YALE JOURNAL OF BIOLOGY AND MEDICINE 97 (2024), pp.529-534. doi: 10.59249/PWON3661

MINI-REVIEW

YALE JOURNAL OF BIOLOGY AND MEDICINE
YJBM

Monkeypox (Mpox): Diagnosis and Emerging Challenges

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Monkeypox (Mpox) has once again been designated a Public Health Emergency of International Concern (PHEIC) as of August, 2024. The severity of the disease is underscored by its significant mortality rate, and unfortunately, there are no targeted therapeutics currently available for this viral infection. Management relies on preventive measures and the use of existing smallpox vaccines due to their genetic similarity to the Mpox virus. Diagnosing a disease is a critical aspect of managing any health condition, and for a highly contagious viral infection like Mpox, it is essential to employ a specific and sensitive diagnostic approach. The lack of adequate diagnostic facilities in laboratories poses a significant challenge, hindering accurate diagnoses and the identification of underlying etiologies, particularly in low-resource settings. Current serology-based diagnostic tests lack specificity for the Mpox virus, leading to cross-reactivity with other orthopoxviruses. With the emergence of new viral variants, molecular and genomic diagnostic methods are far more reliable for accurately confirming Mpox infections. This review focuses on current diagnostic methods approved worldwide and the future challenges that need to be addressed to effectively control and mitigate the spread of Mpox.

MONKEYPOX VIRUS: AN INTRODUCTION

Monkeypox (Mpox) is an emerging zoonotic illness that presents with symptoms similar to smallpox [1,2]. The World Health Organization (WHO) has now designated it as a Public Health Emergency of International Concern (PHEIC) for the second time in 2 years. The first declaration occurred in July 2022 when the virus began to spread across countries where it had not previously been detected. In May 2023, WHO declared the end of PHEIC as there was a decline in cases. The WHO stated in August, 2024 that the current rise in Mpox cases in parts of Africa, combined with the spread of a new sexually transmissible strain of the Mpox virus, is considered an emergency not only for Africa, but for the entire world [3]. This presents a significant challenge for the scientific community in managing the transmission of the disease and developing effective therapeutic interventions for the

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Abbreviations: Mpox, Monkeypox; PHEIC, Public Health Emergency of International Concern; WHO, World Health Organization; DRC, Democratic Republic of the Congo; PCR, Polymerase chain reaction.

Keywords: Mpox, Clade I, Clade II, Zoonosis, PHEIC, Diagnosis, PCR, Vaccines

Author Contributions: BCK, LC, and SCS conceived and designed the study. SK, DT, SM, MY, ABS, and AS analyzed the data, created figures, and prepared the manuscript. BCK, SCS, and LC finalized the manuscript. All authors contributed to the article and approved the submitted version.

public.

Mpox is caused by the Mpox virus, which belongs to the Poxviridae family known for its zoonotic transmissions. It is a double-stranded DNA virus with a genome of ~300 kilobase pairs in size [4]. The first recorded case of Mpox was identified in the Democratic Republic of Congo (DRC) in 1970. It is known to be transmitted zoonotically, with around 72 percent of reported cases originating from zoonotic sources [1-3,5]. The disease is predominantly confined to Africa, with cases reported in countries such as Benin, Cameroon, the Central African Republic, the Democratic Republic of the Congo, Gabon, Liberia, Nigeria, Sierra Leone, Zaire, and Côte d'Ivoire. However, it has now spread beyond Africa and has been reported in other regions globally [6]. The first cases of Mpox outside Africa were reported in the United States in 2003, following exposure to pet prairie dogs that exotic animals of Ghana had infected. Subsequent sporadic cases were noted, and a significant rise in infections began in 2022, with cases emerging in various countries, including India [5].

Mpox transmission occurs via both animal-to-human and human-to-human routes. Humans can contract the virus through direct contact with infected animals by hunting and meat consumption, or through contact with their bodily fluids and even via contaminated surfaces. Human-to-human transmission can occur via respiratory droplets, direct contact with skin lesions or bodily fluids, and aerosolized respiratory secretions. Additionally, transmission can occur through both homosexual and heterosexual exposure [6,7]. The 2022 outbreak saw a surge in Mpox cases, with over 90 percent of transmissions linked to homosexual intercourse among men [8,9]. The emergence of a new virus strain in the DRC, Clade Ib, which seems to be spreading primarily through heterosexual exposure, is particularly concerning and is a key factor behind the declaration of the PHEIC [3]. Clade Ib is a divergent of the Congo Basin Clade (Clade I) that exhibits a case-fatality rate of up to 10 percent [3]. The West African Clade (Clade II) has a much lower case-fatality rate, ~1 percent. The two clades differ in several key aspects, including geographic distribution, disease patterns, and mortality rates. The genomic variations between the clades occur in regions of the genome that code for virulence, which likely account for the differences in clinical severity and mortality [2,10-12].

Currently, three smallpox vaccines are available for protection against the Mpox virus, JYNNEOS™ (also known as IMVAMUNE, IMVANEX, MVA-BN), ACAM2000, and the Aventis Pasteur Smallpox Vaccine (APSV) [13]. These vaccines are recommended for individuals at high risk, including gay, bisexual, or other men who have sex with men, people with multiple sex partners, sex workers, health workers handling orthopoxviruses, and volunteers of outbreak response teams [14]. WHO prequalifies MVA-BN (Modified Vaccinia Virus Ankara – Bavarian Nordic) as the first vaccine against Mpox with an efficacy of 76% post single dose and 82% post dual dose [15]. The vaccine is approved for individuals aged 18 and older and is administered as two injections spaced 4 weeks apart. After being stored at low temperatures, it can be maintained at 2-8°C for up to 8 weeks.

Mpox is a highly contagious disease that has been declared a PHEIC. Currently, there are no specific treatments or vaccines available for the disease, making timely diagnosis crucial for effective management. Early detection enables prompt quarantine of affected individuals, which is essential for minimizing disease transmission. This review offers a comprehensive overview of diagnostic approaches and the challenges that need to be addressed to effectively contain the disease.

DIAGNOSTIC APPROACHES

With emerging evidence of viral adaptation, there is a significant possibility that Mpox could establish endemicity worldwide. Enhancing access to testing is therefore crucial for global eradication efforts [16]. Mpox infection begins with symptoms including fever with chills, body aches, fatigue, and adenopathy. The characteristic symptom is the appearance of rashes similar to smallpox. The incubation period ranges from 7 to 14 days, and up to 21 days [1,2]. Based on syndromic case management rashes, malaise, and adenopathy are more commonly suspected as Mpox cases signs and symptoms [17].

Mpox diagnosis solely on clinical symptoms is difficult; therefore, molecular assays and tests of patient samples are recommended for confirming [1]. The collection of samples should adhere to guidelines for standard precautions. Samples for diagnosis can be serum, urine, nasopharyngeal swab (NPS) or oropharyngeal swab (OPS), or scrapings from lesions [2]. Viral culture, IgG and IgM assays, and real-time Polymerase chain reaction (PCR) are few effective diagnostic methods. PCR, either alone or combined with sequencing, can be employed effectively; when integrated with clinical and epidemiological data, including a patient's immunization history, it provides the most accurate results. Virus culturing produces a live virus and allows for species identification by observing the culture under electron microscope; however, this method requires trained personnel, a Biosafety Level-3 laboratory, and is time-consuming [1]. Serological assays to detect IgM and IgG antibody levels after 5 to 8 days of infection serves as a marker for the Mpox infection. However, due to antigenic cross-reactivity with the viruses of the same family, the assay is not considered as a specific diagnostic test for Mpox [1,2].

Developing a cost-effective, efficient, and rapid diag-

Table 1. List of Primers and Probes used to Detect Mpox Infection

Gene	Sequence (5' – 3')	Reference
E9L	**F:** TCAACTGAAAAGGCCATCTATGA-3′ **R:** GAGTATAGAGCACTATTTCTAAATCCCA **P:** TET-CCATGCAATATACGTACAAGATAGTAGCCAAC-QSY7	[19]
B6R	**F:** ATTGGTCATTATTTTTGTCACAGGAACA **R:** AATGGCGTTGACAATTATGGGTG **P:** MGB-AGAGATTAGAAATA-FAM	[19]
C3L	**F:** TGTCTACCTGGATACAGAAAGCAA-3′ **R:** GGCATCTCCGTTTAATACATTGAT **P:** FAM-CCCATATATGCTAAATGTACCGGTACCGGA-BHQ1	[20]
G2R_WA	**F:** CACACCGTCTCTTCCACAGA **R:** GATACAGGTTAATTTCCACATCG **P:** FAM-AACCCGTCGTAACCAGCAATACATTT-3′BHQ1	[20]
G2R	**F:** GGAAAATGTAAAGACAACGAATACAG **R:** GCTATCACATAATCTGGAAGCGTA **P:** FAM-AAGCCGTAATCTATGTTGTCTATCGTGTCC-BHQ1	[20]
F3L	**F:** CTCATTGATTTTTCGCGGGATA **R:** GACGATACTCCTCCTCGTTGGT **P:** 6FAM-CATCAGAATCTGTAGGCCGT-MGBNFQ	[22]
N3R	**F:** AACAACCGTCCTACAATTAAACAACA **R:** CGCTATCGAACCATTTTTGTAGTCT **P:** FAM-TATAACGGCGAAGAATATACT-MGBNFQ	[22]
ATI Congo	**F:** GAGATTAGCAGACTCCAA **R:** GATTCAATTTCCAGTTTGTAC **P:** GCAGTCGTTCAACTGTATTTCAAGATCTGAGAT-Fluorescein	[22]
ATI West African	**F:** GAGATTAGCAGACTCCAA **R:** TCTCTTTTCCATATCAGC **P:** GCAGTCGTTCAACTGTATTTCAAGATCTGAGAT-Fluorescein **P:** LCRed640-CTAGATTGTAATCTCTGTAGCATTTCCACGGC-Phos	[22]
B7R	**F:** ACGTGTTAAACAATGGGTGATG **R:** AACATTTCCATGAATCGTAGTCC **P:** TAMRA-TGAATGAATGCGATACTGTATGTGTGGG-BHQ2	[22]
Clade Ib assay (dD14-16)	**F:** AAGACTTCCAAACTTAATCACTCCT **R:** CGTTTGATATAGGATGTGGACATTT **P:** FAM-ATATTCAGGCGCATATCCACCCACGT-BHQ	[27]
O2L	**F:** TAGTGAGTTCGGCGACAAAG **R:** GTATCGCATCTCTCGGGTATTC **P:** 6-FAM-ACCGGTAATCTTGTCGAGGAGGACA-ZEN-IBFQ	[24]

F, forward primer; **R**, reverse primer; **P**, probe

nostic method for Mpox is crucial for the timely detection of viral infections. PCR with restriction fragment length polymorphism (RFLP) were originally used to detect orthopoxvirus infection, followed by real-time PCR which is quantitative and sensitive [18]. In 2006, Li et al. [19] first introduced a real-time PCR assay specific to Mpox, targeting the E9L (viral DNA polymerase gene) and B6R (envelope protein) gene. In 2010, another real-time PCR assay that targets the G2R (TNF receptor) gene, was developed by Li et al. [20] and is now referred as a standard detection method in the US. Li et al. [20] also developed two specific assays for the West African strain (G2R_WA) and Congo Basin strain. The C3L (complement binding protein) target was used for Congo Basin strain as the C3L is known to be deleted in the West African strains [20]. PCR targeting the F3 protein, an apoenzyme with a double-stranded RNA-binding domain (dsRBD), is recognized for its higher specificity and lack of cross-reactivity with other Orthopoxviruses [21-23]. Apart from these there are PCR's for generic targets such as B7R, N3R, and O2L (open reading frame) [24-26]. Schuele et al. [27] designed a specific PCR targeting ATI (A-type inclusion bodies) to detect Clade Ib strain of Mpox. Table 1 lists primers and probes used for specific gene targets

Table 2. List of FDA-approved Diagnostic Tests [30]

S. No.	Kit	Type of test	Parent Organization
1	Labcorp Monkeypox PCR Test Home Collection Kit	Home Collection Kit	Laboratory Corporation of America (Labcorp)
2	Non-variola Orthopoxvirus Real-time PCR Primer and Probe Set	Real-time PCR, Single Target	Centers for Disease Control and Prevention (CDC)
3	Cue Mpox (Monkeypox) Molecular Test	Real-time PCR, Single Target	Cue Health, Inc.
4	Alinity m MPXV	Real-time PCR, Multiple Targets	Abbott Molecular, Inc.
5	Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well)	Real-time PCR, Multiple Targets	Quest Diagnostics Nichols Institute
6	Xpert Mpox	Real-time PCR, Multi-analyte, Multiple Targets	Cepheid
7	QuantiVirus MPXV Test Kit	Real-time PCR, Multiple Targets	DiaCarta, Inc.
8	Cobas MPXV for use on the cobas 6800/8800 Systems (cobas MPXV)	Real-time PCR, Multiple Targets	Roche Molecular Systems, Inc.

worldwide. Assays' detection limits and comparative sensitivity can vary significantly between laboratories, primers, and target genes. Most real-time PCR tests offer detection limits and sensitivities ranging from picograms (pg) to femtograms (fg), or approximately 10 to 250 copies per reaction [20,28]. As of now, the US Food and Drug Administration (FDA) has granted Emergency Use Authorization (EUA) for eight commercial diagnostic tests utilizing real-time PCR [29,30] (Table 2). Additionally, the Indian Council of Medical Research (ICMR) has recently validated, and the Central Drugs Standard Control Organisation (CDSCO) has approved, three commercial PCR kits, which are supported by 36 laboratories across India. One of the approved kits, the IMDX Mpox Detection RT-PCR Assay, specifically targets Clade I and Clade II variants [31].

PROSPECTS AND EMERGING CHALLENGES

Dimie Ogoina from the WHO team stated that Mpox, which originated in Africa, was neglected there and later led to a global outbreak in 2022. He emphasized that it is now time for decisive action to prevent history from repeating itself [3]. The resources needed to control the Mpox outbreak like diagnostics, vaccines, antivirals, and robust surveillance systems are not accessible in many African countries. Despite the smallpox vaccine's ability to prevent Mpox, its production was discontinued after the disease eradication in 1980, and if production resumes, it will take time for the vaccine to reach African countries because they are not prioritized like the high-income nations, as seen with the COVID-19 vaccine distribution. In addition to challenges in diagnosis and prevention, there is a significant concern regarding the severe lack of training among healthcare workers, insufficient disease prevention campaigns, and limited awareness within the African population. These factors collectively pose major obstacles in effectively addressing the outbreak. Delayed detection and reporting of outbreaks impede the implementation of prevention and control strategies, which can contribute to the further spread of the disease [27,32,33]. Effective containment of the disease starts with establishing a robust surveillance team, training healthcare professionals, and enhancing diagnostic facilities in the most endemic areas. These measures are crucial for early detection and prompt containment of the disease.

Viral evolution could lead to the emergence of more virulent strains, as well as strains that may evade detection by current diagnostic methods or resist neutralization by existing vaccines. To strengthen preparedness for future viral outbreaks, resources should be dedicated to enhancing and expanding population surveillance, diagnostic testing, and laboratory viral genomic sequencing. Additionally, a standardized protocol and system for rapid laboratory testing and vaccine production should be developed to respond swiftly to the emergence of a new Mpox virus strain [15]. Genomes of Clade IIb show apolipoprotein B editing complex (APOBEC3)-type mutations, which act against viruses. This mutation attacks the single strands exposed during replication which inhibits the virus's ability to replicate, thereby serving as an intrinsic antiviral defense mechanism. Mutations linked to APOBEC3 activity may indicate the virus's evolution and increasing human-to-human transmission [2,16].

The genomes of Orthopoxviruses are highly conserved, resulting in considerable cross-reactivity and cross-protection among antibodies. However, compared to other antigens, the prevalent A29 and M1 antigens elicited stronger cross-neutralizing immune responses against the Mpox virus, making them promising candidates for the development of new orthologous Ortho-

poxviral vaccines. Despite genomic similarities, Mpox contains genes that are either absent, altered, or shortened in vaccinia. These unique gene products can act as distinguishing markers to differentiate between Mpox and vaccinia infections, aiding in the development of novel diagnostic strategies specifically designed to improve Mpox detection [34,35].

DISCUSSION AND CONCLUSION

Mpox is again declared a PHEIC and being a contagious virus, everyone should be responsible for their safety and take necessary precautions. Despite its historical confinement to Africa, Mpox has demonstrated its potential for widespread transmission through zoonotic and human-to-human routes, particularly in emerging strains like Clade Ib. The development of advanced diagnostic methods, especially real-time PCR assays, has greatly improved Mpox detection. Key assays targeting genes like E9L, B6R, and G2R are now standard, with additional targets refining accuracy for specific Mpox strains. To date, there are eight real-time PCR assays approved by FDA that highlight the need for continued innovation to ensure rapid and accurate detection. Vaccine development is in process and targeted global health promotion efforts are required, focusing on at-risk populations. Additionally, ongoing clinical research and pharmacovigilance among vulnerable groups are essential to achieving and maintaining herd immunity against this viral infection. Improved surveillance, advanced diagnostics, strengthened infection prevention and control measures in healthcare settings, and comprehensive social and safety containment strategies are currently required. In addition to developing effective and advanced vaccines specifically for Mpox, substantial global efforts are needed to ensure broad vaccine access. A comprehensive vaccination strategy must be developed to address the increasing cases of Mpox, preventing further exponential growth and mitigating the risk of another pandemic, especially after the COVID-19 era.

Funding: None.

Acknowledgments: We are grateful to the Multidisciplinary Research Unit at Maulana Azad Medical College and Associated Hospitals, the Vector-borne Diseases Group at the International Center for Genetic Engineering and Biotechnology, and the University of Delhi, New Delhi. Additionally, we would like to acknowledge the Delhi School of Public Health, Institute of Eminence for their IoE Fellowships support.

Declaration of competing interest: The authors report no conflict of interest.

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