.5%), and headache (47.5%). The size of skin rashes in 50% of patients was greater than two centimeters. Secondary bacterial infection was observed in all HIV- positive and 32.3% of HIV-negative patients.	Not Reported
Nigeria, 2019 (135)	Evaluated 122 patients (69% male). 26% of the patients were businesspeople, and 20% were students. The incubation time ranged from 3-34 days. The way of transmission was direct contact with infected humans (having signs and symptoms) as well as domestic and wild animals. Skin rash was observed in all patients. Fever (88%), headache (79%), pruritus (73%), and lymphadenopathy (67%) were the highest reported symptoms. 57% of cases had a fever before rashes. Of seven deaths among patients, 4 were HIV positive.	Not Reported
The Democratic Republic of Congo, 2017 (136)	752 patients with monkeypox were evaluated (53% male). The fever had the highest incidence among all symptoms (99.3%). In most patients, fever was reported before skin rashes. Rash (95.7%), lymphadenopathy (76.9%), shivering (79.7%), fatigue (79.7%), and conjunctivitis (17.1%) were the primary symptoms. The skin rash of patients in 88.4% of cases was the same size and in 94.1% was deep. 99.1% of rashes were located on the face, and 97.3% were found on the chest.	Not Reported
Portugal, 2022 (137)	27 patients were evaluated. All patients were male, and 48% were aged 30-39. Fourteen patients were HIV-infected. The incubation period was 21 days. Skin rashes (51.85%), lymphadenopathy (51.85%), fever (48.14%), and headache (25.92%) were the most common reported symptoms.	Not Reported

vaccines are limited, and access strategies are under discussion. Based on the current risks and benefits assessment, the WHO interim guidance concluded that regardless of vaccine supply, at this time, mass vaccination is not required nor recommended for monkeypox (139).

ACAM2000 is a second-generation smallpox vaccine derived from a plaque-purified clone of the same New York City Board of Health strain used to manufacture the Dryvax vaccine. The license for the Dryvax vaccine was withdrawn in 2008. ACAM2000 is an alternative to the JYNNEOS vaccine. ACAM2000 is supplied as a lyophilized preparation of purified live viruses. ACAM2000® is a replication-competent vaccinia virus administered in a single dose by the percutaneous route (scarification). 4 weeks after receiving ACAM2000, the vaccine recipient is considered fully vaccinated. Since 2015, the Advisory Committee on Immunization Practices (ACIP) recommended routine vaccination with ACAM2000 for laboratory personnel who directly handle or culture the vaccinia virus. ACAM2000 is grown in African green monkey kidney (Vero) cells and tested to be free of known adventitious agents. (140-142). Safety data from ACAM2000 clinical trials indicate a safety profile similar to Dryvax. The risk associated with ACAM2000 includes serious adverse effects such as progressive vaccinia, postvaccinal encephalitis, eczema vaccinatum, and myopericarditis. ACAM2000 is not recommended for immunocompromised people, skin conditions like atopic dermatitis/eczema, or during pregnancy (140, 143).

JYNNEOS, also known as MVA-BN, contains a live virus, and it is non-replicating in human cells. Other brand names include Imvamune and Imvanex. This third-generation smallpox vaccine is manufactured by Bavarian Nordic A/S and is indicated for preventing smallpox and monkey pox disease in adults 18 years and older. JYNNEOS is the primary vaccine being used during the recent monkeypox outbreak. JYNNEOS is a modified vaccinia Ankara (MVA), which was derived by more than 570 passages of the strain in primary chicken embryo fibroblast cells and became replication restricted to avian and specific mammalian cells (144, 145). JYNNEOS is provided as a suspension for subcutaneous injection only. Each dose (0.5 mL) is supplied in a single-dose vial. Two doses should be injected four weeks apart. People are considered fully vaccinated about two weeks after their second shot of JYNNEOS. Side effects include injection site

reactions such as itching, pain, redness, and swelling. Severe allergy to any vaccine components (gentamicin, ciprofloxacin, egg protein) is a contraindication of the vaccination. JYNNEOS is safe for vaccination patients with HIV and atopic dermatitis. Animal data do not indicate any reproductive harm. Therefore, pregnancy and breastfeeding are not contraindications (138, 146-148).

Monkeypox vaccine effectiveness

The smallpox vaccine is at least 85% effective in preventing monkeypox based on past reports from Africa (33, 34). The effectiveness of JYNNEOS vaccination against monkey pox was shown by an immunogenicity clinical study and efficacy data from animal studies (149).

Pre-exposure prophylaxis

Based on occupational exposure, ACIP recommends that select personnel such as clinical laboratory personnel, research laboratory workers, and designated healthcare and public health response team members whose jobs may expose them to Orthopoxviruses, such as monkeypox, get vaccinated with either ACAM2000 or JYNNEOS (149).

Post-exposure prophylaxis

In general, the sooner the monkey pox vaccine is given, the more effective it will prevent the disease. Individuals exposed to the monkeypox virus but not received the smallpox vaccine within the last three years are candidates for vaccination. Based on CDC recommendation, the vaccine should be administered within four days of exposure to prevent the onset of the monkeypox infection. If the vaccine is given between 4–14 days after the exposure, it may reduce the symptoms of monkeypox but may not prevent the illness (149).

DIAGNOSIS

Monkeypox is generally not clinically distinguishable from other Pox-like viruses, and clinical observation alone is insufficient for diagnosis. Therefore, rapid laboratory diagnostic methods are crucial to limit the outbreak. Existing laboratory evaluations for monkeypox cases include electron microscopy, immunohistochemistry, the culture of material from rash specimens, serological testing for specific antibodies, and RT-PCR assays. RT-PCR is the method of choice for routine diagnosis of monkeypox virus and other

Orthopoxviruses. Restriction fragment length polymorphism (RFLP) of PCR-amplified genes or gene fragments is also used to detect monkeypox DNA. However, this method is time-consuming and requires virus culture. Whole-genome sequencing, using next-generation sequencing technologies, is the gold standard for the characterization of monkeypox virus and other Orthopoxviruses. However, its use is limited, especially in developing countries, due to its high cost and advanced technology. Although serological tests provide evidence of virus exposure, they have limitations due to cross-reaction with other Orthopoxviruses. Immunological methods include enzyme-linked immunosorbent assay (ELISA) for IgG and IgM antibodies detection of the viral antigen. IgM is detected in serum about five days after the onset of rash, whereas IgG is detected more than eight days after the onset of rash. The presence of both IgM and IgG in a sample is strong evidence of recent exposure to Orthopoxviruses in patients who have been previously vaccinated or exposed to the infection (8, 9, 62-64, 150-152).

CDC's monkeypox virus testing algorithm includes non-Variola Orthopoxvirus testing. Non-Variola Orthopoxvirus is an RT-PCR primer and probe set assay to detect non-Variola Orthopoxvirus. If results are positive for Orthopoxvirus, further characterization testing will be performed at CDC. For effective diagnosis, CDC recommends that clinicians collect two specimens for each patient. Each sample should be collected from multiple lesions, preferably from different locations on the body and from lesions with differing appearances (153). FDA recommends that clinicians must swab the lesion because blood and saliva may give false results (154).

TREATMENT

Most monkeypox patients recover without medical intervention. Currently, there is no specific medication available for the treatment of monkeypox. The main monkeypox management measures are supportive care, antivirals, and Vaccinia Immune Globulin (VIG). Additionally, symptomatic management and treatment of secondary bacterial infections are recommended during the monkeypox infection (126, 155, 156).

Supportive care in monkeypox patients is beneficial in alleviating skin and mucosa complications. There are several clinical supports recommended for patients with monkeypox

infection, including prevention and treatment of secondary bacterial infections, treatment of gastrointestinal symptoms (such as vomiting and diarrhea) to minimize gastrointestinal fluid losses, ensuring adequate hydration and nutrition, and protecting eyes and genitals in severe cases (155, 157).

Although no specific anti-viral treatment is approved for monkeypox infections, antivirals approved for smallpox infection may be effective against monkeypox. Tecovirimat, cidofovir, and brincidofovir are options for treating monkeypox (155, 156).

Tecovirimat, also known as ST-246 or TPOXX (SIGA Technologies), is the treatment of choice for the treatment of smallpox in adults and pediatric patients weighing at least 13 kg. In July of 2018, FDA approved tecovirimat for the treatment of symptomatic smallpox. Tecovirimat was discovered by SIGA Technologies in collaboration with the US Department of Health and Human Services (Biomedical Advances Research and Development Authority) through screening of more than 350,000 unique compounds from a diverse chemical library. Dosage forms include capsules (200 mg of tecovirimat) and single-dose injection vials (200 mg of tecovirimat in 20 mL for further dilution before intravenous infusion). Tecovirimat is an inhibitor of the viral envelope protein VP37, which blocks the final steps in viral maturation and release from the infected cell. Although there is insufficient data to show the efficacy of tecovirimat in humans to treat monkeypox, animal studies have supported its effectiveness in treating diseases caused by Orthopoxviruses. It also improved survival from lethal monkeypox infections in animals. The safety of tecovirimat has been shown in clinical trials in patients; in most cases, only minor side effects had occurred. Tecovirimat injection is contraindicated in patients with severe renal impairment (creatinine clearance below 30 mL/min). Common side effects are headache, nausea, abdominal pain, and vomiting (156, 158-161).

Cidofovir, also known as Vistide (Gilead Sciences, Inc), is an anti-viral medication that works by inhibiting the virus DNA polymerase. In 1996 FDA approved Vistide for the treatment of cytomegalovirus retinitis in patients with Acquired Immune Deficiency Syndrome (AIDS). Currently, there is no adequate data on cidofovir's effectiveness in treating monkeypox in humans. However, in vitro and animal studies showed efficacy against Orthopoxviruses and lethal monkeypox infections in

animals. Severe renal toxicity is the major adverse effect of cidofovir. Vistide administration is contraindicated in patients with a serum creatinine > 1.5 mg/dL or creatinine clearance \leq 55 mL/min. Cidofovir is available as an intravenous solution (75 mg/mL) (156, 162-166).

Brincidofovir, also known as CMX001 or Tembexa (Chimerix Inc), is a prodrug of cidofovir, with an improved safety profile and less renal toxicity. Tembexa was developed in conjunction with the U.S. Department of Health and Human Services' Biomedical Advanced Research and Development Authority and was granted FDA approval on June 4, 2021. Compared to cidofovir, brincidofovir has increased cellular uptake and more efficient conversion to the active form by intracellular enzymes. Brincidofovir is an orthopoxvirus nucleotide analog DNA polymerase Inhibitor. It has been approved for treating human smallpox disease in adult and pediatric patients, including neonates. Currently, there is no sufficient data on the effectiveness of brincidofovir in treating monkey pox in humans. However, in vitro and animal studies have indicated that it is effective against Orthopoxviruses. Based on studies in immune-deficient animals, the efficacy of brincidofovir may be reduced in immunocompromised patients. Available dosage forms include tablets (100 mg) and oral suspension (10 mg/mL). Common side effects are diarrhea, nausea, vomiting, and abdominal pain. Brincidofovir may increase in serum transaminases (ALT or AST) and serum bilirubin. Therefore, monitoring liver laboratory parameters before and during treatment is recommended. Coadministration of brincidofovir and intravenous cidofovir is contraindicated. It may have potential embryo-fetal toxicity and therefore the patients should be advised regarding the potential risk to the fetus. Brincidofovir should be considered a potential human carcinogen. Additionally, based on testicular toxicity in animal studies, it may cause irreversible infertility in male patients (156, 167-169).

Vaccinia Immune Globulin Intravenous (VIGIV) is indicated for the treatment of complications caused by vaccinia vaccination, including eczema vaccinatum, progressive vaccinia, severe generalized vaccinia, vaccinia infections (in individuals who have skin conditions), and aberrant infections induced by vaccinia virus (except in cases of isolated keratitis). VIGIV provides passive immunity for individuals with complications of Vaccinia virus vaccination. However, the exact

mechanism of action is not known. Sufficient data are lacking on the effectiveness of VIGIV in treating monkeypox infection in humans. VIGIV can be considered for prophylactic use in an exposed person with severe immunodeficiency (in T-cell function), as smallpox vaccination following exposure to the monkeypox virus is contraindicated in these patients. Headache, nausea, rigors, and dizziness are the most common adverse reactions reported to VIGIV. Although no serious side effects were reported, there has been a case of severe Vaccinia infection that developed intravascular hemolysis, leukopenia, and thrombocytopenia during VIGIV treatment. VIGIV is available as a sterile solution (15 mL single-use vial containing a dose of \geq 50,000 U/vial). The most common side effects are headache, nausea, rigors, and dizziness. (155, 156, 170, 171).

DISCUSSION

Monkeypox is a zoonotic infection caused by the monkeypox virus, which belongs to the Poxviridae family, the Chordopoxvirinae subfamily, and the Orthopoxvirus genus. The Poxviridae family are double-stranded DNA viruses with large, generally brick or oval-shaped forms (1, 2, 9). Monkeypox virus can infect a wide range of mammalian species, but the natural host is unknown. Monkeypox virus has been isolated from the Gambian pouched rat, tree squirrel, rope squirrel, sooty mangabey monkey, and other primates (9, 172).

Human cases were first reported in the early 1970s, and since then, the confirmed monkeypox cases have increased, or outbreaks of the infection have emerged (2, 20, 173). Monkey pox is an endemic disease; typically, cases are sporadic or occur as localized outbreaks. Central and West African countries, such as Nigeria, Central African Republic, Cote d'Ivoire, Cameroon, the Democratic Republic of the Congo, Gabon, Liberia, the Republic of the Congo, and Sierra Leone are the regions with the most reported cases (9, 174).

Confirmed monkeypox cases from outside the endemic areas are usually due to traveling to infected regions or bites and scratches from infected animals (25, 28). The United States monkeypox outbreak in 2003 was the first reported outbreak outside Africa (21). From October 2018 until mid-2021, monkeypox cases have been reported from the United States, United Kingdom, Israel, and Singapore. In most cases, the patients were international travelers who returned from Nigeria (25-29). In the current monkeypox outbreak, 102

non-endemic countries and territories and seven endemic countries have reported laboratory-confirmed cases. In May 2022, in collaboration with the US state and local jurisdictions, CDC launched an emergency response to identify and monitor additional monkeypox cases in the United States. Some of the most significant measures of the response include releasing a Health Alert Network (HAN) Health Advisory, developing interim public health and clinical recommendations, releasing guidance for Laboratory Response Network (LRN) testing, and establishing a call center to guide states for the evaluation of possible cases. Isolation of the infected patients and practicing good hand hygiene and wearing appropriate personal protective equipment (PPEs) are the measures that the public can take to prevent monkeypox infection (29).

Most clinical characteristics of monkeypox are similar to the smallpox infection except for the timing of the lymphadenopathy that often occurs early at the onset of fever. After exposure to the monkeypox virus, symptoms usually start within three weeks and typically last for 2-4 weeks. Fever, headache, muscle aches, backache, and fatigue are the typical initial symptoms. After the onset of fever and lymphadenopathy, rashes often appear 1-4 days later. The infection can be transmitted from when symptoms start until the rash has healed (112, 113).

Infection with other Orthopoxviruses can induce immunological cross-protection of the same genus, including monkeypox. Currently, no vaccines are specifically designed to protect against monkeypox infection, and the available vaccines, ACAM2000 and JYNNEOS, have been developed to prevent smallpox. JYNNEOS is a non-replicating modified vaccinia Ankara virus vaccine licensed to prevent monkeypox and smallpox in the United States in 2019. According to CDC, JYNNEOS is the primary vaccine against monkeypox. ACAM2000 is a live vaccinia virus with replication competence and is an alternative for JYNNEOS. ACAM2000 is a single-dose vaccine, and maximum immunity occurs four weeks after vaccination. Because of its replication ability, there is a risk of severe adverse effects such as progressive vaccinia, eczema vaccinatum, and myopericarditis. ACAM2000 is not recommended to be administered in immunocompromised individuals. JYNNEOS is a two-dose vaccine; it takes 14 days after receiving the second dose of the vaccine to produce maximum immune protection. Vaccination is recommended for individuals whose jobs may expose them to orthopoxviruses, including laboratory workers who

perform testing or handle cultures or animals with Orthopoxviruses and designated healthcare professionals (138, 140, 144, 175).

Currently, there are no treatments available specifically for monkeypox virus infections. However, anti-viral agents developed against the smallpox virus may be used to prevent and treat monkeypox infection. These medications include cidofovir, brincidofovir, and tecovirimat. Additionally, the FDA has approved VIGIV for treating the vaccination complications, such as progressive vaccinia, eczema vaccinatum, and severe generalized vaccinia. Tecovirimat, cidofovir, and VIGIV are available through the Strategic National Stockpile (SNS) under Expanded Access Investigational New Drug (EA-IND) protocols. Brincidofovir is not currently available from the SNS, but CDC is presently developing an EA-IND to facilitate using brincidofovir in treating monkeypox infection (2, 155, 159, 176-178). The prognosis for monkeypox infection depends on several factors, including previous vaccination, underlying diseases, and other conditions. Patients who should be considered for treatment (following consultation with CDC) might include patients with severe disease, immunocompromised patients, children, pregnant or breastfeeding women, and patients with one or more complications (176).

It is vital not to exclude the possibility of monkeypox infection in the absence of travel history to endemic regions or lack of a specific known close contact with confirmed monkeypox cases. The optimal diagnostic procedure for a suspicious monkeypox case is to obtain specimens from skin lesions and test the samples with RT-PCR. For an optimum result, more than one specimen should be collected from 2 separate lesions on different body parts. In addition to RT-PCR, electron microscopy, immunohistochemistry, cultures of material from rash samples, and serological testing for specific antibodies are laboratory evaluations for monkeypox diagnosis (8, 9, 62-64, 150-152).

CONCLUSION

Monkeypox infection was endemic in central and west Africa except for a large outbreak in the United States in 2003. Several outbreaks in non-endemic countries have occurred in the past five years, raising concern regarding a global pandemic. Developing rapid laboratory diagnostic methods, specific vaccines, and therapies for the prevention and

treatment of monkeypox infection should be a part of the efforts to limit the outbreak.

Vaccination is an important measure to protect people whose jobs may expose them to Orthopoxviruses. Currently, no particular therapeutics are available for monkeypox, and the anti-viral agents developed against the smallpox virus are being used to treat monkeypox infection. In many cases, monkeypox patients develop a mild, self-limiting disease; however, the prognosis for monkeypox may depend on factors such as previous vaccination status, underlying conditions, or comorbidities. Moreover, infected patients must be isolated, and good hand hygiene and wearing appropriate personal protective equipment (PPEs) should be highly considered to prevent the spread of monkeypox infection. In the current monkeypox outbreak, there is a big concern about human-to-human transmission to household members and care providers. Considering the pandemic burden, the public health importance of monkeypox disease should not be underestimated.

CONFLICT OF INTEREST. The authors have no conflict of interest.

AUTHORS CONTRIBUTION. HGK was the principal investigator. MS and HGK conceived the idea of this review. HGK and YM developed the study protocol. HGK and SMMM led the review process and drafted the manuscript. MS contributed to every step of the review process and the creation of the protocol. From the search to the final data extraction, all processes were followed independently by two research experts (MS And SMMM). MS created table 1. SMMM draw Figure 1. SN contributed to drawing Figure 3 and drafting the Molecular virology, Viral genome, and Entrance and replication section. MA and SK contributed to drafting the epidemiology section. YM, HGK, and SMMM contributed to recommendations for the review protocol and provided methodological guidance for the review process and the resulting synthesis. All authors were involved in the interpretation of the results and read and approved the final manuscript.

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