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Review

The Novel Insight into the Emergence of Monkeypox: Old Disease, New Fears?

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Abstract: Monkeypox virus (MPXV), causing zoonotic diseases in humans, is a member of Orthopoxvirus under Poxviridae family. The virus was first reported in monkeys in 1959 in Denmark and in humans in 1970 in the Congo. Outside Africa, the virus first appeared in the USA in 2003 and since then occurred sporadically. The virus reemerged in 2017 and now spreading globally. African wild rodent mammals are thought to be the reservoir of MPXV. Exotic trade of animals and international travel favors the dissemination of MPXV. Genetic analysis shows two clades of the MPXV. Smallpox vaccine shows cross-protection and people who never in contact with Orthopoxvirus affected more than exposed ones. Fever, muscle pain, headache, and vesicle formation are the dominant clinical sign. Guarnieri-like inclusions and Ballooning degenerations are important pathognomic lesion of MPXV. It may produce case fatality rate up to 11%. Genetic materials alterations may favor the reemergence of the virus. The continuing occurrence over 73437 cases in 109 countries shows that MPXV can spread among humans competently and can be a serious issue of global public health concern. Here, we summarize the existing knowledge about re-emergence and insights into MPXV which will be of useful to curb its occurrence.

Keywords: Monkeypox; MPXV; emergence; wild rodents; zoonoses

1. Introduction

Monkeypox (MPX) is a transmissible disease that occurs in humans as well as in animals. MPX was first described in human in 1970s in Democratic Republic of Congo (DRC) and remained endemic throughout the rainforests of Central and Western Africa with no reported cases of outbreak elsewhere [1]. In 2003, MPX was reported in Wisconsin province of the United States [2] and since then it is continuing in multi-country outbreak in nearly all the continents outside the Africa. On the other hand, since 2019 the world is going through a terrible situation due to Covid-19 and till now, is trying to go back in

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normal situation with facing many difficulties in terms of global public health and heavy economic crisis as well [3].

Pondering the current spreading nature of MPX amid the continuing Covid-19 pandemics, SARS-Cov-2 and monkeypox virus (MPXV) might have chances for coinfection that may results in alteration of infectivity patterns, degree of pathogenicity, management practices, and or response to vaccination in one or both cases [4]. The opportunities for interactions between these two viruses could accelerate the emergence of new variants of SARS-CoV-2 with properties that conceivably will further affect the existing pandemic management approaches, i.e. increased abilities to escape host's immune response and hamper health caring system as a whole [5]. As a result, MPXV has been placed in the biosafety level 3 (BSL 3) category, the high threat biodefense category in the EU [6], and in the USA it has been included in the Select Agents and Toxin List [7].

Biological scientists are not paying much more attention to the consequences of MPX as it is evidenced by the inadequate number of research papers in the field of biological, medical and physical science [8]. Occurrences of MPX are poorly managed and hardly reported with little information leading to an incomplete picture of the disease's importance. [8]. However, as the number of occurrences of MPX is increasing worldwide and drawing the attention of the global community feeling, it must need to be addressed by the researchers without delay. Moreover, MPXV might be the next emerging pathogen from Poxviridae family after smallpox that might need significant attention to prevent. In this review, we summarize the latest publicly available information on the origin, emergence, transmission, pathology, diagnosis and preventive measures of MPXV.

2. Global Scenario

The recent MPX occurrence exemplifies the reasons why universal population healthiness cannot be overlooked. The unpredicted existence and widespread topographical dissemination suggest that it might have been going under levels measurable by the surveillance systems. But it can be completed for younger child, immunosuppressed individuals, and for pregnant ladies because of their complex impact of infection in these clusters. The outbreak lasts on to affect primarily younger groups of people than others (**Table 1, Fig. 1**) [9, 10]. World Health Organization (WHO) is closely monitoring and responding to the outbreak and maintaining transnational coordination and sharing of information with the Member States and Partners. Since January 1st to the October 19th, 2022, a total of 73437 MPX cases have been identified based on laboratory-based confirmative tests and reported to the WHO from 109 member countries/ territories/areas in all six WHO Regions (**Table 2, Fig. 2**) [10].

Table 1. MPX in different ages, sex, and ethnic groups [9].

Age Range (Years)	Number of MPX cases				
	Man	Woman	Transgender man	Transgender woman	Another sex/gender
0 to 5	15	7	0	0	0
6 to 10	8	4	0	0	1
11 to 15	11	5	0	0	0
16 to 20	523	53	6	7	7
21 to 25	2554	132	18	32	28
26 to 30	4980	141	16	36	41
31 to 35	6123	115	11	48	46
36 to 40	4428	93	8	30	31
41 to 45	3052	61	5	20	18
46 to 50	1974	46	2	14	11
51 to 55	1387	32	1	2	7
56 to 60	763	16	0	1	0
61 to 65	278	6	0	0	1
66 to 70	99	3	0	0	0
71 to 75	35	1	0	1	0

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76+ years	5	4	0	0	0

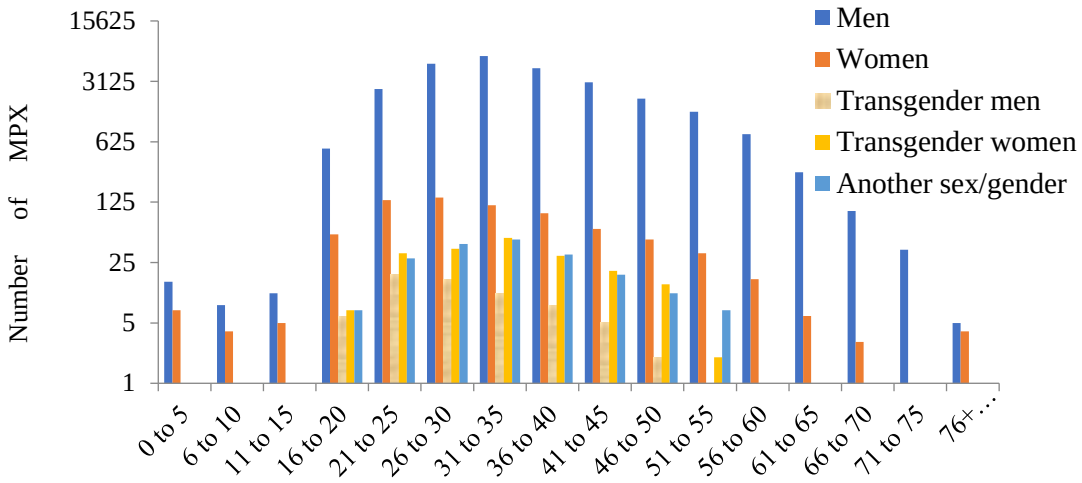

Figure 1. MPX cases of different ages and genders groups reported to CDC as of 19 October 2022. MPX=Monkeypox, CDC=Centre for Disease Control and Prevention.

Table 2. MPX outbreaks in different regions of the world.

	WHO					
Region	**African Region**	**Region of the Americas**	**Eastern Mediterranean Region**	**European Region**	**South-East Asia Region**	Western Pacific Region
MPX cases	869	47215	72	25056	23	202
Death	13	10	1	4	1	0

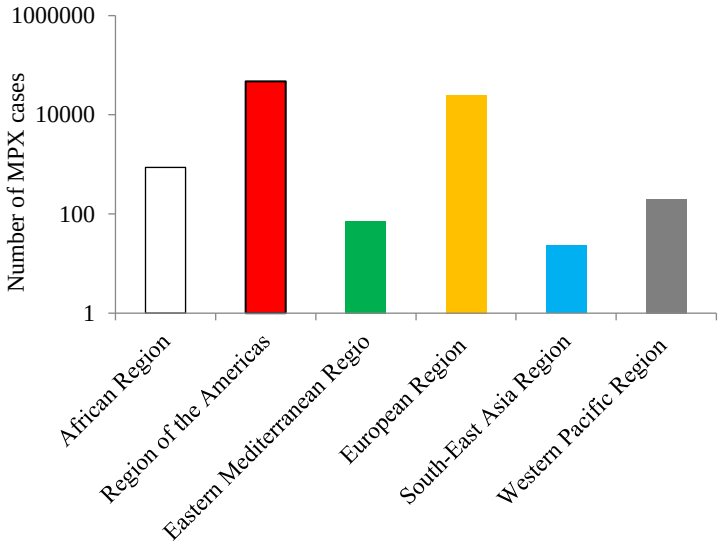

Figure 2. Total number of detected MPX* cases as reported to WHO** on regional basis since January 1st to October 19th 2022. MPX=Monkeypox, WHO=World Health Organization.

3. Origins and Structure of the Virus

MPXV was reported in 1959 as an etiologic agent of sickness when a group of cynomolgus monkeys (*Macaca fascicularis*) were affected in a research station in Denmark [11] and the outbreak continued for a few years elsewhere. It was in 1970s when MPX in a human was identified as a distinct disease revealing smallpox-like illness in DRC [12].

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Since 1970s, the virus was endemic in the African precinct until 2003 when a human case was identified in the USA. The man was infected from prairie dogs (*Cynomys* spp.) that became sick after having contact with some exotic mammals, African rodents (*Funiscuirus* spp., *Heliosciurus* spp., *Cricetomys* spp., *Atherurus* spp., *Graphiurus* spp., and *Hybomys* spp.) imported from Ghana [13]. Subsequently, sporadic occurrences were noted outside Africa but now the world is shivering because of its spreading nature.

Monkeypox (MPX), an emerging zoonotic viral disease becoming common in human beings caused by MPXV, a member of the genus Orthopoxvirus under the family Poxviridae [14]. Along with monkeypox virus (MPXV), Orthopoxvirus genus comprises 12 species of viruses (**Figure 3**) and within these cowpox virus (CPXV), variola virus (VARV), and vaccinia virus (VACV) are able to produce disease in human being [15]. The virus has linear double-stranded DNA (dsDNA) of 170-250 kb genome, measuring 200-250 nm large, brick-shaped, and enveloped cytoplasmic entity which enters into the host cells after binding with glycosaminoglycans [16]. Apoptotic mimicry also mediates the entry of viruses into cellular host for replications [17].

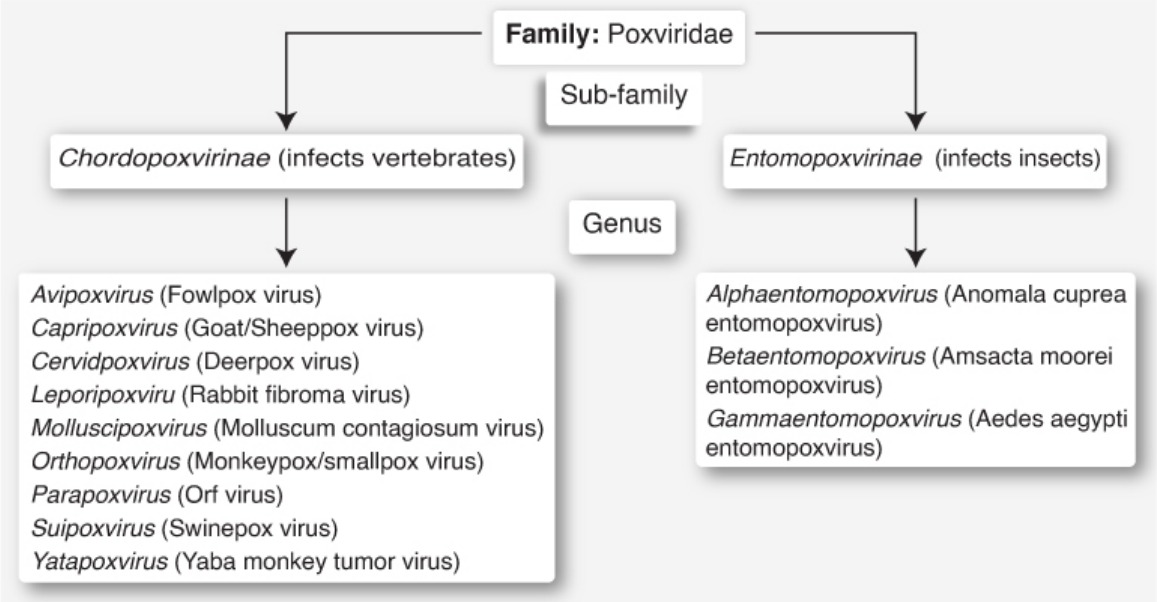

Figure 3. Classification of different types of poxviruses within the family Poxviridae. The Monkeypox virus is classified under the genus: *Orthopoxvirus* within the sub-family: *Chordopoxvirinae* .

The genomic materials of MPXV fall into two distinct clades as revealed by phylogenetic analysis: Central African clades (or Congo Basin) and West African clades and the pathogenicity vary between these two [18]. Usually, Central African one produces more severe infections in human and nonhuman primates [19] with a case fatality rate (CFR) of 10.6% whereas West African strain has 3.6% CFR [20]. These findings led to the assumption that the genomic dissimilarities between these two clades are possibly responsible for variable pathogenesis and transmission pattern. According to some authors, five genes have been implicated for increased virulence properties of Central African Strains. Based on genomic studies, the reported genes were D10L (host range protein), D14L (complement inhibitor), B10R (apoptotic regulator), B14R (interleukin 1 binding protein), and B19R (serine protease inhibitor-like protein) [18, 21]. Among all genes, D14L has been thought to be the main cause explaining the variances in virulence between MPXV clades as it is absent in West African clades [21, 22]. However, further investigations are warranted to understand the molecular and genetic mechanism sitting behind the differences between these two clades.

4. Species Affected and Transmission

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MPXV can cause diseases in a broad range of mammalian hosts even though the natural host is yet to be known. Evidence showed that the virus had been isolated two times from wild animals: a rope squirrel (*Funisciurus anerythrus*) in the DRC [23] and a sooty mangabey (*Cercocebus atys*) in Ivory Coast [24]. It can infect multiple mammalian rodents host in Africa including prairie dogs (Cynomys spp), Gambian pouched rats (Cricetomys gambianus) [25]. In addition, *Funisciurus* spp. (rope squirrels), *Graphiurus lorraineus* (African dormice rodents), *Cricetomys emini* (African giant pouched rats), *Heliosciurus* spp. (sun squirrels), *Oenomys hypoxanthus* (rufous-nosed rats), and *Petrodromus tetradactylus* (elephant shrew) have been found seropositive to MPXV [26]. It is also reported that *Sus scrofa* (domestic pig) and *Macaca mulatta* (rhesus macaque), are vulnerable to MPXV [27]. The virus may also be transmitted through contaminated saliva, respiratory droplets, contact with the exudates of lesions or crust materials, feces, and contaminated patient's environment or items [28, 29].

Inter-human transmissions are favored by some risk factors such as sharing of room and bed, drinking and eating in the same dish, and living in the same household [30]. In addition, sleeping in open-air or on the ground, staying or leading a sylvan life close to the jungle were considered as factors that intensify the risk for animals' exposure which ultimately primes the risk for animal-to-human transmission of the virus [31]. Interestingly, supporting toileting and sanitation, washing, and ironing related to clothing did not have a vital relationship with risk factors for the diffusion of infections. However, the processing of wild animals for feasting or consuming duiker was reported as protective factor (Nolen et al., 2015). MPX has been accredited as hospital-borne infection [32, 33] and venereal transmission has also been postulated for diseased persons with genital lesions to healthy ones [34]. There is also a report of trans-placental transmission during pregnancy and sometimes it could be a cause of foetal death [35] **(Figure 4)**.

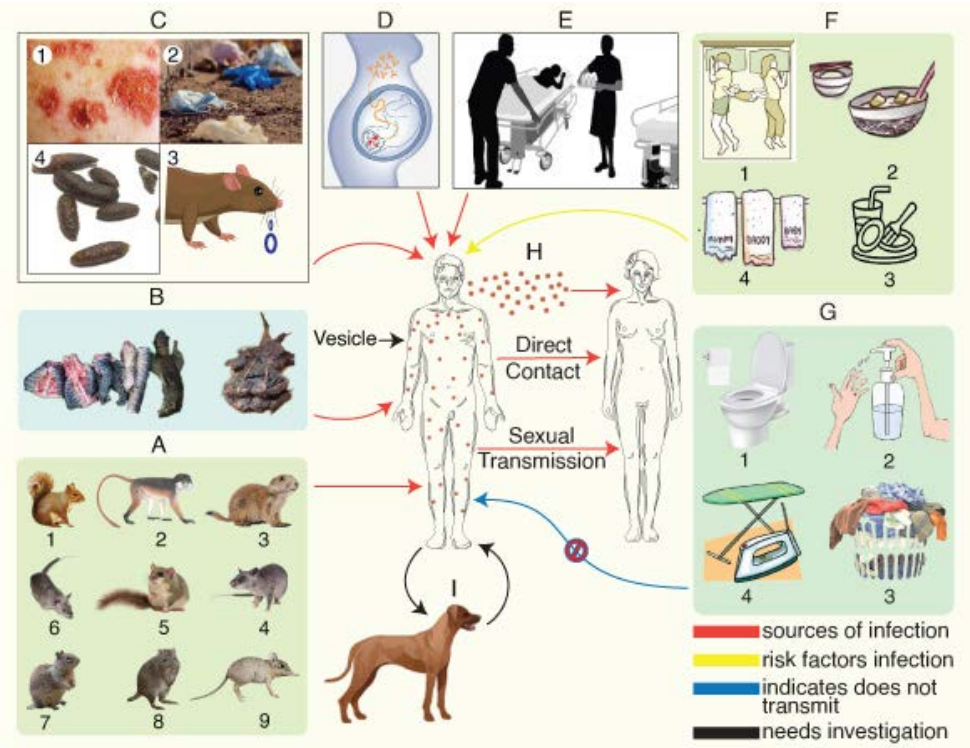

Figure 4. Transmissions of MPX viruses. Schematic illustration to show the different routes of transmission. A=1. Rope squirrel, 2. Sooty mangabey, 3. Prairie dogs, 4. Gambian pouched rats, 5. African dormice rodents, 6. African giant pouched, 7. Sun squirrels, 8. Rufous-nosed rats, 9. Elephant shrew; B= Bush meat; C=1. Skin crust, 2. Patient's used materials, 3. Contaminated saliva, 4. Fecal materials. D=Transplacental transmission. E=Hospital-borne infection. F= Shared 1. bed, 2. food, 3. glass and other utensils, hand towel. H =Respiratory droplets, and G =1. Assisting in toilet, 2. Sanitation and hygiene, 3. Washing clothes and 4. Iron and laundry; I=Dog may be infected from human.

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5. Monkeypox Virus Emergence

Monkeys living in the rain forest of Africa were infected naturally with MPXV and as they were favorite animals to the local villagers, these might have been acting a source of human infection [36]. MPX infection in humans produces very similar lesions as in smallpox [37]. The disease might have been ignored in existence till 1970 when the first human cases were established. Before that time, the virus was circulating among captive primates only [38]. The disease drew the attention of world scientists when massive and intensified steps were taken to eradicate smallpox in Central and West Africa [36].

The viruses are thought to be the prospective pathogens of emerging and or re-emerging infections as they have wider options for host choice that emphasized their zoonotic significance. More than 50% of zoonotic viruses having three or more non-human hosts are considered emerging pathogens [39]. Rodents could be potential reservoir for monkeypox virus as there is a close and consistent relationship between rodent hosts and Orthopoxvirus [40].

6. Evolution of Monkeypox Virus

Gene-related host specificity, subcellular trafficking and immune-modulation of VARV and MPXV have a complement of an open reading frame with unidentified function, non-coding sequence regions, and long inverted terminal repeats (ITR). Alterations in the content of genes i.e. gene gain and lost (**Figure 5**) may offer opportunities for the chances of adaptation for the Orthomyxovirus to another host [41]. The genomic evaluation indicates that it is common for the alteration of genes either by acquired or lost to be related to host-specific properties [41].

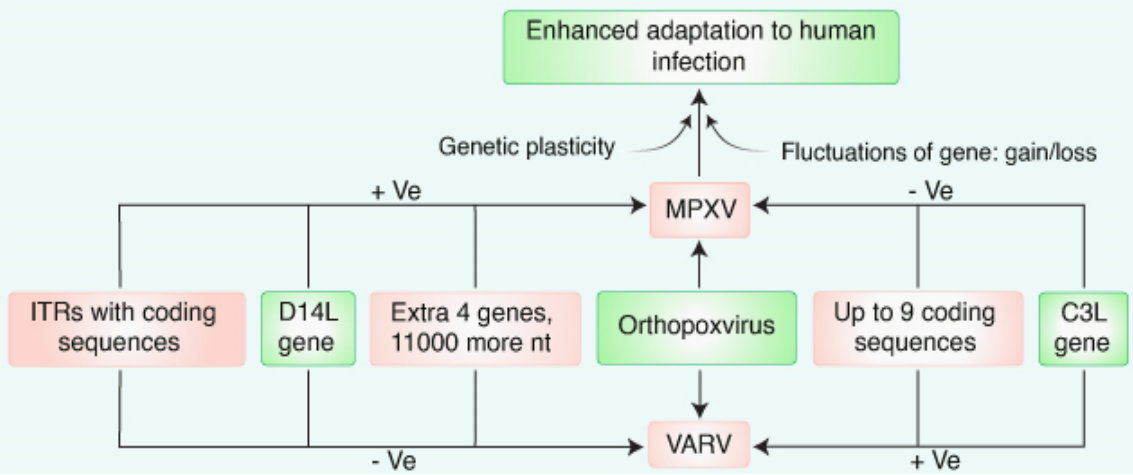

Figure 5. Evolution of MPX viruses. MPX=Monkeypox.

Usually, MPX viral genomes have more DNA content than that of variola [42] and the genome of MPXV contains four extra genes and is almost 11000 nucleotides larger than the variola genome. Moreover, it has nearly 10.5 longer ITRs and additional coding sequences in ITRs whereas variola don't have any of these [43]. Undoubtedly, variola possesses one of the most important size-restricted genomes among all Orthopoxvirus. It has up to 9 established coding sequences which are absent in MPXVs but it retained fragments only [22]. Numerous gene loci present in variola are missing or curtailed in MPXV and are assumed to be responsible for immune evasion and virulence. C3L, a gene responsible for virulence properties present in variola, encodes an inhibitor of complement enzymes. An ortholog of this gene (D14L) is either absent or truncated (but working) protein in MPXV [18, 21], and its adaptations to humans further might happen through the addition of gene or alterations of nucleotide positions and optimizing these non-equivalent unwanted pathways.

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7. Clinical Representations of MPX

MPX shows clinical signs very close to smallpox patients but the brutality is not so severe. The incubation period is mostly 7-14 days but may be extended up to 21 days. Patients usually have a report of contact with animals and people with MPX. MPXV infection largely could be separated into two phases (**Figure 6**): 1) the prodromal phase which lasts for around 02 days and is characterized by fever, stark headache, muscle aching, tiredness, and lymphadenopathy, and 2) the rash phase which lasts for 7-21days and characterized by abnormal changes in texture and color of the skin throughout the body. The patients become contagious after the appearance of rashes that develops 15 days after the onset of fever. The rash usually appears more densely on the face (95%) followed by dissemination to other parts of the body such as palms and soles of the feet (75%), oral mucosa (70%), genitalia (30%), and conjunctiva (20%). The rash persists for around 24 weeks and progresses from plaque to papules, blisters, pustules, scabs, and then shedding off. The number of lesions could be ranged from a few to several thousand [44]. In acute cases, some lesions can coalesce to form a large patch of skin which sometimes falls off. Patients can develop lymphadenopathy, most frequently in the groin region which further may be problematic by secondary bacterial infections, respiratory troubles, bronchopneumonia, encephalitis, corneal infection with loss of vision, dehydration because of vomiting, and diarrhea [44, 45]. Though MPX is a self-limiting illness, it could be dangerous with an unexpected outcome. The degree of exposure of the host to the virus determines the intensity of severity of the disease and also by patients' health status. Children are usually more severely suffered with a case fatality rate of 1-10% [34].

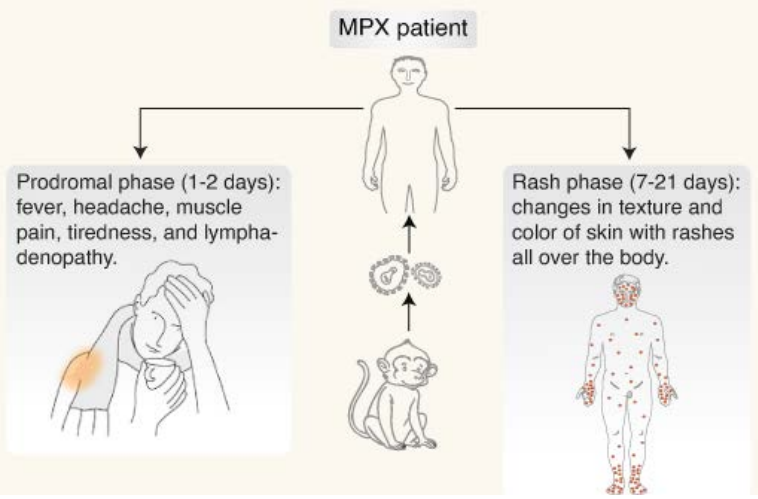

Figure 6. Clinical representation of MPX infection. MPX=Monkeypox.

8. Pathogenesis of MPX

MPXV enters the host through different routes such as oropharynx, nasopharynx, or intradermal routes. Primary replication occurs at the inoculation site and causes primary viremia. Then the viruses go to the zonal lymph nodes followed by secondary viremia and spread to the skin and tertiary organs of body like lungs, eyes, gastrointestinal tract etc. The pathogenesis of MPXV includes viral entry, fusion, replication, and release of the virus from the host cell. At that time virus can produce two infective forms: intracellular mature virions (MV) and extracellular enveloped virions (EV). MVs are membrane-bound which leave the host cell after lysis. It is relatively stable and mainly used for transmission between animals. On the other hand, EVs are specialized MVs bound by a triple membrane which is gained at the time of translocation in Golgi bodies and released by exocytosis [46].

Generally, DNA virus replicates in the nucleus but MPXV completes the replication cycle in the cellular cytoplasm. Similar to other Orthopoxviruses, some enzymes are

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required for viral replication and structural protein synthesis are encoded by genes that are highly conservative and generally positioned in the central part of the genome and those required for interaction with the host are less conserved and located at the terminal part of genome [47]. The proteins necessary for viral DNA replication, transcription, assembly, and release are encoded by these genes [47].

9. Pathology of MPX

Macroscopically, the eyelids of prairie dogs showed yellowish mucoid discharge, ulceration in the middle of the tongue measuring 3 to 4 mm diameter, and red-brown consolidated patchy zones involving 50% of pulmonary parenchyma. Red with a little dispersed, tanned, and spotted areas were observed in livers [48].

On microscopic investigation, necrotic foci in the eyelids, and ulcerated lesions containing necrotic debris and pyknotic epithelial cell, and swollen columnar epithelial cells with Guarnieri-like inclusions were revealed in the palpebral conjunctiva and tongue [48]. Infiltration of varied inflammatory cells, necrosis, and edema was present in the submucosa. Ballooning type degeneration of epithelial cells, acantholysis, and cellular necrosis were noted in the skin and palpebral conjunctiva. Guarnieri-like inclusions and Ballooning degenerations were also shown on the squamous epithelium of lungs. Neutrophils and histiocytes were infiltrated in the lumen and macrophages with intranuclear cytoplasmic inclusions in the lung alveoli formed some syncytia [48].

10. MPXV Mutation and Novelty

Usually, RNA viruses mutates faster than DNA viruses. MPXV does not show many mutations as it is a DNA virus [49]. However, more mutations are thought to have for MPXV isolated from 2022 which indicated that the virus is changing and spreading more competently. Amazingly, sharing 40 mutations of 2022 viral isolates distinguishes it from its nearest variant [50]. Based on a chronological development, it might be expected that a virus like MPXV would pick up a good deal of mutations within the next few decades. However, because of transmission abilities among population, MPXV might have mutations some of which could be harmless or dangerous, and may take advantage of other strains [50]. Still the knowledge is in rudimentary about how MPXV interacts with the host and what consequences of these interactions might have on viral replication. Sometimes, viral mutations are induced by the host immune system or enzymes [51] which lead to the emergence of deleterious mutants. Changing of MPXV might have been taken off in 2017 as evidenced by available literature. The circulating virus among humans demonstrated that MPXV has 10 times mutation rate than the virus's standard mutation rate [50]. Further investigation into how the mutated viruses interact with the host is needed.

High-frequency recombination has been reported in the course of replication in cells within poxviruses [52]. Inter-species recombination in natural condition has also been proved between cowpox virus and ectromelia virus among orthopox viruses [53]. However, there is no report of natural recombination in case of MPXV until now but it could be one of the driving forces for poxvirus evolution. Sasani *et al.* showed that tandem gene duplications are the outcome of recombination [54].

Variola virus changed 1-2 nucleotides per year which is similar to MPXV [55]. The genome isolated from the first West African MPXV differs from that of MPXV of 2022 by 0.06%. Nucleotide of MPXV showed that its AT content is almost two times than GC content [56]. Mammalian DNA and RNA tethering or editing enzymes are reported to wield selective pressures on viral genomes and introduce a biasness in the genomic nucleotide usage. For instance, viral mutation can be augmented by APOBECs which leads to a diminution in the C content and elevation in the T content due to cysteine deamination [57]. MPXV genomes analyses from the enduring 2022 pandemic occurrence showed that APOBEC3 editing enzymes were responsible for ~ 90% of new nucleotide alterations [56].

It is assumed that, near about 10,000 bp genome fragments of MPXV were lost in West Africa due to recombination leading to the divergence of the two MPXV clades [18].

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Neoteric microevolution in the MPXV genome of 2022 emerged a subset of strains containing a 913 bp frameshift deletion homologous of variola virus Ankyrin/Host Range D7L protein liable for IL-18-binding and immune avoidance [56, 58]. How the mutation played role in the 2022 outbreak strains still needs to be answered.

11. Host-MPXV Interactions

Poxviruses have accompanying copious mechanistic structures or approaches to escape and or worsen host's immune response against infection [59]. There is no available information about the innate and adaptive immune response of the host against MPXV infection. Host-MPXV interactions are still now on the blooming stages. One of the most important constituents of innate immunity, the natural killer (NK) cells directly kills virus-infected cells via the secretion of cytokine and regulates the task of other immune cells including T cells and dendritic cells. Activation or inhibition of NK cells are initiated by the interplay among activating or inhibitory receptors on NK cells and their ligand-like major histocompatibility complex-1 (MHC-1) molecules. Perforin and granzymes containing secretory granules and cell to cell interactions facilitate the killing effect of NK cells. During the early stage of infection, NK cells secrete interferon (IFN) and tumor necrosis factor (TNF) that mediate inflammatory response of inflamed tissues. These cytokines dictate dendritic cells to induce T helper (Th1) cells polarity [60]. Lymphocytes number including NK cells are changed in nonhuman primates because of MPXV infection. It also causes lymphadenopathy as well as depletion of lymphoid organs [61]. T cells play an important role in regulating and eliminating viral infections. The immune surveillance fails to detect viral reservoir at the interface when lymphocyte, particularly CD4+ and CD8+ T cell interact with MPXV infected cells and MPXV inhibit the activation of lymphocytes which facilitate dodging of the immune system. Antiviral CD4+ and CD8+ identify MPXV infected monocytes not so strongly which indicated that the production of inflammatory cytokine (IFN or TNF) was not set off by MPXV using virus-specific T cells but the virus can infect primary human monocytes efficiently [62].

Immune bypassing genes such as TNF-receptor, IFN- receptor, MHC class 1, interleukins etc. transferred horizontally within poxviruses are ascribed for avoiding host's immunity and coevolution for many years confers host-restriction among poxviruses [63]. A wide range of host tropisms among the genus of Orthopoxvirus contributes to enhancing host-virus interaction, reduction in pathogenesis and various immune escaping molecules [63, 64]. MPXV uses exclusive immune escaping approaches, MHC independent pathway and depends on MPXV-infected cells to enhance the unresponsiveness of T cells, to avid host's immune surveillance. This immunosuppressant effect necessitates direct cell-to-cell interaction. MPXV codes a novel type of immune modulator that directly or indirectly repress the antiviral activity of host's T cells ($CD4^+$ and $CD8^+$) [62].

12. Monkeypox and Stigmatization

In the US, the vast majority of monkeypox (98%) positive cases have been concentrated among men who have sex with men (MSM). The prevalence of the virus among MSM — combined with its ability to affect anyone. However, as with the HIV, the epidemiologic risk of acquiring monkeypox is greatest at present and is exclusive to MSM or others in the LGBTQIA+ community (Fig. 7). This issue only heightens that stigma or MSM and makes addressing the sexual health of all people even more challenging [65]. This issue cannot be ignored and must be investigated carefully.

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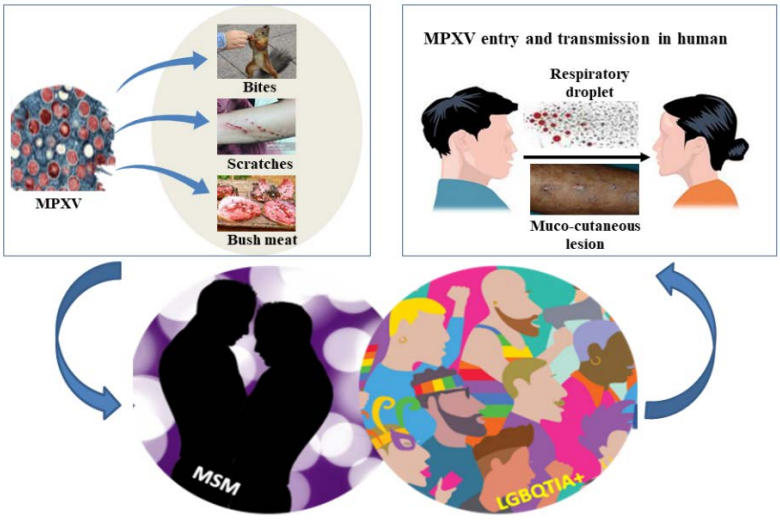

Figure 7. Stigma-a room for Monkeypox thread.

13. Diagnosis

To curb the occurrence of MPX, a speedy but confirmative diagnosis is a must. Based on clinical signs only, MPX can't be diagnosed as it may be confused with smallpox virus infection. Moreover, other viruses such as chickenpox, measles, bacterial skin infection, medication allergies, syphilis etc. can also produce similar clinical manifestation and make difficulties for clinical differential diagnosis [45]. Serological diagnostic methods provide the evidence of viral exposure but these testing suffer from the restrictions of cross-reactivity as it will detect immune responses to other members of Orthopoxvirus exposures. To minimize this, researchers are trying to find highly specific immunoglobulin that could be used for screening test. One such recognized antibody is 69-126-3-7 monoclonal antibody (Mab) developed against MPXV which is very precise and binds with the heparin binding domain of the A27 protein of MPXV [66]. IgM and IgG antibodies could be detected after 5-7 days of infection by enzyme-linked immunosorbent assay (ELISA) and viral antigen identification by immunohistochemistry are of traditional use for diagnosis of the disease. The most important diagnostic methods for MPX comprises PCR, isolation of the virus in cell culture, electron microscopy, immunohistochemistry and immunological methods [45]. This could be used for screening of patients for prioritizing which might be needed for further confirmative methods.

Gene encoding the various types of protein such as Extracellular-envelope (B6R), DNA dependent RNA polymerase subunit 18 (rpo18), DNA polymerase (E9L), and F3L etc. are regularly detected from clinical samples by RT-PCR [67]. Recently, a new assay, recombinase polymerase amplification (RPA) was established to detect G2R gene of the virus within 7 minutes [68]. The test claimed 100% specificity with 95% sensitivity in detecting strains of the virus. Tetracore Orthopox BioThreat Alert®, a rapid and point of care diagnostic tool, has been succeeded for detecting suspected cases of MPX in field condition which could be useful for prioritizing patient which might be looked-for additional confirmative test.

14. Immunoprophylaxis and Chemotherapy

Prevention of disease by producing active or passive immunity is considered the best choice as it is relatively safe. A DNA vaccine, 4pox (L1 and A27 immunogens for Mature virion, A33 and B5 immunogens for enveloped virion of Orthopoxvirus), has been developed with the advancement of gene-based technology and different animals models were used to test its efficiency. It prevents the shedding off the virus from the vaccinated host and decreases the virulence of viral infection [69].

It was demonstrated that immunization with smallpox virus enables cross-protection against other Orthopoxviruses along with MPXV. According to the available

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information, it was indicated that almost 90% of the detected cases are inexperienced to the Orthopoxvirus contagion of which a large number of them were born after the ending of smallpox vaccination program and declaring the eradication of smallpox (70). Almost, 85% protection against MPXV has been found to those people who had been immunized earlier by smallpox [45]. During the MPXV endemic in 2003 in the USA, smallpox vaccine (ACAM2000) suggested by the Center of Disease Control and Prevention (CDC) proved to lessen the symptom but failed to prevent illness [45]. Because of some adverse effect in immunosuppressed patient, the vaccine was not suggested and hence was not available for further use. As an alternate of ACAM2000 for primary use, Advisory Committee on Immunization Practices (ACIP) in 2022 suggested JYNNEOS, a replication-incompetent, attenuated third generation modified vaccinia virus Ankara (MVA) [71]. This vaccine showed relatively less side effects when compared with ACAM2000. It was free from the risk of inadvertent inoculation as there was no cutaneous reaction. The vaccine administration was suggested by a 2-dose regimen with a 28 days interval. Every 10 years for individuals who are exposed to the less virulent Orthopoxviruses and every 2 years for individuals who are exposed to the more virulent Orthopoxviruses has been recommended as booster dose [71].

Some authors have reported that human-chimeric Mab –c7D11 (against MV), and c8A (against EV) enable the host's protection against a lethal dose of MPXV [72]. A recombinant vaccinia virus immunoglobulin (rVIG) has demonstrated a higher neutralizing efficiency against Orthopoxviruses in *in vitro* and *in vivo* when both were administered with a view of preventive and medicinal approach [73]. rVIG considerably saved mice when intraperitoneally inoculated 14 days earlier or 6 days post challenge without showing any known adverse effect. Moreover, the vaccine decreased morbidity in term of weight-change, reduced vDNA levels in blood, ALT (a marker of liver damage), and less infectious virus particles in the liver [73].

Food and Drug Administration (FDA), USA has approved Tecovirimat (ST-246 or TPOXX) for a clinical trial on animal models and found it efficacious in diseased animals. It works by blocking the shedding of intracellular viruses from host cell. No adequate data relating to its effectiveness in treating human MPX cases but the drug was found safe and tolerable. Similarly, cidofovir and brincidofovir (CMX001 or hexadecyloxypropyl-cidofovir) effectively inhibit the DNA polymerase both *in vivo* and *in vitro* model and could be of useful [67]. The productions of extracellular viruses were inhibited by TPOXX and F13L gene coded product interaction with cellular cytotoxicity in some cell line including human origin was >50 μM. F13L gene coded phospholipase is required by the formation of a protein complex that catalyzes the envelopment of intracellular mature virion [67, 74.

However, Tecovirimat resistance can be achieved in viruses due to mutation in the F13L genes which could be overcome by PAV-164, a derivative of methylene blue that potentially inhibited the replication of MPX viruses [75]. The compounds were also able to inactivate virions before causing infection and stopped viral binding, fusion, and entry into host cells. It also showed a potent virucidal action at non-cytotoxic concentrations.

15. What happens next and what to do?

Although MPXV is considered as moderate health hazardous pathogen [10], but anytime, it can be highly pathogenic as it has the capacity for mutation. The decline in herd immunity associated with the ending of smallpox vaccination, greater interactions between human and MPXV reservoirs because of climatic change, disforestation and urbanization, bushmeat ingestion, and poor health condition might have shaped eco-immunological niche for the reemergence of MPXV across the globe. MPXV is an ignored zoonotic virus with a possibility of ill use as bioterrorism. The recent global outbreaks have shade the light for constant and careful surveillance along with the advancement of unique vaccination and chemotherapeutic approaches. The use of smallpox vaccine as a prophylactic measure and declining immunity in a population may pose a potential danger for the

spread of the viruses. Hence, the world biological researchers must need to pay due attention to further research on MPXV keeping in mind with the terrible experiences gathered from the Covid-19 pandemics. We will hopefully see more genomic data and research outcomes published, which will provide us with a better understanding of the 2022 MPXV and the current outbreak.

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