REVIEW

Monkeypox virus from neurological complications to neuroinvasive properties: current status and future perspectives

Ali Sepehrinezhad[1,2] · Rezan Ashayeri Ahmadabad[3] · Sajad Sahab-Negah[2,4]

Abstract

Cases of monkeypox (MPV) are sharply rising around the world. While most efforts are being focused on the management of the first symptoms of monkeypox, such as cutaneous lesions and flu-like symptoms, the effect of the monkeypox virus (MPXV) on multiple organs still remains unclear. Recently, several neurological manifestations, such as headache, myalgia, malaise, fatigue, altered consciousness, agitation, anorexia, nausea, and vomiting, have been reported in patients with MPV. In addition, data from experimental studies have indicated that MPXV can gain access to the central nervous system (CNS) through the olfactory epithelium and infected circulatory monocytes/macrophages as two probable neuroinvasive mechanisms. Therefore, there are growing concerns about the long-term effect of MPXV on the CNS and subsequent neurological complications. This paper highlights the importance of the neuroinvasive potential of MPXV, coupled with neurological manifestations.

Abbreviations

BBB	Blood–brain barrier
CNS	Central nervous system
CSF	Cerebrospinal fluid
ELISA	Enzyme-linked immunosorbent assay
MPX	Monkeypox
MPXV	Monkeypox virus
MRI	Magnetic resonance imaging
PFU	Plaque-forming unit
RT-PCR	Real-time polymerase chain reaction
UK	United Kingdom
U.S.A	United States of America
ZAI-96	Strain Zaire-96

✉ Sajad Sahab-Negah
sahabnegahs@mums.ac.ir

Ali Sepehrinezhad
sepehrinezhad.a@iums.ac.ir

1 Department of Neuroscience, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran

2 Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

3 Shefa Neuroscience Research Center, Khatam Alanbia Hospital, Tehran, Iran

4 Department of Neuroscience, Faculty of Medicine, Mashhad University of Medical Sciences, Pardis Campus, Azadi Square, Kalantari Blvd, Mashhad, Iran

Introduction

Monkeypox virus (MPXV), a double-helix DNA virus, causes zoonotic monkeypox disease that belongs to the family of Poxviridae, a subfamily of Chordopoxvirinae and Orthopoxvirus genera [1, 2]. This genu induces cutaneous manifestation in diseases, such as smallpox (caused by variola virus), cowpox (caused by cowpox virus), camelpox (caused by camelpox virus), and monkeypox in humans [3, 4]. The most common symptoms of monkeypox infection in humans are fever, swollen lymph nodes, exhaustion, chills, back pain, and skin rashes [5, 6]. Furthermore, some neurological complications, such as headache, malaise, myalgia, anorexia, and altered consciousness have been sporadically reported in MPXV patients [7–9]. These neurological manifestations may be related to the invasive potential of MPXV to the brain tissue that has been elucidated in some infected animals [10–12]. There are very few studies in the literature addressing the neuroinvasive potential of MPVX. It is thus crucial to have a mechanistic view on this topic based on the current evidence. Therefore, we highlighted the range of neurological complications associated with MPXV infection

in humans. Furthermore, we determined some possible neuroinvasive properties of MPXV from experimental data.

A brief history of geographical aspects of the monkeypox virus

The MPX was named for the first time when the virus was discovered in monkeys at the State Serum Institute of Copenhagen in 1958 [13]. In August 1970, the first human case of MPX infection was identified in a 9-year-old boy that was admitted with fever to Basankusu Hospital in the Democratic Republic of Congo [14]. Wild animals including primates (mangabey monkeys), Gambian pouched rats, and squirrels are considered as the main reservoirs for MPXV [15]. Direct contact with infected animals' body fluids, touch, bite, scratch, hunting, and cooking are major transmission ways of MPXV from animal to human [16, 17]. Up

to now, two clades of MPXV were phylogenetically identified. One strain originated from Central Africa–Congo Basin [strain Zaire-96 (ZAI-96)] that is more virulent than other strains derived from Western Africa (SL-V70, COP-58, and WRAIR-61) [18, 19]. Between 1970 and 1986 about 404 new cases of MPX had been reported in Central Africa and Western African countries, especially in tropical rainforest areas like Zaire, where cases had direct contact with infected animals [20]. The first outbreak of MPXV in a country outside of Africa was reported in the Midwestern United States in 2003 [9, 21, 22]. In this outbreak, the reservoirs were changed from monkeys and squirrels to prairie dogs which are native animals of the United States (Fig. 1). The source of MPXV in the prairie dogs was imported infected Gambian pouched rats from Ghana into the United States [10, 23]. During the next outbreak, the first case of MPX was confirmed on May 7, 2022, in a British person who travelled to Nigeria and then returned to the United Kingdom [24]. As
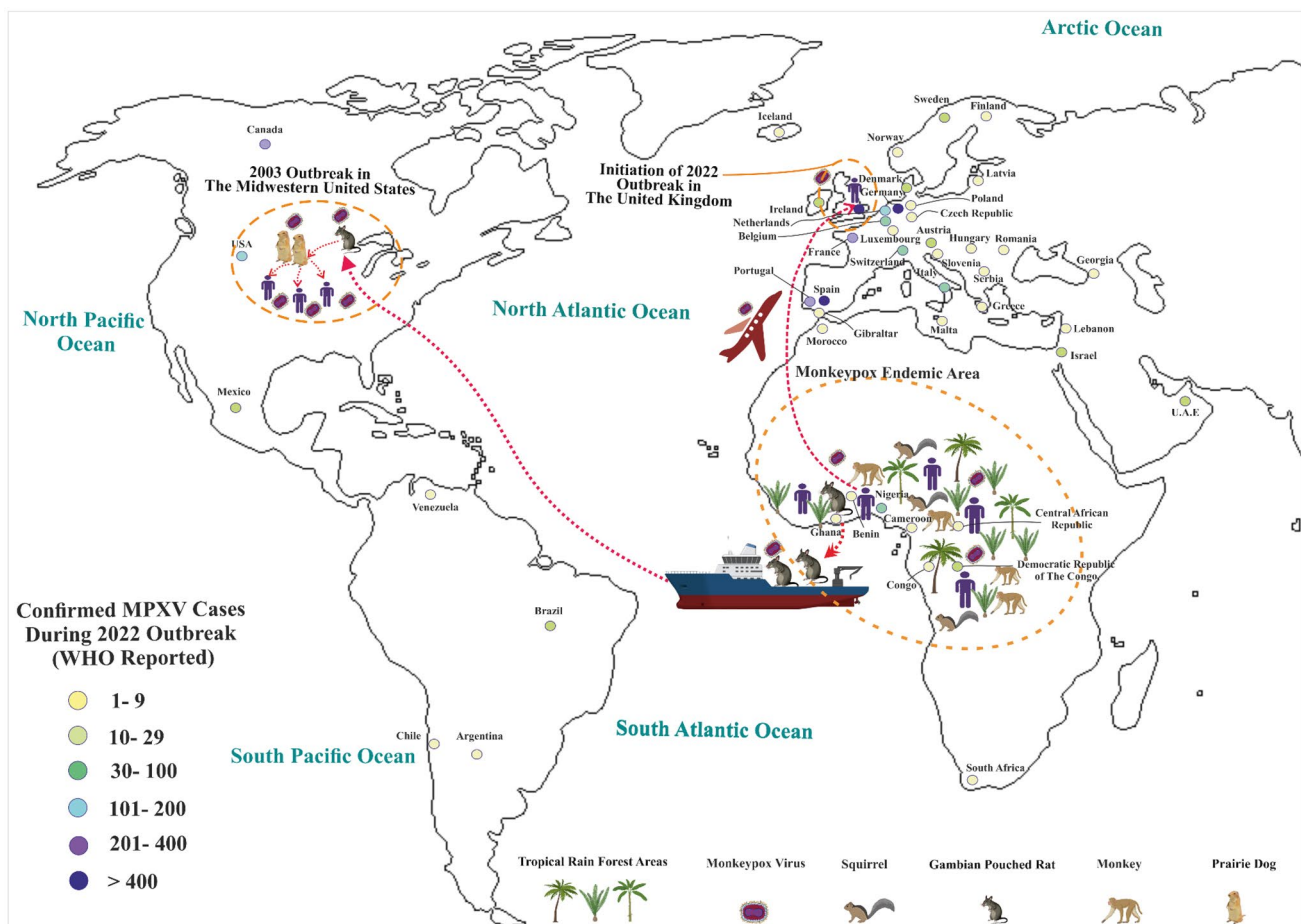

Fig. 1 Schematic overview of initiation, spreading, and distribution of MPXV. MPXV appears mainly in tropical rainforest areas of Central and Western Africa where MPXV reservoirs, such as monkeys, squirrels, and other rodents have a habit. Prairie dogs have been recognized as the main animal reservoirs for transmitting MPV to human during the 2003 outbreak in the U.S.A. Recently, the first confirmed case of MPX was identified in a British person who travelled to Nigeria and returned to the United Kingdom (UK). MPXV: Monkeypox virus; WHO: World Health Organization

of 22 June 2022, 3413 confirmed cases of MPX have been reported from 50 countries. From 17 to 22 June, about 1310 new confirmed cases have been added. The United Kingdom, Germany, Spain, and Portugal with totally of 793, 521, 520, and 317 cases, respectively, had more confirmed cases of MPX worldwide until 22 June 2022 [25].

Detection of MPXV at a glance

Poxviruses have a large linear double-stranded DNA that encodes all enzymes to replicate and assembly, but they use the host cell's ribosomes to conduct translation process in the cytoplasm of infected cells. Morphologically in MPXV, the virions are ovoid or brick-shaped that are covered by a lipoprotein outer membrane about 200 by 250 nm in size in electron micrograph examinations [26, 27]. MPXV is resistant to heat and cold as heating until 40 °C had no strong effect on its infectivity [28], while some chemical agents, such as chloroform, methanol, formaldehyde, and phenol significantly inactivate its pathogenesis [13, 28]. Host infection is mediated through the interaction of viral proteins to host glycosaminoglycans that subsequently initiate the cellular endocytosis process and virus entry. In most poxviruses infection, two types of morphologically different virions, such as intracellular mature virus and extracellular-enveloped virus spread infectious into the host [26]. MPXV transmits from animals to human and human to human. Animal to human transmission (zoonotic transmission) mainly takes place in direct contact with body fluids, touch, bite and scratch of infected live/dead animals [29, 30], just like what happened in the initiation of MPX endemic in Central and Western Africa in 1970. The second form of transmission is human to human which maybe the route responsible for raising the cases of MPXV worldwide. The main human-to-human transmission routes are close contact with a MPX positive or symptomatic person, especially in health workers, sexual partners, and household members. Furthermore, respiratory droplets, contaminated materials, mouth ulcers and lesions as well as other mucosal secretions are considered transmission routes for human-to-human spread [31–34]. MPXV is diagnosed using a set of genetic, phenotypic, immunologic, and electron microscopic methods [26]. For example, a real-time polymerase chain reaction (RT-PCR) test is used to detect the MPX-specific DNA in the skin biopsy specimens [35]. Moreover, immunohistochemistry is applied to reveal the virus antigens in specific tissues and enzyme-linked immunosorbent assay (ELISA) is utilized to detect IgG and IgM antibodies against MPXV in the blood samples. In some cases, electron microscopy may be used to identify poxvirus virions in tissue specimens [36]. It should be noted that MPXV can be identified if the characteristic skin lesions are present and there is a history

of exposure with suspected cases of MPXV [37]. Despite the precise detection of MPXV, the invasiveness and complications of MPXV on the second organs, such as CNS are crucial for basic scientists and clinicians. The CNS infection of MPXV not only can cause long-lasting brain injury but also can induce other neurological manifestations as we reported for SARS-CoV-2. Therefore, presenting current evidence of the neuroinvasive property of MPXV can inform us to focus on this aspect of the virus and make a better decision on clinical management. In the next outlines, we present evidence of neurological manifestations and neuroinvasive properties of MPXV.

Neurological manifestations of MPXV

The preliminary data showed a wide range of neurological manifestations from less serious and nonspecific symptoms including headache, myalgia and fatigue to more severe complications like seizure and encephalitis. The symptoms varied broadly, but the most prevalent were headache, myalgia, fatigue, photophobia, pain and fewer cases of encephalitis and seizure (Table 1). Data for psychiatric symptoms (i.e., depression, anxiety, and suicide) were not included in the current study. Here are brief reviews of published reports on neurological manifestations after MPXV. For example, a severe case of MPXV, who suffered from headache, and myalgia was reported in The United States of America (USA). She was the third reported child there who exposed to an infected prairie dog before admission [38]. In addition, headache, fatigue and myalgia were frequent neurological symptoms in seven other confirmed MPXV patients in the Western hemisphere [9]. A family cluster with MPXV was also reported in the Midwestern USA that had been exposed with prairie dogs. Of three family members infected, two showed mild skin rash only and one presented with severe encephalitis which required hospitalization. The two milder forms had been previously vaccinated with smallpox vaccine. A wide range of neurological manifestations including headache, fatigue, myalgia, confusion and seizure were seen in the severe case. Neurological examination revealed decreased level of consciousness, pupillary dilatation, optic disc edema, loss of corneal reflexes and reduced deep tendon reflexes. Additionally, magnetic resonance imaging (MRI) confirmed hyperintensity in thalamus, brainstem and right posterior parietal cortex consistent with mixed cytotoxic and vasogenic brain edema. Pleocytosis was also detected in the analysis of the cerebrospinal fluid (CSF) [7]. In another study, seizure and confusion accompanied other neurological manifestations such as headache and myalgia in the USA [6]. Furthermore, a variety of neurological complications such as headache, myalgia, pain and photophobia were reported in MPXV confirmed cases in the Bayelsa State of Nigeria

Table 1 Main reported neurological manifestations of patients infected with MPXV

Location	Confirmed MPXV cases	Reported neurological complications (number of cases)	Time period	References
Democratic Republic of Congo (Zaire)	282	Encephalitis (1) and coma (1)	1980–1985	Ježek et al. [40]
U.S.A	1	Agitation, headache, myalgia and fatigue	2003	Anderson et al. [38]
U.S.A	11	Headache (11) and myalgia (1)	2003	Reed et al. [9]
U.S.A	3	Headache (2), seizure (1) and encephalitis (2)	2003	Sejvar et al. [7]
U.S.A	34	Headache (23), myalgia (19), confusion (2), encephalitis (1) and ear pain and seizure (1)	2003	Huhn et al. [6]
U.S.A	19	Headache (13)	2003	Croft et al. [41]
Sudan	10	Myalgia (7), fatigue and headache (5)	2005	Formenty et al. [5]
U.S.A	37	Headache (32) and myalgia (36)	2003	Reynolds et al. [42]
Nigeria	1	Headache and fatigue	2017	Yinka-Ogunleye et al. [43]
Central African Republic	21	Myalgia (5) and headache (5)	2016	Kalthan et al. [44]
Nigeria	18	Fatigue (13), headache (12), myalgia (5), pain (5) and photophobia (3)	2017	Ogoina et al. [39]
Singapore	1	Myalgia	2019	Ng et al. [45]
Nigeria	118	Headache (89), myalgia (74), fatigue (59) and photophobia (38)	2017–2018	Yinka-Ogunleye et al. [46]
Nigeria	40	Headache (19), photophobia (9), encephalitis (3) and seizure (1)	2017–2018	Ogoina et al. [47]
Democratic Republic of Congo	134	Headache (99), myalgia (90) and fatigue (115)	2009–2014	Hughes et al. [48]
Nigeria	2	Headache (2)	2018	Eseigbe et al. [49]
UK	7	Headache (1), severe pain (1), and emotional lability (1)	2018–2021	Adler et al. [9]
U.S.A	21	Headache (21) and fatigue (21)	2022	Charniga et al. [50]
Democratic Republic of Congo	216	Headache (49), myalgia (15), ear pain (14), visual changes (10), dizziness (3) and decreased hearing (2)	2007–2011	Pittman et al. [51]

in a cross-sectional study [39]. Taken together all previous reports we can conclude that currently, the development of CNS and peripheral nervous system complications in MPX patients has not been well established, but there are at least scattered case reports of patients with neurological features and those with severe complications, in particular, require emergent treatments. It is thus imperative to start treatment as quickly as possible while the pathogen spread is mitigated at the population level. To this point, we highlighted available evidence of the neuroinvasive potential of MPXV from experimental data.

Neuroinvasive propensity of MPXV

The neurotropic feature of MPXV on human subjects has not been fully understood, while data from animal studies (i.e., on rodents) have revealed that MPXV can cross the blood–brain barrier (BBB) and showed neuroinvasive capacity as summarized in Table 2. Intranasal and intraperitoneal administration of MPXV strain of 2003 is a well-established disease and caused animal death in ground squirrels after

about 1 week. Necropsy findings indicated high titers of MPXV, using the number of plaque-forming units (PFU) per millimeter of homogenized tissue, in the brain tissues of animals [52]. Furthermore, during the 2003 outbreak, results of MPXV-specific PCR assay of four MPXV suspected rodents (i.e., a prairie dog, a hamster, and two gerbils) showed that the virus penetrated into the brain tissue [53]. Similar findings were reported in rope squirrel, pouched rat, dormice, and again prairie dogs in which MPXV DNA was detected in the brain tissues [10]. As can be seen from Table 2, MPXV can reach the brain parenchyma in animal models; however, considerably more work will need to be done to detect the neuroinvasive and neurotropism of MPXV in human subjects. It is also important to figure out the transmission routes of MPXV to the CNS. Currently, the exact transmission routes of MPXV are not clearly defined; however, previous studies on animal subjects have suggested two probable different routes: (i) olfactory epithelium route and (ii) infected monocytes/macrophages transmission way (Fig. 2).

For instance, the accumulation of MPXV was significantly increased in the nasal septum mucosa and brain tissue

Table 2 Evidence for neuroinvasive property of MPXV in different infected animal species

Species	Rout of infection	Neuroinvasive evidence	Strain	References
Ground squirrel	i.n. and i.p	Virus titration was positive in the brain tissue	MPX 2003	Tesh et al. [52]
Prairie dog, hamster, and gerbil	Suspected animals to infection	Virus was detected in the brain tissues using RT-PCR and electrochemiluminescence assay	Zaire-96-I-16	Kulesh et al. [53]
Prairie dogs	i.n. and i.p.	MPXV was positive in the brain tissue	MPX 2003	Xiao et al. [54]
Rope squirrel, pouched rat, dormice and prairie dogs	Suspected animals to MPXV delivered from Ghana endemic area to the state of Illinois	Viral DNA was detected in the brain parenchyma of some MPXV-PCR-positive animals	–	Hutson et al. [10]
Prairie dog	i.n.	Viral DNA was identified in the brain tissues after inoculation of MPXV	MPXV-USA-2003–044	Hutson et al. [11]
Pouched rat	i.d.	PCR positive and adequate viral titration were seen in the brain	Central African MPXV (ROC 2003–358	Falendysz et al. [55]
Rope squirrel	i.n.	MPXV was detected in the brain tissues of two squirrels using qRT-PCR assay	Recombinant Central African MPXV that expresses firefly luciferase	Falendysz et al. [56]
Prairie dogs	i.n.	The viral load has been reported in the brain parenchyma	MPXV-USA-2003–044	Hutson et al. [57]
Prairie dogs	i.n.	Positive viral load was observed in the brain	MPXV-USA-2003/LUC	Falendysz et al. [12]
CAST/EiJ mice	i.n.	Viral titers increased in brain tissue of infected mice with MPXV	MPX 2003	Earl et al. [58]
Ground squirrel	i.n. and s.c.	Detection of MPXV in brain tissue after necropsy	Central African clade (V79-1–005)	Sergeev et al. [59]
CAST/EiJ mice	i.n.	Bioluminescence imaging in MPXV infected mice revealed that the virus was more replicated in the head and virus plaques quantitatively were seen in brain tissues	Zaire strain (MPXV-z06)	Earl et al. [60]
ICR mice	i.n.	Plaque assay indicated that increased the MPXV values in homogenized animal brain tissues	Central African clade (V79-1–005)	Sergeev et al. [61]

i.n. intranasal, *i.p.* intraperitoneal, *s.c.* subcutaneous, *i.d.* intradermal

after intranasal inoculation of Congo Basin MPXV strain in ground squirrels [59]. Furthermore, intranasal inoculation of MPXV showed that the virus had more replication in the intranasal and brain areas of animals assessed by bioluminescence imaging [60]. Coupled with, the main organs for viral load were the brain, nasal septum, and nasal mucosa after intranasal inoculation of MPXV in mice. These findings represented that the nasal cavity and olfactory epithelium may act as a major route for transmission of MPXV into the brain parenchyma (Fig. 2). MPXV may gain access to the CNS by infecting circulating leukocytes, e.g., monocytes/

macrophages as a second route (Fig. 2). As an example, specific antigens of MPXV-Zaire 79 were identified in circulatory monocytes of macaques after intravenous injection of the virus [62]. Additionally, subcutaneously injection of MPXV in cynomolgus monkeys strongly increased viral particles in the alveolar and mediastinal lymph node macrophages detected by electron microscopy, indicating a high replication process in these cells [63, 64]. Also, the viral particles have been obviously detected after intranasal inoculation of MPXV in activated alveolar macrophages of ground squirrels, mice, and prairie dogs as shown by
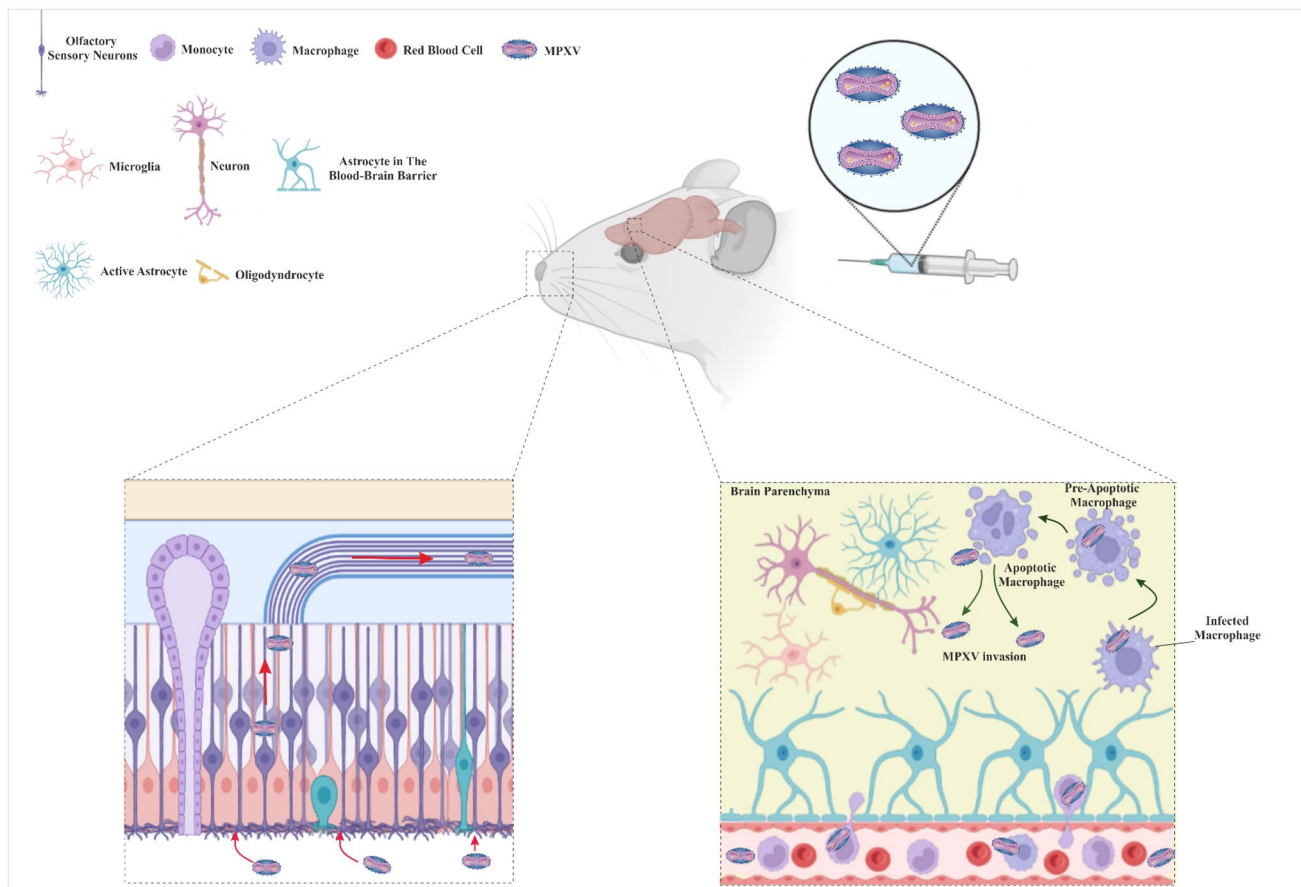
Fig. 2 Probable transmission routes for entry of MPXV into the brain tissue. MPXV can reach the brain tissue through two probable routes, such as olfactory epithelium and infected monocytes/macrophages. After intranasal inoculation, the virus rapidly is replicated in the nasal septum and mucosa and detected in the brain parenchyma. Data also showed that infected monocytes in circulation may cross the blood–brain barrier (BBB) and reach the brain tissue. MPXV can rapidly replicate into the macrophages and release from these cells into the brain. *MPXV* Monkeypox virus

electron microscopy images [16, 59, 61]. It should be mentioned that the current data suggest the possible neuroinvasive potential of MPXV; however, more broadly, research is also needed to determine the exact invasion routes.

Conclusion remarks and future perspectives

To sum up, MPXV may enter the brain parenchyma and show neuroinvasive propensity. This potential can be mediated via two suggested routes, such as olfactory epithelium and hematogenous penetration through infected monocytes/macrophages. In light of the MPXV neuroinvasiveness, it can be explained the onset of neurological manifestations and brain damage in MPX patients. A special focus on the long-term effects of MPXV on CNS using in vitro, in vivo, and postmortem analyses can be helpful to reveal the exact mechanisms of neuroinvasion and even neurotropism induced by MPXV. Finally, neurologists and physicians should be aware of the possible incidence of neurological complications caused by MPXV.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

References

1. Petersen E, Kantele A, Koopmans M, Asogun D, Yinka-Ogunleye A, Ihekweazu C et al (2019) Human monkeypox: epidemiologic and clinical characteristics, diagnosis, and prevention. Infect Dis Clin N Am 33:1027–1043

2. Hraib M, Jouni S, Albitar MM, Alaidi S, Alshehabi Z (2022) The outbreak of monkeypox 2022: an overview. Ann Med Surg 79:104069

3. Petersen BW, Damon IK (2014) Orthopoxviruses: vaccinia (smallpox vaccine), variola (smallpox), monkeypox, and cowpox. Mandell, Douglas, and Bennett's principles and practice of infectious diseases, 8th edn. Elsevier, Philadelphia

4. Diaz JH (2021) The disease ecology, epidemiology, clinical manifestations, management, prevention, and control of increasing human infections with animal orthopoxviruses. Wild Environ Med 32:528–536

5. Formenty P, Muntasir MO, Damon I, Chowdhary V, Opoka ML, Monimart C et al (2010) Human monkeypox outbreak caused by novel virus belonging to Congo Basin clade, Sudan, 2005. Emerg Infect Dis 16:1539–1545

6. Huhn GD, Bauer AM, Yorita K, Graham MB, Sejvar J, Likos A et al (2005) Clinical characteristics of human monkeypox, and risk factors for severe disease. Clin Infect Dis 41:1742–1751

7. Sejvar JJ, Chowdary Y, Schomogyi M, Stevens J, Patel J, Karem K et al (2004) Human monkeypox infection: a family cluster in the midwestern United States. J Infect Dis 190:1833–1840

8. Adler H, Gould S, Hine P, Snell LB, Wong W, Houlihan CF et al (2022) Clinical features and management of human monkeypox: a retrospective observational study in the UK. Lancet Infect Dis 22:1153–1162

9. Reed KD, Melski JW, Graham MB, Regnery RL, Sotir MJ, Wegner MV et al (2004) The detection of monkeypox in humans in the Western Hemisphere. N Engl J Med 350:342–350

10. Hutson CL, Lee KN, Abel J, Carroll DS, Montgomery JM, Olson VA et al (2007) Monkeypox zoonotic associations: insights from laboratory evaluation of animals associated with the multi-state US outbreak. Am J Trop Med Hygiene 76:757–768

11. Hutson CL, Carroll DS, Gallardo-Romero N, Weiss S, Clemmons C, Hughes CM et al (2011) Monkeypox disease transmission in an experimental setting: prairie dog animal model. PLoS ONE 6:e28295

12. Falendysz EA, Londoño-Navas AM, Meteyer CU, Pussini N, Lopera JG, Osorio JE et al (2014) Evaluation of monkeypox virus infection of black-tailed prairie dogs (*Cynomys ludovicianus*) using in vivo bioluminescent imaging. J Wildl Dis 50:524–536

13. Magnus Pv, Andersen EK, Petersen KB, Birch-Andersen A (1959) A pox-like disease in cynomolgus monkeys. Acta Pathol Microbiol Scand 46:156–176

14. Marennikova SS, Seluhina EM, Mal'ceva NN, Cimiskjan KL, Macevic GR (1972) Isolation and properties of the causal agent of a new variola-like disease (monkeypox) in man. Bull World Health Org 46:599–611

15. Brown K, Leggat PA (2016) Human monkeypox: current state of knowledge and implications for the future. Trop Med Infect Dis 1:8

16. Guarner J, Johnson BJ, Paddock CD, Shieh WJ, Goldsmith CS, Reynolds MG et al (2004) Monkeypox transmission and pathogenesis in prairie dogs. Emerg Infect Dis 10:426–431

17. Petersen B, Damon I (2020) Smallpox, monkeypox, and other poxvirus infections. Goldman-Cecil Medicine, 26th edn. Elsevier, Philadelphia

18. Chen N, Li G, Liszewski MK, Atkinson JP, Jahrling PB, Feng Z et al (2005) Virulence differences between monkeypox virus isolates from West Africa and the Congo basin. Virology 340:46–63

19. Likos AM, Sammons SA, Olson VA, Frace AM, Li Y, Olsen-Rasmussen M et al (2005) A tale of two clades: monkeypox viruses. J Gener Virol 86:2661–2672

20. Ježek Z, Fenner F (1988) Human monkeypox: S. Karger Ag

21. Reynolds MG, Davidson WB, Curns AT, Conover CS, Huhn G, Davis JP et al (2007) Spectrum of infection and risk factors for human monkeypox, United States, 2003. Emerg Infect Dis 13:1332–1339

22. Charatan F (2003) US doctors investigate more than 50 possible cases of monkeypox. BMJ (Clinical research ed) 326:1350

23. Enserink M (2003) US monkeypox outbreak traced to Wisconsin pet dealer. American Association for the Advancement of Science, Berlin

24. World Health Organization (2022) Disease outbreak news; monkeypox– United Kingdom of Great Britain and Northern Ireland. Available at: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON381

25. World Health Organization (2022) Disease outbreak news; multi-country monkeypox outbreak in non-endemic countries: Update. Available at: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON396

26. Alakunle E, Moens U, Nchinda G, Okeke MI (2020) Monkeypox virus in Nigeria: infection biology, epidemiology, and evolution. Viruses 12:1257

27. Cho CT, Wenner HA (1973) Monkeypox virus. Bacteriol Rev 37:1–18

28. Rouhandeh H, Engler R, Taher M, Fouad A, Sells L (1967) Properties of monkey pox virus. Arch Gesamte Virusforsch 20:363–373

29. Nworuh B, Iwuoha GN (2019) Factors associated with the practice of monkey pox preventive behaviours among health workers in Yenagoa LGA, Bayelsa state, Nigeria. IOSR-JNHS 8:75–85

30. Ihekweazu C, Yinka-Ogunleye A, Lule S, Ibrahim A (2020) Importance of epidemiological research of monkeypox: is incidence increasing? Expert Rev Anti-Infect Therapy 18:389–392

31. Hutin YJ, Williams RJ, Malfait P, Pebody R, Loparev VN, Ropp SL et al (2001) Outbreak of human monkeypox, Democratic Republic of Congo, 1996 to 1997. Emerg Infect Dis 7:434–438

32. Learned LA, Reynolds MG, Wassa DW, Li Y, Olson VA, Karem K et al (2005) Extended interhuman transmission of monkeypox in a hospital community in the Republic of the Congo, 2003. Am J Trop Med Hyg 73:428–434

33. Jezek Z, Arita I, Mutombo M, Dunn C, Nakano JH, Szczeniowski M (1986) Four generations of probable person-to-person transmission of human monkeypox. Am J Epidemiol 123:1004–1012

34. McMullen CL, Mulembekani P, Hoff NA, Doshi RH, Mukadi P, Shongo R et al (2015) Human monkeypox transmission dynamics thirty years after smallpox eradication in the Sankuru district, democratic republic of Congo. Am J Trop Med Hygiene 93:341

35. Costello V, Sowash M, Gaur A, Cardis M, Pasieka H, Wortmann G et al (2022) Imported Monkeypox from International Traveler, Maryland, USA, 2021. Emerg Infect Dis 28:1002–1005

36. Bayer-Garner IB (2005) Monkeypox virus: histologic, immunohistochemical and electron-microscopic findings. J Cutaneous Pathol 32:28–34

37. McCollum AM, Damon IK (2014) Human monkeypox. Clin Infect Dis 58:260–267

38. Anderson MG, Frenkel LD, Homann S, Guffey J (2003) A case of severe monkeypox virus disease in an American child: emerging infections and changing professional values. Pediatric Infect Dis J 22:1093–1096

39. Ogoina D, Izibewule JH, Ogunleye A, Ederiane E, Anebonam U, Neni A et al (2019) The 2017 human monkeypox outbreak in Nigeria—report of outbreak experience and response in the Niger Delta University Teaching Hospital, Bayelsa State, Nigeria. PLoS ONE 14:e0214229

40. Ježek Z, Szczeniowski M, Paluku K, Mutombo M (1987) Human monkeypox: clinical features of 282 patients. J Infect Dis 156:293–298

41. Croft DR, Sotir MJ, Williams CJ, Kazmierczak JJ, Wegner MV, Rausch D et al (2007) Occupational risks during a monkeypox outbreak, Wisconsin, 2003. Emerg Infect Dis 13:1150

42. Reynolds MG, Yorita KL, Kuehnert MJ, Davidson WB, Huhn GD, Holman RC et al (2006) Clinical manifestations of human monkeypox influenced by route of infection. J Infect Dis 194:773–780

43. Yinka-Ogunleye A, Aruna O, Ogoina D, Aworabhi N, Eteng W, Badaru S et al (2018) Reemergence of human monkeypox in Nigeria, 2017. Emerg Infect Dis 24:1149

44. Kalthan E, Tenguere J, Ndjapou S, Koyazengbe T, Mbomba J, Marada R et al (2018) Investigation of an outbreak of monkeypox in an area occupied by armed groups, Central African Republic. Med Malad Infect 48:263–268

45. Ng OT, Lee V, Marimuthu K, Vasoo S, Chan G, Lin RTP et al (2019) A case of imported Monkeypox in Singapore. Lancet Infect Dis 19:1166

46. Yinka-Ogunleye A, Aruna O, Dalhat M, Ogoina D, McCollum A, Disu Y et al (2019) Outbreak of human monkeypox in Nigeria in 2017–18: a clinical and epidemiological report. Lancet Infect Dis 19:872–879

47. Ogoina D, Iroezindu M, James HI, Oladokun R, Yinka-Ogunleye A, Wakama P et al (2020) Clinical course and outcome of human monkeypox in Nigeria. Clin Infect Dis 71:e210–e214

48. Hughes CM, Liu L, Davidson WB, Radford KW, Wilkins K, Monroe B et al (2020) A tale of two viruses: coinfections of monkeypox and varicella zoster virus in the democratic Republic of Congo. Am J Trop Med Hyg 104:604–611

49. Eseigbe EE, Akude C, Osagie IA, Eseigbe P (2021) Human monkey pox virus infection in Plateau State, North Central Nigeria: a report of two cases. West Afr J Med 38:1242–1246

50. Charniga K, Masters NB, Slayton RB, Gosdin L, Minhaj FS, Philpott D et al (2022) Estimating the incubation period of monkeypox virus during the 2022 multi-national outbreak. medRxiv. 2022:2022.06.23

51. Pittman PR, Martin JW, Kingebeni PM, Tamfum J-JM, Wan Q, Reynolds MG et al (2022) Clinical characterization of human monkeypox infections in the Democratic Republic of the Congo. medRxiv. 2022:2022.05.26.22273379

52. Tesh RB, Watts DM, Sbrana E, Siirin M, Popov VL, Xiao SY (2004) Experimental infection of ground squirrels (*Spermophilus tridecemlineatus*) with monkeypox virus. Emerg Infect Dis 10:1563–1567

53. Kulesh DA, Loveless BM, Norwood D, Garrison J, Whitehouse CA, Hartmann C et al (2004) Monkeypox virus detection in rodents using real-time 3'-minor groove binder TaqMan assays on the Roche LightCycler. Lab Investig 84:1200–1208

54. Xiao SY, Sbrana E, Watts DM, Siirin M, da Rosa AP, Tesh RB (2005) Experimental infection of prairie dogs with monkeypox virus. Emerg Infect Dis 11:539–545

55. Falendysz EA, Lopera JG, Lorenzsonn F, Salzer JS, Hutson CL, Doty J et al (2015) Further assessment of monkeypox virus infection in gambian pouched rats (*Cricetomys gambianus*) using in vivo bioluminescent imaging. PLoS Negl Trop Dis 9:e0004130

56. Falendysz EA, Lopera JG, Doty JB, Nakazawa Y, Crill C, Lorenzsonn F et al (2017) Characterization of Monkeypox virus infection in African rope squirrels (*Funisciurus* sp.). PLoS Negl Trop Dis 11:809

57. Hutson CL, Gallardo-Romero N, Carroll DS, Salzer JS, Ayers JD, Doty JB et al (2019) Analgesia during monkeypox virus experimental challenge studies in prairie dogs (*Cynomys ludovicianus*). J Am Assoc Lab Anim Sci JAALAS 58:485–500

58. Earl PL, Americo JL, Moss B (2012) Lethal monkeypox virus infection of CAST/EiJ mice is associated with a deficient gamma interferon response. J Virol 86:9105–9112

59. Sergeev AA, Kabanov AS, Bulychev LE, Sergeev AA, Pyankov OV, Bodnev SA et al (2017) Using the ground squirrel (*Marmota bobak*) as an animal model to assess monkeypox drug efficacy. Transbound Emerg Dis 64:226–236

60. Earl PL, Americo JL, Cotter CA, Moss B (2015) Comparative live bioluminescence imaging of monkeypox virus dissemination in a wild-derived inbred mouse (*Musmusculus castaneus*) and outbred African dormouse (*Graphiurus kelleni*). Virology 475:150–158

61. Sergeev AA, Kabanov AS, Bulychev LE, Sergeev AA, Pyankov OV, Bodnev SA et al (2016) The possibility of using the ICR mouse as an animal model to assess antimonkeypox drug efficacy. Transbound Emerg Dis 63:e419–e430

62. Song H, Janosko K, Johnson RF, Qin J, Josleyn N, Jett C et al (2013) Poxvirus antigen staining of immune cells as a biomarker to predict disease outcome in monkeypox and cowpox virus infection in non-human primates. PLoS ONE 8:e60533

63. Nagata N, Saijo M, Kataoka M, Ami Y, Suzaki Y, Sato Y et al (2014) Pathogenesis of fulminant monkeypox with bacterial sepsis after experimental infection with West African monkeypox virus in a cynomolgus monkey. Int J Clin Exp Pathol 7:4359

64. Zaucha GM, Jahrling PB, Geisbert TW, Swearengen JR, Hensley L (2001) The pathology of experimental aerosolized monkeypox virus infection in Cynomolgus Monkeys (*Macaca fascicularis*). Lab Invest 81:1581–1600