sciendo Revista Română de Medicină de Laborator 2024;32(1):97-106 REVIEW
DOI:10.2478/rrlm-2024-0005

Understanding the pathogenesis, clinical, laboratory diagnosis and treatment of the recent monkeypox virus outbreak

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ABSTRACT

Human mpox is a zoonotic disease, caused by the mpox virus (MPXV), that can spread either between animals and humans or humans and humans. In 1970 the first human case of mpox was reported in Zaire, Democratic Republic of the Congo (DRC). Other notable human mpox outbreaks in non-endemic countries were identified in June 2003 in the United States, in July 2021 in Dallas (USA), and the most recent one in May 2022 in Europe in the United Kingdom (UK). During the 2022 outbreak, sexual intercourse was observed to be the most prevalent transmission method, although other means should not be ignored, such as the involvement of respiratory droplets, exposure to surfaces and skin suspected of contamination. In the context of the current mpox outbreak, we consider it important and necessary to correctly identify the virus, use the proper laboratory testing for a correct diagnosis of mpox, understand the means of prophylaxis, and apply the correct treatment, highlighting these facts being the aim of this study.

Keywords: Mpox, orthopox virus, diagnosis, laboratory-based diagnosis, treatment

Received: 3 November 2023; Accepted: 4 January 2024; Published: 10 January 2024

INTRODUCTION

Monkeypox (mpox) is a viral infection caused by the monkeypox virus (MPXV). It was first detected in a Cynomolgus monkey at a Danish laboratory in 1958, which gave it its name [1]. Classified under the *Orthopoxvirus* genus (Figure 1) in the *Poxviridae* family and the *Chordopoxvirinae* subfamily [2], MPXV is a double-stranded DNA virus [3]. Out of the 12 different virus genera from the *Chordopoxvirinae* subfamily, the variola virus is considered the deadliest [4].

Geographically, mpox is considered endemic in the Central and Western parts of Africa, because between 1970-2003 all of the identified cases were from that region. In 1970 the first human case of mpox was reported in Zaire, Democratic Republic of the Congo (DRC) in a 9-month-old infant who presented a smallpox-like rash [5]. In 2003, the MPXV spread outside Africa for the first time due to travel-related reasons and the importation of infected animals. Seventy-one total identified cases of mpox were reported from six states in the USA in 2003 [6], the majority of them having been infected with mpox after having contact with pet prairie dogs that were housed near imported rodents from Ghana [7,8].

More recently, two separate cases were notified by the United Kingdom (UK) in 2018. Both of them were symptomatic and had recent travel history to Nigeria. These were the first documented cases in the European Union (EU) [9]. Also, in July and November of 2021, the Centers for Disease Control and Prevention (CDC) reported 2 different cases, in Texas and Maryland, that also traveled from Nigeria [10]. In May 2022, the first case of mpox that marked the beginning of an outbreak outside of Africa was diagnosed in the UK. Between the 1st of January 2022 and the 13th of September 2023, there were 117 locations with clinical cases and 92,546 people diagnosed with mpox at the global level (information

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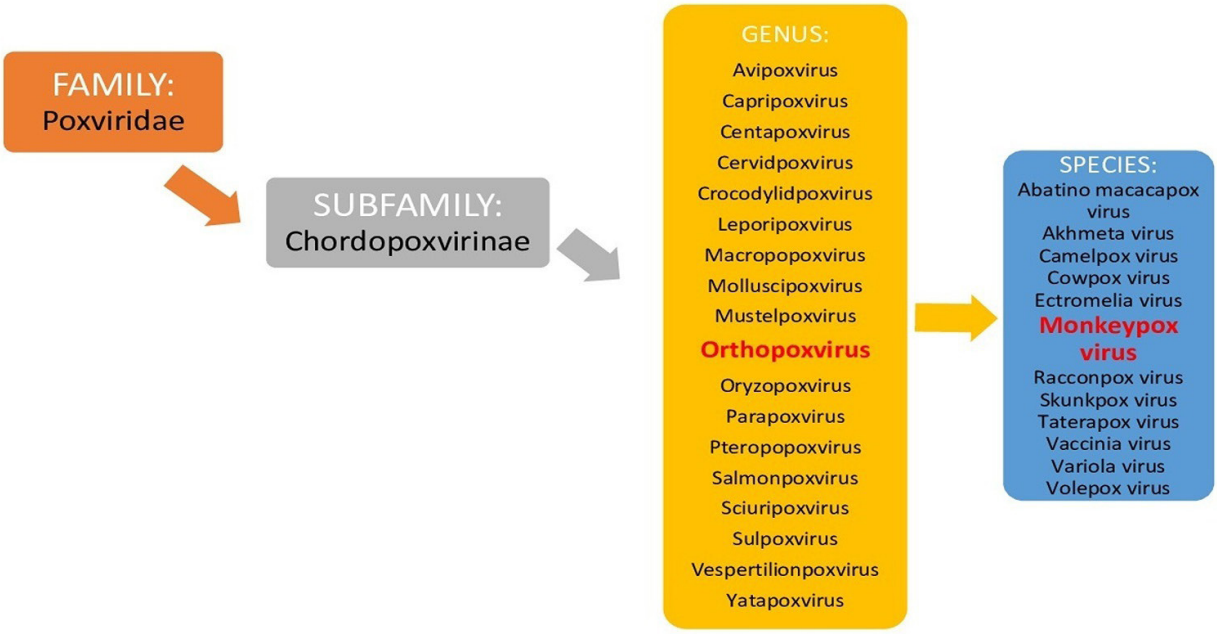


Fig. 1. MPXV position in the Poxviridae Family. Figure adapted from the `International Committee on Taxonomy of Viruses` [2]

correct as of the 27th of December 2023). According to CDC analysis, the majority of cases have been reported in the regions of the Americas and Europe [11].

On the 10th of May 2023, taking into consideration the decline in cases, and after a meeting of The Emergency Committee on mpox it was declared that `the multi-country outbreak is no longer a Public Health Emergency of International Concern (PHEIC)` [12].

METHODS

Research on relevant studies for our review that were published between January 1980 and September 2023 was conducted using the databases of the Web of Science, PubMed, WHO, and CDC. We consider factors like study design, publication date, and patient population.

The following terms were combined in the search terms: mpox; (ii) pathogenesis or spreading mechanism or clinical aspects; (iii) laboratory or microscopy or mpox detection; and (iv) prophylaxis or treatment or vaccine.

To keep the article more concise, we will present the current understanding of the pathogenesis, clinical manifestation, and laboratory diagnosis. The main goal is to reach a minimal understanding that can aid in prophylaxis and treatment of the MPXV infection for clinicians and healthcare personnel.

PATHOGENESIS AND SPREADING MECHANISM

MPXV is a zoonotic virus that is present in a natural animal reservoir in Africa. There have been identified two clades of MPXV: clade I (Central Africa) and clade IIa (West Africa). Out of the two, the first one has a higher mortality rate (10%) and is more easily transmitted from human to human. After spreading outside of Africa in 2003, the MPXV isolate causing these infections is related to clade IIa and was named clade IIb [13].

Morphologically, poxviruses are the largest viruses in animals that can be visualized by light microscopy [14]. Compared to past MPXV genomes (Figure 2), the more recent one (2022) has single nucleotide polymorphism

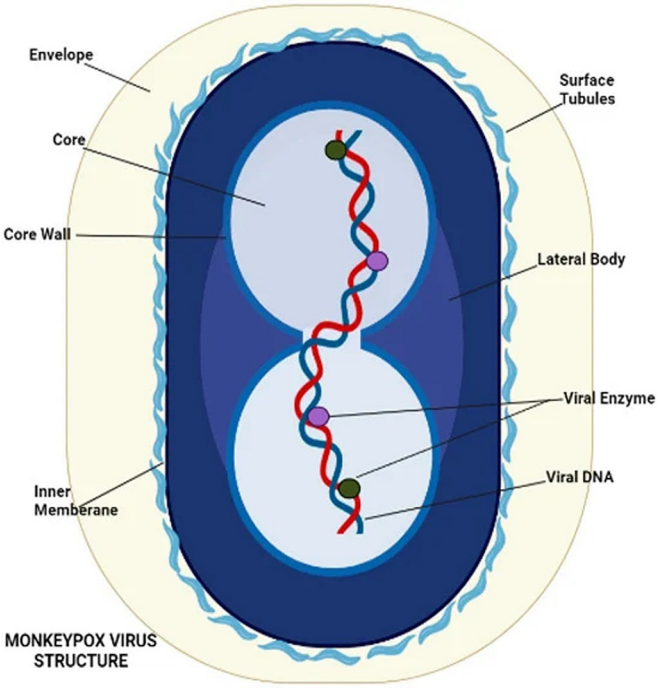


Fig. 2. MPXV structure. *Copyright permission from the publisher [17]

(SNP) which is 6 to 12 folds more than usual. A logical deduction would be that the current strain of the virus has grown to multiply more easily because of transmission between humans. There is a possible association with APOBEC3 signaling of this evolution of the virus [15]. This protein aids the resistance against the virus through genomic mutations. Small genome alterations can affect how severe the infection is and how easily transmissible it becomes. Virus evolution is heavily influenced by gene loss because it can influence the interactions between the virus and the host, which leads to a divergence increase. Compared to previously tested Orthopoxviruses, the 2022 mpox outbreak had higher mutation rates [16].

In a systemic MPXV infection, the means of virus entry is through a respiratory route, a wound, or a mucosal surface. After the initial entry, local replication occurs. Primary viremia occurs afterwards because of virus replication in local lymphatics. The second viremia is usually accompanied by fever and is determined by the replication of the virus in the reticuloendothelial system. The host response always involves the whole immune system [17]. The modulation of the immune response is expected due to the encoding of proteins that poxviruses operate in their genomes. This affects viral survival and, more importantly, disease pathogenesis [18]. As such, the entry of MPXV into the host is characterized by: attachment, hemifusion, core entry, and the nucleoprotein core of the virion deposition into the cytoplasm [19].

The fusion of the virus is activated by a low pH which dissociates a fusion suppressor protein from the entry-fusion complex (EFC). Viral RNA polymerase and early transcription factors regulate the expression of the viral gene and early promoters regulate the synthesis of viral mRNA [20]. After early proteins are produced, which start the core uncoating and DNA replication process, host-derived transcription factors control the intermediate and late transcription steps. Late gene expression is primarily associated with structural proteins, and virion assembly is followed by DNA uptake and condensation through general mechanisms [21].

The MPXV infection can spread between animals and humans or human-to-human (Figure 3). In human-to-human transmission, the virus is contracted through respiratory droplets, contaminated objects, body fluids, and skin lesions. The virus can survive for a long period on objects that can become a vector for transmission [22]. The WHO also suggests that the virus can spread through sexual contact. In the 2022 outbreak, the clinical presentation of the mpox was different from the typical historical appearance of the infection. Traditionally mpox lesions first appear on the face and oral cavity, and then may progress to the hands and feet, and further to other body parts [23].

Recent studies on lesions primarily found in the genital and anal regions suggest that MPXV may be spread through intercourse. The pattern of sexual transmission

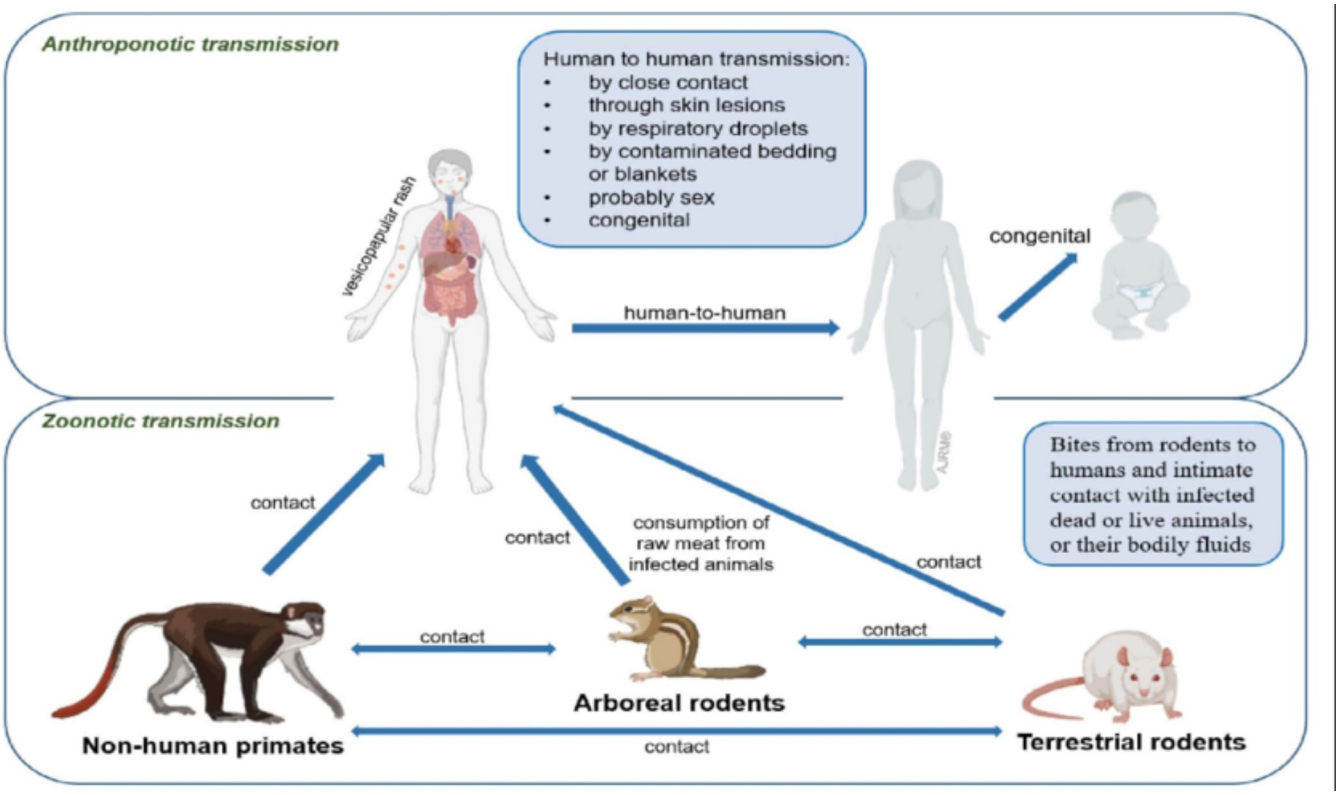


Fig. 3. Visualization of the means of MPXV transmission. *Copyright permission from the publisher [24]

points to the potential for co-infection of the MPXV virus with other sexually transmitted infections [25]. Another very recent systematic review supports the same findings regarding an overlap between sexually transmitted mpox and other sexually transmitted diseases. The findings illustrate the fact that out of all mpox cases, 28.1% were also HIV positive, 2% had syphilis, 2.1% had gonorrhea, 1% had Herpes simplex and 1% had Chlamydia [26].

Direct contact with blood or bodily fluids, sores left by an infected animal, and eating raw meat are all possible ways for animal-to-human transmission to occur. Typically, outbreaks of mpox occur in the population that hunts, handles, and eats infected wild animals [27].

CLINICAL ASPECTS

Clinical forms of the disease can range from mild to fatal. Patients usually report 1 to 4 days of fever, pseudo-influenza-like, followed by a macular-papular rash that progresses towards vesicular. In the end, the rash develops into a pustular with crusted scab lesions [28]. The patients are contagious from the day the rash starts until 4 weeks later when desquamation appears [29].

The incubation period is around 10 to 21 days, with fever, headache, and exhaustion. Especially in the case of current MPXV infection, approximately 90% of patients show severe generalized lymphadenopathy before the onset within 1 to 2 days of the rash. The rash consists of maculopapular lesions, 2 to 5 cm in diameter, which become generalized after centrifugal spread. It goes from papular to crusting, having vesicular and pustular stages in between, over a 14-21 day period, leaving depigmented scars [30]. The most frequently occurring complications include gastrointestinal events, broncho-pneumonia, sepsis, and encephalitis, so bacterial superinfection is quite common, dehydration, sepsis, encephalitis, and even death [25,31].

There are some key points worth mentioning between the classic form and the current 2022 outbreaks. Firstly, in terms of dissemination, the 2022 multicenter outbreak infection occurs through ways of men having sex with multiple partners, whereas the classic form was spread mostly intrafamilial and nosocomial. Also, by way of contraction, the classic form originates from direct contact with an infected animal reservoir that is followed by person-to-person transmission, whilst the current form is more easily transmitted by human-to-human contact [25].

Strictly from a clinical presentation and evolution of the disease, worthy of note are some main differences synthetized by Severino Jefferson Ribeiro da Silva et. al. in a 2023 article, that we condensed in Table. 1.

LABORATORY DETECTION TECHNIQUES

Because the clinical manifestation of MPXV infection is not sufficient for differential diagnosis, healthcare specialists must use laboratory methods [32]. Due to the risk of transmission, especially for healthcare workers who manipulate live viruses (virus culture or isolation), it is important to use standard precautions [33].

A few diagnostic techniques are applicable, including real-time PCR, immunohistochemistry, electron microscopy visualization, viral culture, and anti-orthopoxvirus IgG and IgM. PCR can be used alone, but the result is enhanced when combined with epidemiological data [34]. The least intrusive method of specimen collection is skin lesion material sampling, including swabs from the affected skin, roofs, lesion crust, and exudate. The CDC as well as other regional entities already described specimens and collection procedures [35]. For ease of comprehension, we have compiled a list of possible specimens that aid in identifying the virus [36]:

- Whole blood for PCR testing or serum for serological testing through blood samples,
- Viral culture techniques or PCR from samples of lesion swabs,
- Throat swabs collected during the early stages of the illness as a form of respiratory specimens,
- Cerebrospinal fluid used in severe cases to evaluate neurological symptoms, obtained through lumbar punction,
- Saliva and tears in the acute phase of infection,
- For histopathological analysis, as well as to verify the virus's existence in affected organs, tissue samples can be collected post-mortem,
- Urine and feces samples as it was observed that the

Table 1. Differences in clinical presentations and evolution between the classic and the current form of mpox. Adapted from Severino Jefferson Ribeiro da Silva et. al. Clinical and laboratory diagnosis of monkeypox (mpox): Current status and future directions [25]

Form of mpox	Clinical presentation	Clinical evolution
Classic form	Lesions on the face and extremities (linked with axillary or lymphadenopathy)	Incubation, prodromal stage, eruption phase with skin lesions
Current form	Painful inguinal lymphadenopathy, penile and vesicular rash, perianal lesions, ulcerative lesions, proctitis and pharyngitis	Incubation, prodromal stage (optional), eruption phase with skin lesions (predominantly on genitals)

virus is more likely to be detected for a longer period through this route (25),

- Seminal fluid samples that can help understand the sexually transmissible nature of the virus [36],
- In the case of men who have sex with men in particular, rectal swabs are also important to consider.

Conventional PCR and RT-PCR are the most frequently used laboratory testing techniques among all those mentioned. Two RT-PCR assays have been developed for the detection of MPXV DNA and orthopoxvirus in the 2003 US mpox outbreak. The B6R assay targets the extracellular enveloped protein genes, while the E9L-NVAR assay targets the DNA polymerase of the orthopoxvirus. The aforementioned assays are specific and sensitive (two fg or roughly ten viral genomes). The B6R assay finds MPXV isolates and no other orthopoxviruses, while the first one finds 13 Eurasian orthopoxviruses but not the North American ones. According to these tests, the 2003 US MPXV isolates were more similar to the Clade II strain than the Clade I strain in terms of genomic sequences and clinical symptoms. Although it has one SNP in the Clade II as well, the B6R assay was created to identify the Clade I MPXV. This feature validates the B6R assay's effectiveness in identifying the two recognized MPXV clades. These diagnostic assays are applicable globally in the current outbreak [38].

Histology of lesions and immunohistochemistry (IHC) show the following: spongiosis, ballooning degeneration, epidermal necrosis, viral inclusion, acanthosis, dermal perivascular infiltration, basal vacuolization, and keratinocyte necrosis from vesicular lesions [39].

The gold standard for differentiating MPXV from other orthopoxviruses is whole genome sequencing, so the MPXV associated with the current global outbreak has been sequenced. According to this sequencing, the current MPXV outbreak is associated with Clade II and is more closely linked to the MPXV isolate from the 2018–2019 outbreak in the UK, Singapore, and Israel [40].

Additionally, using the Enzyme-Linked Immunosorbent Assay (ELISA), particular IgM and IgG antibodies in the serum of mpox patients can be found after 5 days for IgM and 8 days for IgG. Regretfully, it is more frequently utilized in epidemiological research and is unable to reliably identify MPXV.

Based on the morphological features of MPXV, electron microscopy is also utilized to aid in diagnosis; however, a significant drawback is that electrons are costly and have complicated operations, making practical detection challenging.

PROPHYLAXIS AND TREATMENT OPTIONS

In May 2022, the first case of mpox was diagnosed in the UK and it marked the start of an outbreak outside Africa. Between the 1st of January 2022 and the 13th of December 2023, there were 117 locations and almost 93,000 people diagnosed with mpox at the global level. According to the CDC analysis, 98% of the reported cases originate in locations that have not historically reported cases [41]. As previously mentioned, mpox is not considered PHEIC due to the decline in cases. The declining number (Figure 4) of cases can be correlated to the core high-risk groups receiving vaccination.

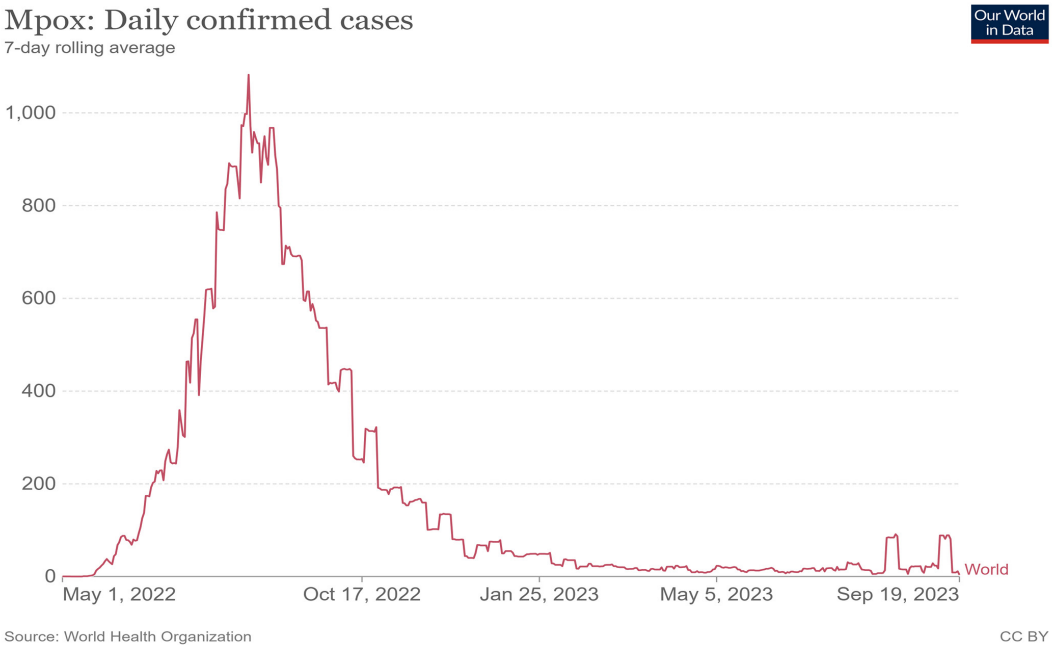


Fig. 4. Cumulative mpox cases 1st of January-13h of September 2023. https://ourworldindata.org/monkeypox [42]

The decline in cases can and should also be attributed to the fact that the disease is self-limiting, meaning it resolves in 2-4 weeks as the host develops immunity to it. Moreover, the nature of sexual transmission of mpox in the current form is still being studied and monitored, but due to the WHO and other health organizations` heeding, limiting sexual encounters, especially in the high-risk groups, may have also contributed prophylactically to the decline in cases.

According to the WHO and the CDC, some preventive actions can be taken on an individual level. For an easier way of visualization, in the following table we have systemized all of the known recommendations as of the date of redacting this article, according to general recommendations, pre-exposure, and post-exposure possibilities (Table 2).

In the US, two vaccines may be used for the prevention of mpox disease: the JYNNEOS™ vaccine (the main one that is currently used) and the ACAM2000™ vaccine. Vaccination is also recommended for people who have had exposure to MPXV as a form of post-exposure prophylaxis. People who have a greater risk of infection through potentially higher exposure to MPXV should be offered vaccination as a form of pre-exposure prophylaxis. In the USA, the ACAM2000™ vaccine is in high supply, but it presents more known side effects [44].

As a form of prevention, JYNNEOS is a Modified Vaccinia Ankara vaccine that was approved by the FDA in 2019 for smallpox and mpox disease prophylaxis. A recent review has shown that this particular vaccine provides significant protection against a possible mpox infection: the incidence of mpox was 7.4 times higher among those who had only received the first dose of the vaccine and 9.6 times higher among those who had received two doses [45]. Also, prophylactic use of Vaccinia immune globulin (VIG) should be considered in patients with immunodeficiencies in T-cell function, in whom smallpox vaccination is contraindicated [25].

Three FDA-approved treatments that showed improvement in MPXV infections are cidofovir, brincidofovir, and tecovirimat [46]. Tecovirimat (TPOXX ST – 246) is an antiviral medication that is approved by the FDA for the treatment of adults and children. The drug is safe for humans with only minor side effects and it is available in oral or injectable form. Brincidofovir was approved by the FDA in 2021 but this drug has very high toxicity and no clinical benefits on the patients treated in the UK, in the last 3 years.

DISCUSSION

Though more and more cases of MPXV infection from person-to-person contact are being reported, generally speaking, the virus is known to spread mostly from animals to humans. Sexual or intimate contact has been identified as the primary mode of transmission during the 2022 outbreak; however, other modes of transmission, such as respiratory particle involvement, contact with contaminated surfaces, and skin-to-skin contact, should also not be disregarded. Numerous studies indicate that contact with contaminated surfaces, healthcare facilities, and other people in the same household can all result in the transmission of MPXV infection [47].

Worldwide, there have been cases of infection, and the most concerning thing to know is that the virus was first spread through person-to-person contact. Because the MPXV is highly contagious, people must understand how to protect themselves and what precautions to take. Simple suggestions for prophylaxis include wearing masks when interacting with any suspected or confirmed case, particularly in medical settings; frequently cleaning surfaces and hands; being extra cautious when handling animals, pets, and raw meat; avoiding multiple partners or engaging in safe sex, etc. [48].

Most countries where the MPXV is more prevalent cannot afford expensive methods of testing and highly developed facilities and laboratories, such as the types needed

Table 2. WHO, CDC, and other authorities' prophylactical recommendations. Adapted from Malik S, Ahmad T, et al. Recent Developments in mpox Prevention and Treatment Options. Vaccines (Basel) [43]

General recommendations	Pre-exposure prophylaxis	Post-exposure Prophylaxis
- Avoidance of direct contact with animals that can carry mpox and with suspected or confirmed mpox infected individuals; - Proper sanitization and hand washing and protection during sexual contact; - Proper preparation and cooking of meat from animals; - Strict isolation of infected animals and humans; - Overall protection in medical environments: proper disinfection of hospital rooms and halls; use of medical masks and gloves.	- Highly recommended for healthcare workers as a means of individual protection and epidemiologically to help prevent the spread of the virus; - Vaccination with Food and Drug Administration (FDA)-approved vaccines such as ACAM2000TM and JYNNEOSTM, especially for medical staff predicted to have direct exposure to the virus; - Although not officially approved, some antivirals are recommended, such as Cidofovir, Brincidofovir and Tecovirimat.	- This type of prophylaxis is recommended in the case of unprotected contact with skin mucous membrane or any other bodily fluids of a confirmed infected patient; - Also recommended for people who closely share space with the infected person, as infection is possible through aerosol secretions; - In the aforementioned cases, post-exposure vaccination is recommended with informed guidance between 4-14 days to avert disease development.

for some diagnostic tools available today. As such, the development of new techniques that have more immunological sensitivity could enhance the diagnosis.

CONCLUSIONS

The last few years have been dominated by the COVID-19 pandemic, which has put a lot of pressure on the overburdened medical system around the world. In this difficult context, mpox prevention and immunization should be a top priority for every country. Thankfully, due to the efficiency of the smallpox vaccine and careful monitoring, the outbreak of mpox is under control at this time. The situation in this case, as opposed to the COVID-19 pandemic, is much more relaxed: we are dealing with a virus on which we have multiple studies and for which we also have treatment plans, including smallpox vaccination, that proves to be 80% effective against the MPXV. It is important to precisely diagnose mpox infection in patients promptly. This is possible through laboratory testing and clinical examination.

ABBREVIATIONS

APOBEC3 – Apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3

CDC – Centers for Disease Control and Prevention

COVID-19 – Coronavirus disease

DNA – Deoxyribonucleic acid

DRC – Democratic Republic of the Congo

EFC – entry-fusion complex

ELISA – Enzyme-linked immunosorbent assay

FDA – Food and Drug Administration

HIV – human immunodeficiency virus

IGG – Immunoglobulin G

IGM – Immunoglobulin M

IHC – Immunohistochemistry

MPOX – Monkeypox

MPXV – Monkeypox Virus

MRNA – Messenger ribonucleic acid

PCR – Polymerase chain reaction

RNA – ribonucleic acid

RT-PCR – real-time reverse transcription polymerase chain reaction

SNP – single nucleotide polymorphism

UK – United Kingdom

USA – United States of America

VIG – Vaccinia immune globulin

WHO – World Health Organization

ACKNOWLEDGMENT

None to declare.

AUTHORS' CONTRIBUTION

AG – Methodology and visualization

AG and AH – Investigation

AG and ML – Conceptualization

AG, SV, and OI – Original draft preparation

AH, SV, and OI – Resources

DM, VM, and RJ – Draft review and editing

ML – Project administration

ML and DM – Supervision

ML, DM, and VM – Validation

All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

There is nothing to declare.

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