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Review Article

Monkeypox; Pathogenesis, Risk Factors, Complications, Laboratory Diagnosis, Prevention and Management: A Concise Review

Erhunmwunse RU[1], Ogbodo EC[2]*

[1]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Benson Idahosa University, Benin City, Nigeria
[2]Department of Clinical Chemistry, Faculty of Medical Laboratory Sciences, Nnamdi Azikiwe University, Anambra State, Nigeria

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***Corresponding Author:** Ogbodo EC
Department of Clinical Chemistry, Faculty of Medical Laboratory Sciences, Nnamdi Azikiwe University, Anambra State, Nigeria

Abstract

Monkeypox otherwise known as Mpox is a zoonotic disease caused by monkeypox virus which belongs to the genus Orthopox virus of the family Poxviridae; a large and diverse family of enveloped double stranded DNA viruses that replicate in the cytoplasm of infected cells. First discovered in 1958 with confirmed case first described in 1970, Monkeypox remains a reoccurrence especially in West African countries such as Nigeria. It occurs primarily in tropical rainforest areas of central and West Africa and is occasionally exported to other regions. There are two distinct genetic clades of the monkeypox virus: the central African (Congo Basin) clade and the West African clade with the earlier historically causing more severe disease and was thought to be more transmissible. In recent times, the case fatality ratio has been around 3–6%. Mpox is transmitted to humans through close contact with an infected person or animal, or with material contaminated with the virus. Clinical presentation resembles that of smallpox, a related orthopoxvirus infection which was declared eradicated worldwide in 1980. Mpox typically presents clinically with fever, rash and swollen lymph nodes and may lead to a range of medical complications. The gold standard method of diagnosis is by polymerase chain reaction (PCR) method with others including viral culture, antibody testing and electron microscopy. This review x-rays the pathogenesis, risk factors, clinical presentations, associated complications, laboratory diagnosis, prevention and management of Mpox highlighting the need for utilization of an integrated approach that include immunization, antiviral treatments, and public health policies tailored to high-risk populations in combatting the disease. The need for training and retraining of Laboratory Scientists and personnels to acquire improved diagnostic skills desired in order to effectively control and reduce the impact of Mpox is highly needed.

Keywords: Monkeypox, Mpox, Pathogenesis, Risk Factors, Clinical Presentations, Associated Complications, Laboratory Diagnosis, Polymerase Chain Reaction, Prevention and Management of Mpox, Hand Washing, Vaccination.

1.0 INTRODUCTION

The term "monkeypox" was changed to "Mpox" in November 2022 by the World Health Organization (WHO). The original name was changed to avoid any potential stigma and to conform to modern best practices, which forbid disease names that are based on living things or places. Monkeypox virus will continue to be used to refer to the virus that causes Mpox until the International Committee on the Taxonomy of Viruses (ICTV) establishes what the virus's official name should be (WHO, 2022).

A zoonotic orthopoxvirus called monkeypox (Mpox) accidentally causes a smallpox-like disease in people, however with noticeably reduced mortality (Patel and Patel, 2023). The fact that this virus is endemic to western and central Africa, with outbreaks in the Western Hemisphere linked to the exotic pet trade and international travel, makes it clinically significant (Patel and Patel, 2023). The vaccinia vaccine originally provided coincidental immunity against the mpox virus, but the eradication of smallpox and the consequent lack of immunization campaigns allowed monkeypox to develop clinical relevance (Moore, Rathish and Zahra, 2022).

According to Suu-Ire *et al*., (2021), monkeypox is a viral zoonosis disease that has a harmful impact on

both human and animal health. Its symptoms are comparable to those of smallpox, although they are clinically less severe. Monkeypox has emerged as the most significant orthopoxvirus for public health despite the elimination of smallpox more than 40 years ago and the consequent end of smallpox immunization (Silva *et al*., 2020). Monkeypox is mostly found in Central and West Africa, frequently close to tropical rainforests; however, it has started showing up in cities more and more recently (Silva *et al*., 2020).

The monkeypox virus, which belongs to the Orthopoxvirus genus and family Poxviridae, is what causes the disease (Peter *et al*., 2021). The majority of these are found near tropical rain forests in remote communities in Central and West Africa (Peter *et al*., 2021). The reemergence of MPXV in Nigeria in 2017 (at Bayelsa state), 39 years after the last case was reported there, and the export of travelers' monkeypox (MPX) from Nigeria to other regions of the world in 2018 and 2019, respectively, have sparked concern that MPXV may have emerged to fill the ecological and immunological niche left by smallpox virus (Alakunle *et al*., 2020).

Additionally, genome-wide phylogenetic analyses show that the isolates of the monkeypox virus (MPXV) from the most recent outbreak in Nigeria in 2017 are monophyletic with the isolate that was exported to Israel from Nigeria, but they do not find any isolates that have a common ancestor with isolates from earlier outbreaks in 1971 and 1978, respectively (Alakunle *et al*., 2020).

Monkeypox can live for several months on soil, crusts, and clothing and is resistant to drying, low temperatures, and abrasion. The virus can be inactivated by heating to 56°C for 30 minutes or 60°C for 10 minutes because it is heat sensitive. It is susceptible to sodium hypochlorite, chloroxylenol, glutaraldehyde, formaldehyde, and paraformaldehyde, as well as to ultraviolet light and common disinfectants (DaAnGene, 2022). Since May 2022, outbreaks of the monkeypox virus have been reported in numerous nations, including the UK, Portugal, Spain, Australia, Germany, and France. This has drawn much public interest. The World Health Organization (WHO) declared the monkeypox pandemic a "Public Health Emergency of International Concern (PHEIC)" on July 23, ranking it on same level with polio and COVID-19.

Up until September 5, 2022, there were 53027 verified cases of monkeypox worldwide, and it appears that the number of reported cases is continuing to rise (DaAnGene, 2022). The accurate diagnosis is a crucial step in controlling the monkeypox virus outbreak, and the WHO advised using the nucleic acid amplification testing (NAAT) method to identify the virus (DaAnGene, 2022). Real-time or conventional polymerase chain reaction (PCR) is the gold standard for identifying monkeypox virus DNA (DaAnGene, 2022).

The absence of smallpox virus reduces the population-wide immunity coverage during these eradication efforts and increases the re-emergence of Monkey pox virus infection, despite the fact that smallpox vaccine provided effective protection against smallpox virus and monkeypox virus, respectively (Reynolds *et al*., 2020).

2.0 Background on Monkey Pox Virus

Taxonomy of Monkey Pox

Family: Poxviridae
Subfamily: Chordopoxvirinae
Genus: Orthopoxvirus
Species: mpox virus.

The monkeypox virus, a member of the Orthopoxvirus genus and Chordopoxvirinae subfamily of the Poxviridae family, is the virus that causes monkeypox. Other significant human and animal viruses, such as the smallpox virus, vaccinia virus, cowpox virus, etc., can also be found in the genus Orthopoxvirus (Ilic *et al*., 2022). 96.3% identity was observed in the center part of the genome, which codes for necessary enzymes and structural proteins, in a genomic comparison of the monkeypox and smallpox viruses in 2001. However, significant differences were found in the end regions, which code for virulence and host-range parameters. It is well established that smallpox immunization confers cross-immunity with up to 85% protection against infection with human monkeypox (Ilic *et al*., 2022).

Two separate genetic clades of the monkeypox virus exist based on genetic, geographic, and phenotypic variations: Clade I, formerly known as the Central African (Congo Basin) clade, and Clade II, formerly known as the West African clade.

The ones attributed to the Congo Basin are more virulent, associated with a more severe disease, higher mortality, and greater human-to-human transmissibility (Alakunle and Okeke, 2022). The WHO (2022) argues that the Congo Basin group is deadlier than the Western African clade. Subclades IIa and IIb make up Clade II.

These have epidemiological and clinical distinctions. The size of the mpox virus under electron microscopy is between 200 and 250 nanometers. Poxviruses have a brick-like structure and a linear double-stranded DNA genome that is enclosed in a lipoprotein envelope (Alakunle *et al*., 2020). Poxviruses have all required replication, transcription, assembly, and egress proteins in their genome in addition to their dependency on host ribosomes for mRNA translation (Alakunle *et al*., 2020).

When monkeys shipped from Singapore to a research center in Denmark became ill, the mpox

(monkeypox) virus was first isolated and identified in 1959. However, the virus was isolated from a young kid in the Democratic Republic of the Congo who was thought to have smallpox in 1970, making that the first known case of the disease in humans (Moore *et al*., 2022). The vaccinia vaccine formerly provided coincidental immunity against the mpox virus, but the eradication of smallpox and the consequent lack of vaccination campaigns allowed mpox to develop clinical relevance (Nguyen *et al*., 2021). Additionally, given that the majority of mpox cases in Africa occur in rural areas, it is possible that underreporting of these cases is occurring (Sklenovská and Van Ranst, 2018).

Poxviruses are large, linear, double-stranded DNA viruses that replicate in the cytoplasm of vertebrate or invertebrate cells. Their genome sizes range from 130 to 360 kbp. In contrast to DNA viruses, which normally replicate and express their genomes in the nucleus while heavily relying on cellular proteins, pox viruses do not do this. Poxviruses differ from other viruses in that they heavily rely on virus-encoded proteins to facilitate cytoplasmic replication (Kaler *et al*., 2022). Genes important in crucial processes like transcription and virus assembly are found in the genome's core region, whereas those near the termini are responsible for interactions between viruses and their hosts.

More than 150 genes are encoded by poxviruses, 45 of which are shared by all sequenced members of this family and 90 of which are shared by the chordopoxvirus subfamily. The majorities of these genes that are conserved across viruses are involved in viral function and make up the bulk of the genome (Okyay *et al*., 2022).

The larger size of the virus makes it difficult for the virus to replicate quickly, and orthopoxviruses need a more comprehensive strategy to survive within the host (Kaler *et al*., 2022). The larger size of the orthopoxviruses alerts the immune system of the individual very early on and therefore, generates an immune response very easily.

Orthopoxviruses are equipped with a collection of chemicals expressed by virulence genes that will operate as modulators by being directed against the host's immune system's components in order to be able to avoid the host immune system. According to whether they operated intracellularly or extracellularly, these proteins that are in charge of modulatory effects against the host's immune response can be divided into two groups (Okyay *et al*., 2022).

These proteins can be divided into two groups based on their modulatory effects on the host's immune response. Virotransducer proteins and virostealth proteins are examples of intracellular proteins. The oxidative burst and apoptotic pathways, which the cell uses to respond to the infection, are interfered with by the virotransducer proteins (Kaler *et al*., 2022). The major histocompatibility complex class 1 (MHC 1) and CD+4 are immune recognition molecules that the virostealth proteins, which also function intracellularly, downregulate in order to decrease the likelihood of the host immune system detecting the virus.

Viromimic proteins are the only type of external protein, whereas two other types of intracellular modulatory proteins help monkeypox evade the host's immune response (Okyay *et al*., 2022). As a result, virokines create viral mimics of host cytokines, chemokines, and growth factors that are successful in both obstructing host responses that are harmful for virus survival and encouraging responses suitable for viral replication and dissemination. In order to enable viral replication, these modulatory proteins simultaneously strive to subvert the host's immune response. Monkeypox and other orthopoxviruses would be unable to subvert the immune system without the presence of these proteins (Okyay *et al*., 2022).

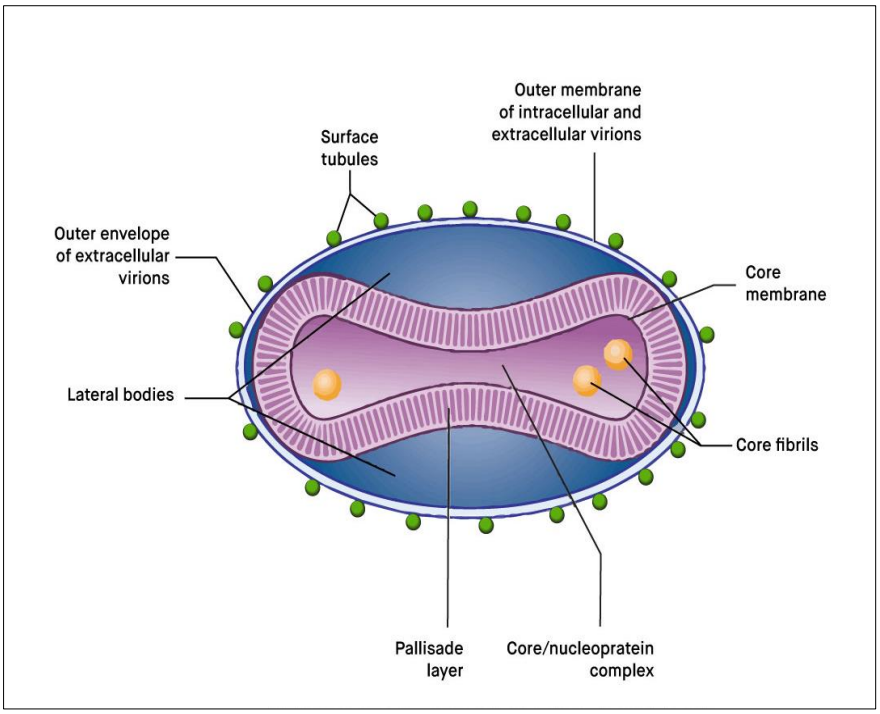

Fig. 1: Structure of Monkey pox virus (Vitrosens, 2022)

2.1 History of Monkeypox

In 1958, an outbreak of pox-like illness among captive cynomolgus macaque monkeys (hence the name monkeypox) occurred in an animal facility at a laboratory in the State Serum Institute in Copenhagen; these monkeys had been imported to Denmark from Singapore for the purpose of polio-vaccine research. Even though several rodents, including rats, mice, and squirrels, later showed signs of monkeypox infection, the disease's origin is still unknown (Reynolds *et al*., 2018). As a result, the term "monkeypox" might not be applicable given that the precise reservoirs and methods of virus transmission are yet unknown (Xiang and White, 2022).

In 1970, nine months after the nation had eradicated smallpox, the first case of human monkeypox was discovered in Zaire (now the Democratic Republic of the Congo). A 9-month-old baby was hospitalized on September 1st, 1970, with a condition resembling smallpox. The WHO Smallpox Reference Centre in Moscow received samples from the patient, and it used those samples to isolate the monkeypox virus. The patient was released from the hospital after recuperating; unfortunately, on October 23, he contracted the measles, and six days later he died. The normal scars from smallpox immunization were present on every single family member. In the hospital as well as the village, there was no sign of any additional infection transmission.

Since then, human monkeypox has expanded throughout Africa, primarily in Central and West Africa, becoming endemic in the Democratic Republic of the Congo. In 2003, the United States of America saw the first cases of human monkeypox recorded outside of Africa. Numerous cases of human monkeypox were reported in the years that followed (Bunge *et al*., 2022).

2.2 Aetiology

Mpox is a zoonotic disease that humans can contract from animals. Squirrels, rats, monkeys, primates, prairie dogs, hedgehogs, pigs, mice, and other animals found in the African locations where mpox was formerly extensively documented are believed to be the disease's animal reservoirs (Alakunle *et al*., 2020). However, human-to-human transmission via respiratory droplets, fomites, and direct contact with lesions of an infected person is the main cause of the ongoing epidemic (Alakunle *et al*., 2020). According to a recent investigation, large viral loads have been discovered in bodily fluids such urine, saliva, semen, and feces as well as in swabs from the oropharynx and rectum, indicating that sexual transmission is a significant contributor to the spread of the disease (Peiró-Mestres *et al*., 2022).

2.3 Epidemiology

Mpox is a zoonotic illness that is most prevalent in the Democratic Republic of the Congo and is indigenous to central and western Africa. The research suggests that African rodents are the natural reservoir, despite the fact that the disease was initially discovered in captive monkeys (thus the name). Squirrels, rats, mice, monkeys, prairie dogs, and humans have all contracted infections (Sklenovská and Van Ranst, 2018). There are currently two clades that are genetically distinct from one another. In contrast to the West African clade, the Congo Basin (Central African) clade is reported more frequently and has documented examples of human-to-human transmission (Sklenovská and Van Ranst, 2018). Human

mpox cases and sporadic clusters have been reported outside of Africa.

In 2003, Gambian giant rats imported from Ghana infected co-habitant prairie dogs sold as household pets in the Midwestern United States. This resulted in fifty-three human cases of mpox. In October 2018, one case occurred in a man who traveled from Nigeria to Israel (Erez *et al.*, 2019). In May 2019, one case occurred in a man who traveled from Nigeria to Singapore (Yong *et al.*, 2020).

Three members of a family who had visited Nigeria in May 2021 and returned to the United Kingdom were infected with the mpox virus (Hobson *et al*., 2021). The orderly onset of symptoms in each case within the family (day 0, day 19, day 33) may indicate transfer from person to person. One case included a man who traveled from Nigeria to Texas in July 2021 (Rao *et al*., 2022). One case included a man who traveled from Nigeria to Maryland in November 2021 (Costello *et al*., 2022). As of May 2022, there was one incidence of human mpox in a man who had traveled from Canada to Massachusetts, and there were also clusters of cases in the United Kingdom.

Given alleged flaws in disease reporting and confirmation, precise prevalence and incidence are challenging to establish. Nevertheless, both metrics have increased after the routine smallpox immunization was stopped (Sklenovská and Van Ranst, 2018). Living in densely forested and rural parts of central and western Africa, handling and preparing bush meat, caring for someone who is sick with the mpox virus, and not having received the smallpox vaccine are all proven risk factors for mpox infection (Petersen *et al*., 2019). The likelihood of infection has also been linked to male gender. The societal expectation that men routinely hunt and interact with wild animals may, however, complicate this.

In 2022, there has been currently a widespread Mpox outbreak affecting several nations on various continents, mostly in the community of men who have sex with men (MSM), with a presentation that primarily consists of genital lesions (Velavan and Meyer, 2022). 99% of cases were found to be in the MSM community in a cohort of 595 confirmed cases of mpox in Spain in 2022, with the lesions primarily affecting the genital, perineal, or perianal areas. Inguinal lymphadenopathy was also shown to be a common feature in the study, supporting the idea that sexual transmission was the primary method of transmission. As of July 6, 2022, Germany reported 1304 confirmed cases, primarily among men that have sex with men (MSMs) (Selb *et al*., 2022).

The West African lineage of the Mpox virus is thought to be the cause of the 2022 outbreak, according to sequencing data from multiple nations. However, recent information indicates that a new lineage may be responsible for the current outbreak (Luna *et al*., 2022). Direct contact with bodily fluids, skin lesions, or respiratory droplets of infected animals can result in transmission, as can indirect contact with contaminated fomites. Mathematical modeling in the setting of declining herd immunity to orthopoxviruses reveals a rising threat of disease transfer between humans, even if human-to-human transmission has historically been limited (Grant, Nguyen and Breban, 2020). In the hospital sector, the Centers for Disease Control and Prevention (CDC) advises isolation in a negative pressure chamber as well as standard, contact, and droplet precautions with escalation to airborne precautions if possible.

According to Nolen *et al*., (2015), since 1970, human instances have primarily been documented from remote, rain forest regions of the Congo Basin, mainly in the Democratic Republic of the Congo. However, cases have also been steadily increasing throughout Central and West Africa. Benin, Cameroon, the Central African Republic, the Democratic Republic of the Congo, Gabon, Cote d'Ivoire, Liberia, Nigeria, the Republic of the Congo, Sierra Leone, and South Sudan are the 11 African nations where human cases of monkeypox have been documented since 1970 (Petersen *et al*., 2019). For instance, in the Democratic Republic of the Congo, an outbreak was recorded in 1996–1997 with a lower case motality ratio and a greater attack rate than typical.

In this example, where most travelers serve as the primary vehicle for the transmission of this virus to non-endemic areas, a concurrent monkeypox outbreak was discovered, which may explain real or perceived changes in transmission dynamics (Alakunle *et al*., 2020). Over 500 suspected cases, over 200 confirmed cases, and a case fatality rate of about 3% have been reported in Nigeria since 2017. Cases are still being reported today. Within these endemic zones, animal-human interaction may be the main means of viral transmission. According to a recent analysis, the Democratic Republic of the Congo, Nigeria, and Cameroon had the highest rates of monkeypox cases in Africa, while Central African Republic had the lowest rates (Guagliardo *et al*., 2020).

Monkeypox cases in Nigeria from September 2017 to February 28th, 2022 are reported by the Nigeria Center for Disease Control and Prevention (NCDC, 2022) as follows: With 23% of the instances, Rivers State was at the top of the list, followed by Lagos and Bayelsa States with 19% and 14% of the cases, respectively. While the Federal Capital Territory (FCT) recorded 3% cases, the other states are Delta (13%), Cross River (6%), Edo (5%), Imo (4%), Akwa Ibom (3%), Oyo (3%), Enugu (2%), Abia (1%), Plateau (1%), Nasarawa (1%), Benue (1%), Anambra (1%), Ekiti (1%), Ebonyi (0%), Niger (0%), Ogun (0%) and Adamawa (0%).

2.4 The Course of the Disease

An incubation period of between 7–12 days and possibly up to 21 days follows monkeypox virus infection (CDC, 2022). The 2-4 day smallpox-like prodromal stage is characterized by fever, lymphadenopathy, headache, chills, muscle aches, back pain, and an overall feeling of discomfort. A well-circumscribed rash with a fast, centrifugal pattern that resembles smallpox appears 1 to 3 days after the onset of fever (over the head, body, extremities, including the palms and soles, oral mucosa, and genitalia). The rash appears as macules at first, then changes to papular, vesicular, and pustular lesions, and finally crusts over a period of 14 to 28 days. Human monkeypox often manifests as a self-limited illness that clears up completely within 2 to 4 weeks.

Respiratory distress, subsequent bacterial infections, bronchopneumonia, encephalitis, sepsis, and ocular infections with consequent blindness are among the sequelae of monkeypox (Ogoina *et al*., 2020). Children, patients with comorbidity (immune deficiencies including HIV) (Obimakinde *et al*., 2020), pregnant women, and people who have not received a smallpox vaccination are more likely to experience severe illness and worse outcomes (currently, those between the ages of 40 and 45 may be more susceptible to monkeypox due to the cessation of smallpox vaccination following the disease's global eradication). Miscarriage may occur as a result of infection during pregnancy (Mbala *et al*., 2017).

Monkeypox case fatality rates varied between 0% and 1% for the West African strain and up to 10% for the Congo Basin strain. The frequency of the asymptomatic infection is unknown, but serological studies showed that both some people who had had the smallpox vaccine and some who had not had it had asymptomatic human monkeypox infections. While some writers believe that the infectivity phase begins on the first day of the rash, according to the Centers for Disease Control and Prevention, a person can occasionally be contagious even during the prodromal stage (Obimakinde *et al*., 2020).

Patients are most contagious when lesions develop. An infected person is contagious until all scabs have fallen off and new skin covers all the monkeypox spots (lasting for a period of 2 to 4 weeks). Monkeypox virus is highly resistant in dry environments and at high temperatures and persists on infected surfaces for a longer period (about 2 weeks) (Obimakinde *et al.*, 2020).

2.5 Pathophysiology

Viruses enter the body through any channel (oropharynx, nasopharynx, or intradermal), infect immune system cells, especially dendritic cells and macrophages, and multiply there (McCollum and Damon, 2014). Following their migration to lymph nodes, these cells start to infect the endothelial cells there, which cause the development of tiny blood clots. The virus subsequently travels through the bloodstream to various tissues and organs, resulting in a variety of symptoms. This is referred to as the incubation period, which typically lasts seven to fourteen days but can last up to 21 days. This means those seven to fourteen days after infection, monkeypox symptoms usually start to manifest.

Similar to smallpox, the infectious process for monkeypox virus starts with exposure to the host's oropharyngeal or respiratory mucosa. The monkeypox virus replicates at the inoculation site after viral entrance; in human-to-human transmission, the inoculation site is the respiratory and oropharyngeal mucosa. In primary viremia, the viral load multiplies and then spreads to the neighborhood lymph nodes. In secondary viremia, the viral load travels through circulation to distant lymph nodes and organs. The entire process is the incubation period, which can last up to 21 days and normally lasts seven to fourteen days (Moore and Zahra, 2022). Monkeypox does not have clinical manifestation during the incubation stage; hence this phase is not communicable.

The prodromal stage can be associated to monkeypox symptoms and clinical manifestation. Secondary viremia spreads from the lymphoid organs to the skin and tertiary organs including the lungs, eyes, gastrointestinal tract, etc, during the prodromal stage. An individual is thought to be the most contagious during the prodromal stage. This is mostly because of symptoms including lymphadenopathy and mucocutaneous lesions, among other non-specific symptoms (Moore and Zahra, 2022).

The mpox virus replicates at the site of inoculation after viral entrance by any route (oropharynx, nasopharynx, or intradermal), and subsequently spreads to nearby lymph nodes. Following an initial viremia, the virus spreads and seeds additional organs. This is the incubation period, which can last up to 21 days and normally lasts 7 to 14 days.

One to two days before lesions may be noticed, prodromal symptoms can include chills, lethargy, lymphadenopathy, headache, myalgia, backache, and fever brought on by secondary viremia. A rash then follows, frequently starting on the face before spreading to other parts of the body. Before becoming a scab and subsequently healing, this rash develops and goes through several stages. Infected patients may be contagious at this time. The oropharynx is where skin lesions start to form. The virus damages and inflames the epidermis, respiratory system, and other organs as it keeps replicating. Monkeypox can cause pneumonia, encephalitis, or septicemia in extreme instances, all of which can be fatal (Soheili *et al*., 2022). When lesions start, serum antibodies are frequently detectable (Hutson *et al*., 2015).

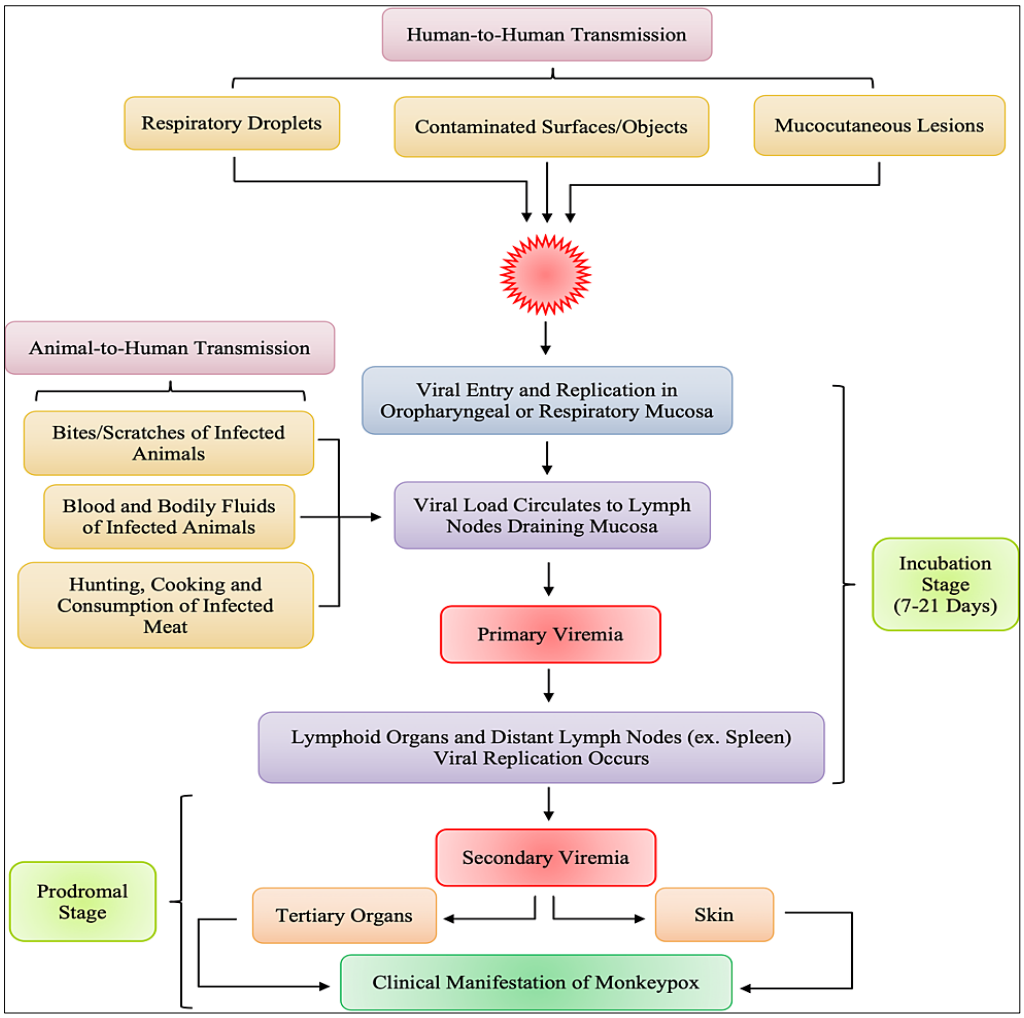

Fig. 2: Proposed Pathogenesis of Monkeypox (Kaler *et al.*, 2022)

2.6 Natural Reservoir of Monkeypox Virus

The monkeypox virus (together with other Orthopoxviruses) can infect a variety of animal hosts and can transmit to people, in contrast to the variola virus, which only infects humans (Kmiec and Kirchhoff, 2022). Three virus-positive African species, including rope squirrels (Funisciuris species), giant pouched rats, and dormice, were linked to the importation of monkeypox into the USA in 2003. Serological studies revealed that a variety of wild animals, including rodents (mice, rabbits, squirrels, hamsters, and groundhogs), anteaters, prairie dogs, southern opossums, marmosets, and hedgehogs, can become infected under natural circumstances. The natural animal reservoir is still unknown, despite the fact that several animals have been identified as being prone to the monkeypox virus (Silva *et al.*, 2020).

2.7 Transmission

A human can contract the monkeypox virus through coming into contact with an infected person or animal, or by coming into contact with contaminated objects (CDC, 2022). A total of 54 documented occurrences of human monkeypox between 1970 and 1979 suggested that the monkeypox virus still exists in animals and that humans are merely incidental hosts (Alakunle *et al.*, 2020). Animals can transmit diseases to humans through damaged skin, respiratory droplets, or mucosal membranes (CDC, 2022). The intake of undercooked meat and other animal products coming from diseased animals is a potential risk factor. Prior to the epidemic in 2022, interaction with animals was thought to be the primary method of animal-to-human transmission for human monkeypox illnesses (CDC, 2022).

Human monkeypox outbreaks with the index case appearing to have been transmitted by an animal were detected in some endemic nations, yet the transmission persisted for up to a generation. According to the CDC, the main ways that human-to-human monkeypox transmission happens are through direct contact with an infected person's skin lesions or body fluids (such as an infectious rash, sores, or scabs), through respiratory droplets (during prolonged and close face-to-face contact), or through contact with recently contaminated surfaces, objects, or fabrics (such as bedding, clothing, or towels).

Hugs, massages, kisses, prolonged face-to-face contact, and touching fabrics and objects during sex that

a person infected with monkeypox used and which were not disinfected (such as bedding, towels, sex toys, and fetish gear) are all examples of direct contact. Intimate contact is defined as oral, anal, and vaginal sex as well as touching the genitals or anus of an infected person. Healthcare workers, family members, and other close contacts of the monkeypox case are more at risk of infection due to respiratory droplet transmission (Atkinson *et al*., 2022). There are few facts about monkeypox infection transfer from mother to child during pregnancy and lactation (CDC, 2022). Neonatal monkeypox infection, miscarriage, stillbirth, and premature delivery are among the unfavorable effects of monkeypox virus transfer from mother to child (CDC, 2022).

In the 2017 human monkeypox outbreak in Nigeria, a majority of cases were young adult males presenting with genital ulcers, and among them, some cases had concomitant syphilis and HIV infection (Ogoina *et al*., 2019). However, sexual transmission of human monkeypox is not established, and the role that genital secretions might have in transmission requires further research (CDC, 2022). Animal-to-human (zoonotic) transmission can occur from direct contact with the blood, bodily fluids, or cutaneous or mucosal lesions of infected animals. In Africa, evidence of monkeypox virus infection has been found in many animals including rope squirrels, tree squirrels, Gambian pouched rats, dormice, different species of monkeys and others.

Although rodents are most likely, the natural monkeypox reservoir has not yet been found. A potential risk factor is consuming undercooked meat and other animal products from infected animals. Living in or close to forests can expose people to diseased animals indirectly or at a low level. Infected skin lesions, respiratory secretions, or recently contaminated objects can all cause human-to-human transmission (CDC, 2022). Health professionals, household members, and other close contacts of active cases are more at risk because droplet respiratory particle transmission typically necessitates lengthy face-to-face contact. However, in recent years, the number of person-to-person infections in a community's longest known chain of transmission has increased from 6 to 9.

The absence of smallpox vaccination may be the cause of the diminishing immunity seen in all cultures. Congenital monkeypox may result from transmission through the placenta from the mother to the fetus, as well as from intimate contact during labor and after delivery. Despite the fact that close physical contact is a known risk factor for transmission, it is currently unknown whether or not monkeypox can particularly spread through sexual contact. The CDC (2022) states that more research is required to fully comprehend this risk.

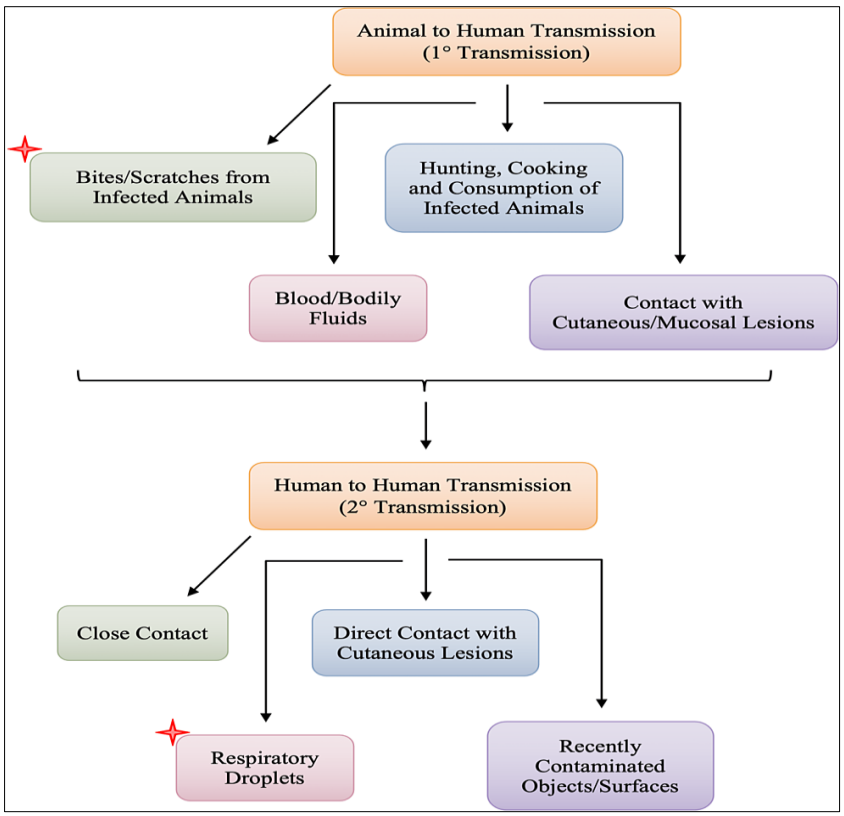

Fig. 3: Suspected Modes of Transmission of Monkeypox to Humans (Kaler *et al.*, 2022)

2.8 Risk factors

The following have been identified as risk factors for monkey pox;

i. Age
ii. Nosocomial infection
iii. Zoonotic infection

Direct contact with the blood, body fluids, or cutaneous or mucosal lesions of infected animals can result in animal-to-human (zoonotic) transmission (NCDC, 2022). Numerous animals in Africa have shown signs of infection with the monkeypox virus, including rope (Yinka-Ogunleye, Aruna, Dalhat, Ogoina and McCollum, 2019) and tree squirrels, Gambian robber rats, dormice, various kinds of monkeys, and others. Rodents are the most plausible candidates for the monkeypox natural reservoir, though this has not yet been determined. A potential risk factor is consuming undercooked meat and other animal products from infected animals (Petersen *et al*., 2019). People who live in or close to forests may be indirectly or minimally exposed to diseased animals.

Human to Human Transmission

Close contact with respiratory secretions, skin sores on an infected person, or recently contaminated objects can cause human-to-human transmission. Health care providers, family members, and other close contacts of active patients are more at risk because droplet respiratory particle transmission typically necessitates extended face-to-face contact (Nguete *et al*., 2018). The number of person-to-person infections in a community's longest documented chain of transmission has increased from 6 to 9 in recent years. This might be an indication of a general decline in immunity brought on by the end of smallpox vaccination campaigns.

History of Travel to Endemic Countries or Regions

The Orthopoxvirus genus of the Podxviriae family includes the enveloped double-stranded DNA virus known as the monkeypox virus (Guagliardo *et al*., 2020). The central African (Congo Basin) clade and the West African clade are two separate genetic clades of the monkeypox virus. According to Guagliardo *et al*., (2020), the Congo Basin clade has historically been associated with more severe disease and was assumed to be more transmissible. The sole nation where both virus clades have been discovered, Cameroon, serves as the geographic dividing line between the two clades thus far (NCDC, 2022).

Cameroon, the Central African Republic, the Democratic Republic of the Congo, Gabon, Ghana (found only in animals), Cote d'Ivoire, Liberia, Nigeria, the Republic of the Congo, and Sierra Leone are some of the nations where monkey pox is endemic. There have been recorded importations into South Sudan and Benin in the past. Nigeria and Cameroon are the nations currently reporting cases of the West African clade (WHO, 2022).

2.9 Complications Associated with Monkey Pox

Patients with monkeypox may experience severe and perhaps life-threatening effects, although this is rare. For instance, bacterial skin and soft tissue infections might spread from the skin sores. The sores may be highly irritating, and if scratching causes a secondary bacterial infection, careful local wound care and, in some circumstances, antimicrobial medication, may be necessary. Additionally, lesions in the eye or oral mucosa may be present (WHO, 2022). Secondary skin bacterial infections, dehydration, conjunctivitis, keratitis, pneumonia, sepsis or septic shock, and in rare instances, encephalitis and death are among the complications in endemic countries. As a result, clinical care should, as and when necessary, concentrate on the management of clinical syndrome, preservation of nutritional and hydration status, and prevention of complications and sequelae.

The majority of patients experience a prodromal headache, usually a generalized or frontal headache, which is the most typical neurological presentation. Myalgias and asthenia are two additional typical prodromal symptoms. Additionally, mental disorders and neuralgia may appear. Conjunctivitis can occur in some people, and corneal lesions can result in scarring and vision loss (Ardler, Gould and Hine, 2022). Encephalitis with seizures might sporadically happen. In immunocompromised people, viral neuroinvasiveness can be of special concern.

2.10 Signs and Symptoms

Monkeypox typically takes 6 to 13 days to incubate, although it can take anything from 5 to 21 days for symptoms to appear.

There are two phases to the infection:

1. The 0–5-day invasion phase, which is marked by fever, severe headache, lymphadenopathy (lymph node swelling), back pain, myalgia (muscle aches), and severe asthenia (lack of energy). In contrast to other illnesses (chickenpox, measles, and smallpox) that may initially seem similar, monkeypox is distinguished by lymphadenopathy.
2. The skin eruption often starts one to three days after the onset of the fever. Instead of the trunk, the rash is more frequently found on the face and limbs. In 95% of cases, it affects the face, and in 75% of cases, it affects the palms of the hands and the bottoms of the feet. The eye and oral mucous membranes are also impacted (in 70% of instances), along with the genitalia (30%), conjunctivae (20%), and genitalia (WHO, 2022). The progression of the rash goes from macules (flat, firm lesions) to papules (slightly raised, firm lesions), vesicles (clear fluid-filled lesions), pustules (yellowish fluid-filled lesions), and crusts that dry up and break

off. Lesions can range in number from a few to several thousand.

The symptoms of monkeypox typically persist between two and four weeks and are self-limited. Children are more likely to experience severe cases, which are correlated with the level of patient health, the severity of the viral exposure, and the nature of complications. Poorer results could result from underlying immunological weaknesses. Although smallpox immunization provided protection in the past, currently those under the age of 40 to 50 (depending on the country) may be more susceptible to monkeypox due to the cessation of smallpox vaccine campaigns worldwide after the disease's eradication (WHO, 2022).

Complications of monkeypox can include secondary infections, bronchopneumonia, sepsis, encephalitis, and infection of the cornea with ensuing loss of vision. The extent to which asymptomatic infection may occur is unknown. The case fatality ratio of monkeypox has historically ranged from 0 to 11 % in the general population and has been higher among young children. In recent times, the case fatality ratio has been around 3–6% (WHO, 2022). A person with mpox can spread it to others from the time symptoms start until the rash has fully healed and a fresh layer of skin has formed. Some people have been found to have infection but no symptoms (CDC, 2022).

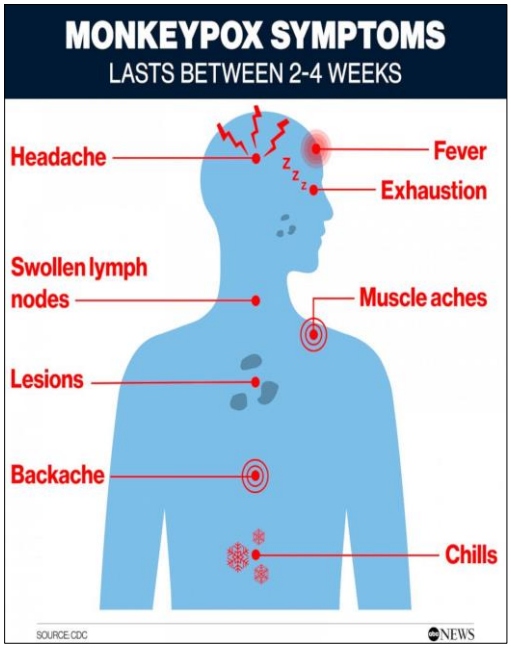

Fig. 4: Diagrammatic representation of signs and symptoms associated with monkey pox and regions of the body affected (CDC, 2022)

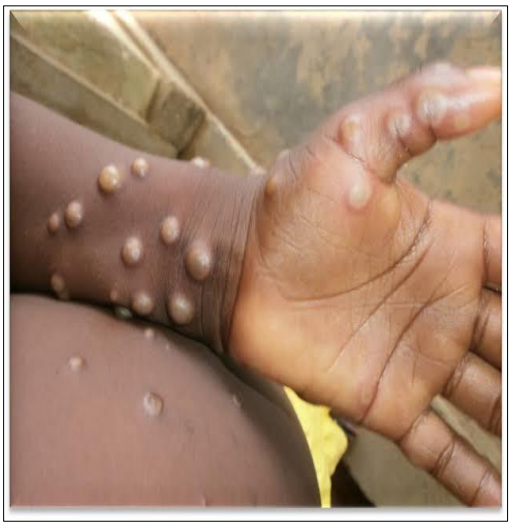

Fig. 5: Typical rashes seen in monkey pox (WHO, 2022)

2.11 Host Immunological Response in Mpox Viral Disease

The MPXV-mediated immune injury leads to poor clinical outcomes in patients with monkeypox. MPXV infection not only causes skin lesions with the genital rashes as emerging clinical symptoms but also triggers a strong immune response that results in sepsis, deep tissue abscess, and severe respiratory disease (Adler *et al.*, 2022). Besides, MPXV infection also causes damage to multiple immune organs, including diffuse myeloid hyperplasia, thymitis, tonsillitis, splenic injury, and lymphadenopathy.

Monkeypox virus (MPXV) might enter the body via the respiratory (panel a) or skin (panel b) route. In the respiratory tract, the virus can infect airway epithelial cells such as ciliated cells. Antigen-presenting cells such as dendritic cells and macrophages are also susceptible to MPXV infection. Upon inoculation in the skin, the virus infects keratinocytes and fibroblasts. Skin-resident immune cells such as Langerhans cells, dendritic cells and macrophages are also targeted. In both scenarios (panels a and b), it is hypothesized that infected antigen-presenting cells travel to nearby draining lymph nodes and facilitate its spread through the lymphatic system (panel c). Direct viral access to the lymphatics has been also speculated. A common feature of human monkeypox is swelling of lymph nodes (lymphadenopathy). The abnormal proliferation and retention of natural killer cells might be one of the causes. Following its spread through lymphoid tissue, MPXV may target other large organs such as the spleen and liver (panel d). Of note, MPXV antigens have been previously been detected in both hepatocytes and Kupffer cells in non-human primate (NHP) models. The viraemia wave could then allow the virus to further spread to distant organs such as the skin and gonads. Recently, MPXV was isolated from semen of infected individuals, highlighting the possibility of sexual transmission (panel e). The infection of skin and mucosae leads to the appearance of infective pustules (panel f) and ulcers (panel g). The latter release high quantities of virus into the saliva, which potentially leads to aerosolized transmission of MPXV (panel h). See figure 6.

Like other viruses, the genus of *Orthopoxvirus* has developed various mechanisms to evade the host's defense systems. This ability facilitates the entry of the virus without being detected or recognized by the systems. Some of those mechanisms are described briefly below.

It has been demonstrated that orthopoxviruses can disturb the pattern recognition receptors (PRRs) expressed by the innate immune cells. These proteins consist of several subfamilies, including the Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-1-like receptors (RLR), and C-type lectin receptors (CLRs). They are responsible for recognizing various microbe-related molecules or molecules released by impaired cells (Amarante-Mendes *et al*., 2018). Once the PRRs bind to the microbial ligands, the subsequent cascades occur, including activation of inflammation-related transcription factors such as nuclear factor kappa B (NF-κB), interferon regulatory factors (IRFs), and activating protein-1 (AP-1) (Shchelkunov, 2012). It has been known that signal transduction of TLRs involves several types of intracellular adaptors proteins such as MyD88, MAL, TRAM, TRIF, and SARM, which are pivotal for triggering intracellular immunologic reactions (Amarante-Mendes *et al*., 2018). Any disruption in those adaptor proteins may cause problems in exerting adequate immunologic response towards viral infections. At this point, orthopoxviruses contain genes encoding proteins that could interact and damage the functionalities of those adaptor proteins. For example, MPXV could produce a protein called A47R, which can interact with MyD88, TRIF, and TRAM (Shchelkunov, 2012). Consequently, these adaptor proteins' physiological functions are disturbed, followed by the inhibition of the transcription factors associated with inflammation, i.e., NF-κB (Shchelkunov, 2012). Ultimately, this condition leads to the failure of the innate immune systems to recognize the viruses.

As a member of pattern recognition receptors (PRRs), TLR plays a critical role in recognizing various noxious molecules. Upon its interaction with those molecules, the cytoplasmic domain (TIR) of TLR recruits the appropriate adaptor proteins (e.g., MyD88, TRIF, or TRAM). This interaction induces the subsequent molecular pathways that will eventually upregulate the expression of NF-κB, which is critical in modulating the innate immune system. It has been found that this mechanism can be impaired by the action of a MPXV protein called A47R. This viral protein can interact with the adaptor proteins leading to the impairment of viral recognition by the immune system. TLR (Toll-like receptor); TIR (Toll/interleukin-1 receptor); MyD88 (myeloid differentiation primary-response gene 88); TRIF (TIR-domain-containing adaptor protein inducing IFNβ); TRAM (TRIF-related adaptor molecule); NF-κB (nuclear factor kappa B). The figure was created by Biorender.

The development of viral proteins showing properties as apoptosis inhibitors becomes another strategy utilized by orthopoxviruses, including MPXV, to evade the host's defense systems (Shchelkunov, 2012). Apoptosis is a common and essential mechanism found in multicellular organisms to prevent viral proliferation and diminish the spread of infection to the other cells by killing the infected cells. As mentioned above, the ability of MPXV to inhibit NF-κB activity results in the failure of the immune system to recognize the virus. It has been clearly documented that NF-κB also plays a fundamental role in regulating apoptosis (Albensi, 2019).

In addition to the disruption of NF-κB regulation, other specific mechanisms are also proposed to explain the ability of *Orthopoxviruses* to inhibit cellular apoptosis. It has been reported that orthopoxviruses, including MPXV, might hinder the activities of caspase-1, caspase-8, and caspase-9, which are essential in executing apoptosis (Shchelkunov, 2012). In MPXV strain Zaire-I-96, this inhibitory action might be mediated by several viral proteins (e.g., B12R and C7L) (Shchelkunov, 2012).

Furthermore, like other orthopoxviruses, MPXV also has a gene that encodes protein mimicking activity of Bcl-2 proteins, which have been known to play a critical role in regulating apoptosis (Shchelkunov, 2012). The viral protein P1L has been revealed to have activity similar to B-cell lymphoma-2 (Bcl-2)-like proteins in MPXV strain Zaire-I-96 (Shchelkunov, 2012). Molecularly, this viral protein interacts with the IκB kinase (IKK) complex, which is vital for facilitating the activation of NF-κB (Shchelkunov, 2012). This action also leads to the failure to induce cellular apoptosis. It has also been demonstrated that MPVX and other orthopoxviruses could produce proteins acting as an inhibitor of interferon, which is pivotal in tackling viral infection. This activity is mediated by the ability of MPVX to block the production of interferon regulatory factors (IFRs), which are known as the initial cascade in interferon production (Shchelkunov, 2012).

In addition to the mechanisms mentioned above, orthopoxviruses have other multiple genes encoding proteins used to perturb various stages of the host's inflammatory cascade. They could disturb the production of cytokines and chemokines, the activity of the complement system, the activity of the ubiquitin–proteasome pathway, and several other targets (Goetzke *et al*., 2021).

Following its success in avoiding the host's immune system, MPXV is capable of attacking many sites within the human body. In this case, clinical manifestations of monkeypox are remarkably similar to those of smallpox. However, although these infectious diseases share many uniformities in their signs and symptoms, several manifestations are used to differentiate smallpox and monkeypox. For example, lymphadenopathy is closely associated with monkeypox but is not a characteristic of smallpox (McCollum and Damon, 2014). The enlargement could occur in lymph nodes located at various sites. Nevertheless, the nodes in submandibular, cervical, or inguinal areas seem to be the primary sites of MPXV-related lymphadenopathy (Weinstein *et al*., 2005).

The enlargement of lymph nodes may indicate that the immune response activated by the host following MPXV infection is more effective than the infection caused by the other orthopoxviruses (McCollum and Damon, 2014). To date, no clear explanation for this phenomenon has been reported. However, this might be caused by different viral proteins produced among the orthopoxviruses, and this could be seen in the case of a viral protein called the vaccinia complement control protein (VCP) produced by orthopoxviruses (Shchelkunov, 2012).

The VCP consists of four short consensus repeats (SCRs); each consists of approximately 60 amino acids, resembling a regulator of complement activation. VCP can bind to several complement components (e.g., C3b and C4b), followed by disturbance of the subsequent complement cascades (Shchelkunov, 2012). Ultimately, VCP suppresses inflammatory response (Miller, Shchelkunov and Kotwal, 1997).

The intact structure of VCP protein is found in other orthopoxviruses (viruses of variola, cowpox, and vaccinia). In contrast, in MPXV, the structure of VCP is either truncated (Clade 1 or Congo Basin/Central Africa Clade) or deleted (Clade 2 or Western African Clade) (Shchelkunov, 2012). The unique structure of VCP in MPXV causes the functional activity of VCP in repressing the host's inflammatory response to be inadequate. Consequently, in MPXV infection, the immune response generated by the host is more intense, and this possibly causes the event of lymphadenitis (Shchelkunov, 2012).

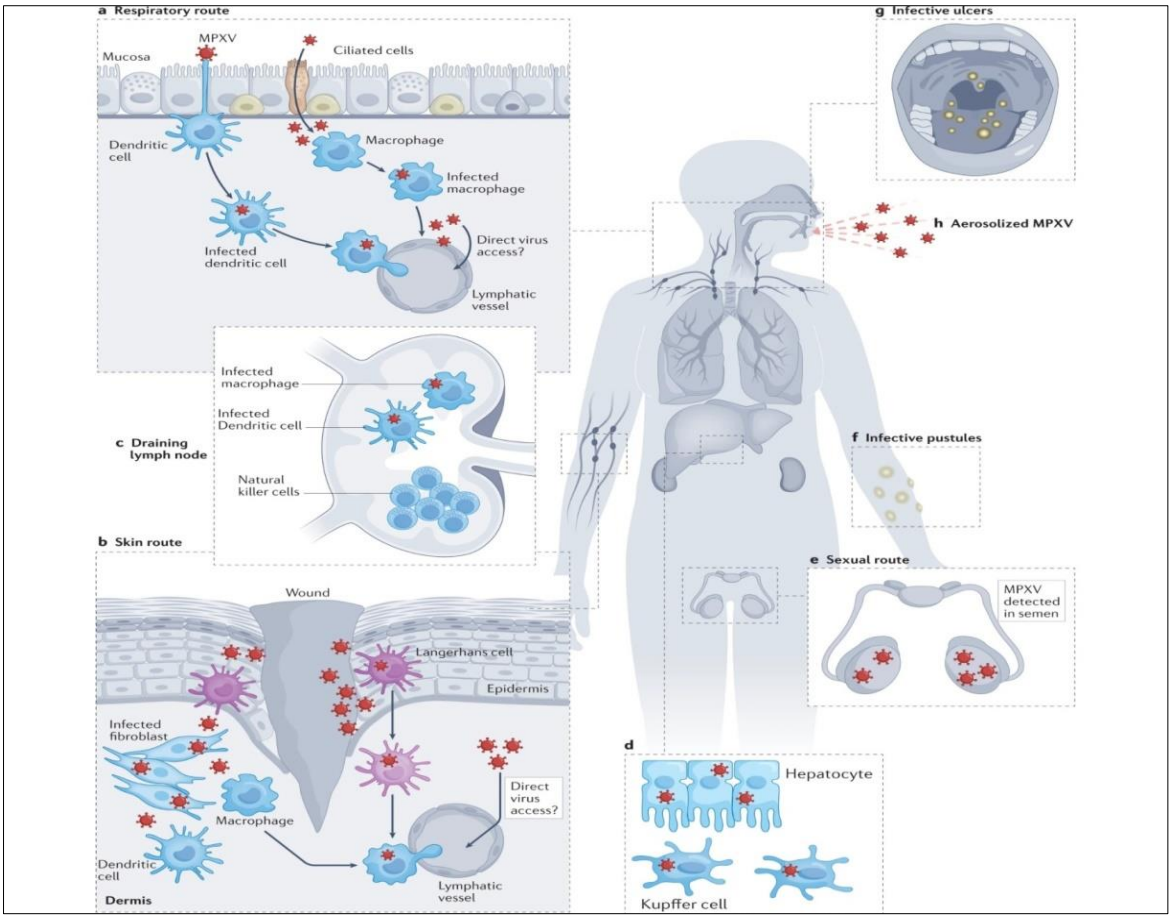

Fig. 6: Immunopathogenesis of human monkeypox (Lum *et al.*, 2022)

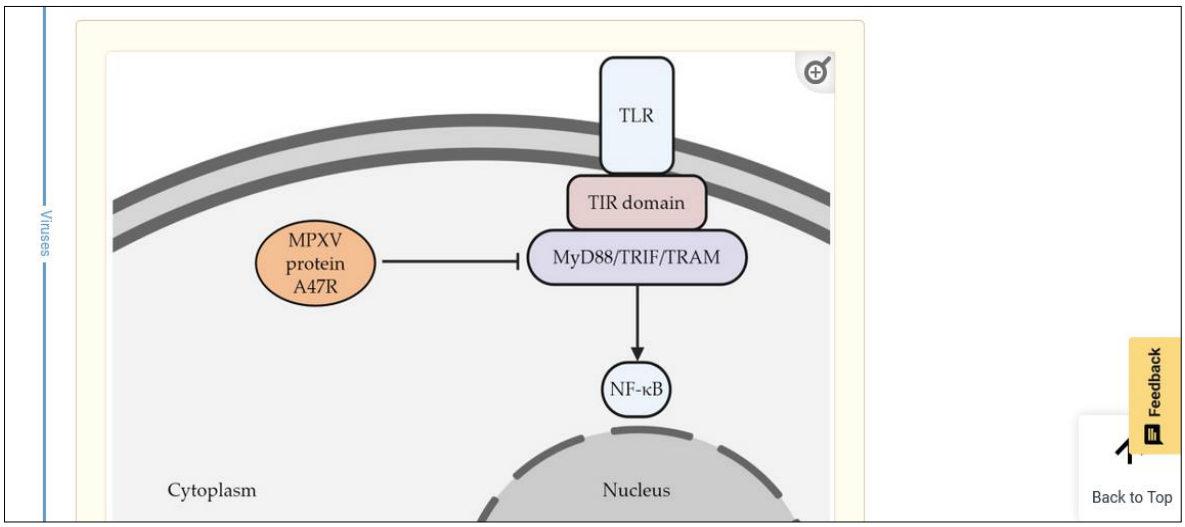

Fig. 7: The figure was created by Biorender, as referenced by Harapan *et al*. (2022)

As a member of pattern recognition receptors (PRRs), TLR plays a critical role in recognizing various noxious molecules. Upon its interaction with those molecules, the cytoplasmic domain (TIR) of TLR recruits the appropriate adaptor proteins (e.g., MyD88, TRIF, or TRAM). This interaction induces the subsequent molecular pathways that will eventually upregulate the expression of NF-κB, which is critical in modulating the innate immune system. It has been found that this mechanism can be impaired by the action of a MPXV protein called A47R. This viral protein can interact with the adaptor proteins leading to the impairment of viral recognition by the immune system. TLR (Toll-like receptor); TIR (Toll/interleukin-1 receptor); MyD88 (myeloid differentiation primary-response gene 88); TRIF (TIR-domain-containing adaptor protein inducing IFNβ); TRAM (TRIF-related adaptor molecule); NF-κB (nuclear factor kappa B).

2.12 Diagnosis of Monkey Pox

2.12.1 Clinical Diagnosis

The incubation period for monkeypox is 8 days (4-14 days), and symptoms last for two to four weeks (Cheema *et al*., 2022). With a viral febrile prodromal phase marked by headache, malaise, backache, exhaustion, lethargy, and low-grade fever, the earliest signs and symptoms are typically non-specific. A vesiculopustular rash then develops 12–16 days after exposure and starts on the face and trunk before centrifugally spreading to other body areas, such as the palms and soles. Rash develops morphologically in stages, including macular, papular, vesicular, and pustular lesions.

The pustules eventually develop crusts, which after one to two weeks desquamate (Cheema *et al*., 2022). The initial signs and symptoms of MPXV infection resemble smallpox, however unlike smallpox, lymphadenopathy is a significant hallmark, with sensitive maxillary, cervical, and inguinal lymphadenopathy (1-4 cm), found in 84% of unvaccinated individuals, and 54% of patients who had received vaccinations. The presence of lymphadenopathy suggests that MPXV may be more strongly recognized by the immune system than variola.

Patients with immunosuppressed states, prolonged viral particle exposure, and the development of comorbidities such bronchopneumonia, encephalitis, and vision loss owing to corneal infection typically have worse clinical outcomes. Hypo-hyperpigmentation, scarring, dehydration (caused by nausea and vomiting), and a subsequent bacterial infection that results in septicemia are some additional risks (Cheema *et al*., 2022).

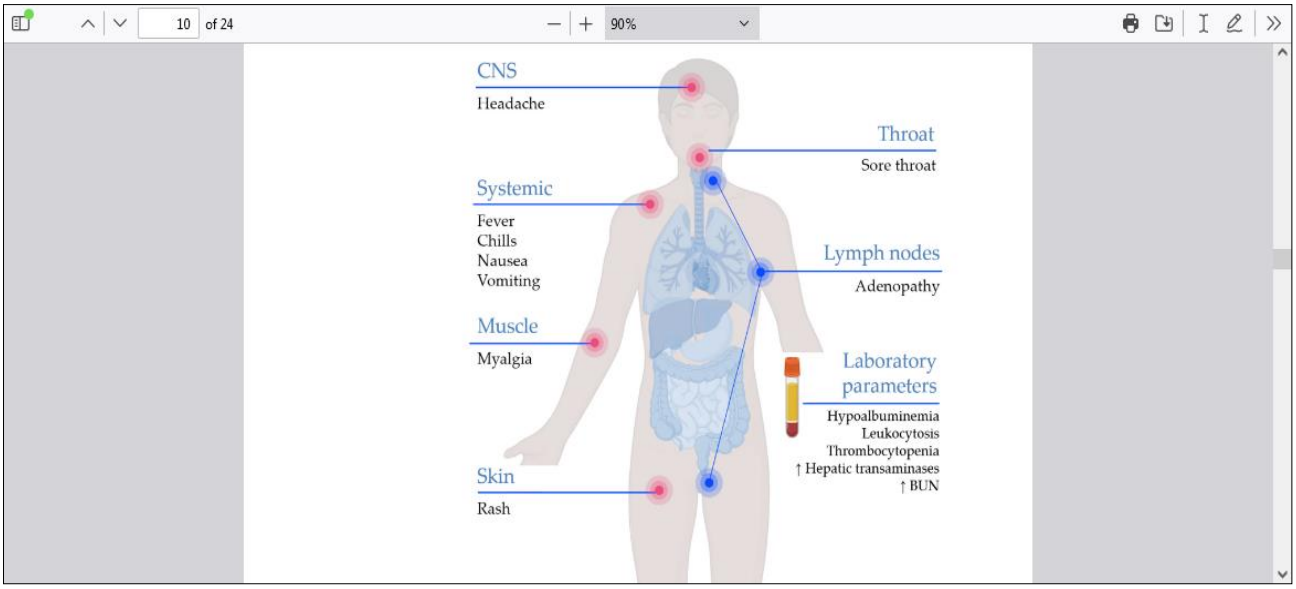

Fig. 8: The clinical and laboratory features of monkeypox. BUN: blood urea nitrogen. The figure was created by Biorender, as referenced by Harapan *et al*., (2022)

2.12.2 Laboratory Diagnosis of Monkey pox

An individual with the aforementioned symptoms may have monkeypox, especially if they have traveled or had contact with people who have the disease.

There are several laboratory techniques used to identify monkeypox, including:

i. Polymerase chain reaction (PCR) method
ii. Viral culture
iii. Electron microscopy
iv. Antibody testing (IgG and IgM)

The polymerase chain reaction (PCR) test can confirm a suspected case of monkeypox (Cheema *et al*., 2022). For diagnosing monkey pox, PCR is considered the gold standard.

1. PCR Technique in the Diagnosis of Monkey Pox

The preferred laboratory test is the polymerase chain reaction (PCR), as recommended by the WHO due to its accuracy and sensitivity. Using real-time or traditional polymerase chain reaction (PCR), such as that provided by the monkeypox detection kit, is favored for detecting specific viral DNA sequences and can be used alone to confirm PCR results for monkeypox virus infection. The amplification of nucleotide sequences is made possible by the polymerase chain reaction (PCR), a method that has revolutionized molecular biology and other fields. As a result, the PCR approach can both qualitatively and quantitatively detect the mpox virus.

Background on PCR

Kary Mullis, a biochemist from the United States, had an inspiration in 1983 while late-night traveling home. He penned the concept that ultimately earned him the 1993 Nobel Prize for Chemistry on the

back of a receipt. Simple reproduction of the cell's DNA replication mechanism in a test tube was the idea at hand. The end result is the same: the creation of new complementary DNA (cDNA) strands based on the ones that already exist. Sanger's DNA sequencing methodology served as the foundation for Mullis' innovative method. He understood that DNA polymerase's repeated application set off a series of events that led to the amplification of a particular DNA segment (Ferrini, 2022).

His theory was founded on the 1976 isolation of Taq, a thermostable DNA polymerase from the bacteria Thermus aquaticus that was found in hot springs. Taq DNA polymerase can withstand several denaturation cycles since it has a temperature optimum of 72 °C and can endure lengthy exposure to temperatures as high as 96 °C (Ferrini, 2022). Molecular biologists were striving to perfect cyclic DNA amplification procedures prior to the discovery of Taq polymerase, but they had to add new polymerase at each cycle since the enzyme could not resist the high temperatures required for DNA denaturation. The use of a thermostable enzyme allowed them to perform multiple cycles of amplification without needing new polymerase, which made the entire process more efficient and less time consuming.

Science published the first explanation of this Taq polymerase-based polymerase chain reaction (PCR) in 1985. The first PCR kit to receive FDA approval hit the market in 1993. PCR has since seen consistent, methodical improvement. It has changed the game in a variety of fields, including illness monitoring, genetic engineering, and the examination and diagnosis of forensic evidence. Unquestionably, it is regarded as one of the most significant scientific breakthroughs of the 20th century (Ferrini, 2022).

Principle of PCR

PCR makes it possible to obtain, by in vitro replication, multiple copies of a DNA fragment from an extract. Matrix DNA can be genomic DNA as well as complementary DNA obtained by RT-PCR from a messenger RNA extract (poly-A RNA), or even mitochondrial DNA. It is a technique for obtaining large amounts of a specific DNA sequence from a DNA sample (Kadri, 2019). This amplification is based on the replication of a double-stranded DNA template. It is broken down into three phases: a denaturation phase, a hybridization phase with primers, and an elongation phase. The products of each synthesis step serve as a template for the following steps, thus exponential amplification is achieved (Kadri, 2019).

The primers, Taq polymerase, template DNA, four excess deoxyribonucleoside triphosphates (dNTPs), and DNA extract are all combined in a reaction mixture to perform the polymerase chain reaction (Kadri, 2019). In the heating block of a thermal cycler (an apparatus with an enclosure where the sample tubes are deposited and in which the temperature can vary, very quickly and precisely, from 0 to 100°C by Peltier effect), the tubes containing the mixture reaction are repeatedly subjected to temperature cycles several tens of times. The device enables programming of the number of cycles between temperature steps as well as their duration. Three phases lasting a few tens of seconds each make up a cycle.

Use of PCR in Disease Diagnosis

The PCR is an excellent diagnostic tool. Genetic illness detection already makes extensive use of it. It is feasible to identify the detrimental mutation(s), their locations, sizes, and natures by amplifying all or a portion of the gene that causes a genetic condition. As a result, deletions, inversions, insertions, and even point mutations can be found using either PCR alone or in combination with other methods (Kadri, 2019). However, PCR can still be used to identify infectious disorders (viral, bacterial, parasitic, etc.), as it is already done for infections like chlamydia, hepatitis C, and AIDS.

PCR has the great benefit of delivering extremely accurate and quick results from minute biological samples, where the presence of the pathogen is not always apparent with other techniques (Kadri, 2019), despite the fact that other diagnostic instruments are effective at detecting certain diseases.

Standard PCR Experiment Overview

A specific DNA segment is amplified using the PCR method from a complex mixture of starting material known as template DNA. The starting material, including the sample matrix and accessibility of target DNA, affects the sample preparation and purification methods. Most of the time, just minimum DNA purification is necessary, and some procedures, such direct PCR or extraction-free PCR, do not even need to purify their DNA or RNA first. Nevertheless, for PCR to function, the DNA sequence information bordering the DNA fragment to be amplified (also referred to as target DNA) must be known (Ferrini, 2022). Practically speaking, a PCR experiment is simple to do and can be finished in a few hours. A PCR reaction typically requires five essential reagents:

(a) **DNA to be amplified:** Also known as template DNA or PCR template. This DNA may come from any source, including plasmid DNA, cDNA, and genomic DNA (gDNA).

(b) **DNA polymerase**: A DNA polymerase capable of operating at high temperatures is necessary for all PCR operations. Commonly used Taq polymerase can amplify templates up to 5 kb and integrate nucleotides at a rate of 60 bases per second at 70 °C, making it appropriate for ordinary PCR without the need for specific conditions. Polymerases are being developed in new generations to enhance reaction efficiency. To reduce non-specific amplification at the start of the reaction, some, for instance, are designed

to only activate at high temperatures. Others include a "proofreading" feature, which is crucial, for instance, when it's essential that the amplified sequence exactly matches the template sequence, as it is when cloning (Ferrini, 2022).

(c) **Primers:** To signal where to begin amplification, DNA polymerases need a short sequence of nucleotides. These small segments of single-stranded DNA, or primers, are used in PCRs and have a length of roughly 15 to 30 bases. A pair of primers, one on the forward strand and one on the reverse, is precisely designed to flank the target area of DNA when constructing a PCR experiment. The researcher first identifies the DNA region to be amplified. A crucial step in any PCR experiment is the design of the primers, which must be done with care. The unique DNA of interest must be the target of the primer sequences, which must be chosen to prevent binding to a comparable sequence (Ferrini, 2022). Given that the annealing process occurs simultaneously for both strands, they ought to have comparable melting points. The proportion of bases that are guanine (G) or cytosine (C) compared to adenine (A) or thymine (T) might affect a primer's melting temperature, with higher GC contents raising melting temperatures. In order to match a primer pair, this might be taken into account while adjusting primer lengths. Additionally, because they will decrease PCR performance, sequences with a propensity to create secondary structures or primer dimers should be avoided. Primer creation can be aided by a variety of free internet tools (Ferrini, 2022).

(d) **Deoxynucleotide triphosphates (dNTPs):** Deoxynucleotide triphosphates (dNTPs) are the four fundamental DNA nucleotides (dATP, dCTP, dGTP, and dTTP) that are used as building blocks for new DNA strands. For the best base incorporation, dNTPs are often given to the PCR reaction in equimolar quantities (Ferrini, 2022).

(e) **PCR buffer**: The PCR buffer makes sure that ideal conditions are kept throughout the PCR reaction. Magnesium chloride (MgCl2), tris-HCl, and potassium chloride (KCl) make up the bulk of PCR buffers. Tris-HCl and KCl help to keep the reaction's pH steady while MgCl2 acts as a cofactor for the DNA polymerase. The reagents stated above are mixed, and the tube is then subjected to a heat cycle to perform the PCR reaction (Ferrini, 2022).

The denaturation, annealing, and extension steps of the PCR amplification are shown in the figure below as three distinct sets of periods and temperatures collectively known as steps.

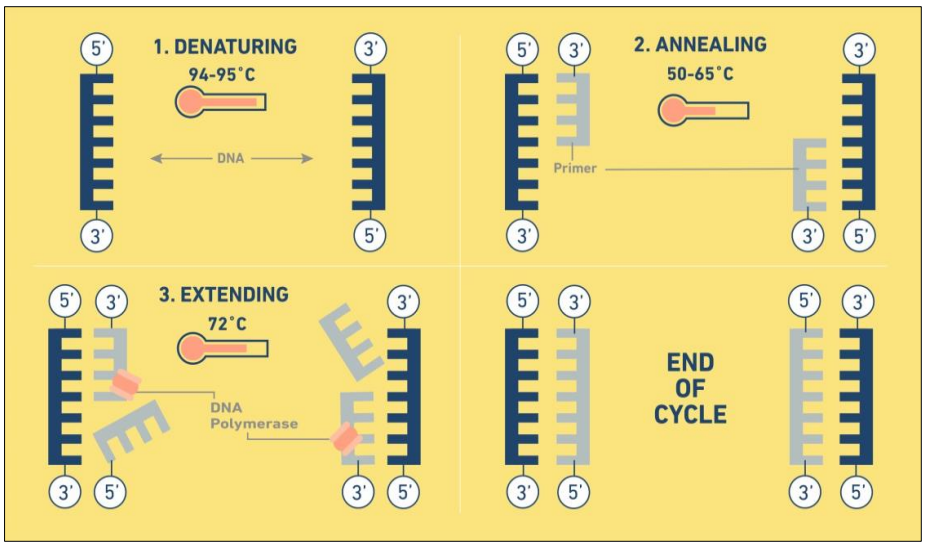

Fig. 9: Steps of a single PCR cycle (Ferrini, 2022)

Each of these steps, termed cycles, is repeated 30-40 times, doubling the amount of DNA at each cycle and obtaining amplification as illustrated in the figure shown below.

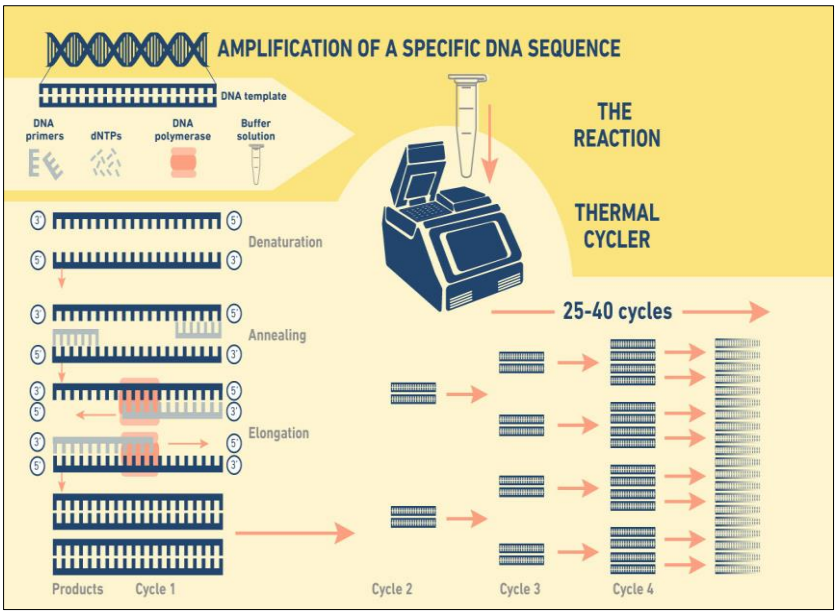

Fig. 10: The different stages and cycles of DNA molecule amplification by PCR (Ferrini, 2022).

Steps in a PCR Cycle

1. Denaturation:

Denaturation, the initial step of PCR, involves rapidly breaking the hydrogen bonds between the two DNA strands while heating the template DNA to 95 °C for a brief period of time (Ferrini, 2022).

2. Annealing

The reaction mixture is then cooled for 30 seconds to 1 minute. Annealing temperatures are usually 50 - 65 °C however, the exact optimal temperature depends on the primers' length and sequence. It must be carefully optimized with every new set of primers. The two DNA strands could rejoin at this temperature, but most do not because the mixture contains a large excess of primers that bind, or anneal, to the template DNA at specific, complementary positions. Once the annealing step is completed, hydrogen bonds will form between the template DNA and the primers. At this point, the polymerase is ready to extend the DNA sequence (Ferrini, 2022).

3. Extension

The temperature is then raised to 72 °C, or 74 °C in the case of Taq, which is the recommended working temperature for the DNA polymerase that is present in the mixture. Each primer is joined at one end by the DNA polymerase, which then creates new DNA strands that are complementary to the template DNA. Instead of the two DNA strands that were initially present, we now have four (Ferrini, 2022). When the temperature is increased to 94 °C, both the "original" and newly produced double-stranded DNA molecules denature once more into single strands. The second cycle of denaturation-annealing-extension starts at this point.

At the end of this second cycle, there are eight molecules of single-stranded DNA. By repeating the cycle 30 times, the double-stranded DNA molecules present at the beginning are converted into over 130 million new double-stranded molecules, each one a copy of the region of the starting molecule delineated by the annealing sites of the two primers (Ferrini, 2022).

To determine if amplification has been successful, PCR products may be visualized using gel electrophoresis, indicating amplicon presence/absence, size and approximate abundance. Depending on the application and the research question, this may be the endpoint of an experiment, for example, if determining whether a gene is present or not. Otherwise, the PCR product may just be the starting point for more complex downstream investigations such as sequencing and cloning (Ferrini, 2022).

Nature of Specimen for Diagnosis of Mpox Using PCR

The recommended specimen type for laboratory confirmation of monkeypox is skin lesion material, including swabs of lesion surface and/or exudate, roofs from more than one lesion, or lesion crusts. Where feasible, biopsy is another specimen option (WHO, 2022).

Unsuitability of Blood for Diagnosis of Mpox

PCR blood tests are usually inconclusive because of the short duration of viremia relative to the timing of specimen collection after symptoms begin and should not be routinely collected from patients. As orthopoxviruses are serologically cross-reactive, antigen and antibody detection methods do not provide monkeypox-specific confirmation. Serology and antigen detection methods are therefore not recommended for diagnosis or case investigation where resources are limited. Additionally, recent or remote vaccination with a vaccinia-based vaccine (e.g. anyone vaccinated before

smallpox eradication, or more recently vaccinated due to higher risk such as orthopoxvirus laboratory personnel) might lead to false positive results (WHO, 2022).

Sample Collection

Skin lesion material, including swabs of lesion surface, exudate, or lesion crusts are the recommended specimen types for laboratory testing of mpox virus specimens. Procedures and materials used for collecting specimens may vary depending on the phase of the rash (e.g., swabs from lesion surface or crust from healing lesion). Only sterile, synthetic swabs (including but not limited to polyester, nylon, or Dacron) with plastic, wood, or thin aluminum (wire) shafts should be used to collect suspected or confirmed mpox specimens for diagnostic testing. Cotton swabs are not to be used (CDC, 2022). Using two swab sticks, specimens are collected from each lesion, preferably from different locations on the body or from lesions that differ in appearance (e.g., a pair of swabs for each lesion with a total of 2-3 lesions). Each lesion is vigorously swabbed, avoiding contamination of gloved hands, to ensure that adequate viral DNA is collected. Unroofing or aspiration of lesions (or otherwise using sharp instruments for mpox testing) before swabbing is not necessary, nor recommended due to the risk of sharps injury. The two swabs used in collecting specimens from different lesions, crusts, and exudates are placed in separate tubes (CDC, 2022).

Sample Labelling

The specimen container is clearly labelled with the patient identifiers and the appropriate specimen information included prior to specimen collection. Identifiers must be visible, and labels cannot cover the identifiers. Patient identifiers should include at least 2 of the following:

i. Patient name (full first and last name, no initials)
ii. Patient date of birth (MM/DD/YYYY)
iii. Patient sex assigned at birth
iv. A unique ID generated at the time of collection (e.g., a medical record number).

Specimen information should include but is not limited to:

i. Collection site (e.g., left arm, upper left groin, right cheek, etc.)
ii. Collection date
iii. Specimen type (CDC, 2022).

Preservation of Specimen

Lesion samples are stored in a dry, sterile tube and kept cold. Each swab is inserted into a sterile container such as a sterile tube or urine container. Glass containers are not recommended. The swab's shafts is carefully bent or broken off to fit inside the sterile container. After completely securing the lid, the container is wiped with an EPA-approved disinfectant for emerging viral pathogens (CDC, 2022). Placing parafilm around the lid of the container is recommended for additional leak-proof protection, but not required. If specimen testing does not occur promptly after specimen collection, the specimens are refrigerated at (2-8°C) or frozen at (-20°C or lower) until testing occurs. Dry swabs, swabs in viral transport media (VTM) or lesion crust(s) that are stored at 2-8°C can be tested up to 7 days from collection. Swab specimens in VTM and lesion crust(s) that are stored frozen (-20°C or lower) can be tested up to 30 days from collection whereas dry swabs that are stored frozen (-20°C or lower) can be tested up to 60 days from collection (CDC, 2022).

Specimen Transport

When specimens are to be sent out to a reference laboratory for mpox testing, the following are considered.

When preparing specimens for shipment, individual bagging of specimens is recommended so that if a leak does occur, it does not cause the rejection of all specimens. Specimens are transported in a container of dry ice. Specimens are not to be shipped at room temperature. Specimens received outside of acceptable temperature ranges will be rejected. The primary receptacle and secondary packaging should maintain their integrity at the temperature of the refrigerant used, even if the refrigerant's temperature changes. Packages containing dry ice should be designed to prevent pressure buildup, package rupture, and allow gas release (CDC, 2022).

When shipping the specimen to a reference laboratory for testing, the outer package is appropriately marked and labelled with identifiers such as these:

i. Sender's name and address
ii. Recipient's name and address
iii. Biological substance label
iv. Proper shipping name, Biological substance, Category B etc.

Procedure for Detection of Monkey Pox Virus Using PCR

Materials Needed

1. Disposable gloves
2. Disposable laboratory coat
3. Ice and ice bucket
4. Microcentrifuge rack
5. Microcentrifuge tubes (sterile and nuclease free)
6. Optical 96-well reaction plates
7. Optical adhesive covers and applicator or strip caps and capping tool
8. Pipette tips (aerosol-resistant filter) (CDC, 2022).

Reagents

Reagents (Light Sensitive)

- Real-time PCR master mix
- Positive controls

a. Monkeypox virus controls
b. Human DNA
c. Extraction control (such as human cell lines)

- Primer and probe sets
  a. Monkeypox virus primers and probe
  b. Human DNA primers and probe (e.g., RNase P, β-actin, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH))

Reagents (Non-light Sensitive)

a) TE buffer (10mM, pH 8.1 ± 0.2)
b) Water (molecular grade)
c) Acceptable surface decontaminants
d) Ethanol (70%)
e) RNase AWAY

Protocol

To prepare positive controls and extraction control, dilute DNA or make aliquots of human cells (extraction control) with a known cut-off to create a positive control for use when performing real-time PCR (CDC, 2022).

a. In a 40 cycle PCR, a positive control should have a CT cut-off value between 22–28 and (if used) a second low positive control should have a cut-off value between 30–36.

A standard curve can also be used and can be prepared using DNA with a known concentration. For example, logarithmic dilutions from 0.1 ng/µL to 1 fg/µL are created from the known positive control and run-in triplicate with the specimens to be tested.

Assay primers from stock are diluted to 20 µM and probes to 10 µM in TE buffer or molecular grade water.

Human DNA control primer is diluted to concentrations established by the laboratory's validation data (CDC, 2022).

a. Example, RNaseP primers can be diluted to 12. 5 nmol and probe diluted to 2.5 nmol.

Store in the dark at 2–8°C and use within 6 months. Alternatively, single use aliquots can be prepared and stored frozen up to 24 months (manufacturer instructions should be followed for storage conditions, most frequently ≤-20°C).

DNA Isolation

DNA isolation is performed according to laboratory's DNA extraction SOP**.**

Plate Layout

i. Real-time PCR worksheet is used to establish the plate layout.
ii. Each specimen and control may be tested in duplicate or triplicate.
iii. Reagent lot numbers are recorded on worksheet.

Workspace Preparation for Real-Time PCR

PCR workstation is decontaminated e.g., RNase AWAY followed by ethanol (70%).

Reaction Mixture Preparation and Plating Best Practices (Master Mix Workstation)

i. One microcentrifuge tube is labelled per reaction mixture.
ii. Frozen reagents are thawed on ice and gather remaining reagents from refrigerator.
iii. Reagents are briefly vortexed and centrifuged (5 seconds). Reagents are added according to volumes as calculated before test, to the appropriately labeled microcentrifuge tube.
iv. Reaction mixture tubes are briefly vortexed and centrifuged (5 seconds).
v. 15 µL master mix reaction is dispensed into each assigned well.

Specimen and Control Plating (Specimen Workstation)

i. 5 µL of molecular grade water is pipetted into all NTC-labeled wells.
ii. Each DNA specimen tube is vortexed and centrifuged.
iii. 5 µL specimen DNA is pipettes into each specimen-labeled well (both for Monkeypox virus assay and for Human DNA assay).
iv. Previous step for both positive controls is repeated.

Best Practices for Performing Real-Time PCR

i. The protective covering of the optical adhesive cover is peeled off and placed over the wells, making sure all wells are covered.
ii. Using the plastic optical adhesive cover applicator, the edge of the applicator is firmly run over the cover, ensuring a tight seal.
iii. Wells are inspected for bubbles and liquid drops on the sides of the wells above the reaction mixtures. If either bubbles or drops are seen, the tray is gently tapped or flicked repeatedly until the bubbles are dispersed and drops have fallen back into the main reaction mixture. Small drops that do not move after repeated tapping of the plate will not affect assay performance. If possible, use a tabletop centrifuge with plate adapter and centrifuge the plate for 1 minute at 500 x g and room temperature (CDC, 2022).
iv. The reaction plate is placed into the specimen block of the real-time thermocycler. Make certain that plate orientation is correct, and that the tray is fully inserted into the specimen block.
v. Program The real-time PCR instrument is programmed according to manual for the

appropriate cycling conditions and volume of the assay run.

Data Retrieval and Review

After run completion, results are analyzed. Threshold should be set above background as determined by your laboratory's SOP from previous validation data.

Calculations

Calculations are adjusted if not using triplicates Average CT value = (Replicate 1 + Replicate 2 + Replicate 3) ÷ 3

Reference / Alert values

i. The general population is expected to be negative for Monkeypox virus DNA.
ii. All positive results are to be considered an alert value for clinical testing.
iii. After PCR results are available, follow laboratory post-analytical SOPs for approval and reporting. For diagnostic testing, it is recommended to report results to the state or local department of health (CDC, 2022).

Advantages of PCR

I. Valuable for detecting specific pathogens that are difficult to culture in vitro or require a long cultivation period
II. Significantly more rapid in providing results compared to culturing
III. Enables earlier informed decision making.
IV. Rapid diagnosis of bacteremia, parasitaemia or viraemia particularly when their levels are low in specimens.
V. Useful in detecting cases in extra pulmonary specimens which may be missed by smear and/or culture
VI. Valuable screening tool
VII. PCR is still considered an adjunct test for certain diagnostic tests that still rely on smear and culture, such as tuberculosis (Karki, 2022).

Disadvantages

I. PCR testing alone may be limited as a diagnostic tool
II. Still need culture for testing for drug/antibiotic susceptibility and genetic typing
III. Post treatment diagnosis may be challenging.
IV. PCR detects dead organisms that may be shed for weeks after the patient stops showing symptoms. Unclear regarding persistence of infection. Detecting dead organisms at this stage may have no clinical relevance
V. PCR results should not be used as the sole basis of a patient treatment management decision. All results should be interpreted by a trained professional in conjunction with review of the patient's history and clinical signs and symptoms (Karki, 2022).

Precautions in the use of PCR Machine

i. Only tubes and plates designed for the PCR machine should be used.
ii. Before running, double-check tubes and, in particular, plates are tightly sealed.
iii. Solution spills should be cleaned up, and biohazard containers should be used for disposal.
iv. Be cautious when using a PCR machine lid. If you drop or bang lids, this could get broken.
v. Once, the use of the PCR machine is finished, it should be turned off.
vi. Prior to beginning a run, make sure the PCR heating block is clean. Before beginning, inspect every tube holder.

To ensure that the lid sits flat against the top of the tubes for even heating and sealing, distribute the tubers evenly over the block (Karki, 2022).

2. Viral Culture as a Method of Detecting Mpox Virus

For viral culture, MK2 cells, LLCMK2 cells, and Vero E6 cells can be used to acquire oropharyngeal or nasopharyngeal swabs for viral culture. The presence of cytotoxic effects, such as multinucleated syncytial keratinocytes, can be used to identify growth. This cytopathic impact, however, is also present in the Vaccinia and cowpox viruses, making it difficult to distinguish these viruses in cell culture. The virus from the culture may be identified by DNA restriction analysis (Pal *et al*., 2017). Swabs of the lesion's exudates, scabs, a skin biopsy of the vesiculopustular rash, or a sample of the roof of an intact vesiculopustule can all be used to make the diagnosis.

Even though the practice of viral culture has largely been replaced by nucleic acid amplification tests, circumstances still exist in which the availability of viral culture will allow for the diagnosis of infections not included in a provider's differential diagnosis. Viral culture can detect a wide range of pathogens not covered by common NAATs and isolate viruses that may not have been part of a provider's original differential diagnosis. Furthermore, culture growth is less impacted by strain variability or genetic mutations that may contribute to false negative results by NAATs (WHO, 2022). Viral culture is necessary for phenotypic antiviral susceptibility testing and culture results can be useful in determining infectivity in situations of prolonged viral shedding (Mileto *et al.,* 2021). Few studies have documented viral culture of MPXV with most being limited to the Congo Basin lineage. Moreover, the appearance of cytopathic effects (CPE) caused by MPXV has been poorly characterized from the standpoint of the practicing clinical microbiologist. Some of the CPEs associated with mpox culture include: cell discohesion (either globally or in foci with a central clearing), cell rounding, or syncytia etc. formation. Diagnosis of mpox in viral culture with typical CPE, is confirmed by direct immunofluorescent (DFA) or

indirect immunofluorescent (IFA) staining of the shell vial (WHO, 2022).

Safety Procedures

Use of adequate standard operating procedures (SOPs) must be ensured and laboratory personnel must be trained for appropriate donning and doffing of personal protective equipment (PPE), specimen collection, storage, packaging and transport. All specimens collected for laboratory investigations should be regarded as potentially infectious and handled with caution (WHO, 2022). Measures should be taken to minimize the risk of laboratory transmission based on risk assessment when testing routine clinical specimens from confirmed or suspected monkeypox patients. These may include limiting the number of staff testing specimens only to staff with proven competency, wearing appropriate PPE, using rigorously applied standard precautions, and avoiding any procedures that could generate infectious aerosols. Where appropriate and available, consideration of vaccination among staff is encouraged. Effective disinfectants include quaternary ammonium compounds and 0.5% (or 200ppm) bleach (freshly made) (WHO, 2022).

Specimen Type

The recommended specimen type for laboratory confirmation of monkeypox is skin lesion material, including swabs of lesion surface and/or exudate, roofs from more than one lesion, or lesion crusts. Swab the lesion vigorously, to ensure adequate viral DNA is collected. Both dry swabs and swabs placed in viral transport media (VTM) can be used. Two lesions of the same type should be collected in one single tube, preferably from different locations on the body and which differ in appearance. Lesions, crusts and vesicular fluids should not be mixed in the same tube (WHO, 2022). If resources permit it, two tubes may be collected to minimize risk of poor sampling or inhibitors, however only one should be tested and the second should only be tested in case the first provides inconclusive results. In addition to a lesion specimen, the collection of an oropharyngeal swab is encouraged. However, data on the accuracy of this specimen type for diagnosis is limited for monkeypox, therefore a negative throat swab specimen should be interpreted with caution (WHO, 2022).

Specimen Storage

Specimens collected for MPXV investigation should be refrigerated (2– 8 °C) or frozen (-20 °C or lower) within one hour after collection. If transport exceeds 7 days for the sample to be tested, specimens should be stored at -20 °C or lower. Longer term specimen storage (>60 days from collection) is recommended at -70°C (WHO, 2022). Viral DNA present in skin lesion material is relatively stable if kept in a dark, cool environment, which can be considered when cold chain is not readily available but room temperature shipment is not recommended until further studies provide evidence that sample quality is not compromised. Repeated freeze-thaw cycles should be avoided because they can reduce the quality of specimens. Aside from specific collection materials indicated in the annex, other requisite materials and equipment may include: transport containers and specimen collection bags and triple packaging, coolers and cold packs or dry ice, sterile blood-drawing equipment (e.g. needles, syringes and tubes), labels and permanent markers, PPE, and materials for decontamination of surfaces (WHO, 2022).

Packaging and Shipment of Clinical Specimens

Specimens should be stored refrigerated or frozen within an hour of collection and transported to the laboratory as soon as possible after collection. Correct handling and storage of specimens during transportation is essential for accurate diagnostic testing (WHO, 2022). Transport of specimens should comply with any applicable national and/or international regulations, including the UN Model Regulations and any other applicable regulations depending on the mode of transport being used. For international transport, specimens from suspected probable or confirmed cases of MPXV, including clinical samples, viral isolates and cultures should be transported as Category A, UN2814 “infectious substance, affecting humans”. All specimens being transported should have appropriate triple packaging, labelling and documentation. Shipping requires a dangerous goods certified shipper (WHO, 2022).

3. Antibody Testing (IgG and IgM) in the Diagnosis of Monkey Pox

The use of ELISA to detect antibodies such as immunoglobin M (IgM) or immunoglobin G (IgG) in serum or plasma is an efficient way to diagnose monkeypox infection (Karem *et al*., 2005). IgM is detectable in the serum approximately 5 days after the rash appears, whereas IgG is discovered after more than 8 days. Increased titre values in paired sera for IgG and IgM titers can be used to identify seroconversions, which are used as a sign of recent monkeypox infection (Fowotade *et al*., 2018).

Antibody detection from plasma or serum should not be used alone for diagnosis of monkeypox. However, IgM detection from recent acutely ill patients or IgG in paired serum samples, collected at least 21 days apart, with the first being collected during the first week of illness, can aid diagnosis if tested samples yield inconclusive results. Recent vaccination may interfere with serological testing (WHO, 2022). A number of rapid antigen and antibody tests have been developed in the format of lateral flow assay (LFAs) for a range of specimen types (serum, plasma, lesion fluid, oropharyngeal swab). To date, both the analytical and clinical performance of these assays are unclear.

Serological assessment of mpox infection is useful in a number of settings, for example, identification of self-attenuated infection, assessing population seroprevalence to determine asymptomatic infection, and assessment of population immunity. However, mpox serology is not extensively utilized in diagnostic laboratories due to lack of commercial assays. Despite this, a number of in-house assays (e.g., immunofluorescence assay [IFA], enzyme-linked immunosorbent assay [ELISA], hemagglutination inhibition assay [HAI]) have been developed over the years to meet the demand for surveillance and to differentiate vaccination from natural infection. Previous serodynamic studies using ELISA suggest that IgM may persists for weeks to months, whereas IgG persists for years (Brown and Leggat, 2016).

Nature of Sample

Whole blood collected in EDTA container, lesion fluid and oropharyngeal swab.

EDTA blood may support detection of MPXV but may not contain the high level of virus found in lesion samples, as any viremia occurs early in the course of infection, usually in the prodromal period, and before skin lesions become manifest. Collection of a lesion biopsy during the macular stage should be considered only if clinically indicated and only be performed by personnel with appropriate training (WHO, 2022).

Advantages

i. Faster turnaround time
ii. Large numbers of individuals can be screened within a short period.

Disadvantages

There are two major challenges in mpox serological development;

i. Limited availability of MPXV antigens/inactivated viral particles and
ii. Serological cross-reactivity between Orthopoxviruses.
iii. False positive or negative results are possible.

Diagnosis via Electron Microscopy

On electron microscopy, round-to-oval intracytoplasmic inclusions with core sausage-shaped features spanning 200–300 nm can also be detected. These inclusions are widespread in Orthopox viruses, making it easier to distinguish them from herpes and parapox viruses (Pal *et al*., 2017).

Inverse electron microscopy with negative staining can reveal a huge brick-shaped particle with rounded corners, which is indicative of a poxvirus in biopsy specimens from lymph nodes or scab material, vesicular fluid, blood specimens, or viral culture (Mullendore *et al*., 2016).

Diagnostic electron microscopy (DEM) was an essential component of viral diagnosis until the development of highly sensitive nucleic acid amplification techniques (NAT). The simple negative staining technique of DEM was applied widely to smallpox diagnosis until the world-wide eradication of the human-specific pathogen in 1980. Since then, the threat of smallpox re-emerging through laboratory escape, molecular manipulation, synthetic biology or bioterrorism has not totally disappeared and would be a major problem in an unvaccinated population (Gelderblom and Madeley, 2018). Other animal poxviruses may also emerge as human pathogens. With its rapid results (only a few minutes after arrival of the specimen), no requirement for specific reagents and its "open view", DEM remains an important component of virus diagnosis, particularly because it can easily and reliably distinguish smallpox virus or any other member of the orthopoxvirus (OPV) genus from parapoxviruses (PPV) and the far more common and less serious herpesviruses (herpes simplex and varicella zoster) (Gelderblom and Madeley, 2018).

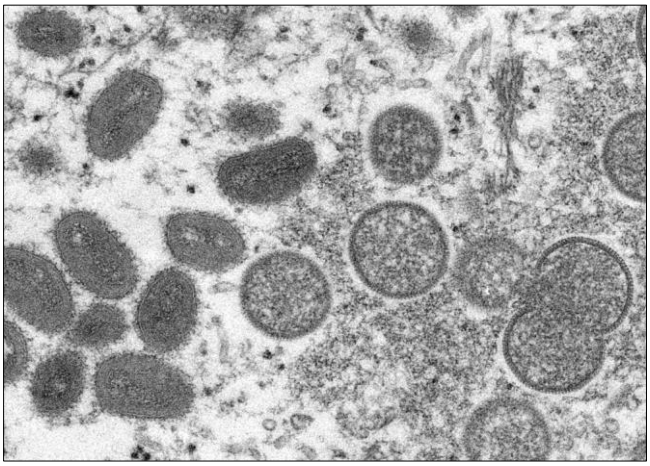

Fig. 11: An electron-microscope image of various virions (virus particles) of the monkeypox virus taken from human skin (Anthony, 2022)

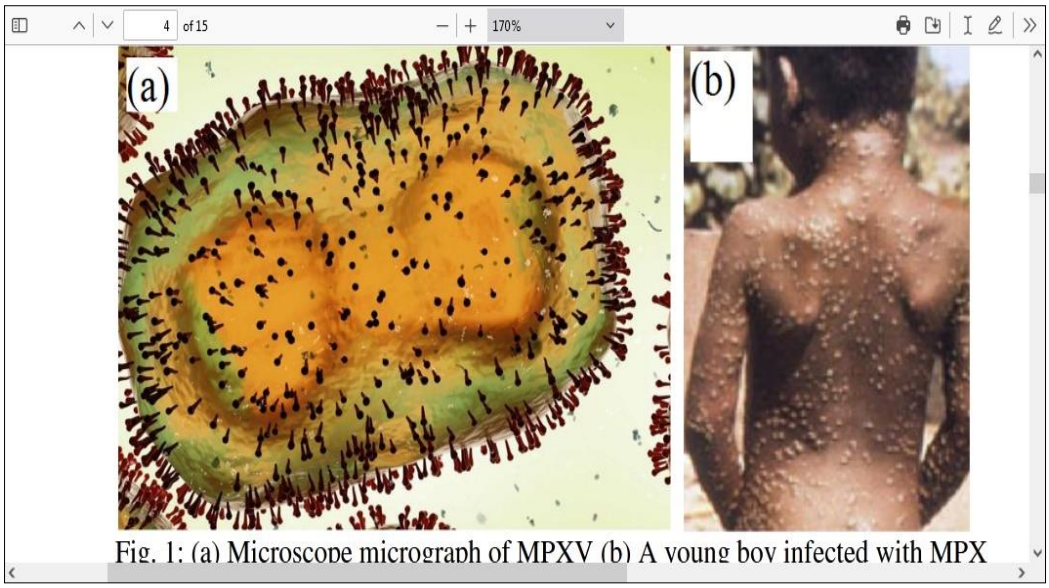

Fig 12: (a) Microscope micrograph of monkeypox virus (b) A young boy infected with monkeypox (Barnett, 2021) and referenced by Igiebor *et al.* (2022)

Nature of Specimen

These can be used in the diagnosis of mpox using electron microscopy;

i. Skin lesion material, including: swabs of lesion surface and/or exudate
ii. Roofs from more than one lesion, or
iii. Lesion crusts.

Scanning Electron Microscopy (SEM) Sample Preparation Techniques

Visualizing a biological specimen with an electron microscope is not a trivial task, mostly because of the intrinsic nature of the electron and matter interactions that are responsible for the image formation. In order to be imaged using SEM, the specimen needs a conductive surface and has to be placed inside high vacuum. Thus, biological specimens cannot be imaged in their native state and need to be heavily processed. Accordingly, sample preparation is a crucial step, which represents an essential activity.

Step 1: Primary Fixation with Aldehydes (Proteins)

Proteins are crosslinked by glutaraldehyde and formaldehyde to stabilise the ultrastructure before further processing.

Step 2: Secondary Fixation with Osmium Tetroxide (Lipids)

Bi-lipid membranes are fixed to prevent their extraction by solvents during dehydration. The black osmium precipitates which is formed during this process increases sample conductivity and minimizes image distortions resulting from charging.

Step 3: Dehydration Series with Solvent (Ethanol or Acetone)

A fixed specimen is dehydrated by incubation in a series of ethanol or acetone solutions. Solvent concentration is increased gradually so that water is removed gently, without causing specimen shrinkage.

Step 4: Drying

Allowing acetone or ethanol to simply evaporate from sample surface would create artefacts as these solvents have relatively high surface tension and would create micro-ripping of the surface upon leaving. To prevent this, dehydration solvents are replaced either with Hexamethyldisilazane (HMDS) or liquid CO_2. HMDS can be used in cell preparations and after a short (3 minute) incubation it is removed and excess is left to evaporate. Liquid CO2 on the other hand is applied to tissues in a critical point drier where it is brought to a critical temperature and pressure point at which it vaporises (Davey, 2019).

The specimen is mounted on a metal stub using a sticky carbon disc which increases conductivity. Silver-containing glue can additionally be applied for even more conductivity.

Step 6: Sputter Coating with Conductive Material

To prevent charge buildup on specimen surface, it is coated with a conductive material, most commonly gold. The metal is applied in a controlled manner in a sputter coater. It is critical that the coating is thick enough to prevent charging (typically around 10 nm) but not thick enough to obscure specimen surface details.

Advantages of Electron Microscopy

1. Magnification and Higher Resolution

As electrons rather than light waves are used, it can be used to analyze structures which cannot otherwise be seen. The resolution of electron microscopy images is in the range of up to 0.2 nm, which is 1000x more detailed than light microscopy.

2. Diverse Applications

Electron microscopy has a diverse range of applications in many different fields of research including technology, industry, biomedical science and chemistry. Examples of applications include semiconductor inspection, computer chip manufacture, quality control and assurance, analysis of atomic structures, and drug development.

3. High-Quality Images

With proper training, an electron microscope operator can use the system to produce highly detailed images of structures which are of a high quality, revealing complex and delicate structures that other techniques may struggle to reproduce (Davey, 2019).

Disadvantages of Electron Microscopy

1. Inability to Analyze Live Specimens

As electrons are easily scattered by other molecules in the air, samples must be analyzed in a vacuum. This means that live specimens cannot be studied by this technique. This means that biological interactions cannot be properly observed, which limits the applications of electron microscopy in biological research.

2. Black and White Images

Only black and white images can be produced by an electron microscope. Images must be falsely colorized.

3. Artefacts

These may be present in the image produced. Artefacts are left over from sample preparation and require specialized knowledge of sample preparation techniques to avoid (Davey, 2019).

4. Cost

Electron microscopes are expensive pieces of highly specialized equipment. As most projects have limited budgets, it may prove detrimental to use an electron microscope in the research. However, running costs can be similar to alternatives such as confocal light microscopes, so the investment in a basic electron microscope is still worth considering even if budgetary concerns are a major factor in decisions against utilizing the technology.

5. Size

Despite the advantages in technology over the years, electron microscopes are still large, bulky pieces of equipment which require plenty of space in a laboratory. Also, as electron microscopes are highly sensitive, magnetic fields and vibrations caused by other lab equipment may interfere with their operation. Consideration must be given to this if the researcher is looking to install an electron microscope in their laboratory (Davey, 2019).

6. Training

Specialist operators are required to operate electron microscopes, and these can undergo years of training to properly use this technology.

2.13 Prognosis of Monkey Pox

There are two distinct clades of the mpox virus, with a third possible clade potentially being described in the present outbreak (Luna *et al.,* 2022). The West African clade has a more favorable prognosis with a case fatality rate below 1%. On the other hand, the Central Basin clade (Central African clade) is more lethal, with a case fatality rate of up to 11% in unvaccinated children. Aside from potential scarring and discoloration of the skin, the remainder of patients typically fully recovers within four weeks of symptom onset (Sklenovská and Van Ranst, 2018). In a cohort of 1119 confirmed cases of mpox from the ongoing outbreak in Spain, Germany, Italy, and the United Kingdom, there have been no reported deaths even though it included a subset of patients with HIV, suggesting that the circulating strain may be less virulent. However, the quality of medical care could also play a factor in this (Selb *et al.*, 2022).

2.14 Prevention

Raising awareness of risk factors and educating people about the measures they can take to reduce exposure to the virus is the main prevention strategy for monkeypox. Scientific studies are now underway to assess the feasibility and appropriateness of vaccination for the prevention and control of monkeypox. Some countries have, or are developing, policies to offer vaccine to persons who may be at risk such as laboratory personnel, rapid response teams and health workers (WHO, 2022).

Use of Biosafety Cabinet in Prevention of Disease Spread amongst Laboratory Professionals

Routine diagnostic specimen processing is performed in Biosafety Level 2 (BSL-2) laboratory facilities following standard and special practices, safety equipment, and facility specifications recommended for BSL-2 according to site-specific and activity-specific biosafety risk assessments. Additional precautions to reduce exposure risk may include, but are not limited to:

i. Solid-front gowns with cuffed sleeves
ii. Double gloves
iii. Eye protection (safety glasses, snugly fitting goggles) or face protection (face-shield)
iv. NIOSH-approved particulate respirator equipped with N95 filters or higher
v. Limiting the number of laboratory personnel who work during specimen manipulation.
vi. Laboratory with directional airflow

Diagnostic specimens are manipulated in a certified Class II Biosafety Cabinet (BSC) or other containment devices, especially if there is a potential to generate aerosols (e.g., vortexing or sonication of specimens in an open tube) (CDC, 2022). Open vessels

on bench tops should not be worked with unless it is safe to do so based on site and activity-specific risk assessments (i.e., the specimen has been fully inactivated utilizing an approved inactivation method). If one cannot perform a procedure within a BSC, a combination of PPE and other containment devices (e.g., glove box, centrifuge safety cups, or sealed rotor) which are designed to create a barrier between the specimen and the laboratory personnel are to be used (CDC, 2022).

Other Preventive Measures Include

1. Reducing the Risk of Human-To-Human Transmission

Surveillance and rapid identification of new cases is critical for outbreak containment. During human monkeypox outbreaks, close contact with infected persons is the most significant risk factor for monkeypox virus infection. Health workers and household members are at a greater risk of infection. Health workers caring for patients with suspected or confirmed monkeypox virus infection, or handling specimens from them, should implement standard infection control precautions. If possible, persons previously vaccinated against smallpox should be selected to care for the patient. Samples taken from people and animals with suspected monkeypox virus infection should be handled by trained staff working in suitably equipped laboratories. Patient specimens must be safely prepared for transport with triple packaging in accordance with WHO guidance for transport of infectious substances (WHO, 2022). Avoiding sexual contact with someone with a localized anogenital rash and limiting the number of sex partners; avoiding close contact with someone who has symptoms consistent with possible monkeypox infection; keeping hands clean with water and soap or alcohol-based gels; and maintaining respiratory etiquette are other ways to prevent transmission of the virus. If a person develops symptoms such as a rash with blisters on face, hands, feet, eyes, mouth, and/or genitals and peri-anal areas; fever; swollen lymph nodes; headaches; muscle aches; and fatigue they should contact their health care provider and get tested for monkeypox (WHO, 2022). If someone is suspected or confirmed as having monkeypox, they should isolate, avoid skin-to-skin and face-to-face contact with others and abstain from sex, including oral sex, until the scabs have fallen off. During this period, cases can get supportive treatment to ease symptoms. Anyone caring for a person sick with monkeypox should use appropriate personal protective measures as mentioned above (WHO, 2022).

2. Reducing the Risk of Zoonotic Transmission

Over time, most human infections have resulted from a primary, animal-to-human transmission. Unprotected contact with wild animals, especially those that are sick or dead, including their meat, blood and other parts must be avoided. Additionally, all foods containing animal meat or parts must be thoroughly cooked before eating (WHO, 2022). Residents and travellers to monkeypox-endemic countries should avoid contact with sick mammals such as rodents, marsupials, non-human primates (dead or alive) that could harbour monkeypox virus and should refrain from eating or handling wild game (bush meat) (WHO, 2022).

3. Preventing Monkeypox through Restrictions on Animal Trade

Some countries have put in place regulations restricting importation of rodents and non-human primates. Captive animals that are potentially infected with monkeypox should be isolated from other animals and placed into immediate quarantine. Any animals that might have come into contact with an infected animal should be quarantined, handled with standard precautions and observed for monkeypox symptoms for 30 days (WHO, 2022).

4. Restricting or Monitoring Travelling to Monkey Pox Endemic Countries

Movement to regions with high rate of monkey pox should be monitored. Any rash-like illness during travel or upon return should be immediately reported to a health professional, including information about all recent travel, sexual history and smallpox immunization history. Individuals who have been identified as contacts of monkeypox cases and, therefore, are subject to health monitoring, should avoid undertaking any travel, including international, until completion of their health monitoring period (WHO, 2022).

5. Large Gatherings

Large gatherings may represent a conducive environment for the transmission of monkeypox virus if they entail close, prolonged and frequent interactions among people, which in turn could expose them to contact with lesions, body fluids, respiratory droplets and contaminated materials (WHO, 2022). Therefore, such large gatherings are to be avoided.

How to Handle the Remains of a Patient Who Died from Monkeypox Virus

During outbreaks, any unprotected handling of the dead bodies of infected patients constitutes a biosafety hazard. This poses a major risk of transmission, as the dead body remains contagious several days after death. The management of the burial is therefore the responsibility of the safe burial teams. The family and members of the community are also at risk especially if the burial rites involve manipulation and cleaning of the body (Nigeria Centre for Disease Control, 2017). The burial team is to oversee the safe burial of the victims. This team must adhere to the following key principles:

i. Verbally convey condolences and sympathy to the victim's family
ii. Clearly but emphatically explain the procedure for handling of remains and outline how and why the procedure for body preparation and burial will differ from the normal/local tradition.

iii. If a psychologist is available, collaborate with him/her in engaging with the family.
iv. If necessary, employ the support of security agents.
v. Avoid conduct of funeral ceremonies during the burial
vi. Ensure that the patient's home is disinfected.
vii. The burial must take place as soon as possible after preparation of the remains at the hospital.

The Safe Burial team should:

i. Prepare the body with care to avoid the risk of transmission.
ii. Strive to respect the cultural practices and religious beliefs of the family, as long as they do not result in a risk of transmission. Let the family understand that certain practices that entail a risk of transmission will be abandoned.
iii. Advise the family and the community on actions to take in order to protect themselves against the disease.
iv. If the body is prepared without information or support to the family and the community, the members of the community would not be willing to bring other relatives to the hospital for fear of not receiving the dead body once the patient has died.

Find and use an influential member of the family in ensuring that dangerous practices like touching and washing the dead body are avoided (Nigeria Centre for Disease Control, 2017).

2.15 Vaccination against Monkey Pox

Vaccination against smallpox was shown in the past to be cross-protective against monkeypox. However, any immunity from smallpox vaccination will only be present in persons over the age of 42 to 50 years or older, depending on the country, since smallpox vaccination programmes ended worldwide in 1980 after the eradication of smallpox (WHO, 2022). The original (first generation) smallpox vaccines from the eradication programme are no longer available to the general public. In addition, protection for those who were vaccinated may have waned over time (WHO, 2022).

Smallpox and monkeypox vaccines, where available, are being deployed in a limited number of countries to manage close contacts. While smallpox vaccines have been shown to be protective against monkeypox, there is also one vaccine approved for prevention of monkeypox (WHO, 2022). This vaccine is based on a strain of vaccinia virus (known generically as modified vaccinia Ankara Bavarian Nordic strain, or MVA-BN). This vaccine has been approved for prevention of monkeypox in Canada and the United States of America. In the European Union, this vaccine has been approved for prevention of smallpox. An antiviral to treat orthopoxviruses has been also recently approved in the United States of America and in the European Union. WHO has convened experts to review the latest data on smallpox and monkeypox vaccines, and to provide guidance on how and in what circumstances they should be used (WHO, 2022).

There is a vaccine for monkeypox recently approved by some countries for which supplies are limited. Some countries may hold smallpox vaccine products which could be considered for use according to national guidance (WHO, 2022). Vaccine products may be available in limited quantities through national authorities, depending on the country. Regardless of vaccine supply, mass vaccination of the population is not required nor recommended for monkeypox; every effort must be made to control human-to-human spread of monkeypox through early case-finding and diagnosis, isolation and contact-tracing (WHO, 2022).

The JYNNEOS vaccine is approved for prevention of smallpox and mpox. It is the primary vaccine being used in the U.S. during this outbreak.

Post-exposure prophylaxis (PEP) is recommended for contacts of cases with an appropriate second- or third-generation smallpox or monkeypox vaccine, ideally within four days (and up to 14 days) of first exposure to prevent onset of disease.

Pre-exposure prophylaxis (PrEP) is recommended for health workers at high risk of exposure, laboratory personnel working with orthopoxviruses, clinical laboratory personnel performing diagnostic testing for monkeypox, and response team members as may be designated by public health authorities (WHO, 2022).

All decisions around immunization with smallpox or monkeypox vaccines should be based on an assessment of risks and benefits on a case-by-case basis, using shared clinical decision-making. Implementation of vaccination should be accompanied by robust pharmacovigilance, and the conduct of vaccine effectiveness studies under clinical trial protocols is strongly encouraged (WHO, 2022).

2.16 Treatment/ Management of Monkey Pox

Currently, there are no specific clinically proven treatments for mpox infection. As with most viral illnesses, the treatment is supportive symptom management. There are, however, prevention measures that can help prevent an outbreak. The infected individual should remain in isolation, wear a surgical mask, and keep lesions covered as much as reasonably possible until all lesion crusts have naturally fallen off and a new skin layer has formed. For severe cases, investigational use can be considered for compounds with demonstrated benefit against orthopox viruses in animal studies and severe vaccinia vaccine complications (Moore *et al*., 2022).

The oral DNA polymerase inhibitor brincidofovir, oral intracellular viral release inhibitor tecovirimat, and intravenous vaccinia immune globulin have unknown efficacy against the mpox virus. Dual therapy with tecovirimat and brincidofovir can be trialed in severe cases. Tecovirimat inhibits viral envelope protein VP37, thus blocking viral maturation as well as the release of the virus from infected cells (Rizk *et al.*, 2022). Brincidofovir is approved for the treatment of smallpox in the US. Normal saline and probenecid should be given concurrently with cidofovir (Rizk *et al.*, 2022).

Vaccinia Immune Globulin (VIG) is a blood product that is high in antibodies against the Vaccinia virus and is made from the pooled blood of people who have had the smallpox vaccination. It is licensed by the FDA to treat complications of vaccinia vaccination. The effectiveness of VIG against smallpox and mpox is uncertain, and VIG has not been trialed in humans for smallpox or mpox (Rizk *et al*., 2022).

For individuals exposed to the virus, temperature and symptoms should be monitored twice daily for 21 days because that is the accepted upper limit of the mpox incubation period. Infectiousness aligns with symptom onset; therefore, close contacts need not isolate while asymptomatic. In some cases, post-exposure vaccination with modified vaccinia, Ankara vaccine (smallpox and mpox vaccine, live, non-replicating) is recommended. Contact between broken skin or mucous membranes and an infected patient's body fluids, respiratory droplets, or scabs is considered a "high risk" exposure and warrants post-exposure vaccination as soon as possible. According to the CDC, vaccination within four days of exposure may prevent disease onset, and vaccination within 14 days may reduce disease severity (Moore *et al.*, 2022).

The replication-defective modified vaccinia Ankara vaccine is a two-shot series, four weeks apart, with a superior safety profile compared to first and second-generation smallpox vaccines. Unlike live vaccinia virus preparations, administering modified vaccinia, Ankara does not create a skin lesion or pose a risk of local or disseminated spread. In addition, clinical trials have shown that modified vaccinia Ankara is safe and stimulates antibody production in patients with atopy and compromised immune systems, which are known contraindications to live vaccinia administration (Petersen *et al.,* 2019).

Identifying the potential benefits and drawbacks of preventative mpox vaccination in endemic communities requires more thorough data collection and feasibility analysis. Access to medical care, testing capabilities, and infrastructure limits the ability to make informed decisions about best addressing this neglected tropical disease (Petersen *et al.,* 2019).

3.0 CONCLUSION

Monkeypox is an uncommon viral illness that mostly affects Central and West Africa. Mpox infections have been documented in recent years all over the world. It can spread from person to person as well as from infected animals to humans. Exposure to the excretions and secretions of an infected animal or person has been related to the spread of disease to people. The illness is marked by fever, exhaustion, and a rash of lesions that resemble smallpox; it can also cause complications like pneumonia, encephalitis, and sepsis, which can be fatal. The rapid spread of the monkey pox has made the inadequacy of preventative measures clear. Animals traveling internationally must be confined and screened for the monkeypox virus. A high index of suspicion must be in place when examining patients, especially the high-risk population. High-risk populations like the immunocompromised, men having sex with men (MSM), and those who handle animals that have traveled internationally, especially rats and other small mammals, should begin receiving the mpox immunization. Medical professionals must be aware of the early clinical signs of the monkeypox virus to make an early diagnosis and begin prompt therapy. Patients with confirmed or suspected monkeypox infections must be quarantined until all lesions have healed. Thus, key emphasis is the need for utilization of an integrated approach that include immunization, antiviral treatments, and public health policies tailored to high-risk populations in combatting the disease. furthermore, the need for training and retraining of Laboratory Scientists and personnels to acquire improved diagnostic skills desired in order to effectively control and reduce the impact of Mpox is highly needed.

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