A Descriptive Review on Monkeypox Virus from Past to Present

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

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Ethics Statement

This study did not involve any kind of human participants, human specimens or tissue, vertebrate animals or cephalopods, vertebrate embryos or tissues, or field research.

Abstract:

Monkeypox infection is caused by an oval-shaped, double-stranded DNA virus called Monkeypox virus (MPXV) belonging to the Poxviridae family and Orthopoxvirus genus. Previously, this virus used to infect only in regions of Central, East, and West Africa. Recently, its outbreak outside Africa at an extreme level has made the World Health Organization (WHO) declare the MPox infection a Public Health Emergency of International Concern (PHEIC) on July 23, 2022. The review covers a detailed discussion of the history, evolution, current situation, and possible future outcome of MPXV. Additionally, MPXV characteristics, replication mechanism inside the host cell, and symptoms related to the infection are also explained here. Other than the advantages and disadvantages of different identification methods, particularly the importance of Genome Sequencing is meticulously pointed out in this article. Besides, the review also highlights the approved antiviral treatment, preventive measures, and challenge-resolving strategies for better management of MPXV outbreaks.

Keywords: Virions, Polymerase Holoenzyme, Polymerase Chain Reaction, Genome Sequencing, Vaccines, Lineage, Apolipoprotein B.

Dedication:

Dedicated to my Late cousin Carmi Rocky Halder who has always pushed me to grow as a better version of myself every day and never be afraid of learning from mistakes.

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First and foremost, I would like to thank the Almighty for his endless blessings, which have given me the ultimate strength and assistance in completing the project.

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List of Acronyms

MPXV	MonkeyPox Virus
WHO	World Health Organization
PHEIC	Public Health Emergency of International Concern
CDC	Centers for Disease Control and Prevention
IRT	Inverted Terminal Repeat
OD	Adenylation Domain
OB	OB-fold Domain
CTD	C-Terminal Domain
NTD	N-Terminal Domain
Exo	Exonuclease
dsDNA	Exogenous double-strand DNA
EPA	Environmental Protection Agency

IMV	Intracellular Mature Virions
IEV	Intracellular Enveloped Virus
CEV	Cell-Associated Enveloped Viruses
EEV	Extracellular Enveloped Virions
GARP	Golgi-associated Retrograde Protein
COG	Conserved Oligomeric Golgi
VPS	Vacuolar Protein Sorting
PCR	Polymerase Chain Reaction
ATI	A-Type Inclusion Body Protein
LAMP	Loop-Mediated Isothermal Amplification
RPA	Recombinase Polymerase Amplification
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CBIA	Competitive Binding Inhibition Assay
MAb	Monoclonal Antibody

IND	Investigational New Drug
OPG	Orthologous Poxvirus Genes
ORF	Open Reading Frame
APOBEC3	Apolipoprotein B mRNA Editing Enzyme Catalytic Subunit 3G
SNP	Single-Nucleotide Polymorphism
IPC	Infection Prevention and Control
LMICs	Low and Middle-Income Countries

Chapter 1

Introduction

1.1 Background

The Monkeypox virus (MPXV) was first discovered in 1958 in the laboratory monkeys of Denmark. This zoonotic MPXV, a subtype of the genus Orthopoxvirus belonging to the Poxviridae family, causes monkeypox infection in humans. The first human case was discovered in 1970 in a nine-month-old child from the Democratic Republic of the Congo (WHO, 2023, “Mpox (monkeypox)"). MPXV is a double-stranded DNA virus with an outer covering, or lipid envelope, with protein spikes on top holding inside all of its genetic information-carrying materials. The symptoms of MPXV infection have many similarities with smallpox but with a much lower mortality rate. Earlier the vaccine made of vaccinia virus used for smallpox prevention was able to give protection against monkeypox. MPXV is available in two variants: Clade I and Clade II. Till now many lineages of each clade have been discovered. Originating in Central Africa, Clade I has been responsible for about 10.6% of deaths whereas the West African origin Clade II caused around 3.6% of deaths (H. Li et al., 2022, “Evolving Epidemiology of MPXV”). Meanwhile, the Clade II variety, or more precisely the Clade IIb subtype, which originated from West Africa has been found to affect humans more during the years 2022–2023. Scientists found that one of the main reasons behind the difficulties of treating this infection nowadays is every single lineage is undergoing numerous mutations. Another subtype, the Clade IIb variety rarely kills humans; typically, more than 99% of infected individuals by Clade IIb recover (CDC, 2023, “About Mpox”).

1.2 Epidemiology

Since the first discovery of MPox in 1970, till now, the Clade I variant affected part of Central and East Africa mostly whereas the Clade II variant primarily affected West Africa (WHO, 2023, "*Mpox (monkeypox)*"). Within the years 1981-1986, around 37 cases of MPox infection were identified in the Democratic Republic of Congo, in most parts of the Zaire area. Between the years 1996-1997, the infection was vastly spread in the region of Zaire. From February to August of 1996, there were 71 clinical cases reported, including 6 fatalities, in 13 communities, mostly in the Zaire region (Ligon, 2004).

This disease, which was formerly restricted to the continent of Africa, has now spread outside its borders causing harm to other nations. In the year 2003, the outbreak of MPox widely gained global attention as it was the first outbreak of the disease outside of Africa. The outbreak affected 47 individuals throughout six U.S. states: Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin (CDC, 2022, "Past U.S. Cases and Outbreaks"). The probable reason was considered the shipment of MPXV-infected mammals from Ghana & West Africa to the US (Ligon, 2004). In October 2018 and May 2019, one traveler in Israel and another in Singapore were found to be affected by MPox respectively, during their travel from Nigeria (WER et al., 2023). In the Democratic Republic of the Congo, over 3,000 suspected cases were recorded annually in 2018. By 2020, there were 6216 cases and 222 fatalities. Moreover, 78 cases in the Central African Republic (CAR), 9 cases in Cameroon, 138 cases in Nigeria, 2 cases in Sierra Leone, and 8 cases involving men traveling from Nigeria between the ages of 30 and 50 have been reported to be affected by MPXV from the period of 2018 to 2021 (WHO, 2023, "WER").

Similarly, cases of MPox infections were reported in numerous nations in 2021 and 2022, mostly affecting travelers who had recently traveled to parts of Africa. During this MPox outbreak in 2022, predominantly this infection was prevalent in a group of males who were

engaged in sexual activities with other males. This referred MSM group had some common symptoms like inguinal lymphadenopathy and lesions in the genital, perineal, or perianal areas. A cohort study of MPox infection conducted in Spain has shown that out of 595 confirmed cases, 99% were among the MSM group. Similarly, out of 1304 confirmed cases as reported in Germany on 6th July 2022, most of the infection was within the MSM population (Moore et al., 2023). The rapid global spread of MPox prompted the World Health Organization (WHO) to designate it as a Public Health Emergency of International Concern (PHEIC) on July 23, 2022. The declaration highlighted how critical it is to address the problem and place safety measures forward to stop MPox from spreading and becoming a pandemic (WHO, 2023, “Mpox (monkeypox)").

An article published by Cambridge University has brought out how the factors of the environment are affecting the MPox spread. The ongoing deforestation is making the MPox spread more likely to happen due to increased human and infected animal interaction. Besides, the article pointed out that the increased amount of pollutants is also another reason for the spread. A study conducted in the United Kingdom, Spain, France, Germany, Italy, the Netherlands, Switzerland, and Portugal showed that the MPox spreading chance multiplies by 29.6%, 9.7%, 13%, and 80.6% for every 10-unit rise in PM2.5, PM10, NO2, and O3 levels respectively. So, rapid industrialization or fuel consumption is also to some extent responsible for Mpox (Singh & Shaikh, 2023). Currently, the MPXV spread is more common in human-to-human compared to animal-to-human. A MaxEnt model was used for detecting which populations are at high-risk zones and it showed that the MPox infection has spread at an alarming rate in Europe and North America. Other than that Central Africa plus East and South Asia regions are also also affected by MPox infection compared to other regions. Spain, France, Britain, and Germany from European countries and the United States, Canada, and Mexico of North America have a high susceptibility towards MPox infection as confirmed cases are

increasing in these areas. The possible reasons might be MPox transmission from transportation hubs or greater population travel and migration from one country to another. Additionally, the weather also to some extent affects the MPXV spread. Enhanced precipitation in the driest season, increases the chances of the infection (Gao et al., 2023).

1.3 Structure of MPXV

MPXV is a large, oval-shaped virus with a size range of 200-250 nm when observed under an electron microscope. The virion is formed of 5 regions made of nucleic acids (DNA), a double-concave dumbbell-shaped core, a palisade layer, a bilayered inner and outer membrane made of lipoproteins, and 10 nm long surface tubules. The double-stranded 197 kb long linear genome of MPXV consists of a central region of approximately 101 kb and two terminal variable regions with an inverted terminal repeat (ITR) of 6.4 kb at each end. The central region codes for structural proteins and essential enzymes whereas the ITR consists of ORFs, hairpin loops, and short tandem repeats that are responsible for virtual transcription and replication (H. Li et al., 2022, *"*Landscape Immune Response of MPXV*"*).

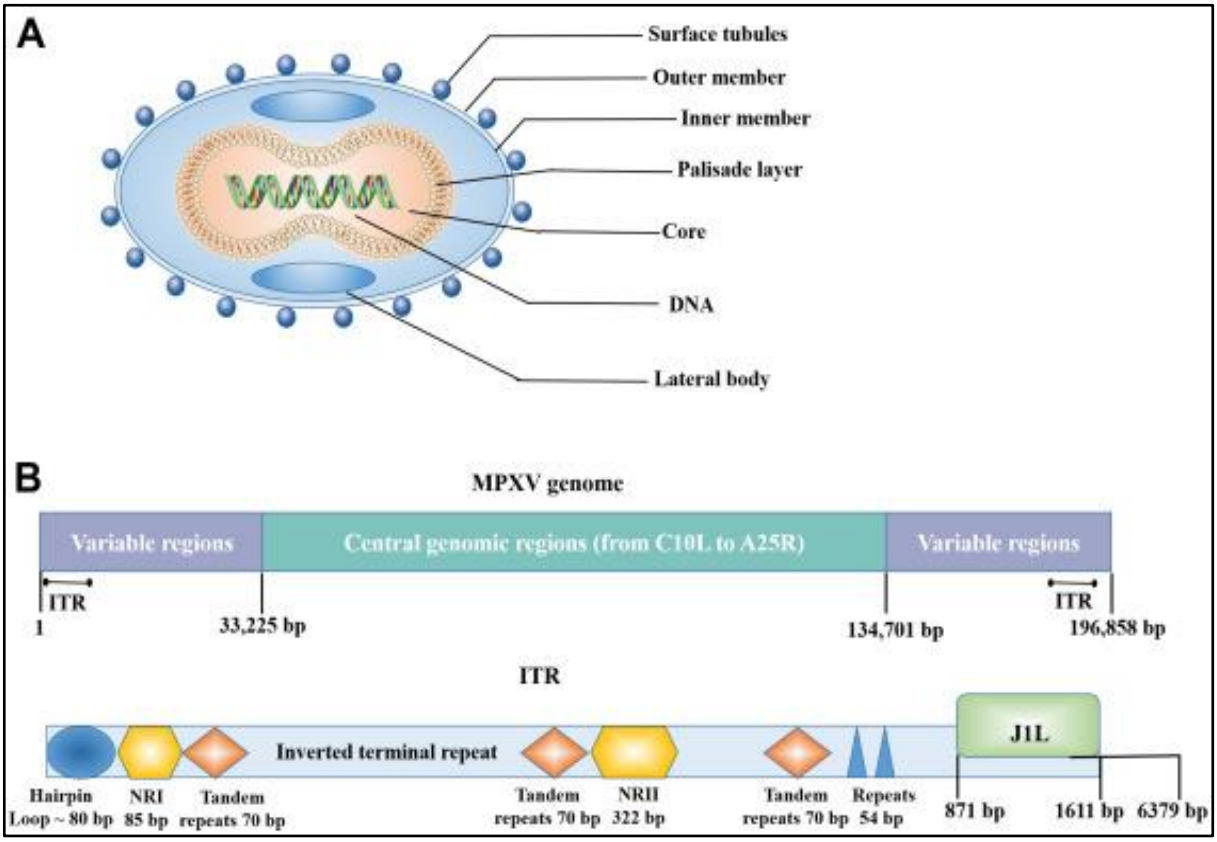

Figure 1: Structure and genomic construction of MPXV. ***(A) 5 distinct parts*** *of MPXV include the core and palisade layer, inner membrane, outer membrane, surface tubules, and deoxyribonucleic acids (DNA).* ***(B)*** *The* ***197-long*** *genomic construction consists of the central region extending from C10L to A25R and an inverted terminal repeat (ITR) is present at the two ends of the variable regions (H. Li et al., 2022, "Landscape Immune Response of MPXV").*

1.3.1 Replicating MPXV DNA Polymerase Holoenzyme Framework

The DNA polymerase holoenzyme of MPXV needed for genome replication consists of the following components: DNA polymerase F8 for catalyzing viral DNA synthesis, heterodimeric processivity factor consisting of A22, and uracil-DNA glycosylase E4. The 426 residues present in the A22 structure of MPXV consist of three domains: the N-terminal domain (NTD), the middle domain (Mid), and the (CTD). The mid-region is further differentiated into two regions: an adenylation domain (OD) and an OB-fold domain (OB). Altogether one F8, one

A22, one E4, a primer-template DNA, and an incoming dTTP substrate are present in the replicating holoenzyme-DNA complex (Peng et al., 2023).

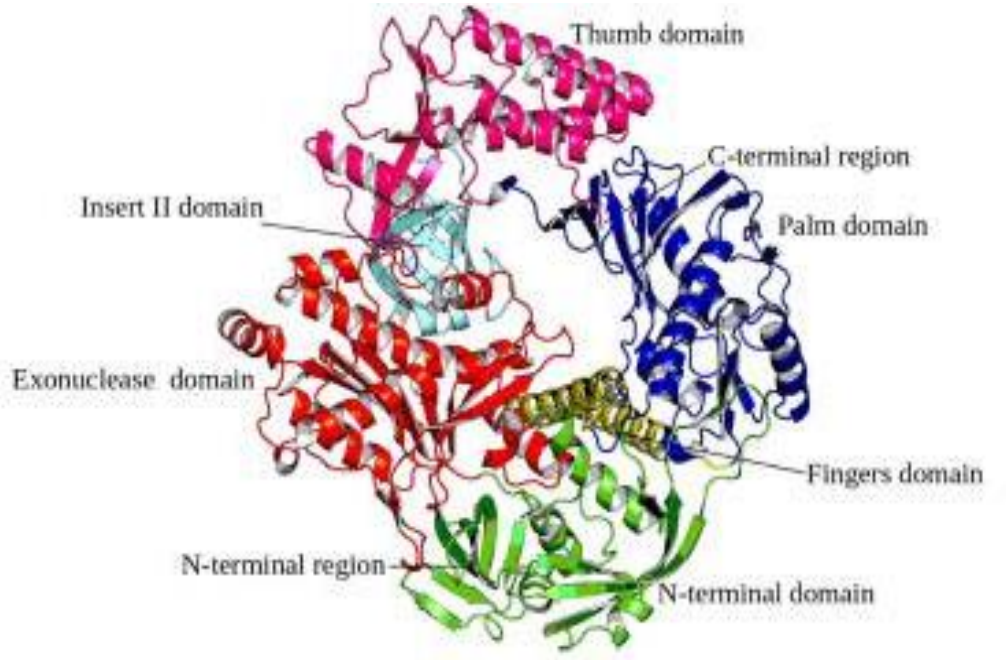

Figure 2: Domain Structure of MPXV DNA Polymerase Holoenzyme. ***(A)*** *The* ***Green region*** *denotes the* ***N-Terminal Domain*** *having residues 1–157 and 497–523* ***(B)*** *The* ***Red region*** *denotes the* ***Exonuclease Domain*** *having residues 158–353 and 435–496* ***(C)*** *The* ***Blue region*** *denotes the* ***Palm Domain*** *having residues 524–618 and 676–829* ***(D)*** *The* ***Cyan region*** *denotes the* ***Insert II Domain*** *having residues 354–434* ***(E)*** *The* ***Pink region*** *denotes the* ***Thumb Domain*** *having residues 830–1006* ***(F)*** *The* ***Yellow region*** *denotes the* ***Finger Domain*** *having residues 619–675. The inhibitor drugs block the activity of MPVX DNA Polymerase Holoenzyme by reacting with the* ***SP549, ASP753, TYR550, ASN551, SER552, and ASN665*** *residues of the major active site (Kumari et al., 2023).*

1.3.2 Interaction of Components within the Replicating MPXV DNA Polymerase Holoenzyme

According to Y. Li et al. (2023), the pairwise interaction between F8, A22, and E4 subunits is responsible for the stabilization of replicating MPXV DNA polymerase holoenzyme. In this holoenzyme, the two domains of A22 act as a bridge where the N-terminal domain (NTD) of A22 binds to E4 and the C-terminal domain of A22 interacts with the F8. The main reason behind the interaction of F8 with the C-terminal domain (CTD) of A22 is due to the insertion of poxvirus-specific short helices of insert 3 regions into the hydrophobic pocket of A22. The expanded hydrophobic network comprises amino acid blocks from both F8 (Leu578 and Ile582) and A22 (Phe354, Val372, Phe377, Ile379, Val384, Phe407, and Phe414). In opposition, Asn576 and Arg577 of F8 form Hydrogen bonds (H-bond) with Asn373 of A22. Moreover, Val178, Phe179, and Leu278 of F8 and Val33, Trp36, Ile135, and Tyr136 of E4 also interact by hydrophobic bonding.

1.3.3 Primer-Template DNA Strand Interaction with Replicating MPXV DNA Polymerase Holoenzyme

The working mechanism of two different states of the DNA replication holoenzyme, the F8-A22-E4 complex collected from MPXV was observed. It was found that the trimeric form of the complex was more active compared to the hexameric form. The hexameric form couldn’t undergo the replication process because the DNA binding site of F8 and E4 was concealed. Conversely, the addition of an exogenous double-strand DNA (dsDNA) substrate or another factor to the holoenzyme complex causes a conformational change and turns it into an active trimeric form. This exposes the dsDNA binding region in the thumb domain site of F8 and thus

the primer and template strand get the position to bind for initiating the further replication process (Y. Li et al., 2023).

The binding of dsDNA in a groove formed in the middle of the palm and thumb domain of F8 results in ~17° rotation of the finger domain towards the palm domain. This rotation activates the replication state by bringing positively charged Arg634 and Lys661 of the finger domain of F8 close to the active site for further binding with the arriving triphosphate dNTP. The rotated fingers domain further liaises with the Exonuclease (Exo) domain of F8 polymerase to stabilize its closed conformational structure. In addition, by unique rotation, the thumb domain wraps around the duplex of primer and template DNA to fit the complex within its positively charged minor groove. The vast protein-DNA network observed in the primer-template DNA sequence and F8 includes 18 residues, 29 residues, and 47 residues from the primer strand, template DNA strand, and F8 respectively. This protein-DNA network is created by interaction between the phosphodiester backbone of the DNA directly with the phosphate groups of F8. The primer strand interacts with 9 residues of F8 mostly by both electrostatic and hydrogen bonds (H-bond). Correspondingly, the phosphates of the template strand by hydrogen bonding (H-bond) interact with 13 residues present in the main or side chains of F8 (Peng et al., 2023).

1.3.4 Incoming Nucleotide dTTP Interaction with the MPXV DNA Polymerase Holoenzyme

The incoming dTTP binds at the active site of polymerase right after the primer is present at the 3′ end of the strand. This active site is mainly the groove formed by the palm and finger domain residues. This incoming dTTP joins with polymerase by interacting with aspartate residues (D549 and D753); Y550, S552, and L553 of main chains; and positively charged R634 and K661 of finger domains from side chains (Peng et al., 2023).

1.4 Transmission of MPXV

The MPXV is less contagious than other viral infections like smallpox but its risks can't be unseen. The MPXV usually enters through the nasopharynx, oropharynx, or intradermal pathways when a healthy person comes into very close contact with an infected animal or person (Moore et al., 2023). Moreover, scientists found that the prime reason for global outbreaks of MPox in 2022 was mostly due to unprotected sexual contact (WHO, 2023, "mpox spread").

1.4.1 Person-to-Person

Humans can contract MPox through skin-to-skin contact, sexual contact (oral, anal, or vaginal), or other prolonged face-to-face interactions with infected humans**.** Rashes, scabs, saliva, respiratory droplets, or secretions like mucus or snot are carriers of this virus. People who contracted MPox can spread this virus 1-4 days before the appearance of symptoms. So, a person can't be termed as fully recovered until the rashes disappear and fresh skin starts to develop (CDC, 2023, "How It Spreads"). The WHO further refers people who fall under certain categories—like young children, children or infants of breastfeeding mothers, eczema sufferers, and those with severely compromised immune systems, like HIV-positive individuals—are more likely to suffer from severe illnesses due to MPXV and this might prove fatal (WHO, 2023, "Symptoms of mpox"). According to a report from the CDC (2023) on pregnant or breastfeeding mothers, the MPXV can be transmitted to fetuses from infected expectant mothers through the placenta, and evidence has shown this has led to many pregnancy complications including stillbirth and miscarriages. Interaction of infants before and during birth with an infected mother can lead to MPXV transmission but transmission through breast milk or amniotic fluids is still not known yet.

1.4.2 Animal-to-Person

Coming in contact with the rash, scab, saliva, or other fluid through a bite or invasive scratch from an infected animal can also spread MPox infection. Urine and feces of infected animals can also carry the virus. Wild animals like squirrels, rats, and mice living in the endemic regions (West and Central Africa) are mostly in close contact with humans. Small mammals can be carriers of MPXV without showing any symptoms. Hunting, trapping, skinning, processing, or ingesting such MPox-infected wild animals can lead to the dissemination of the virus among people (CDC, 2023, "How It Spreads"). Contact or consumption of such dead or sick animals must be avoided to stop further infections from spreading.

1.4.3 Object-to-Person

Indirect transmission from fomites like infected cloths or linens or even the surface of a room where an infected person has stayed can be a source of MPXV transmission (Tiecco et al., 2022). Beddings, towels, objects, electronics, etc. can contain the MPXV if used or touched by an infected person. According to Morgan et al. (2022), MPXV can survive up to 15 days on any inanimate object. Their findings pointed out that objects with porous layers like bedding or clothing have more presence of MPXV compared to any non-porous layers like metal or plastic.

Close contact with such contaminated items can infect healthy individuals if the person touches his nose, mouth, eyes, or other mucus membrane without washing hands. The entry of MPXV through cuts or abrasions is also possible. The only way to prevent such object-to-human transmission is to disinfect surfaces and objects and clean hands every time after handling any infected items. Disinfectants registered by the Environmental Protection Agency (EPA) can act

against emerging viral pathogens and these recommended disinfectants should be used according to the manufacturer's instructions on the label.

1.4.4 Person-to-Animal

MPXV transmission from human to animal is less likely to occur but the possibility of it can't be denied. Both infected persons or pet animals can transmit MPXV among each other while petting, cuddling, hugging, kissing, licking, or sharing sleeping areas, and foods. It's better to separate pets into a safer place unless the MPXV-infected person fully recovers (CDC, 2023, "Mpox in Animals and Pets").

1.5 Pathogenesis of MPXV

MPXV is a big double-stranded DNA virus that replicates in the cytoplasm of the infected cells. 16 proteins present in the virion membrane help in the entry of the virus into the host cell through plasma membrane fusion or by endocytosis process. Upon entry into the host cell, the viral gene transcription and viral DNA synthesis begin at the perinuclear sites (Peng et al., 2023).

The MPXV pathogenesis includes 4 steps: viral entry, fusion, replication, and release of newly formed virus from the cell. Two types of infectious forms of virus get released after replication inside the cell one is intracellular mature virions (IMV) and another is extracellular enveloped virions (EEV) type. The IMV is simply single membrane bound whereas the EEV is enclosed antigenically by a triple membrane. This step from IMV to EEV conversion includes the double-layered wrapping of IMV by the Golgi body to make an intracellular enveloped virus (IEV). This Golgi-wrapped IEV by interacting with actin-tail loses the outermost Golgi wrapping. Further unwrapping IEV by fusing with cell membrane produces cell-associated enveloped viruses (CEVs) which then get released from the cell as EEV eventually. This

additional double lipid layer of EEV develops through translocation in the Golgi apparatus. This extra layer of EEV helps the new virion to remove itself from the intact cell and further proceed to infect other cells within the host body. On the other hand, IMV gets released through cellular breakage, and it's responsible for virus transmission among hosts. The same article discusses the role of GARP, COG, and two distinct Central African MPXV strains (R1 and R2) responsible for MPXV pathogenesis (H. Li, 2022, "Landscape Immune Response of MPXV").

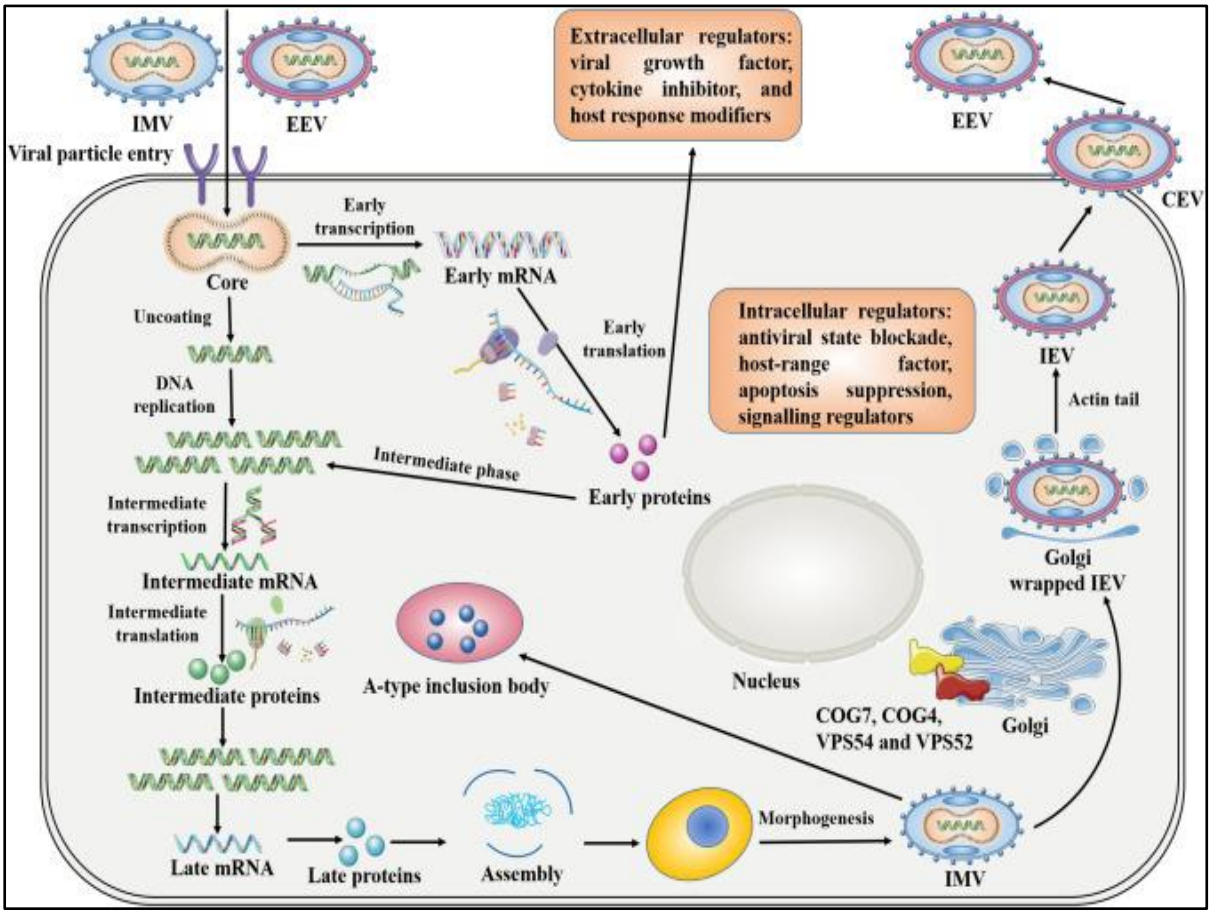

Figure 3: Invasion and growth of MPXV inside the cell. The replication of MPXV is formed of three stages: early, intermediate, and late viral mRNA synthesis. The next step is protein synthesis which then assembles with other viral components and undergoes morphogenesis to form new ***intracellular mature virions (IMV)****. IMV is further wrapped inside two membranes by Golgi to form an* ***intracellular enveloped virion (IEV)****. IEV then fuses with the cell membrane and releases new virions outside the cell (H. Li et al., 2022, "Landscape Immune Response of MPXV").*

1.5.1 Function of GARP and COG in MPXV Pathogenesis

Golgi-associated Retrograde Protein (GARP) and Conserved Oligomeric Golgi (COG) complexes are two essential factors for the function of the viral infectious cycle. The GARP complex involved in retrograde endosomal transport, contains four vacuolar protein sorting (VPS) genes, VPS51, VPS52, VPS53, and VPS5. Among these proteins, VPS54 and VPS52 are responsible for the formation of extracellular enveloped virions (EEV). Another multisubunit tethering complex COG acts in the regulation of glycosylation enzymes, membrane trafficking, and managing Golgi structure. This COG complex comprises of (Lobe A – COG1, COG2, COG3, COG4, and Lobe B – COG5, COG6, COG7, COG8). Particularly knockout of COG7 and COG4 results inhibition of the virus from interacting with cells and thus prevents further virus entry, fusion, and spread (H. Li et al., 2022, "Landscape Immune Response of MPXV").

1.5.2 Function of Two Distinct Central African MPXV Congo Strains: R1 and R2 in Viral Pathogenesis

Deleting two different strains of Central African MPXV Congo R1 (Open Reading Frame 17-31) and R2 (Open Reading Frame 181–192) has shown significant ways to suppress the virulence. R2 encodes for important immunomodulatory proteins which include viral replication inhibitors of type I and type II interferons, an inhibitor of IL-1β, and two apoptosis modulators (Lopera et al., 2015). Therefore, the deletion of R2 directly affects the pathogenesis of MPXV. On the other hand, deletion of R1 stops viral replication. Thus, suppressing both results in a more effective response against MPXV than suppressing one (H. Li et al., 2022, "Landscape Immune Response of MPXV").

1.6 Clinical Manifestation

As per the CDC (2023) described “MPox Symptoms” report, it first starts to develop during the 3rd week after being exposed to the virus. MPox has an incubation period of 5- 21 days and then the symptoms start appearing. Usually, this infection is divided into 2 phases the prodromal phase which lasts up to 0-3 days with fever, chills, headache, enlarged lymph nodes, back pain, muscle soreness, and fatigue. The symptoms of the prodromal phase have similarities with the secondary viremia. Later with the above symptoms, the rash phase appears lasting from 7-21 days (Tiecco et al., 2022).

Lesions first appear on the oropharynx then they start spreading all over the body. During the rash phase, severe or itchy rashes on the face, palms of hands, soles of feet, groin, genital areas, and anal areas are observed that resemble pimples or blisters. There may also be lesions on the rectum, vagina, anus, mouth, throat, or eye. Usually, rashes last up to 2-4 weeks, and providing proper medication other common symptoms can be handled. Within this period the lesions progress through many stages like macular, papular, vesicular, and pustular phases before disappearing completely. During this time the lesions simultaneously keep changing and form a firm solidified structure that can male up to 2 to 10 mm in size. For 5-7 days the lesions remain in the pustular phase before the crusts start forming. The crusts form and shed over the next 7-14 days. The condition gets cured within the next 3-4 weeks from the time the symptoms appear. Patients are no longer considered as a threat of spreading infection once all the crusts are shed off (Moore et al., 2022).

The severity of MPox infection can vary from person to person but cautiously it needs to be handled to prevent death. Based on WHO, in serious cases, the common symptoms are accompanied by a bacterial infection in the brain, skin, blood, lungs, or genitals causing encephalitis, myocarditis, pneumonia, balanitis, proctitis, urethritis, and sometimes eye

problems. In this case, hospitalization, supportive care, and antiviral medication are recommended (WHO, 2023, “Symptoms of mpox”).

1.7 Sample Collection and Storage Procedure for MPXV Detection

There are some specific conditions for the collection and storage of specimens for diagnostic testing and accurate identification. The below table contains the directions published by WHO regarding the handling of body fluids (Urine, Vitreous fluid, Cerebrospinal fluid, and Semen) and PAHO regarding the sample (Serum, Skin, and Oropharyngeal swab) handling:

Sample	Collection
Urine, Vitreous Fluid, Cerebrospinal Fluid, Semen	Sterile Collection Tube
Plasma	Sterile Collection Tube with EDTA
Serum	Serum Separating Tube
Oropharyngeal Swab	Dacron or polyester fiber swabs with VTM (Viral Transport Media) / dry swab
Lesions Swab: Skin surface, exudates or crusts	Dacron or polyester fiber swabs with VTM (Viral Transport Media) / dry swab

Table 1: Sample Handling (WHO, 2023, “Diagnosting Testing for MPXV”; PAHO, 2022, “Laboratory Guidelines for MPXV”)

According to WHO and PAHO, Urine, Vitreous fluid, Cerebrospinal fluid, Serum, Skin, and Oropharyngeal swabs can be refrigerated at 2-8°C or the samples can be frozen at -20°C or lower. This cold temperature needs to be applied within 1 hour of the specimen collection.

Further, after the 7 days, the samples must be stored at -20°C or lower. In contrast, the semen can be stored at room temperature for less than 1 hour but for longer storage, the temperature needs to be switched to -20°C or lower. When specimens are needed to be stored for a very long time approximately 60 days or more from the day of collection then -70°C is recommended for the preservation of the sample. It should be noted that samples should not undergo repeated freeze-thaw cycles as it enhances the chances of sample constituent degradation.

1.8 Use of Different Methods for Diagnosis of MPXV

As per a report by WHO, published on April 18, 2023, detection of MPox infection can be difficult due to its similarity with other infections like chickenpox, measles, scabies, syphilis, herpes simplex, or any other bacterial or allergic reaction. A person infected with MPox may also have infections like chicken pox or any other sexually transmitted disease. Early detection is necessary to prevent the spreading of highly contagious infections like MPox. The sample for diagnosis is collected from the rash, fluid, or crust of skin lesions by vigorous swabbing. In the absence of skin lesions, the sample is collected from oropharyngeal, anal, or rectal swabs (WHO, 2023, “Mpox (monkeypox)").

In the recent article of Zhou & Chen (2023), few MPXV detection techniques are mentioned and the next six methods are discussed based on their advantages and disadvantages.

1.8.1 Polymerase Chain Reaction

The laboratory Polymerase Chain Reaction (PCR) test is mainly performed to identify the viral MPox DNA. Previously, haemagglutinin protein was recognized as a means of MPXV detection. However, since most Orthopoxviruses have the same protein so specifically MPXV can’t be distinguished. Later, the gene encoding the A-type inclusion body protein (ATI) was

specifically identified to recognize MPXV by conventional PCR. Experimentally another method for particularly MPXV identification by traditional PCR was found by deleting the 8-bp in the ATI gene.

For better specificity, higher detection speed, and sensitivity real-time PCR can be performed by using the LightCycler DNA-Master HybProbe kit and the LightCycler 480. G2R (plasmids encoding gene), B6R (extracellular envelope protein gene), E9L-NVAR gene (DNA polymerase gene), RPO18 gene (DNA-dependent RNA polymerase subunit 18), B7R gene and complement binding protein genes C3L, F3L, N3R are used in real-time PCR for identification. Moreover, Loop-Mediated Isothermal Amplification (LAMP) uses the D14L or ATI gene whereas Recombinase Polymerase Amplification (RPA) and GeneXpert method use G2R to identify MPXV.

1.8.2 Isothermal Amplification

An *in vitro* method in which nucleic acids are amplified rapidly using enzymes and specific primers at a steady temperature. Zhou and Chen (2023), highlighted in their article that at present, Loop-Mediated Isothermal Amplification (LAMP) and Recombinase Polymerase Amplification (RPA) are the two main Isothermal Amplification approaches used for MPXV detection.

1.8.2.1 Loop-Mediated Isothermal Amplification (LAMP)

Using LAMP, rapid amplification of the targeted region of the virus within 1 hour for specifically MPXV identification was possible. For this at 60-65°C in the presence of *Bst* DNA polymerase, 4-6 primers are needed to bind to specifically six target regions of the MPXV. Among all other genes, A27L-1 and F3L-1 are recognized as the fastest, most sensitive, and most reliable primers for effective MPXV identification by LAMP. Another two important

genes, the D14L gene of clade I (Congo Basin) and the partial ATI gene of clade II (West Africa) have been identified as a target to be used as a source of MPXV identification.

According to Iizuka et al. (2009), the sensitivity and specificity of LAMP were tested against the Nested PCR by collecting peripheral blood and throat swab specimens. The percentages that were shown for Nested PCR vs COM-LAMP, C-LAMP, and W-LAMP were 80% and 100%; 79% and 100%; and 70% and 100% respectively.

One of the main challenges in carrying out the LAMP technique is primer design. Primers for binding to six independent locations of target DNA/RNA are difficult to design. The target locations must be close together within 2-3 pairs but too close can lead to error results.

1.8.2.2 Recombinase Polymerase Amplification (RPA)

Compared to LAMP, RPA gives a similar level of amplification at a faster rate and shortest time. MPXV-RPA-assay has shown 95% sensitivity using only two primers and one probe whereas LAMPS requires six primers. RPA is performed at 37-43°C in 15 minutes in the presence of three main protein factors: recombinase, recombinase loading factor, and single-stranded binding proteins. Besides, due to the advantage of storing RPA reagents by freeze-drying in an environment above 30°C, this technique can be run in those areas where well-resourced labs aren't available. However, due to a lack of primer or probe specificity, many non-target sequences in DNA/RNA can undergo amplification leading to erroneous results.

1.8.3 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

Once introduced into the sample, the CRISPR array with the help of guide RNA can specifically identify and bind with the MPXV genome. Afterward, the highly selective endonuclease enzyme and Cas12 protein precisely cleave the part of DNA that is present at the

later part of the targeted CRISPR-MPXV genome binding. Next, a fluorescent reporter system made of F3L and N3R as reporter genes attaches to the target MPXV genome sequence. Once attached this specific targeted part glows and helps in the identification. The scientist after MPXV genome identification can further manipulate it for research purposes. This CRISPR-Cas12 base method is quite specific and sensitive for rapid identification of the MPXV virus (Zhou and Chen, 2023).

1.8.4 Immunological Methods

As per Zhou and Chen (2023), immunological methods are less reliable compared to others due to the cross-reactive nature of Orthopoxviruses that is antigen/antibody binding to other molecules rather than the targeted one. Sometimes it can lead to false-positive results as well. Though these methods have some limitations, they can be used for future epidemiological studies.

Immunohistochemistry identifies the viral antigens in tissues or cells whereas immunoassays target A29, an MPXV envelope protein for virus recognition. Again, ELISA detects serum antibodies: specifically, IgG and IgM from the sample. Usually, these antibodies in infected individuals can be detected 7 or 21 days after the appearance of the rash phase. MPXV infection can be identified if the IgG titer is at least four times higher in the recovery phase compared to the acute phase. To prevent the cross-reactivity, in the plasma cell samples around 6×10^8 PFU equivalents/mL inactivated monkeypox or vaccinia whole-cell lysate was added. This helped to differentiate between the presence of the vaccinia virus and MPXV (Dubois & Slifka, 2008). If the ELISA test is run based on MPXV protein B21R after 2-6 months of infection then 100% sensitivity and 92% specificity of test result can be obtained i.e. confirming the presence of MPXV in the sample. As per Ichihashi & Oie (1988), when the competitive binding inhibition assay (CBIA) test was used to analyze the serums of MPXV-infected patients then it was found

that the MPXV interacted plus formed a bond with monkeypox-specific monoclonal (MAb) antibody H12C1 but not with MAb G6C6 which is a vaccinia-specific monoclonal antibody. So, it's an effective technique for distinguishing MPXV from another Orthopox vaccinia virus.

Nowadays "Monkeypox Antigen Rapid Test Kits" from different brands are available in the market. The main advantages of these kits are they are highly sensitive, accurate, and can be easily operated to give results within 10-20 minutes. There are also a few other methods available for MPXV detection but compared to PCR, RT-PCR, or isothermal amplification processes these are less sensitive. Western Blot (WB) techniques mainly identify the specific MPXV proteins present in the sample. As per Hughes et al. (2014), an experiment was conducted in which MPXV proteins A29, A35, B6, and M1 along with VACV containing A27 were tested for binding with the monoclonal antibody (mAb 69-126-3-7). The result showed that the monoclonal antibody binding was specific for only MPXV protein A29. Moreover, another serological test method Haemagglutination Inhibition (HI) detects the presence of haemagglutinin protein on viruses. MPXV has hemagglutinin proteins which can clump the RBCs. Using antibodies RBCs can be inhibited from aggregating in the serum sample for further MPXV diagnosis. In converse, Radioimmunoassay (RIA), an ultramicroscopic analytical method uses both labeled and unlabelled antigens to competitively bind with specific antibodies for quantifying the MPXV antigens in a sample (Zhou and Chen, 2023).

1.8.5 MPXV Isolation and Culture

MPXV can be cultured well in mammalian cells and other cell lines like HeLa, Vero, BSC-1, and RK-13. This virus can also mature in chicken embryos and cause cytopathic lesions in them. In contrast, MPXV in Vero cells can multiply faster and detach themselves as new virions within 24 hours. These new virions of MPXV can be detected using immunofluorescence and specific antibodies. The main disadvantages of this culture method in MPXV identification are

this requires more time and a safer environment to get accurate experimental results. Further, experienced personnel are needed to carry out the processes in the laboratory with a biosafety level ≥ 3 to avoid contamination. Isolation and culture of MPXV might need special attention but it can help in the development of effective antivirals by allowing the study of the MPXV origin, genomic sequences, and mutations (Zhou and Chen, 2023).

1.8.6 Whole Genome Sequencing (WGS)

One of the methods for a very precise and detailed study of MPXV's entire genomic sequence is the computational-based lab analysis technique called Whole Genome Sequencing (WGS). This technique is important for the identification of specific strains, genetic variants, lineage mutations, and unknown transmission modes of MPXV. In particular, by manipulating the collected WGS data, the genetic changes of MPXV over the years can be found and the possible threats related to it can also be determined. Moreover, a notion of MPXV survival and adaptability throughout the ecological changes from the past to now can also be perceived. Despite all these advantages, the major drawbacks of this method are the need for high storage and accurate processing mode of data which can lead to expensive operational charges. Though the WGS method isn't manageable in large-scale operations still for research purposes of MPXV, it can bring revolutionary outcomes in the future (Zhou and Chen, 2023).

1.9 Treatment of MPXV

Currently, vaccines and antiviral drugs like Cidofovir, and Brincidofovir are popular for MPXV infection treatment. However, continuous mutations of MPXV are leading to drug resistance.

1.9.1 Tecovirimat

In January 2022, the European Medicines Agency (EMA) authorized the utilization of the oral or intravenous antiviral drug Tecovirimat for severe MPox infection, due to the similarity of MPox with smallpox. Tecovirimat mainly interacts with the MPXV DNA Polymerase Holoenzyme by forming a Hydrogen bond (H-bond) with the ASN665 and ASP753 residues. (Kumari et al., 2023). This drug might be given in some serious cases like bleeding from sores, the spread of smaller sores into larger ones, or in case of severe immunocompromised conditions during MPXV infection. However, before the use of Tecovirimat consent from the patient is taken since this investigational drug is still not approved by the FDA for MPXV infection (CDC, 2023, “Mpox Treatment with TPOXX”). This drug is also available in pill form and commonly this drug inhibits the viral envelope protein VP37 from developing wrap-around intracellular mature virions (IMV) and thus doesn’t let enveloped virions (EEV) from producing further. This prevents both the replication and mutation of the MPXV inside the cell (Tiecco et al., 2022). The use of tecovirimat is not recommended for everyone due to the possibility of MPXV growing resistant to this drug.

1.9.2 Second-Generation Vaccine

ACAM2000, a second-generation, live attenuated, vaccinia virus-containing vaccine that was approved in 2015 by the FDA to be used for people of age between 18 to 64 years old. It is a single-dose vaccine that has been shown to develop immunity against MPXV within 28 days

from the date of administration (Abdelaal et al., 2022). However, one of the disadvantages related to this vaccine is it can trigger some cardiac conditions like myocarditis and pericarditis (Ghosh et al., 2023).

1.9.3 Third-Generation Vaccines

Currently, three vaccines MVA-BN, LC16, and OrthopoxVac have been approved to treat MPox in case of serious conditions or those who are more exposed to the virus but the use of these vaccines at the mass level has not been recommended yet. Moreover, the FDA has authorized a standard dosage regimen of the JYNNEOS, a 3rd generation MVA-BN vaccine for use in the USA. In Canada and the European Union, this same vaccine is approved under the trade names of IMVAMUNE and IMVANEX respectively (Panda & Mukherjee, 2022). 0.5 ml of this JYNNEOS vaccine can be given to people below 18 years or above 18 years, subcutaneously in two doses, separated by a period of 28 days. An alternative regimen has also been authorized in which individuals who are 18 years of age or older can receive 0.1 ml of it intradermally in two doses spaced between 28 days apart (CDC, 2023, "JYNNEOS Vaccine"). After the first dose around four weeks (28 days) need to be waited before the administration of the second dose. In fact, two weeks after the second dose, maximum protection is achieved against MPXV. However, it is carefully observed if any serious allergic reaction is evident after the first dose of JYNNEOS is injected. If a condition like anaphylaxis is observed in that case, a second dose isn't recommended (CDC, 2023, "Mpox Vaccination Basics").

Another third-generation, attenuated vaccine licensed in Japan has been developed based on VACV LC18m8. This vaccine has shown fewer side effects than the administered conventional Lister strain vaccine. The attenuation of this virus is achieved due to a B5R gene mutation which specially codes for extracellular virion protein (Shchelkunova & Shchelkunov, 2022).

In November 2022, a live VACdelta6-based culture vaccine, named OrthopoxVac was licensed in Russia and has been approved for immunization against MPox as well as for other Orthopoxvirus infections.

1.9.4 Vaccinia Immune Globulin Intravenous (VIGIV)

VIGIV and CNJ-016 are approved by the FDA to be used in children and the elderly in serious cases of MPox under the Expanded Access IND protocol. VIGIF contains IgG antibodies produced against the vaccinia virus with some inactive components like maltose and polysorbate 80. Within the first 10 minutes of intravenous administration, symptoms like back or abdominal pain, nausea, and vomiting may appear. Some other common symptoms like chills, fever, headache, muscle pain, and exhaustion may happen as well after the needle is removed. However, in case of severe conditions like anaphylactic shock or hypotension, the treatment should be discontinued (CDC, 2023, “Use of (VIGIV, CNJ-016)”).

1.9.5 Brincidofovir and Cidofovir

Under special consideration, physicians recommend the use of investigational medication Brincidofovir in emergencies. Even though Brincidofovir is the prodrug of cidofovir, cidofovir isn't normally advised because it is less tolerable and safe compared to Brincidofovir. However, both aren’t recommended at the same time.

Application of Cidofovir both topically and intravenously has provided a positive efficacious response against MPXV in immunocompromised people. Cidofovir complex binds with MPXV DNA Polymerase Holoenzyme at the SER552, ASP753, and GLU792 by Hydrogen bond (H-bond) and ARG634 plus LYS638 by salt-bridge (Kumari et al., 2023). This drug gets activated into the phosphorylated form by cellular kinases. The active diphosphate derivatives

act as an inhibitor to terminate functions of viral DNA polymerase and DNA polymerase 3′–5′ exonuclease enzyme (Ghosh et al., 2023).

Brincidofovir is the lipid conjugative of Cidofovir and for this lipidic nature, it penetrates better in the cell membrane and provides greater oral bioavailability. Brincidofovir binds with the DNA Polymerase Holoenzyme to exhibit its antiviral action. Mainly Brincidofovir interacts with residues SER552, ASP549, and ASP753 by Hydrogen bond (H-bond), and ARG634 plus LYS638 interacts with it by salt-bridge (Kumari et al., 2023). Once the Brincidofovir enters the targeted cell, it liberates the cidofovir molecules after being cleaved by the phospholipase enzyme. The liberated Cidofovir molecule further gets activated into Cidofovir diphosphate by two phosphorylation steps to give the therapeutic effect against MPXV (Ghosh et al., 2023).

1.10 Prevention of MPXV

It is always known that prevention is better than cure so the risk of getting affected by MPox can be prevented by getting the full course of the JYNNEOS vaccine, avoiding skin-to-skin contact or any kind of sexual activity with an MPox-infected person, avoiding touch of any object used by an MPox-infected person, limiting contact with MPox carrier wild animals and avoiding raw meat consumption. Lastly, building the habit of washing hands often with soap and water or an alcohol-based sanitizer after interaction with infected patients is very important. It’s better suggested to completely isolate an infected person into a separate room with necessary items like utensils, bedding, clothes, electronics, soap, etc. for his use. It would be better if a separate bathroom is also used unless the infected person fully recovers. Especially, healthcare professionals must be extra cautious during the diagnosis of MPox, especially when handling needles or swabs as well as during the time of providing treatment (WHO, 2023, “Protect Other People From Mpox”). Besides, the deceased body should be transferred to the morgue as early as possible by properly wrapping it with cloth or shroud to

prevent fluid leakage or lesions open to the environment (WHO, 2022, “Clinical Management and Infection Prevention and Control”).

To prevent the spread of MPox from hospitals other than patient isolation or gown, gloves, PPE use, instructions on room cleaning, or specimen handling were adopted in many hospitals. For eg., collected swabs were inserted into a closed-cap container and kept inside two plastic bags. The outermost plastic pack had a biohazard sign and then it was put inside a plastic container with a screw top for ensuring maximum safety and better transport into the laboratory. Besides, the cleaning protocol recommended the use of hypochlorite solution (1,000 ppm) for cleaning the occupied or suspected MPox patient rooms. Other than this shared equipment was rubbed with chlorine wipes and those that are susceptible to chlorine were cleaned two times with Quaternary ammonium wipes (Safir et al., 2023).

1.11 Mutations Leading to Future Threats

The sudden and extensive appearance of the Clade IIb strain of MPXV on a global scale provoked substantial concerns regarding the health and safety of people all around the world. However, the cases of MPox infection declined gradually on May 23, 2023, and it was no longer a public health emergency of international concern (PHEIC) (PAHO, 2023, “End of MPox Emergency”). Even though the number of cases has declined in the present, the potential threats associated with it in the future can’t be overlooked.

The monkeypox virus has been divided into three clades. Clade I represents the lineage from the Congo Basin, Clade II is the lineage from West Africa, and Clade III causes the current MPox infection outside of Africa. The Clade III is further divided into two categories A.1 and A.2. The A.1 is again divided into two lineages A.1.1 and B.1. The viruses responsible for the 2017-2018 outbreak in West Africa have converged to form hMPXV-1A. hMPXV-1A group has more similarities in the genomic background towards Clade IIa compared to Clade I.

hMPXV-1A was apparent in the 2017-2018 outbreak in Nigeria and later this showed up in the UK, Singapore, and Israel as A.1 lineage and US with A.2 lineage. Further, it's revealed that the A.1.1 lineage detected in the US in 2021 has evolved by having characteristics of both the A.1 group and the B.1 group. Genomic analysis has shown that A.1.1 has 0.007, 0.019, 0.027, and 0.035% genetic variation respectively with B.1, A.1, A, and A.2 lineages. Further, the A.1 lineage showed 0.019, 0.023, 0.007, and 0.016% genetic diversity with the A.1.1, B.1, A, and A.2 lineages (Desingu et al., 2022). The alteration of the coding region involving the H3L and B21R antigens in the virus is primarily responsible for the evolution and variations across these Clades. The B.1 lineage which is primarily linked to the present cases of MPox infection has undergone microevolution giving rise to numerous clusters including B.1.1, B.1.2, B.1.3, B.1.4, B.1.5, B.1.6, B.1.7, and B.1.8. (H. Li et al., 2022, "Evolving Epidemiology of MPXV"). The genomic analysis of B.1 has exhibited 0.007, 0.023, 0.031, and 0.039% genetic variation with A.1.1, A.1, A, and A.2 lineages (Desingu et al., 2022). Another finding from an article demonstrated that the mean nucleotide substitution rate for the A.2 lineage is almost close to 5.53×10^{-5} substitutions per base/year, while the B.1 lineage has 1.13 × 10-4 substitutions per base/year. This suggests that the B.1 lineage has undergone more alterations than the A.2 lineage (Azzi, 2023).

Moreover, 10 proteins OPG210, OPG188, OPG153, A27L-like, D2L-like, OPG109, OPG105, OPG071, OPG047, and OPG023 were observed to be more susceptible to changes due to assigning mutation in 187 open reading frames (ORFs) of the MPXV genome. Particularly OPG210 and OPG105 have several nucleotide substitutions in the recent strains of MPXV and got mutated along many lineages of the MPXV (H. Li et al., 2022, "Evolving Epidemiology of MPXV").

The Apolipoprotein B mRNA Editing Enzyme, Catalytic Subunit 3G (APOBEC3), a cytidine enzyme, has caused around 42 nucleotide changes in the virus by conversion of GA to AA or from TC to TT. This evolution is also leading to human-to-human transmission of the monkeypox virus. The influence of APOBEC3 has caused around 46 B.1-specific single-nucleotide polymorphisms (SNPs) till now. Among these polymorphisms, 4 intergenic, 18 synonymous, and 24 non-synonymous mutations are found. Among 10 genes particularly these 6 genes OPG003, OPG093, OPG098, OPG110, OPG185, and NBT03_gp174 genes related to the B.1 lineage have undergone mutation under the influence of APOBEC3 enzyme. These 6 proteins are responsible for determining the virulence factors plus immune evasion. Other non-APOBEC3 mutations include changes in D209 N, P722S, and M1741I amino acids present in genes responsible for creating OPG210 or B22R immunogenic surface glycoproteins. Likewise, in the MPox virus, a mutation in the OPG176 gene coding for a BCL-2-like protein that disrupts the host immune response is also observed (Luna et al., 2023).

The 2022 outbreak of MPox contained the mutation in the L108 residue of the MPXV F8L gene. The phenyl group of L108F is closer to the flipped nucleotide and this enhances the binding affinity of polymerase enzyme and triphosphate due to the rise in hydrophobicity. Thus this whole phenomenon enhances the processivity, alters the sensitivity towards the nucleoside inhibitors, and helps in further DNA synthesis. Emerging since 2018, the W411 mutation has managed to keep evolving up to the 2022 outbreak. In the F8L insert 2, W411 remains exposed to the surface and influences insert 2 to more feasibly interact with the regulatory factors of MPXV (Kannan et al., 2022). S30L and D88N mutations in the G9R gene were evident in the 2022 outbreak. There are possibilities that this mutation may affect the G9R and E4R (uracil DNA glycosylase) and cause functional variations in other proteins (Jin et al., 2024).

For the past decades, MPXV has been seen to survive and adapt even after multiple mutations in its genes. The multiple mutations are responsible for the transmission of this infection rapidly in non-endemic areas. In comparison to RNA viruses, DNA viruses like MPXV are slow and less fatal. Nevertheless, mutational features of MPXV can't be neglected since mutations can lead to the risk of immunity evasion, increased virulence, and drug/vaccine resistance. Therefore, it is important to look into the risks of future MPXV mutations to stop massive outbreaks and prevent MPox infection from becoming a worldwide health problem.

1.12 Present Scenario

The World Health Organization (WHO) published a report on 19 September 2023, affirming that 90,439 Mpox cases have been found globally in 115 countries up to September 11, 2023, from 1 January 2022. In that report, 22 countries among 115 were newly affected within 21 days (WHO, 2023, "External Situation Report 28"). Another recent report by the World Health Organization (WHO) published on October 20, 2023, stated that since January 1, 2022, up to September 30, 2023, there have been 91,123 confirmed cases of MPox infection in 115 countries, resulting in 157 deaths overall. Among these, the highest number of majorly affected ten countries are the United States of America, Brazil, Spain, France, Colombia, Mexico, Peru, The United Kingdom, Germany, and China (WHO, 2023, "External Situation Report 29"). Due to the sudden rise of MPox infection in recent years, some therapeutic approaches have been fully or partially approved under some restrictions and guidance for treatment purposes. Moreover, these measures aim to offer patients effective treatment options considering the condition and progression of infection in the patient. According to another recent report by WHO, in total 668 cases from 29 countries have been confirmed globally till October 2023. The most cases were observed from the Western Pacific and European regions. Apart from this, 8 cases new cases from African Regions and 1 new case from the Eastern Mediterranean

Region have been recorded. Through the latest global observance, it's been revealed that the MPXV infection has less propagation in the European and American countries compared to Western Pacific and Southeast Asia areas. Apart from this, in the Democratic Republic of the Congo, 12,569 cases have been suspected and 581 deaths are documented (WHO, 2023, "External Situation Report 30"). The latest report of the World Health Organization (WHO) published that around 92,783 confirmed cases with 171 deaths have been recorded from January 1, 2022, to November 30, 2023. Among this in November, 906 cases of MPox infection were confirmed from 26 countries. As per the report, the infection spread extensively in the European Region and in the Region of the Americas with 26, 654, and 60, 400 confirmed cases respectively (WHO, 2023, "External Situation Report 31").

To discuss the current situation, another crucial aspect comes to the forefront which is vaccine distribution. The disparity between rich and LMICs (Low and Middle-Income Countries) is once again evident due to the inequality in vaccine distribution. The wealthy countries like the US, European Union, Britain, and Canada have seemed to be at the forefront when it comes to having access to MPXV vaccines. One of the main reasons for the unavailability of such vaccines is due to its limited production. Africa doesn't have the proper infrastructure and qualified personnel for its vaccine production. Out of 12 production facilities, only 6 countries South Africa, Egypt, Algeria, Senegal, Rwanda, and Morocco are contributing to vaccine production which is also less than 1%. Around 1.2 billion people in South Africa have no access to the MPox vaccine yet. Again only Japan is responsible for the current production of LC16m8 and no other countries are producing it in the global market. Moreover, even after the availability of 100 million doses of ACAM2000, it raises concerns about immunocompromised people's safety as previously many side-effects have been recorded for this particular vaccine. As per WHO, 35 countries are fighting for 16.4 million currently available doses of the JYNNEOS vaccine. The only European manufacturer of this vaccine,

Bavarian Nordic has halted production in 2022. This highlights the fact that amidst the rising confirmed cases of MPox, it's still uncertain how much percentage of people in low and middle-income countries will have the opportunity to get vaccinated (Ogunkola et al., 2023).

Chapter 2

Methodology

The review study uses data from renowned websites, current research papers, and articles. Most of the articles and peer-reviewed journals belong to top-notch and remarkable sources like PubMed, Science-Direct, The Lancet, SpringerLink, etc. Besides, the data from renowned websites are collected mostly from WHO (World Health Organization), and CDC (Centers for Disease Control and Prevention). Each of the papers from different sources was carefully read and analyzed before collecting recent and reliable information to create the review article named **"A Descriptive Review on Monkeypox Virus from Past to Present"**.

Chapter 3

Result & Discussion

3.1 Comparison Between ACAM2000, JYNNEOS, and LC16 Based on Contradictions

The comparison between the three vaccines shows that the non-replicating, third-generation, JYNNEOS (MVA-BN) vaccine and minimally replicating, third-generation, LC16 vaccine are less restricted and much safer to be administered in people with certain health issues like immunocompromised, atopic dermatitis, cardiovascular disorder. Particularly, pregnant and breastfeeding women need special attention in case of live vaccine administration, and replicating ones like ACAM2000 must be avoided to protect the infant's health and prevent any sort of pre-birth and post-birth complications. JYNNEOS (MVA-BN) vaccine is notably a safer one compared to other vaccines but one of the shortcomings is that the recipient needs to be above 18 for the administration of this specific vaccine (Abdelaal et al., 2022). Table 2 compares the contradictions related to the administration of different generations of vaccines in people:

Contradictions	ACAM2000 (2nd Generation)	MVA-BN (JYNNEOS, 3rd Generation)	LC16 (3rd Generation)
Immunocompromised	✓	–	–
History of Atopic Dermatitis	✓	✓	✓
Pregnancy	✓	–	–
Breastfeeding	✓	–	–
Allergy to one of the vaccine components	✓	✓	✓
Underlying Heart Conditions (eg. coronary artery disease, cardiomyopathy)	✓	–	–
Cardiac Risk Factors (eg. hypertension, diabetes, smoking)	✓	–	–

Table 2: Contradictions in Administration of 2nd and 3rd-Generation Vaccines in Different Health Conditions (Abdelaal et al., 2022).

3.2 Comparison of Adverse Effects Noticed After Administration of 2nd-Generation Vaccine and 3rd-Generation Vaccine

Few adverse effects were noticed after the administration of ACAM2000 or JYNNEOS. It was found that receivers of the replicating ACAM2000 vaccine faced more challenges compared to the receivers of the non-replicating JYNNEOS vaccine. This makes the JYNNEOS (MVA BN) vaccine safer due to not replicating or producing a severe cutaneous response in the human host (Abdelaal et al., 2022). Table 3 shows a thorough notion of adverse effects faced by the people after the administration of the third-generation vaccines:

Adverse Reactions	ACAM2000	JYNNEOS
Lymphadenopathy	**Yes**	**–**
Lymph Node Pain	**Yes**	**–**
Headache	**Yes**	**Yes**
Dyspnoea	**Yes**	**–**
Nausea	**Yes**	**Yes**
Vomiting	**Yes**	**–**
Diarrhea	**Yes**	**–**
Constipation	**Yes**	**–**
Inadvertent Inoculation	**Yes**	**–**

Adverse Reactions	ACAM2000	JYNNEOS
Inject Site Purities	**Yes**	**Yes**
Fatigue	**Yes**	**Yes**

Table 3: Comparison between third-generation vaccines, ACAM2000 and JYNNEOS in case of Adverse Reactions (Abdelaal et al., 2022).

3.3 Preparedness and Response to Handle MPXV Outbreak

The World Health Organization (WHO) published guidance on 10 June 2022 to clinically manage, prevent, and control the MPXV infection. For example, the WHO recommends hospitalization for patients such as small children, expectant mothers, and immunocompromised individuals so that they continue to receive clinical and supportive care while being appropriately isolated from spreading the virus. As well as, suspected or confirmed patients are recommended to undergo continuous follow-up and counseling to monitor any changes in their physical or mental health.

In the case of mild and non-complicated patients, isolation at home is possible by maintaining some instructions related to Infection Prevention and Control (IPC). This involves having a skilled health worker inspect and confirm first if the house is suitable to isolate the infected patient with necessary amenities such as enough food, water, and a sanitary system. This also includes checking the facility of contacting the patient by phone call or telemedicine. Lastly, it includes careful assessment of the danger remaining to infect healthy or vulnerable people even after the patient has been isolated. If the chances of spreading infection remain then the patient must be kept in an alternate location away from healthy individuals. Moreover, only one

caregiver should be appointed and the caregiver must maintain at least a 1-meter distance from the patient while being present in the isolation room. Even if isolation isn't possible then also at least 1 meter distance needs to be kept between patients in a single room. In case, the caregiver needs to get considerably closer during an examination or sample collection from patients then proper masks, disposable latex gloves, shoe covers, goggles, respirators, and well-fitted clothes like PPE should be worn. Furthermore, patients should notify the hospital before the visit so that all required arrangements can be made in advance to properly deliver necessary treatment to the patient along with limiting the access of the virus to the surrounding area. Besides, the formation of a MPox management team in the hospital, compromising young health workers below ≤45 years of age with proper training and experience is suitable for dealing with MPox patients. The hospital staff based on their expertise can be divided into numerous groups to ensure health workers' safety and round-the-clock service for patients' betterment (Ahmed et al., 2023).

Management of MPox would be a lot easier if mass people were made aware and given proper education and instructions related to stress managing techniques, self-management ways, stigma mitigating mentality, and details concerning home care. Continuous on-site, online, or hybrid training for healthcare practitioners is very much necessary in this case to learn the best possible ways to provide excellent care and treatment to patients. The developed MPox readiness and response curriculum plus a free e-learning program needs to be delivered through online sessions in a way so that everyone can have access to the learning opportunities (Ahmed et al., 2023). In addition to this, the MPXV, evolution, susceptibility to existing treatments, stability of new variants, unknown symptoms appearance, duration, and transmission mode need to be continuously tracked to find better ways to fight off the infection (WHO, 2022, "Clinical Management and Infection Prevention and Control").

Chapter 4

Conclusion

Summary

Monkeypox has been a part of global talk after it suddenly started spreading in non-endemic areas in the year 2022. Due to its huge outbreak outside Africa, it was formerly considered and declared as a matter of global health concern. Again, many new variants of MPXV have emerged throughout recent years which also has put forward possible risks of adverse conditions in the upcoming future. Yet, due to being less lethal as well as having a slower transmission rate, MPox is no longer considered an upcoming reason for pandemics like COVID-19. Additionally, the availability and approval of the use of vaccines and oral medication have made it possible to treat the MPox viral infection in a much more efficient manner. Proper maintenance of the guidelines provided by the World Health Organization (WHO) for both patients and healthcare providers, without a doubt can reduce the MPox infection in large communities. In brief, indeed the prevailing cases of Monkeypox are not as deadly as the COVID cases but by ensuring proper treatment and awareness among the mass population it is possible to control the infection to a greater extent.

Future Prospect

Extensive surveillance in the MPXV-infected area is necessary for figuring out the geographical distribution and new symptomatic alterations of this virus. Among the diagnostic methods of MPXV, RT-PCR has been the most effective till now due to its higher sensitivity, specificity, rapidity, and accurate outcomes. Nevertheless, for better understanding and further study of MPXV transmission and mutation, the Genome Sequencing method needs to be

applied in a more accessible and cost-effective manner. For handling such delicate viruses laboratories need to be occupied with vital equipment along with skilled personnel. In addition, primary protection against MPXV infection can be achieved if mass immunization against smallpox in every nation is ensured. Apart from this, proper vaccine distribution among high to low-income countries is a major step in protecting the humans from the alarming effects of MPox infection.

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