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Review Article

Monkeypox; A Virus with a History, a Present, and a Future

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Abstract

Monkeypox is caused by a virus of the genus *Orthopoxviruses*. It is a double stranded DNA virus with typically a slow mutation rate. More than 40,000 years of evolution have shaped the current genome. That same line of evolution also gave rise to other viruses, such as variola that causes smallpox and vaccinia that was used for the first smallpox vaccine. Monkeypox virus is a human pathogen with symptoms that are similar to those of smallpox, but symptoms, disease progression, and mortality are much reduced. Transmission originally happened primarily from animals to humans, but human to human transmission is also possible and lately more pronounced. Since the first identification of monkeypox virus in humans in 1970, sporadic outbreaks happened in Africa by two clades of the virus, depending on the location of the outbreak on the west coast or in central Africa.

The 2022 outbreak changed the landscape for the virus. The mutation rate is now much faster and some 40 mutations have been found in the current isolates of monkeypox virus that differentiate it from isolates from past outbreaks. These mutations may have improved the transmission rate from human to human. This author believes that there is justified concern that this may become one of the pathogens of the future.

Keywords: *Monkeypox Virus; Smallpox Virus; Vaccinia Virus; Orthopox Viruses; Virus-Host Interactions; Host Range*

Abbreviations

ANK: Ankyrin; BTB: Bric-a-Brac; IFN: Interferon; MPXV: Monkeypox Virus; NF: Nuclear Factor; ORPV: Orthopox Viruses; TNF: Tumor Necrosis Factor; VARV: Variola Virus; VACV: Vaccinia Virus

Introduction

Monkeypox virus (MPXV) is a member of the genus *Orthopoxvirus* (ORPV) in the sub-family *Chordopoxvirinae* and the family *Poxviridae*. MPXV was first discovered in 1958 in Copenhagen. It was identified in laboratory monkeys that had been transported from Singapore to Denmark [1]. The first transmission to humans did not happen until 1970 in the Democratic Republic of Congo [2]. MPXV is now recognized as an emerging zoonotic disease with a moderately high fatality rate in humans [3,4] and an amazing broad spectrum of animal hosts [5,6]. Two most remarkable members of the ORPV genus are the variola virus (VARV) that is the causative agent of Smallpox and has caused more fatalities than any other infectious disease and vaccinia virus (VACV) that was used as the vaccine that eradicated smallpox around 1980 [5].

MPXV is a double-stranded (ds) DNA virus that replicates in the cytoplasm of the host. Genomes are from 170 to 210 kb (kb) in length with approximately 200 encoded proteins [7]. Of these 200 genes, some 90 are found in the sub-family of *Chordopoxvirinae*, while the remaining genes are unique to specific viruses [8]. None of the poxviruses are known to accumulate point mutations [9], instead their evolution is characterized by many events of gene gains, gene losses and gene duplications that led to the variety of ORPV we see today [10]. Chapter I of this review article will focus on those of the events that led to the evolution of MPXV some 600 years ago.

Chapter II will then discuss the current genome of MPXV in comparison to VARV with an emphasis on accessory genes that are not involved in viral replication or virion morphogenesis, but instead impact virus-host interactions and host range [6]. The genus ORPV contains a total of 12 virus species. The ectromelia and camelpox viruses are highly host-specific. MPXV, cowpox and buffalopox viruses can infect non-reservoir species; in addition to their name giving host, MPXV can infect prairie dogs [11] and cowpox infects domestic cats [12] and elephants [13]. VACV has a wide host range, including rodents, cattle and humans [14] and VARV is entirely limited to humans. A study of host range genes in *Poxviridae* yielded six host range genes from VACV, including K1L, K3L, E3L, B5R, C7L and SPI-1 and additional host genes from other ORPV [15]. This list will be used to browse MPXV genes for host range genes and compare their absence/presence to that of VARV. The outcome of Chapter II will be two questions that could lead to testable hypotheses in the future.

Seeing as MPXV and the other ORPV have undergone lengthy evolution that stretches more than 40,000 years and are today recognized as effective human and/or animal pathogens, chapter III will briefly summarize symptoms and transmission of MPXV in humans and chapter IV will focus on recent outbreaks across the world in the context of previous smallpox vaccinations, as well as their cessation. A systematic review [16], supplemented with the most recent outbreaks, was used to construct the worldwide map of MPXV outbreaks since 1980 that is presented as part of chapter IV. The conclusion of these two chapters will be that MPXV is an emerging zoonotic pathogen with significant epidemic potential.

The potential threat posed by MPXV is of course subject to current research, which manifests itself in 16 ongoing clinical trials (www.clinicaltrials.gov; 10/12/2022), which will be summarized in chapter V. Topics are diverse and include effectiveness of vaccines; the use of tecovirimat as a drug against MPXV, disease characteristics, viral shedding and pharmacist knowledge of the virus and effective drugs.

Chapter I: Evolution of an effective virus

MPXV belongs to the genus of ORPV that also includes VARV (Smallpox) and VACV (vaccine) [17]. The double-stranded DNA genomes are from 170 to 210 kb (kb) in length and encode about 200 proteins [7]. Approximately half the genome contains core or essential genes, encoding functions such as replication and virion morphogenesis [18]. The other half of the genome consists of accessory genes that encode proteins involved in host range determination and virus-host interactions [15]. These genes have undergone lengthy evolution, encompassing many events of gains and losses, which will be detailed below.

About half the accessory genes can be categorized into four groups, based on their protein-protein interaction domains. The BTB (+/- Kelch) proteins [19] contain a Kelch domain consisting of 44 to 55 amino acid repeats at their C-terminus and a Bric-a-Brac (BTB) on their N-terminus. The domains point towards a function as substrate adaptor for cullin 3 ubiquitin ligases. The ANK (+/- Pranc) proteins [20] are encoded by genes at the C-terminus of pox viruses. The domain consists of repeats of ANKyrin. The Bcl-2 domain [21] has a function in the control of apoptosis. The final group of proteins contain the chordopoxvirus-specific chemokine-binding PIE domain, which functions in immune evasion [22]. To understand the significance of these proteins, one needs to go back 40,000 years and take a look at the evolution of *orthopoxviruses*.

A very recent study by Babkin., *et al*. provided information on the evolution of 80 ORPV [23]. A general timeline for the evolution of the modern ORPV based upon the Babkin study is provided in figure 1. Most of the Old World OPRV split up from the New Old World ORPV

around 40,000 years ago, though the Abhmeta and Alaskapox viruses are also considered Old World ORPV. The vast majority of the modern ORPV evolved between 5,000 and 1,600 years ago. Exceptions are VARV and MPXV which evolved 300 AD and 600 years ago, respectively (Figure 1). Interestingly, VARV split into a P1 and a P2 clade in 1654; the rather precise time was determined based upon sequence homology (95% HPD) [23]. In 1874, the P2 clade split up further into the minor alastrim strains from South America and the West Africa strains.

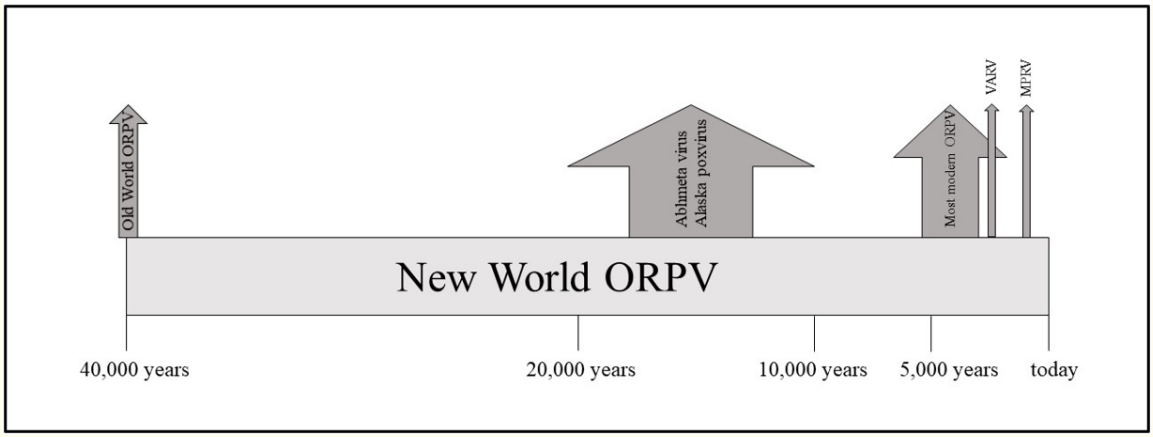

Figure 1: *General timeline of ORPV evolution. The figure was constructed with data from [23].*

Gains and losses of genes during the evolution of ORPV were studied by Senkevich., *et al* [24]. The ORPV are one genus within the sub-family of *Chordopoxvirinae* that are capable of infecting tetrapods. One wave of extensive gene gain was determined for the time after the split of the genus *Salmonpoxvirus* from the common ancestor for both, *Salmonpoxvirus* and ORPV. At this time, 41 core genes were acquired, only one of which was involved in virus-host interaction [24]. A second wave of gene gain happened after the split of the *Crocodilpoxvirus* from the branch that would lead to the ORPV. This gain encompassed 43 genes, completing the core genome and introducing the first accessory genes, including the ones that encode 11 ANK (+/- Pranc) proteins [24]. Note that both, *Salmonpoxvirus* and *Crocodilpoxvirus* split from the *Chordopoxvirinae* before the time that is depicted in figure 1.

The third wave of gene gain happened around the time of the evolution of the ORPV. This was primarily a duplication of genes previously gained, in particular those encoding Bcl-2 and BTB (+/- Kelch) proteins [24]. Further duplication of such genes happened during the fourth wave when the ORPV diverged from the *Centapoxviruses* [24]. Interestingly, two genes were acquired from the host during this fourth wave, a guanylate kinase (OPG186, COPA57R) and an inactivated protein kinase (OPG198, COPB12R) [24].

While the early evolution of the ORPV is characterized by extensive gene gain, the evolution of some of the ORPV that are relevant today included immense gene loss. The branch that leads to the ancestor of MPXV is characterized by the loss of 37 genes [24]. VARV lost a total of 37 genes as well, 19 right at the branch and 18 later. VACV lost 19 genes at the base. A block of 10 genes at the left end of the genome was lost in all three of the MPXV, VARV and VACV branches. While the early gene gains were either entirely (first wave) or mostly (second wave) core genes, gene losses were primarily in accessory gene that are located at both ends of the genome. In that context, it is noteworthy that core genes encode proteins of essential functions, such as replication and virion morphogenesis. In contrast, accessory genes encode proteins that are required for virus-host interaction and host range. At this point, it is hypothesized that the extensive gene

losses that some of these viruses have gone through may impact host specificity. This hypothesis is based on the observation that all the modern orthopoxviruses are characterized by solid gene losses, while being rather host specific.

Chapter II: Current genome of MPXV

Through the above described series of gene gain and loss events, MPXV has acquired its genome of today, which of course differs among the different strains. At both ends of the genome, MPXV and other ORPV possess terminal inverted repetitions that include short tandem repeats [25], as well as terminal hairpins [26]. Alignment of the MPXV-ZAI sequence with that of other ORPV revealed 190 open reading frames that were larger than 60 bp [27]. The central region of the genome was occupied by genes that were considered essential for other ORPV. Species and strain specific differences resided in the two terminal regions. For example, MPXV-ZAI was missing 25 open reading frames relative to other ORPV and 19 relative to VARV. Many of these missing genes encode proteins with functions in immune evasion and host range [28]. Figure 2 summarizes the MPXV genome sequence.

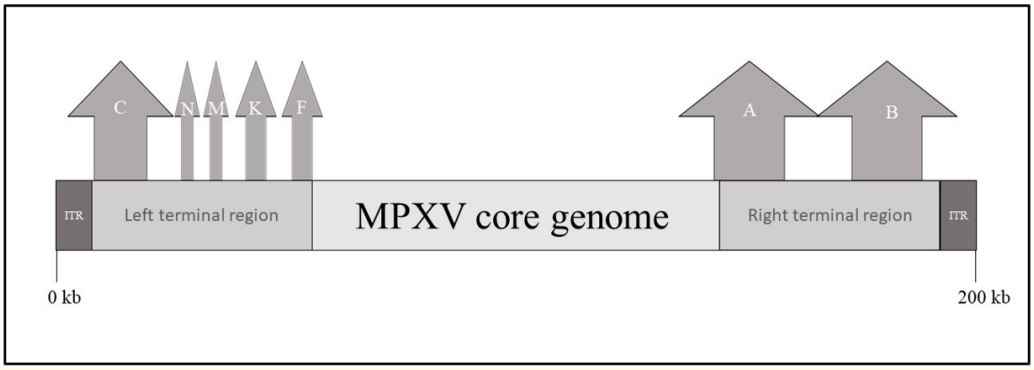

Figure 2: *MPXV genome. The genome contains approximately 200 kb, encoding 200 proteins. The genome is flanked by the inverted terminal regions, followed by the left and right terminal regions that contain the accessory genes. The core genes are in the center of the genome. The arrows designate regions from the Copenhagen VACV nomenclature for the accessory genes.*

Since the strain specificity resides within the genes that determine host range and immune evasion, a table was compiled that took advantage of data presented in supplemental tables S1 and S2 from Senkevich., *et al* [24]. The table compares gene losses of MPXV to those of VARV with an emphasis on those of the accessory genes that harbor BTB (+/- Kelch) domains, ANK (+/-Pranc) domains, Bcl-2 domains, or PIE domains. Annotations were taken from Senkevich., *et al.* [24] for the OPG nomenclature and supplemented with the Copenhagen nomenclature for the VACV genes. For this manuscript, we will use the OPG nomenclature and provide the COP nomenclature in parentheses (when available). For the location of the genes on the genome, see figure 2.

A cluster of genes of three concomitantly lost genes are OPG6 from the BCl-2 family, OPG8 from the BTB family, and OPB13 from the PIE family. These three genes are lost in both MPXV and VARV (Table 1). It has been postulated they could be part of a species specific host signal transduction pathway [24]. Another pair of genes that got lost together four times within the ORPV evolution is OPG32 (C3L) and OPG33 (C2L) [24]. OPG32 is not included in table 1, because it does not carry either of the four common domains. It has been described as a secreted complement control factor. OPG33 has the BTB and Kelch domains and is missing in both, MPXV and VARV (Table 1).

Gene/protein	Orthopoxviruses and VACV annotations[1]	MPXV[2]	VARV[2]
BTB (+/-Kelch)	Total of 8	Has 2/8	Has 0/8
Specifics	OPG 8, BTB domain	0	0
	OPG 11, BTB/Kelch domains	0	0
	OPG 30, COP C5L, BTB domain, lysophospholipase [29]	1	0
	OPG 33, COP C2L, inhibits inflammation [30]	0	0
	OPG 47, COP E3L, IFN resistance, larger lesion size [31], host range [15]	1	0
	OPG 184, COP A55R interacts with Cullin 3		
	OPG 196, COP B10R, BTB/Kelch domains, shope fibroma [32]	0	0
		0	0
ANK (+/-Pranc)	Total of 13	Has 7/13	Has 4/13
Specifics	OPG3, COP C19L, binds to NF-κB [33]	1	1
	OPG4, COP C17L, ANK/Pranc domains [34]	0	0
	OPG9, ANK/Pranc domains	0	0
	OPG14, inhibits NF-κB activation	0	0
	OPG15, ANK/Pranc domains	1	0
	OPG17, inhibits NF-κB activation	0	0
	OPG23, host range	1	0
	OPG25, COP C9L, IFN resistance [35]	1	0
	OPG37, COP M1L, inhibits intrinsic apoptosis	1	1
	OPG39, COP K1L, inhibits NF-κB, host range	1	0
	OPG203, COP B18R, binds to SCF ubiquitin ligase [32]	0	1
	OPG205, COP B20R, ANK/Pranc domains	1	1
	OPG211, COP C15/B21, ANK/Pranc domains	0	0
Bcl-2	Total of 12	Has 8/12	10/12
Specifics	OPG5, COP C16L, virion morphogenesis, host range factor [36]	1	1
	OPG6, Bcl-2 domain	0	0
	OPG29, COP C6L, inhibits type IFN production, signaling	1	1
	OPG34, COP C1L, Bcl-2 domain, host range [37]	1	1
	OPG35, COP N1L, inhibits NF-κB activation	1	1
	OPG36, COP N2L, IRF2 activation inhibitor, α amanitin target	1	1
	OPG44, COP K7R, blocks IFN-ß promoter induction	1	1
	OPG45, COP F1L, blocks mitochondrial apoptosis [38]	1	1
	OPG176, COP A46R, inhibits TLR- and NF-κB activation [39]	1	1
	OPG179, COP A49R, inhibits NF-κB activation [40]	0	1
	OPG182, COP A52R, NF-κB pathway inhibitor, blocks TLR signaling	0	0
	OPG203, COP B18R, NF-κB inhibitor [32]	0	1
PIE	Total of 13	Has 7/13	Has 4/13

Specifics	OPG1, COP C23L, chemokine binding	1	1
	OPG2, COP C22L, TNF-α receptor homolog [34]	1	1
	OPG12, PIE domain, SCP-2	0	0
	OPG13, prevents CD30/CD153 interaction, TNF receptor homolog	0	0
	OPG26, COP C8L, secreted	0	0
	OPG38, COP M2L, blocks SD28 mediated T cell activation	1	1
	OPG170, COP A41L, binds chemokines	1	1
	OPG183, COP A53R, TNF receptor homolog CrmC, secreted, prevents TNF	0	0
	binding to receptor	1	0
	OPG192, COP B7R, ER resident, SCP-3, influences lesion size in mice	1	0
	OPG195, COP B9R, ER localized, prevents antigen presentation [41]	1	0
	OPG209, COP C13L/C14LPIE domain, SCP-1	0	0
	OPG212, TNF receptor homolog CrmD	0	0
	OPG213, TNF receptor homolog CrmE		

Table 1: *MPXV and VARV accessory genes.*

All data for the construction of this table were taken from [24]. Additional references regarding functions of some of the open reading frames are supplemented as available. Locations of the genes from the COP nomenclature are included in figure 2.

[1]Data for protein annotations were taken from table S1 [24].

[2]Data for presence/absence in MPXV and VARC were taken from table S2 [24].

OPG: ORPV Nomenclature; COP: Copenhagen VACV Nomenclature.

Another trio of genes that are not included in the MPXV or VARV genome are OPG183 (A53R), OPG212, and OPG213, all from the PIE family and encoding the tumor necrosis factor (TNF) receptor homologs CrmC, CrmD, and CrmE, respectively (Table 1). TNF was early described as a tumor necrosis factor in mice [42] and has later been recognized as a proinflammatory cytokine that serves as one of the primary regulators of inflammation in humans [43]. The gateways to the complex signal transduction pathway are the two differentially expressed receptors TNFR1 and TNFR2 [44]. CrmC, CrmD and CrmE are decoy receptors that poxviruses have evolved to mimic the extracellular domain of TNFR1 and TNFR2, hence inhibiting the binding and activity of TNF [45]. While these decoy receptors contribute to virulence of VACV [46], neither MPXV nor VARV possess either of these three genes (Table 1). The same is true for TNF receptor homolog OPG13 (Table 1). In contrast, the TNF α homolog OPG2 (C22L) is present in both, the MPXV and VARV genomes. It seems like MPXV and VARV have at least one TNF receptor decoy in OPG2 to interfere with the TNF dependent signaling pathways in the host.

Staying with the topic of interference with human host signaling, a reasonable large group of genes (Table 1) involves the activation of nuclear factor (NF)-κB. NF-κB is part of a proinflammatory signal transduction pathway in humans that was described as the prototype of such a pathway, leading to the production of cytokines and chemokines [47]. One of the two pathways that lead to activation of NF-κB activation involves TNF α and IL-1 [48], the second pathway depends on the NF-family cytokine lymphotoxin β [49]. Table 1 includes three genes from the ANK (+/-Pranc) family and five genes from the Bcl-2 family; all these inhibit NF-κB or its activation. OPG14, OPG17 and OPG182 (A52R) are lacking in both, MPXV and VARV. OPG35 (N1l) and OPG176 (A46R) are present in the genomes of both MPXV and VARV. MPXV possesses OPG39 (121L) and the VARV genome includes OPG179 (A49R) and OPB203 (B18R). Altogether it seems like both MPVX and VARV possess genes that would allow them to interfere with NF-κB signaling in the human host.

Continuing with genes that are differentially possessed between MPVX and VARV, MPVX possess two genes that promote interferon (IFN) resistance, these are OPG47 (E3L) from the BTB (+/-Kelch) family and OPG25 (C9L) from the ANK (+/-Pranc) family. The VARV

genome is lacking these genes. In VACV, OPG47 (E3L) was described as a double-stranded RNA-binding protein that inhibits the IFN-induced, double-stranded RNA-activated protein kinase in the human host [31]. This promotes resistance towards IFN. OPG25 (C9L) is a second protein that fulfills this same function. Deficiencies in IFN signaling have been described as a driver of uncontrolled viral replication and increased disease symptoms during pandemics that were caused by novel pathogens, such as H1N1 influenza and SARS-CoV-2 [50]. The reason for this is that novel pathogens do not encounter pre-existing antibodies and the human body needs to rely on the innate immune system that depends on INF. Note that VARV that does not harbor OPG47 (E3L) and OPG25 (C9L) is older as a pathogen (2,300 years) than MPXV (600 years) which does possess these two INF resistance genes. Furthermore, VARV has throughout the millenia likely infected many more human hosts than MPXV (human or monkey hosts) has and still does. Is it possible that the continued existence of MPXV in both the human and many animal populations is dependent on INF resistance? Maybe. Intriguingly, OPG47 (E3L) was also described as one of several host range genes [15]. The same is true for OPG39 (K1L) [15], which was also present in MPXV and not in VARV. Could these two genes contribute to the larger host range of MPXV relative to VARV? Possibly. At this point, there are many more questions than there are answers, which may lead to hypotheses to be tested in the future.

Chapter III: Monkeypox disease

Throughout many years of evolution, MPXV has turned into a virus that can persist. With low case numbers, there have been continuous outbreaks, primarily in Africa, but lately also in other parts of the world. The disease symptoms and disease progression are not as lethal as for Smallpox [51]. Symptoms take between 7 and 14 to develop post infection, at which time the first serum antibodies can be detected. Early symptoms include fever and chills, accompanied by body aches and fatigue (Figure 3). The characteristic 'pox' symptom is the development of a rash on face and body, followed with lesions across the entire body [51].

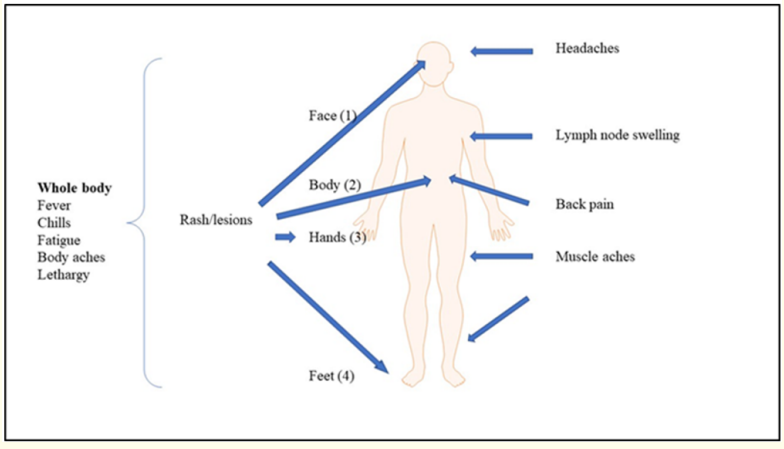

Figure 3: *Symptoms of MPXV. The information was taken from [51], the schematic of the human from Motifolio (www.motifolio.com). The numbers in parenthesis indicate the order of the lesions.*

Mortality varies dependent on the clade and available healthcare, but is typically low in the 1 to 10% range. In a sense, MPXV is a milder version of VARV, which may contribute to its persistence throughout time, especially after Smallpox vaccination cessated.

The animal reservoir for MPXV transmission is suspected to be African rodents, with transmission occurring through contact with animal body fluids, respiratory droplets or skin lesions [52]. On the human side, transmission from animals involves mucous membranes, broken skin, and the respiratory tract [53]. Human to human transmission involved body fluids as well, including respiratory droplets, mother to child transmission, and sexual transmission. MPXV has an R_0 slightly larger than 1, this means there is a potential for MPXV causing an epidemic [53].

Chapter IV: Monkeypox outbreaks

Throughout many years of evolution, MPXV has turned into a virus that can persist. With low case numbers, there have been continuous outbreaks, primarily in Africa, but lately also in other parts of the world. A 2019 systematic review [16] summarized MPXV outbreaks from 1970 to 2019. For the construction of figure 4, data that were included in supplemental table S6 from this article were added to the world map from the CDC that demonstrated the 2022 MPXV outbreak (www.cdc.gov).

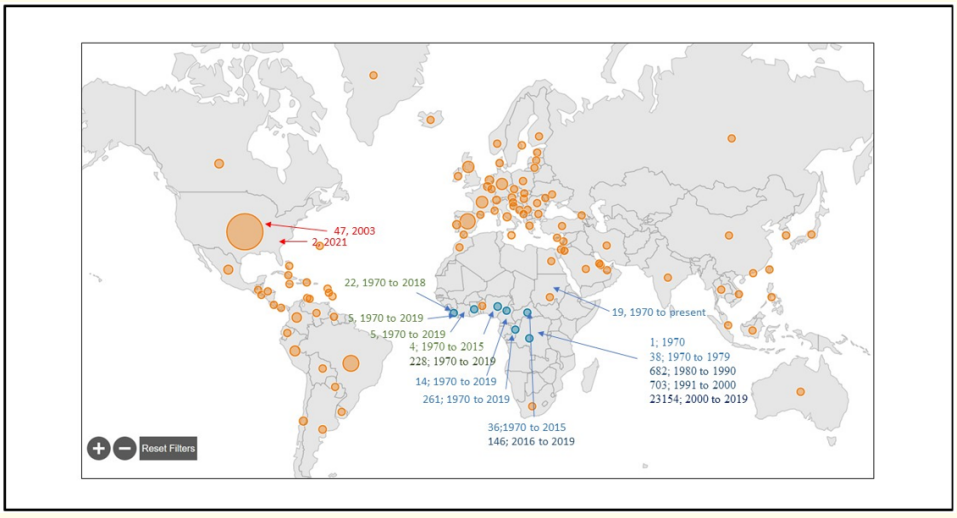

Figure 4: *MPXV cases from 1970 to today. The map was downloaded from the CDC (www.cdc.gov) and supplemented with data from supplemental table S6 [16].*

The blue circles from this map are countries that had a previous history of outbreaks. Orange circles were used for countries for who MPXV was new. Circle size increases with case numbers. Outbreaks prior to 2022 were added with case numbers and time periods. Blue font and arrows were used for outbreaks from the Congo Basin clade, green font and arrows for outbreaks caused by MPXV from the West African clade. Red font and arrows were used for two outbreaks in the US.

As mentioned in the introduction, the first human case of MPXV was identified in 1970 from the Democratic Republic of Congo [2]. Since that time, the number of outbreaks has slowly but steadily increased until recently [16] (Figure 4). During the time period from 2006 to 2007, the case number in the Democratic Republic of Congo increased 20 fold as compared to the time period from 1981 to 1986 [54]. Another increase was reported between 2008 and 2013 from 21.3 cases per population of 10,000 to 28.4. Rimoin and coworkers attributed these increases to the cessation of the Smallpox vaccine 30 years earlier [54]. Many of the outbreaks in the Democratic Republic

of Congo happened in rural areas within tropical forests [55-57], others in areas of armed conflict [58]. Among the risk factors, sleeping on the floor and preparing wild animals to cook ranked high [59].

Outside the Democratic Republic of Congo, outbreaks were accompanied by single to double digit case numbers until 2015 (Figure 4). As one example, the single outbreak in Sudan in 2005 was caused by the move of an individual from the Democratic Republic of Congo [60]. An outbreak in the United States in 2003 included 47 individuals across several states [61]. This was the first reported outbreak of MPXV in the Western world. However, after 2015, case numbers went up. A noteworthy outbreak happened between 2017 and 2020 in Nigeria. At that time, the vaccination rate against Smallpox in the population was only about 10.1%, serum immunological levels were low, especially in the unvaccinated population. It is believed that the resurgence of MPXV was driven by a decline in immunity to Smallpox that used to be acquired by vaccination [62].

Figure 4 distinguishes between countries whose MPXV outbreaks were caused by the West Africa clade (green font) and those whose outbreaks were associated with the Congo Basin clade (blue font). Genome sequence analysis of isolates from Western and Central Africa, as well as the isolates from the 2003 US outbreak confirmed the two clades, with the West Africa and the US isolates being slightly less virulent than the Congo Basin isolates [63]. A total of nine proteins were identified that exhibited differences across the two clades. These are the essential proteins H5R (transcription factor [64]), A9L (virion morphogenesis [65]), A50R (DNA ligase [66]) and A36R (actin tail nucleation [67]), as well as the accessory proteins interleukin-1ß receptor [68], Spi-1 (apoptosis and host range [69]), C7L (host range [70]), MT4 (apoptosis [71]) and the complement control protein CCP [72]. Most remarkably, the isolates from the West African were completely devoid of the CCP protein [63]. The ability of the Congo Basin MPXV to block complement enhanced viral neutralization might explain the prolonged viraemia and increased virulence of these isolates [63]. Likewise, isolates from the West African/US clade do not possess a functional interleukin-1ß ortholog, which may lead to an increase in immune recognition and viral clearance [63]. Altogether, differences in these nine key proteins can explain the difference in disease severeness and mortality between the two clades.

While these previous outbreaks were sporadic, limited to Africa, and typically associated with international travel or animal transport, the landscape of MPXV infections changed drastically in 2022 [51,73]. A small number of cases were reported in 2021, including one individual in 2021 who had travelled from Nigeria to Texas in July and another one to Washington DC in November. In May 2022, case numbers climbed [74]. Starting on May 2, when the first case of MPXV was reported in the UK in an individual who had traveled from Nigeria [75]. On May 18, 14 cases were reported from Portugal, 7 from Spain, and 13 from Canada. On May 19, the first cases were confirmed in Belgium, Sweden and Italy. On May 20, Australia reported their first cases in patients returning from Europe, also France, Germany and Netherlands reported their first cases. Meanwhile, 71 cases were reported from Great Britain. On May 21, the first cases were recorded from Switzerland and Israel, on May 23 from Denmark, on May 24 from the Czech Republic. At this time, 19 countries had reported cases of MPXV with transmission either from human to human or animal to human. India reported their first four cases on July 25 [76]. By October, the World Health Organization (WHO) reported 68,900 cases worldwide [77].

While DNA viruses are generally slow to mutate [78], the MPXV virus from the 2022 outbreak differs significantly from previous isolates of the virus. It contains many more mutations now, which indicates that it evolves to spread more effectively within the human population. A total of 40 mutations have been found in the 2022 strains that distinguish the current virus from its closest related variant. It is assumed that the virus is able to mutate faster because its ability to transmit among humans. This may be due to some human's immune system, where specific enzymes induce mutations in viruses [79]. Genome sequence analysis indicates that MPXV evolution took off in 2017 and currently exhibits a mutation rate that is about 10 times of what is used to be. This also included point mutations, whereas previous evolutionary events were primarily the gain and loss of entire genes or cluster or genes. The current isolates are believed to belong to clade 3, which is closely related to the virus that caused the outbreak in the US in 2021 [80]. Contradictory research proposed that the current MPXV virus exists in at least two clades [81], as evidenced by genome sequence analysis of 10 isolates, all of which differed from isolates from Europe. Of these 10 isolates, three differed from the remaining seven and were distinct from one another. Altogether,

evidence accumulates that these new isolates of MPXV are different from previous ones, mutate more effectively, and transmit better among humans.

Chapter IV: Clinical trials

Given the evidence towards MPXV potentially causing epidemics in the future, the question arises what the newest state of research might be. A search on Clinicaltrials.gov yielded 16 clinical trials on MPXV on October 12, 2022 (Table 2). This includes five vaccine trials, five drug trials primarily investigating tecovirimat, and six trials on diverse topics, including virus clearance, virus shedding and Pharmacist knowledge. The trials are held across the world, with hot spots in Africa, Europe, and the US. The current status of the majority of them is 'recruiting'. This author was not able to find any published articles on results from any of the trials. However, trial NCT00728689 has results included from their phase I trial on ST-246 on Clinicaltrials.gov.

Trial ID	Title	Status	Location
Vaccines			
NCT05512949	Trial to evaluate the immunogenicity of dose reduction strategies of the MVA-BN monkeypox vaccine	Recruiting	US
NCT05522296	Break-through infection following monkeypox vaccination	Recruiting	Germany, Spain
NCT05562323	Characterization of vaccine-induced responses against monkeypox (MoVIHvax) an observational prospective cohort study	Recruiting	Spain
NCT02977715	IMVAMUNE® smallpox vaccine in adult healthcare personnel at risk for monkeypox in the Democratic Republic of the Congo	Active, not recruiting	Congo
NCT03745131	Cohort study of healthcare workers receiving Imvanex®	Completed	UK
Antiviral drugs			
NCT05559099	New tecovirimat for treatment of monkeypox virus	Recruiting	Congo
NCT05534984	Study of tecovirimat for human monkeypox virus	Recruiting	US
NCT05534165	Tecovirimat in non-hospitalized patients with monkeypox	Not yet recruiting	NI
NCT02080767	Tecovirimat (ST-246) treatment for orthopox virus exposure	Available	NI
NCT00728689	Phase I trial of an investigational smallpox medication	Completed	US
Miscellaneous			
NCT05476744	Viral clearance and epidemiological characteristics in patients with monkeypox	Recruiting	Spain
NCT05443867	Monkeypox asymptomatic shedding: evaluation by self-sampling MPX-ASSESS	Recruiting	Belgium
NCT05543577	Assessing the preparedness and knowledge of pharmacists in the current monkeypox outbreak	Recruiting	Egypt
NCT05567939	Clinical, virological, immunological, psychosocial and epidemiological consequences of human monkeypox virus (ProMPX)	Recruiting	Netherlands
NCT05438953	Follow-up of contact at risk of monkeypox infection: a prospective cohort study	Recruiting	France
NCT05058898	A one health study of monkeypox human infection	Recruiting	Central African Republic

Table 2: *Clinical trials on MPXV.*

Data were taken from www.clinicaltrials.gov on October 12, 2022.

NI, not indicated on website.

Thanks to the large degree of sequence similarity between the ORPV [27], vaccines against one member of the ORPV family typically protect against other ORPVs. This is aided by some 24 membrane and structural proteins that antibodies can target [82]. In this context, the increased occurrence of MPXV might be due to a decreasing immunity against Smallpox in our populations due to cessation of the Smallpox vaccine [54,62]. Dryvax by Wyeth Laboratories (PA, USA) is an example of a first generation vaccine, was generated from VACR, and cross protected against Smallpox in humans and Monkeypox in many animals [83]. ACAM2000 (Emergent Product Development Gaithersburg, MD, USA) is an example of a second generation vaccine that is licensed in the US against Smallpox. This vaccine was derived from a single clonal viral Dryvax isolate and is characterized by reduced neurovirulence in several animal models [84] and similar safety and immunogenicity as Dryvax [85]. JYNNEUS (Bavarian Nordic, Hellerup, Denmark) is based on the VACR virus Ankara which is non-replicating. In two doses, this vaccine has an effectiveness as Dryvax [86]. The vaccine has a US license against both Smallpox and Monkeypox. Alternative names for JYNNEUS are Imvamune, Imvanex, and MVA-BN.

Five clinical trials are ongoing to test third generation vaccines based on the non-replicating VACR virus (Table 2). NTCC05512949 tests two subcutaneous doses of 2 x 10^7 or 1 x 10^8 viruses (MVA-BN) on 210 participants and determines the peak humoral immune response. NCT05522296 tests single doses of the same vaccine to address limited availability of the vaccine. Their outcome is PCR confirmed Monkeypox infection. NCT05562323 also uses the one dose vaccination of MVA-BN, but the vaccine is administered intradermally. NCT02977715 uses the same vaccine under the name Imvamune in the only ongoing phase III trial on 1,600 participants. The fifth and final vaccine trial NCT03745131 specifically tests health care workers who received the vaccine as pre-exposure or post-exposure prophylaxis. Their outcome is the determination of antibody titers after first and second dose.

Five clinical trials are ongoing to test the effectiveness of tecovirimat against MPXV (Table 2). Tecovirimat inhibits the viral envelope protein VP37. This serves to block viral maturation and release from the human host cell. As a consequence, spread of the virus within the host is reduced [87]. Tecovirimat is primarily used for patients with severe disease progression after infection with VARV. In mice, tecovirimat improved survival from lethal MPXV infection [88]. Note that tecovirimat is also known under the names TPOXX and ST-246.

NCT05559099 is a phase III trial that tests 600 mg doses twice daily over the course of 14 days and determines time to lesion reduction and time to a negative throat test. NCT05534984 is a phase II trial that tests the effect of a number of 200 mg capsules that is dependent on body weight and determines time to lesion reduction, number of days to first two consecutive negative tests, mortality, and number of days to first negative PCR test. NCT05534165 is a phase III trial that tests the effect of 400 or 600 mg tecovirimat dependent on body weight, administered for 14 consecutive days. This trial determines time to lesion reduction, pain, and virus levels in blood, lesions, oropharynx, and rectum. NCT02080767 by the US Army administers 600 mg doses daily and does not provide much other information on clinicaltrials.gov. Data are listed as available but could not be found on clinicaltrials.gov. NCT00728689 is a phase I trial with 12 and 11 patients for the single (day 1 to 3) and second (days 11 to 13) dose of 400 mg tecovirimat. This study determined pharmacokinetic parameters and safety parameters. Data are included on clinicaltrials.gov and include no reports of adverse advents in all patients, underarm tenderness in one patient and neck pain and headache in one patient.

The six trials that are listed in table 2 under 'others' cover miscellaneous topics. NCT05476744 studies viral clearance and epidemiological characteristics in 75 patients infected with PMXV. They determine viral clearance (time to undetectable virus load) in lesions, blood, oropharynx, rectum, and others. NCT05443867 studies asymptomatic virus shedding in 140 patients with MPXV. Virus shedding has led to the detection of MPVX in the rectum, nasopharynx, semen, urine, and feces in the past [89]; virus shedding can contribute to virus transmission. Among the outcomes of the trial are the proportion of seroconversion in PCR positive contacts vs PCR negative contacts. NCT05543577 compares knowledge of pharmacists to that of intern physicians with 380 participants. NCT05567939 determines time to resolution of symptoms in MPXV infected patients, as well as demographic, sexual, and other clinical characteristics. NCT05058898 determines the proportion of MPXV cases after interhuman or animal exposures, as well as a number of secondary outcomes, including

viral mutations and genetic proximity between viral strains. Altogether, the 16 clinical trials involving MPXV cover a wide range of topics and a bulk of new information will result from trial completion that will help us understand, prevent, and treat infection with MPXV.

Conclusion

In conclusion, MPXV has several thousands of years of evolution behind its current genome, and is causing a current, but declining worldwide outbreak. The future looks brighter for the virus than this author would wish for. The current outbreak gave the virus the opportunity to mutate faster than ever before and transmission from human to human is becoming more efficient. I do think that this is a virus to watch out for in the future and that worldwide monitoring is necessary. This opinion is supported by the many new research projects and clinical trials, investigating the characteristics of the virus, its prevention, and treatment.

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Conflict of Interest

The author declares no conflict of interest.

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