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Unusual Monkeypox virus outbreak in 2022: Phenotypic and molecular characteristics

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ABSTRACT

Monkeypox virus, first identified in 1958 in Asian monkeys employed for experiments in a laboratory in Denmark and then in 1970 in humans in Africa, in the Democratic Republic of Congo (DRC), has continued to circulate for about 50 years in some regions of Africa, indicated as Congo Basin (CB) and West Africa (WA) where it has become endemic. Rare outbreaks have occurred outside endemic countries, linked to importation of the virus from endemic areas. Suddenly, since early May 2022, cases of MPX developed outside the endemic areas and their number increased rapidly. Important differences in the epidemiology of 2022 MPX compared to previous MPX spread have begun to be observed soon. First, the 2022 cases could not be traced to contacts with infected cases or animals from endemic countries. The 2022 cases are due to human-to-human transmission and not to contact with infected animals; among the transmission routes the sexual route seems to predominate, particularly among men who have sex with men (MSM). Affected countries are located on several continents, mainly in America and Europe, but also in Asia and Australia. As of mid-November 2022, 110 countries have reported MPX cases, for a total of more than 79,000 confirmed cases and 50 deaths. What is behind this new MPXV behavior and what consequences might it have? This review aims to clarify the possible underpinnings of this 2022 MPX outbreak, with a focus on the molecular mechanisms, through an analysis of the literature. Most of the studies undertaken for this purpose are concerned with the molecular genetics of MPXVs and have been based on analysis and sequence comparison of the different species of the OPXV genus, of isolates of the two different MPXV Clades, of MPXVs in circulation before and during 2022, as well as of MPXVs identified from May 2022 onwards. These studies, reveal some variations mainly in the sequences of the Inverted Terminal Repeats (ITRs), known, on the other hand, as more variable regions of the viral genome. These are variations mainly in the genes involved in the virus-host relationship, virulence and immune evasion. However, further studies are needed to confirm the real significance of these variations in virus evolution. Of particular interest is the observation, shared by many authors, of the frequency of mutations in the MPXVs 2022 genome associated with APOBEC activity. These mutations may in fact represent a marker of human-to-human transmission that characterizes the new MPXV isolates. Overall, the variability of the MPXVs 2022, grouped in the B.1 lineage of Clade IIb, is not particularly high compared, for example, to many RNA viruses. However, it is still much higher than that of the previously circulated MPXV. Even if the epidemiological curve has changed trend in the past 3 months, it remains important to shed full light on the causes the multinational MPX outbreak of 2022.

1. Introduction

According to some researchers (Tiee et al., 2018), the first evidence of the existence of the monkeypox virus (MPXV) could date back to the end of the 19th century. With certainty, the first report of this virus in monkeys dates back to 1958 (Magnus et al., 1959) and in humans to 1970 (Ladnyj et al., 1972). Later MPXV infections have been documented in other animal hosts such as mainly rodents, and several non-human primate species (Khodakevich et al., 1986; Arita and Henderson, 1968). Several cases or outbreaks in humans have been described in Africa. More rarely, Monkeypox (MPX) cases have been signaled outside Africa, always basically related to contacts with animal sources in Africa or imported from Africa. In May 2022, the scenario changed completely and in less than 7 months, from early May to November 13, there were 79.411 confirmed cases in 110 countries, 50 of which died (Monkeypox Outbreak, 2022). Only 982 cases, with 14 deaths, occurred in Africa, where the virus is endemic, all the others occurred in non-endemic

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Abbreviations

OPXV	Orthopoxvirus
VacV	vaccinia virus
CPXV	cowpox virus
MPXV	Monkeypox virus
MPX	Monkeypox virus disease
VARV	variola (smallpox) virus
ITR	Inverted Terminal Repetition
MV	mature virion
EV	extracellular virion
FDA	Food and Drug Administration
EMA	European Medicines Agency
MSM	men who have sex with men
MOPICE	Monkeypox inhibitor of complement enzymes
CAR	Central Africa Republic
CB	Congo Basin
WA	West Africa
APOBEC	apolipoprotein B mRNA editing enzyme, catalytic polypeptide;
MVA	modified vaccinia Ankara

countries and predominantly in the United States and Europe. Moreover, the transmission of the virus is no longer linked to contact with infected animals but is mainly human-to-human. This worrying new epidemiological picture prompted the World Health Organization to declare the escalating global monkeypox outbreak a Public Health Emergency of International Concern (PHEIC) (WHO Director, 2022) on July 23, 2022.

1.1. Aim of this review

This new epidemiological picture raises several questions. The first: What is the origin of this worrying new outbreak? Does it have a single origin and are the episodes recorded in the various countries somehow connected or do they have different origins and are they independent from each other? Is the monkeypox virus that has been circulating since May 2022 similar to the one responsible for the previous episodes and to which clade does it belong? Are there any differences between the viruses identified in the different countries affected? Are there signs of evolution between the viruses at the beginning of the episode and those that appeared in the following months? Therefore, this review aims to see if there are answers to these questions in the literature to date that can help address and resolve this new and worrying outbreak of MPX.

2. The monkeypox virus - who is it?

2.1. Taxonomy and structure of the virus (Moss, 2012; Tesgera et al., 2019; Lansiaux et al., 2022)

MPXV belongs to the *Poxviridae* family, *Chordopoxvirinae* subfamily, *Orthopoxvirus* (OPXV) genus, which includes several species. The most famous member of the genus is the smallpox virus. Although in the late 1970s the virus was eradicated and smallpox vaccination ceased, studies have continued and continue to do so because the virus continued and continues to pose a risk due to its possible use in bioterrorism. Other, closely related, members of this genus which can infect humans, are the vaccinia virus (VacV) and the cowpox virus (CPXV). OPXV genus includes also camelpox virus, horsepox virus, ectromelia virus and still others. The members of the genus *Orthopoxvirus* obviously share the fundamental characteristics and are antigenically and genetically similar so that they are serologically cross-reactive and cross-protective. But they are also clearly distinguishable for many aspects that justify, among other things, the differences in host spectrum and in pathogenicity. They are large viruses (200–400 nm in diameter), with characteristic oval or brick particles, observed under the electron microscope. Their genome consists of a linear double-stranded DNA of about 200 kb, containing about 200 ORFs, of more than 180 nucleotides or 60 amino acid residues. A unique feature of these viruses is that, although they are DNA viruses, they replicate in the cytoplasm. Therefore, all the proteins necessary for viral replication are encoded by the viral genome. The Orthopoxvirus genome is organized in a central, more conserved portion, that comprises about 75% of the viral genes encoding essential functions for viral replication such as proteins involved in DNA replication, transcription, virion assembly and release. All essential genes common to all members of the genus are present in this central region of MPXV, from ORFs C10L to A26R, nucleotide positions around 56.000–120.000, with homology around 90%. This region is flanked by more variable sequences, known as Inverted Terminal Repetitions (ITRs), containing tandem repeats, with genes implicated rather in host relationship, pathogenicity, and immunomodulation. At least 4 ORFs are present in the ITR of MPXV, whereas VARV lacks ORFs in this region. The viral DNA, together with DNA-dependent RNA polymerase and transcriptional enzymes are protected within a core protein. The core in turn is covered by a lipoprotein envelope and possibly by a second envelope different from the previous one. In fact, there are two types of viral particles or virions, the mature intracellular particles, MV, which are released commonly by cell lysis, and the extracellular particles, EV, which are released from the infected cell commonly by exocytosis, equipped with an additional envelope. Both types of particles are infectious. MVs are responsible for transmitting the virus from one host to another, while EVs are responsible for spreading the virus from cell to cell within the same host. The membrane that surrounds the MVs is composed of at least 20 proteins and 6 other proteins are associated with the outer membrane of the EVs.

2.2. Life cycle (Lansiaux et al., 2022) (Gong et al., 2022) (Kaler et al., 2022)

MPXV is able to infect nearly all mammalian cell types by recognizing and binding ubiquitous cell receptors such as glycosaminoglycans and extracellular matrix components. Four viral proteins are involved in the attachment of MVs of Vaccinia virus (D8, A27, H3, A26). Corresponding proteins in MPXV are E8, A29, A28 and H3 (Gong et al., 2022). Only H3 is present in all orthopoxviruses. In contrast, the EVs attachment proteins are not yet known. At least 11 proteins, which are conserved in all poxviruses, (A16L, A21L, A28L, F9L, G3L, G9R, H2R, J5L, L1R, L5R, ORL) forming a transmembrane complex, are involved in the subsequent entry step. Virus entry may occur by fusion at the cellular plasma membrane at neutral pH or by endocytic route at low pH. Entry of EVs requires the loss of the outer membrane at the cell surface. Then, the core is released in the cytoplasm and transcription by the DNA-dependent viral RNA polymerase begins. Poxviruses DNA replication occurs in particular structures of the cytoplasm called factories, once referred to as Guarneri bodies. Expression of early viral genes occurs first. The products of early genes include a range of extracellular and intracellular modulators that contrast the host's defenses at different levels and in different ways (Kaler et al., 2022). Early genes expression is followed by intermediate genes and finally late genes. Host cell transcription factors also contribute to a more efficient development of the intermediate and late phases. In this regard, two host gene complexes have been identified, GARP (Golgi-Associated Retrograde Protein) and COG (Conserved Oligomeric Golgi) complexes, that act at the level of the Golgi apparatus. These complexes play an important role in the completion of the viral cycle and in the release of the virus. As the late viral gene products accumulate, morphogenesis and viral particle assembly begins. Intracellular Mature Virions (IMV) are formed which migrate through the microtubule system towards the cell periphery and through the Golgi apparatus acquire a membrane thus forming Internal extracellular virions (IEV). IEVs by merging with the cell membrane lose one of their outer membranes and become cell associated EVs (CEV). These particles can be

pushed towards neighboring cells through actin polymerization and escape from the cell.

2.2.1. Inhibitors of virus replication (Lansiaux et al., 2022; Gong et al., 2022; Brown et al., 2022; Akazawa et al., 2022)

Antiviral drugs, in most cases, are intended to interfere with certain phases of the virus' replicative cycle. This requires a thorough knowledge of the virus' life cycle.

One of the antiviral compounds used, albeit to a limited extent, in the most serious MPXV infections, is Tecovirimat, trade name Tpoxx (TPOXX, ST-246). This compound was approved by the FDA in 2018 for use against smallpox and by the EMA in 2022. Its efficacy against MPX has not yet been demonstrated. Its target is the VP37 protein; by binding to this protein, the compound hinders the formation of the envelope.

Cidofovir is an acyclic phosphonate nucleoside analog that inhibits viral DNA polymerases and is used in the treatment of many DNA virus infections. It can only be administered intravenously and may have important side effects, such as nephrotoxicity. Instead, Brincidofovir, which is a lipid conjugate of cidofovir, approved by the FDA in 2021 against smallpox, is more suitable.

Under the pressure of the current epidemiological situation of MPX, compounds already approved for use against other infection agents were evaluated for their possible activity towards MPXV. In Akazawa's study (Akazawa et al., 2022), three compounds were identified atovaquone (anti-Pneumocystis jiroveci), mefloquine (anti-malarial), and molnupiravir (anti SARS-CoV-2) which also appear to be active against MPXV where they act at different phases of the replication cycle.

Many other antiviral compounds, which may interfere with various stages of viral replication, are being tested, but are not yet available for the treatment of MPXV infections. The use of Vaccinia Immune Globulin Intravenous has also been proposed and attempted (VIGIV), but its efficacy is unproven.

3. MPXV pathogenesis

MPX is a viral zoonosis. Despite the name, monkeys are not the only or the main reservoir of the virus. Other mammals, especially rodents, can become infected with the virus and transmit the infection to humans.

Table 1
Animals in which natural MPXV infection has been demonstrated by virus isolation in cell culture or by viral nucleic acid detection (Parker and Buller, 2013) Much more data are available on experimental MPXV infections and serosurveys in different animal species, mainly rodents and monkeys, in the African endemic countries. In most cases, however, serological investigations are based on the search for anti-OPXV antibodies that could also have been induced by other members of the genus and not specifically by MPXV. Although relatively unlikely, this hypothesis must be taken into account.

Animal host	Year	Geographical Location
Asian monkey (*M. Fascicularis*)	1958	Copenhagen[a]
Asian monkey (*M.Fascicularis*)	1959	US
	1962	
Giant anteaters (*Myrmecophaga tridactyla*)	1964	Rotterdam Zoo[b]
Asian orangutan (*Pongopygmaeus)*		
African gorillas (*Gorilla gorilla*)		
several other monkey species		
Wild squirrel (*Funisciurus anerythrus*),	1985	Zaire
Prairie dogs (*Cynomys ludovicianus*),	2003	US
Gambian-pouched rats (*Cricetomys* sp.)		
Southern opossum(*Didelphis marsupialis*)		
African hedgehogs (*Atelerix* sp.)		
Woodchucks (*Marmota monax*)		
Jerboas (*Jaculus* sp.),		
Short-tailed opossums (*Monodelphis domestica*)		
Sooty mangabey (Cercocebus atys)	2012	Côte d'Ivoire

[a] Monkeys imported from Singapore.
[b] At the origin of the outbreak Giant anteaters of Central/South America recently imported at the ZOO.

Table 1 shows, by way of example, animals found with active virus infection as a result of a natural infection (Parker and Buller, 2013).

MPXV can penetrate the human body and be transmitted from host to host via several routes. The classic routes, more frequent at least until May 2022, when the source of infection was predominantly infected animals, are the respiratory, nasopharyngeal, and oropharyngeal routes, via infected respiratory droplets, and the intradermal route through direct contact with skin and mucosal lesions. Cases of maternal-fetal transmission have also been demonstrated (D'Antonio et al., 2022). In the current 2022 outbreak , where human-to-human transmission predominates, the sexual transmission route is predominant with particular reference to transmission between homosexuals or among men who have sex with men (MSM). Many of the later stages in the infection pathogenesis are also linked to the route of entry of the virus into the host.

According to a classical pathogenetic pattern, similar to that of smallpox virus, MPXV initially replicates at the site of penetration in the host. This initial replication is followed by a low-grade primary viremia by which the virus reaches the local lymph nodes where it replicates further. As a result, a secondary, more massive, viremia develops, which carries the virus to distant lymph nodes and other organs. These steps take place during the so-called incubation period through which the virus reaches local lymph nodes where it replicates further. As a result, a secondary, more massive viremia develops, which carries the virus to distant lymph nodes and other organs. These phases occur during the so-called incubation period. After this period, non-specific symptoms characterizing the prodromal phase appear, while the secondary viremia proceeds and carries the virus to the lymphatic organs and characteristic target organs, such as the skin and sometimes others (such as the lungs, eyes, gastrointestinal tract, etc.).

Some preliminary studies also suggest a possible role of altered levels of cytokines (particularly Th2-type) in the severity of infection (Johnston et al., 2015).

3.1. Clinical manifestations (Lum et al., 2022; Benites-Zapata et al., 2022)

MPX has an incubation time of 5–21 days followed by a prodromal phase, with non-specific symptoms, of about two days and then by the appearance of specific manifestations, similar to those of smallpox. Fever, chills, malaise, headache, sore throat, may appear during the prodromal phase. In a high percentage of MPX cases, swollen lymph nodes are also observed in submandibular, cervical, axillary, and inguinal regions, which are absent in smallpox. The prodromal phase is followed by the appearance of a characteristic maculopapular rash. Lymphadenopathy may occur either in the prodromal phase or during the rash. The rash occurs in 95% of cases, fever in 72%, itching in 65% and lymphadenopathy in 62%. Other less frequent manifestations may be fatigue, sore throat, headache, cough, myalgias, photophobia, arthralgia, difficult breathing, conjunctivitis, nausea/vomiting and diarrhea. Possible complications of MPX may be ocular lesions in 9% of cases, secondary bacterial infections in 18%, hemorrhagic pustules in 1%, ulcerative or necrotic lesions in 10%. There are also cases of MPX characterized exclusively by genital lesions (Patrocinio-Jesus and Peruzzu, 2022). The rash in the current outbreak most frequently affects the pelvic and inguinal region (in 75% of cases compared to 30% in African patient). It is also worth noting that in the current epidemic, transmission between homosexuals or men who have sex with men (MSM) is very frequent (De Baetselier et al., 2022).

In contrast to previous episodes, in the cases observed in the current outbreak sometimes the appearance of the rash is not even preceded by the prodromal phase. Furthermore, the rash can also be characterized by a single lesion in the genital area. This could lead to misdiagnoses, suggesting another sexually transmitted infection (Thornhill et al., 2022). Cytokines modulation could also affect the severity of MPX (Johnston et al., 2015).

An index of the severity of the disease is often considered the number

of skin lesions. When> 100 (>100–250) are indicative of a severe form (Lum et al., 2022).

Finally, it is important to note that asymptomatic infections may also occur, whose frequency is unclear (De Baetselier et al., 2022). This phenomenon could have an important effect on virus transmission.

3.2. Immune evasion

Like other OPXV, the Monkeypox virus encodes for numerous virulence factors. Prominent among these are immune evasion factors which enable the virus to overcome some of the host's immune defenses (Table 2). Not all the proteins involved are known. Among those whose function has been identified are proteins that interfere with the signaling pathways induced by the activation of recognition receptors and proteins, like F3, that can inhibit the antiviral response of IFN (Lum et al., 2022; Arndt et al., 2015); MPXV encodes BCL-2 like proteins of VACV that block activation of NF- κB and IRF3; in addition, MPXV encodes a number of Ankyrin-like proteins also able to control NF- κB activation; the BR-203 protein is reported to play a role in blocking apoptosis of infected lymphocytes, BR-209 encodes an interleukin-1β binding protein that hinders the bond of IL-1β to its receptor; the gene C3L (of VACV, D14L in MPXV) encodes a protein that inhibits the first steps of the complement cascade. In MPXVs this is also referred to as MOPICE, an inhibitor of monkeypox complement enzymes. Interestingly, it is absent in the less virulent MPXV clade II strains (Weaver and Isaacs, 2008). A list of 32 genes and proteins associated with virulence and immune evasion in MPXVs and VACV is reported by Fok-Moon Lum et al. (2022). A unique characteristic of MPXV is its ability to evade both CD4 + and CD8 + antiviral responses, in an MHC I independent manner. However, the mechanisms underlying this particular phenomenon have yet to be elucidated (Hammarlund et al., 2008).

4. History and epidemiology of human Monkeypox virus

As reported in the Introduction, Monkeypox virus (MPXV) was first identified in 1958 in Denmark in a research center for the study of polio vaccines (Statens Seruminstitut, Copenhagen) (Magnus et al., 1959). For these studies the facility was regularly supplied with Asian monkeys, both Macaca fascicularis (cynomolgus) and Macaca mulatta (rhesus monkey). Among a group of M. fascicularis monkeys imported from Singapore for about 2 months, a first episode of disease occurred in a low percentage of animals. A subsequent episode occurred again in M. fascicularis monkeys a few months after the first one. Other episodes of MPXV infection in monkeys imported from various Asian countries were documented in the following decade in the US and the Netherlands.

It was not until 1970 that the first case of MPXV infection in humans was identified, in a 9-month-old child, in the territory of Basankusu in the Democratic Republic of Congo. The source of contagion in this case was not identified (Ladnyj et al., 1972).

Subsequently, as Table 3 shows, 47 cases were documented in Africa in the 1970s (Breman et al., 1980). However, it is necessary to specify the number of cases in the various outbreaks, in this period as in later ones, reported by the various available sources does not always agree (Sklenovska and Van Ranst, 2018; Bunge et al., 2022; Kozlov, 2022). In the 1980s, more than 300 cases are documented (Jezek et al., 1987), in most cases in the RDC (former Zaire). Even in the 90s the MPXV mainly affects the DRC where 71 cases are reported and 4 cases are described in Gabon (Mukinda et al., 1997). The Democratic Republic of the Congo has been the country most affected by the Monkeypox virus since it was first detected in humans in 1970, but the circulation of the virus increased significantly after the cessation of smallpox vaccination in 1980. Active surveillance programs were conducted between 1981 and the 1986 and between 2005 and 2007, to assess the intensity of the circulation of MPXV in this geographical area after the cessation of smallpox

Table 2
Immune evasion activities of MPXV (Lum et al., 2022; Weaver and Isaacs, 2008) Most of these activities are shared by other OPXVs. Modifications in the proteins involved, or the lack of their expression, may modify the virulence of the strains.

Immune evasion activity	Gene/viral protein involved	Protein function
Interference with cellular signaling pathways	Several (BCL-2)- like proteins	inhibition of NF-kB and IRF3 activation inhibition of PK-mediate pathway
Apoptosis escape	BCL-2-like proteins, B12R, B19R, C7L, D5R	Inhibitors of apoptosis
Prevention IFNα/β signaling	B16R	IFNα/β binding proteins
Antagonism of immune mediators	B19R, D14L (MOPICE)	IFNγ binding proteins Inhibition of the Complement system
	B14R J3R and A41L	IL-1β binding protein CC and CXC chemokine binding proteins
Reduction of immune cells activation	N3R, B10R	inhibitor of natural killer cell- mediated NKG2D- dependent cell lysis Inhibitor of intracellular trafficking of MHC class I molecules

Table 3
Number of human cases of MPX in the different decades before May 2022 (Bunge et al., 2022; Kozlov, 2022; Guarner et al., 2004) The circulation of the virus until 2022 occurred almost exclusively in certain areas of Africa, defined as MPXV endemic regions. The hardest hit are the DRC and, since 2017, the Nigeria. In most cases where both data are available, a large difference is observed between the number of suspected cases and the number of confirmed or probable cases. This difference is attributable to several factors, among which, first of all, the unavailability of all samples for laboratory confirmation tests. The eradication of smallpox in the late 70s and the cessation of smallpox vaccination had important consequences for the spread of MPXV.

	Endemic regions	Non endemic regions	Number of cases	
Years	Geographical Locations		confirmed [c]	Suspected[c]
1970–1979	DRC		39	NA°
	Nigeria		3	
	Other Africa Countries[a]		6	
Eradication of smallpox and cessation of vaccination				
1980–1989	DRC		343	404
	Nigeria		0	0
	Other African Countries[a]		14	NA°
1990–1999	DRC		60	511
	Nigeria		0	0
	Other African Countries[a]		9	NA°
2000–2009	DRC		51	10,027
	Nigeria		0	
	Other African Countries[a]		60	>150
2003		US	47	
2005		Sudan	19	
2010–2019	DRC		NA°	>18,000
	Nigeria		184	
	Other African Countries[a]		42	
2018		UK[b]	2	
2018		Israel[b]	1	
2018		Singapore[b]	1	

°not available.
[a] Central Africa Rep., Liberia, Sierra Leone, Congo Rep., Cameroon.
[b] These cases have been imported from Nigeria as described in the section 4.
[c] Cases are considered suspected on the clinical basis, are confirmed after laboratory diagnosis (virus isolation, PCR, detection of specific IgM).

vaccination. Thirty years after smallpox eradication, the incidence of MPXV infection has increased 20-fold. However, the protection given by the smallpox vaccine is long lasting. In fact, individuals vaccinated for even 25 years still have a lower risk of contracting MPX. Moreover, the incidence of infection may also be affected by a combination of several factors (ecological and socials, like deforestation, conflicts, displacement), improved surveillance and diagnostic capacities (Rimoin et al., 2010). In the early 2000s, in addition to new episodes of Monkeypox virus in the Republic of Congo and the Democratic Republic of Congo, MPXV cases occurred for the first time outside the endemic areas (Guarner et al., 2004) in 2003, 47 cases of MPX were documented in the US, linked to importation from Ghana of infected rodents (Gambian pouched rat, dormice and rope squirrels). During transport to the US., these rodents infected prairie dogs. The latter in turn transmitted the infection to humans. There have been no fatalities and no human-to-human transmission was observed. Center for Disease Control and Prevention (CDC) recommended the smallpox vaccine (ACAM2000TM), during the 2003 MPXV outbreak in the US, that shows to reduce the symptoms. In addition, in 2005 for the first time 19 cases of MPX were reported in Sudan (Damon et al., 2006). In the new millennium the circulation of MPXV continues in the endemic areas of Africa. In 2017 in Nigeria, where no cases of MPX had been reported for decades, the virus re-emerged on a massive scale. From September to December 2017, 197 suspected cases were recorded in 13 countries, 68 of which were confirmed. In 2018, 104 suspected cases were recorded in 19 states, of which 38 were confirmed. In 2019, 113 suspected cases were reported 46 of which were confirmed (Alakunle et al., 2020). These cases are of particular importance as travelers from Nigeria have exported the virus and disease to non-endemic areas such as Israel, Singapore, and the UK (Mauldin et al., 2022) with evidence of human-to-human transmission. The case described in Israel in 2018 (Erez et al., 2019), imported from Nigeria, involved a 38-year-old man. Before leaving Nigeria, he had had contact with two rodent carcasses in his Nigerian residence on 17 September 2018. Back in Israel, on 29 October he noticed the appearance of some itchy lesions on his penis. The following day fever and chills also appeared. The clinical manifestations increase again in the following days. The patient remained in isolation at home until complete recovery. There were no secondary cases of transmission of the infection to contacts in the home or between healthcare workers. The case imported into Singapore (Yong et al., 2020) also involved a 38-year-old man resident in Nigeria. This man had attended a wedding in Nigeria from 21 to 23 April and ate barbecued bushmeat, which, in the absence of other risk factors, could have been involved in the transmission of the virus. In the following days the man travelled to Singapore on business and here on April 30 the first symptoms of MPX appeared. He was practically isolated for a few days in the hotel before being admitted to hospital. All possible contacts were identified and monitored for 21 days. In no case did the disease or a secondary infection developed. Two cases of MPX were imported from Nigeria to the UK again in September 2018 (Vaughan et al., 2018). A first case involved a 32-year-old Nigerian naval officer, who had travelled to London from Nigeria to attend a training course in Cornwall. Arrived in London on September 2, he travelled by train to the naval base and already on September 3 he manifested the first symptoms that had already appeared a few days before leaving his country and that increased in the days immediately following. The second case concerned a 36-year-old British citizen returning from a 22-day trip to Nigeria. Even before he left Nigeria, a rash had appeared on his face and he had been suffering from malaise for about a week, so much so that he was treated with antibiotics. In the days following his return to the UK, the symptoms worsened and the patient was hospitalized and isolated. The patient reported possible contact in Nigeria with a person who had suspicious skin lesions and the consumption of potentially risky foods. A secondary case of MPX occurred 18 days after contact among the healthcare workers who assisted this patient. No other suspected cases were identified among other contacts. Another case of MPX imported from Nigeria to the UK was recorded in 2019. In addition, two further cases of MPX imported from Nigeria were reported in the UK in 2021 resulting in to two cases of human-to-human transmission, one secondary and one tertiary (Adler et al., 2022). Since 7 May 2022, an increase in MPXV cases has been observed in the UK, most of which can no longer be proven to have been imported from Nigeria or other African countries where MPXV is endemic. Furthermore, as reported in the Section 1. Introduction, a similar situation has developed rapidly in several other countries mainly in Europe and the US and also in Australia and the Middle East.

The epidemic curves with the number of cases in the various regions show a strong increase until mid-August. Then, a sharp decrease begun so that fewer cases were reported each week than in the previous week. For example, in week 45, between 7 and 13 November 1114 cases were reported, while in week 44, between 31 October and 6 November, there were 1348 cases, a decrease in new cases of 17.4%. In 63 countries, no new cases were reported in the last 21 days (Monkeypox Outbreak, 2022).

5. Variability of MPXV. Phenotypic and molecular aspects

The current outbreak of MPX 2022 differs from previous episodes in several respects: the unprecedented increase in the number of cases in a few months (May to at least August 2022), the more frequent routes of transmission and target population of infection, and also some clinical differences. For this reason, numerous studies have been undertaken to understand the basis of the different pattern of this epidemic compared with epidemics that have occurred in past years. Studies on the genomic differences between the various members of the Orthopoxvirus genus have also been helpful in beginning to understand the bases of some of the differences observed among MPXVs.

Several molecular mechanisms may underlie the differences between different members of the genus such as the appearance of point mutations or small insertions or deletions, the acquisition of new genes through horizontal transfer of genetic material and recombination and again the fragmentation and loss of genetic material with loss of related functions (Hendrickson et al., 2010).

It is interesting to note that cowpox virus which has the widest host spectrum among the Orthopoxviruses, possesses the greatest number of genes, while the variola virus that infects only humans has a limited number of genes despite being the most pathogenic compared to the other species in the genus. Cowpox virus genomes range from 228,250 to 223,666 bp and two isolates of variola virus were found to be 188,062 and 185,853 bp long, respectively (Table 4). There are numerous VACV strains with different genome sizes as a result of laboratory manipulations, introduced to improve vaccine performance. For instance, the genome of vaccinia virus strain Ankara is of 177,923 bp and strain WR is of 194,711 bp. As for MPXV, it has been reported that the MPXV strain named ZAI-96-I-16 (Clade I), isolated during an outbreak in Zaire (later

Table 4
Comparison of genome length of few species of OPXV (Hendrickson et al., 2010) As it can be seen, ITRs in smallpox virus (VARV) are small in size and do not contain ORFs. Variable sized ITRs are observed in MPXVs with varying numbers of ORFs.

Species	Name (abbrev.)	Genome length	ITR length	ITR genes
Cowpox virus	CPXV-Ger	228,250	7374	5
	CPXV-Gri	223,666	8303	5
Variola virus	VARV-BRZ	188,062	518	0
	VARV-KUW	185,853	522	0
Vaccinia virus	VACV-WR	194,711	10186	6
	VACV-MVA	177,923	9644	2
Monkeypox virus	MPXV-WR[a]	199,195	8749	6
	MPXV-ZAI[b]	196,858	6378	4
	MPXV-LIB[b]	200,263	NA[c]	NA [c]
	MPXV-USA[a]	198,780	NA[c]	NA[c]

[a] Clade IIa.
[b] Clade I
[c] not available

named Democratic Republic of Congo), has a genome of 196,858 bp, and the strain MPXV WR (Clade II) isolated in the US during the outbreak of 2003 has a genome of 199,195 bp. The latter data could be related to the broad host spectrum of MPXVs and to the different virulence that characterizes the two MPXV clades.

Epidemiological and clinical observations showed that MPX cases in geographically distinct regions of Africa had a different pattern; in Central Africa Republic (CAR), in the Congo Basin area (CB), more cases of MPX were documented and the disease was characterized by more severe forms with higher mortality (about 10%), while in West African countries (WA) fewer cases were observed, the disease was mild with lower mortality (3.6%), sometimes absent. In addition, more frequent human-to-human transmission, with longer transmission chains, was documented in the Netherlands than in WA, where almost exclusively animal-to-human transmission cases were observed. These observations have stimulated more and more extensive studies, many of them based on comparing the genomic sequences of viral isolates of different origins, in order to identify the genetic basis of these differences (Likos et al., 2005; Chen et al., 2005).

Genome sequence analysis has led to the division of MPXVs, according to a recent revision of WHO classification, into two clades, Clade I and Clade II. Clade I was previously referred to as clade 1, and also referred to as CB, Congo Basin, or CAR, Central African Region, in several papers. Clade II was previously referred to as West African, WA. The latter includes two subclades IIa and IIb. The viruses involved in the 2022 outbreak belong to clade IIb (Monkeypox: experts give virus variants new names, 2022).

Studying the genetic basis of the observed differences between different clades of MPXV may provide useful data to elucidate the genetic basis and origin of the new 2022 outbreak.

Several studies have focused on comparing viral sequences and identifying individual virulence and pathogenicity genes. A different approach has been followed by Lopera et al. (2015) to identify the genetic basis of the different virulence of the two clades of MPXV, clade I (CB or CAR) and clade IIa (WA). First, they identified in the genome of the two clades and other related OPXV regions with high variability in the frequency of mutations, deletions and insertions, the presence of truncated genes. In particular, they identified two regions at the 5′ and 3′ ends of the viral genome (R1- Open Reading Frame 17 to 32 and R2- Open Reading Frame 179 to 193) containing immunomodulatory genes and genes for the host spectrum. They then constructed three recombinant viruses with deletions in these regions and tested them in vitro and in vivo in a mouse model. Deletions in the R1 region or the R2 region alter virulence and pathogenicity, respectively, while deletions in both regions lead to an attenuation of the virus. Instead, the comparison of virus sequences of the CB clade (now clade I) and of the WA clade (now clade IIa) allowed to hypothesize possible genes involved in the different pathogenicity found between the two clades (Likos et al., 2005; Chen et al., 2005) Chen et al. studied the virulence of both clades using an experimental animal model, by aerosol infections of cynomolgus monkeys. In this experimental model the isolate of clade I (CB) proved to be more virulent than that of clade II a (WA). They genomic sequences of WA isolates were also compared with those of a CB strain. The results showed that the WA isolates and the CB strain are genetically distinguishable and belong to different clades. Then they tried to identify genes that might be related to virulence. The sequences of orthologous genes from strain SL-V70, chosen as the prototype of West African isolates, were compared with the sequences of ZAI-96, representative of CB isolates. This comparison revealed 5 genes that could be associated with the virulence of CB isolates: the D14L sequence of the ZAI-96 strain, which codes for an inhibitor of complement enzyme, is absent in SL-V70 due to a DNA deletion. – ZAI-96 D10L sequence, encoding a host range function, has a 4 bp deletion in SL-V70, which could eventually result in an alteration of function – ZAI-96 B10R encodes a protein of 221 aa whose function is not yet well defined, but could be related to virulence. Modifications in this ORF in SL-V70 would lead to a fragmented protein. The same would occur in the case of B14R which encodes an IL-1 binding protein. – ZAI-96 B19R could encode a serine protease inhibitor; changes observed in the ortholog in SL-V70 could alter the protein function. However, it is clear that further studies are needed to confirm and to correctly interpret these observations. That is, it must be shown that the modified proteins are actually capable of changing the behavior of the virus by modifying its virulence.

Genomic variability was also studied within the same Clade, the Clade I, circulating in the DRC. Four distinct lineages were identified in isolates obtained between 2005 and 2007, correlating with primary and secondary cases of human infection. These different lineages are characterized by deletions and loss of genes which could be related with increased transmissibility and pathogenicity (Kugelman et al., 2014).

Comparison of the genome sequences of the viral strains from previous outbreaks with the sequences of the viral strains identified so far in 2022, together with the collection of epidemiological and clinical data, can significantly contribute to understanding the origin of the 2022 multinational outbreak, the prevalent modes of transmission, and pathogenicity.

In an attempt to explain the origin and characteristics of the 2022 episode, Wassenaar et al. (2022)) compared the genomic sequences of 5 strains identified in Nigeria in 2017, 6 strains of 2022, 5 from different European countries and one from US, and two strains dating back to 1970. Several differences emerged, of particular interest were changes in intermediate gene transcription factor VITF-3 and three amino acid changes in the helicase gene that could have important consequences on viral replication. However, more studies are needed to establish the real impact of these observations.

Gigante et al. (2022) analyzed the genome sequences of 9 MPXV isolates of the ongoing outbreak (within the Clade IIb) in United States. These turn out to constitute two different lineages: the main one, indicated as B.1, and a minor variant A.2. The sequences of the United States. isolates of lineage B.1 constitute a monophyletic group with the European MPXV sequences; there appears to be considerable similarity between the sequences of this group and those of an isolate from a 2021 Maryland case imported from Nigeria. In contrast, two other US sequences with about 80 nucleotide substitutions in comparison to the previous ones B.1 seem more similar to an isolate from a case imported from Nigeria to Texas in 2021. This could indicate a different introduction in the United States of these two lineages. The differences between B.1 and A.2 lineages were studied also by other authors (Jolly and Scaria, 2022). From these studies it emerges that the appearance of lineage A.2 dates back to July 2021 and therefore would greatly precede the appearance of lineage B.1. It was calculated that A.2 would have a mean nucleotide substitution rate of $5.53 \times 10 -5$ substitutions per base/year, lower than that calculated for B.1 ($1.13 \times$ 10-4). A.2 would have continued to circulate cryptically until the outbreak occurred in May 2022.

Furthermore, Gigante's study (Gigante et al., 2022) highlights another peculiarity that seems to characterize the 2022 strains, namely the presence of mutations indicative of human APOBEC3 cytosine deaminase activity.

APOBEC3 proteins are a family of cytidine deaminases, which through the deamination of cytidine lead to its conversion into uracil. They can thus induce mutations in the viral genome, up to blocking viral replication, thus exercising an innate antiviral defense activity. The antiretroviral activity of APOBEC3 enzymes has been much studied, in particular against HIV. However, antiviral activity of APOBEC3 has also been documented against some DNA viruses such as HBV, HPV and others. APOBEC3 enzymatic activity can lead to the blockade of viral replication with genome degradation or the development of viral variants and virus evolution. In the course of HIV infection, APOBEC3 expression has been shown to increase. However, some viruses, including HIV, are able to evade this host defense mechanism.

In this regard, Gigante et al. (2022) documented frequent mutations 5′ GA-to-AA, characteristic of APOBEC3 activity in the sequences of 2022

isolates of the B.1 lineage since 2017, that were not found in isolates of the CB or WA clade before 2017. An initial report of O'Toole and Rambaut (O'Toole and Rambaut, 2022) documented that 42 of 47 nucleotide changes, observed in the 2022 MPXV isolates are of a particular type, i.e. from GA to AA or from TC to TT, attributable to the activity of the APOBEC3 enzyme. Instead, mutations associated with APOBEC3 activity were rare in MPXV isolates before 2017. Several other observations in the literature agree on this point (Isidro et al., 2022; Wang et al., 2022; Colson et al., 2022; Chen et al., 2022). Mutations linked to APOBEC3 activity could be a marker of evolution of MPXVs and of increasing human to human transmission.

In addition, several other changes are signaled as characteristic of the new lineage of 2022 isolates, B.1. Isidro et al. (2022) report another interesting difference between the 2022 MPXVs and a 2018 reference strain, the presence of 3 amino acid substitutions in protein B21, known as an important target of the antibody response. Similar results are also reported by Wang (Wang et al., 2022) who confirms, among other things, the presence of 3 amino acid substitutions in the OPG210 protein (homolog of B21R), an important target of the immune response and also reports the presence of 4 mutations in the OPG105 protein.

Jaydee Sereewit et al. (2022) analyzed clinical samples from cases of MPX diagnosed in Washington state and Ohio in 2022. They focused on finding mutations capable of ORF disruption by whole genome sequencing. Such mutations of different magnitude were found in different ORFs in 25 genomes. These mutations affected different genes, all non-essential, located in the terminal regions, with the exception of one for the DNA-dependent RNA polymerase subunit rpo132 which could take advantage of an alternative start codon. The other non-essential genes would be part of those coding for immunity evasion factors.

To understand the evolution of MPX during the 2022 outbreak, some authors (Scarpa et al., 2022) compared the whole genome sequences of 1271 strains of the clade IIb, B.1 lineage The results of the analysis performed suggest that genetic variability does not significantly increase during the outbreak, as can be expected for DNA viruses compared to RNA viruses. Small clusters have been observed that appear to be related to geographic distribution. Furthermore, no positive correlation emerges between divergence in the sequences and collection dates of the relative samples.

The limited variability even of the 2022 MPXVs does not entail a selective advantage of the virus and can justify the containment of the spread already reported by the WHO. However, it should be emphasized that the variability of the B.1 lineage is still higher than that of the A.2 lineage and of the MPXVs prior to this outbreak.

6. Vaccination

Members of the genus OPXV, as initially reported (section 2), in particular MPXV, VARV and VACV are antigenically related and therefore infection with one usually confers protection to the others as well. Several studies indicate that smallpox vaccination protects against MPXV in 85% of cases.

Because of this, the vaccines proposed to prevent MPXV infection have actually been developed to prevent smallpox, the most feared among members of the genus. First generation vaccines against the smallpox virus, the classic vaccines that led to the eradication of smallpox, based on the use of live, replicating, viruses, are no longer used for their important side effects. Second and third generation vaccines have been developed with the aim of obtaining effective and safer products compared to the first-generation ones. Second generation vaccines have been introduced, derived from the previous ones; ACAM2000 is a single plaque-purified vaccinia virus, derivative of Dryvax, a classical first-generation vaccine, aseptically propagated in cell culture. ACAM2000 (see also section 3) vaccine is still proposed in the US today and has been approved in 2019 by the FDA for the prevention of smallpox and also monkeypox. Third generation vaccines have also been developed. They are derived from an attenuated vaccine strain, Ankara (MVA), which does not replicate in mammalian cells. A third-generation smallpox vaccine, IMVAMUNE, was tested as a MPX vaccine in 2017 in the Democratic Republic of Congo to protect health personnel at risk (Jones, 2008). IMVAMUNE has now been approved also in Europe. JYNNEOS, similar to IMVAMUNE is also available in US in limited quantities. Some, after being initially approved for the prevention of smallpox, begin to be extended to the prevention of MPX as well. In addition to the use of preventive vaccines (pre-exposure prophylaxis), the use after possible exposure to the virus (post-exposure prophylaxis) was also proposed, for administration to take place within a few days of exposure (McCollum and Damon, 2014; Harris, 2022; Poland et al., 2022). Other types of new generation vaccines are being studied and tested.

7. Discussion and conclusions

The Monkeypox virus outbreak that affected many countries around the world in 2022 certainly represents a new event in the history of this virus. Fortunately, the phenomenon now appears to be declining since the end of July and mid-August, even if we are unable to predict if and what traces it will leave. The decrease in cases is more evident in the US and Europe while in some countries in South America and Africa the trend is different.

Could the sudden increase in MPX cases be due to intrinsic modifications of the virus or rather to the virus encounter a susceptible population capable of rapidly spreading the virus through risky behaviors? Both phenomena may have influenced the evolution of this outbreak.

After reading these pages, are we able to answer the questions we posed in the section 1.1?

Regarding the origins of 2022 outbreak, thanks largely to phylogenetic analyzes, which now make use of new and increasingly improved technologies, the 2022 MPX outbreak seems to date back to cases imported to nonendemic countries from Nigeria around 2021, as reported in sections 3 and 4. The viruses in the new outbreak have been documented to belong to the less virulent clade IIb (ex-West Africa). However, these viruses appear to belong to two different lineages A.2 and B.1 that would have originated as a result of two separate introductions (Section 4). Regarding the detection of signs of evolution among viruses identified since May 2022, many mutations have been documented in the B.1 lineage viral strains compared to MPXVs circulating before 2017, indicating a high mutation rate, unusual for MPXVs, indicative of accelerated evolution. However, the mutation rate of the B1 lineage over time is lower compared to other viruses, especially among RNA viruses. Many of these mutations could be related to the host's APOBEC3-like enzymatic activity which could be correlated with a progressive adaptation to human-to-human transmission of lineage B.1 MPXVs. However, these are hypotheses to be confirmed through further in-depth studies. This phrase relating to the need for further studies often recurs in the papers cited as, consequently, in this review. Therefore, the studies conducted so far have allowed us to formulate suggestive and interesting hypotheses, but there is still an important gap to be filled.

While the increase in MPX cases in endemic countries of Africa can be attributed to the vaccine-induced decline in immunity against MPXV, along with ecological and social factors, the decline in vaccine-conferred immunity cannot be responsible alone of the development of the new outbreak of 2022 in non-endemic countries in which intrinsic factors of the virus are possibly involved, as suggested by the various studies reported.

But now that the epidemiological picture seems to be changing again, another question arises, regarding what factors may be or have been determinants in the reversal of the epidemic trend. Can the observed decrease in cases be attributed to the use of the vaccine promptly adopted in the most affected countries, in the US and Europe? In addition to vaccine administration, other factors that may have counteracted this epidemic include changes in risk behaviors and natural immunity to infection developed in the populations most at risk. And, perhaps, even a

combination of these three factors. Among these possibilities, according to Kupferschmidt (2022) the main role in the reduction in the number of cases of MPX would be due more to changes in sexual behavior than to the spread of natural immunity to infection or the use of the vaccine. If this hypothesis is confirmed, Kupferschmidt concludes, there could be a risk of a resurgence of the epidemic, since it is likely that by decreasing the feeling of danger, people at risk tend to reduce the precautions taken in sexual intercourse.

The name of the disease is likely to be changed from MPX to MPOX, as recently determined by WHO. It is possible that the name of the virus will also undergo changes, a decision that is the responsibility, however, of the ICTV. Changes have already been made to clade names (Monkeypox: experts give virus variants new names, 2022) to remove references to African countries, which evidently are not the only ones in which these clades circulate.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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