Chapter 2
Monkeypox Virus

Nikola Sklenovská

Abstract *Monkeypox virus* is a member of the *Orthopoxvirus* genus in the *Poxviridae* family. Monkeypox virus causes monkeypox, an emerging zoonotic disease recognized as the most important orthopoxvirus infection in humans in the smallpox post-eradication era. Many animal species, including rodents and monkeys, can transmit the virus. However, the animal reservoir has not been identified yet. Human-to-human transmission exists, and the longest reported chain of transmission is six generations. The clinical presentation of monkeypox is very similar to the presentation of smallpox, i.e. the febrile prodrome is followed by a skin eruption period. Lymphadenopathy is a typical sign of monkeypox. The case fatality of monkeypox depends on the virus clade, and it falls between 1% and 11%. Monkeypox was reported in 11 countries of Central and West Africa. The disease was also exported outside of the African continent to the USA, the UK, Israel, and Singapore. The frequency and geographical spread of human monkeypox cases have increased in recent years, with little understanding of the disease's emergence, epidemiology, and ecology. Monkeypox can be diagnosed by polymerase chain reaction performed on lesion specimens. Serological tests and antigen detection do not provide a definitive diagnosis given the orthopoxvirus serological cross-reactivity. Modified vaccinia Ankara vaccine was recently approved in the USA for monkeypox prevention in adults at high risk of infection. There is currently no specific treatment for monkeypox infection.

Keywords Monkeypox · Orthopoxvirus · Emerging infectious diseases · Zoonosis · Disease outbreaks · One health

Abbreviations

CA	Central African
CEV	Cell-associated virion

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dsDNA	Double-stranded DNA
DRC	Democratic Republic of the Congo
EEV	Extracellular enveloped virion
EV	Enveloped virion
IEV	Intracellular enveloped virion
IMV	Intracellular mature virion
ITR	Terminal inverted repetition
mRNA	Messenger RNA
MPXV	Monkeypox virus
MPXV-ZAI	Monkeypox virus Zaire-96-I-16 Strain
MV	Mature virion
MVA	Modified vaccinia Ankara
OPV	Orthopoxvirus
PCR	Polymerase chain reaction
VZV	Varicella-zoster virus
WA	West African
WHO	World Health Organization

2.1 Preamble

Monkeypox virus (MPXV) was discovered and described in the Statens Serum Institut (Copenhagen, Denmark) in 1958 when two outbreaks of pox-like disease were observed in cynomolgus monkeys. The institute was receiving a continuous supply of monkeys from Singapore, which was used for polio vaccine research and production (Magnus et al. 1959). Subsequently, multiple other laboratory outbreaks of monkeypox were recorded in Europe and the USA in captive monkeys imported from Asia (Arita et al. 1972). Seroprevalence studies in Asia did not find evidence of monkeypox on the continent (Arita et al. 1972). Later it was suggested that grivets (MPXV-susceptible monkeys also exported in large scale to Europe and North America) could have been the source of infection of Asian monkeys during co-transportation (Jezek and Fenner 1988). The first human monkeypox case was reported in August 1970 in the remote village of Bokenda, in the equatorial province of the Democratic Republic of the Congo (DRC) (Ladnyj et al. 1972).

With the eradication of smallpox in 1980 and subsequent cessation of smallpox vaccination, monkeypox has emerged as the most important orthopoxvirus pathogenic for humans. Monkeypox was considered a rare sporadic zoonotic disease with a limited capacity to spread between humans in the past (WHO 1984). However, the number of reported cases and their geographical range has increased after the cessation of the smallpox vaccination and the disease can be life-threatening in the DRC and other countries in West and Central Africa (Meyer et al. 2002; Rimoin et al. 2010). Additionally, multiple exportations of the virus outside of Africa in the past years have highlighted its global importance.

2.2 Classification

MPXV is a member of the genus *Orthopoxvirus* (OPV) and the family *Poxviridae*. MPXV is one of the five OPV species pathogenic for humans, together with variola virus, the causative agent of smallpox, now eradicated in nature, cowpox virus, camelpox virus, and vaccinia virus (Shchelkunov et al. 2005).

Poxviruses infect most vertebrates and invertebrates, causing a variety of diseases of veterinary and medical importance. The family *Poxviridae* is divided into the subfamily *Chordopoxvirinae* whose viruses infect vertebrates and the subfamily *Entomopoxvirinae* which infect insects. The subfamily *Chordopoxvirinae* is divided into 11 genera, one of which is OPV (Table 2.1). All OPV species, except variola virus which is an exclusively human pathogen, have animal reservoirs and are therefore classified as zoonotic pathogens.

2.3 The Virus

2.3.1 Morphology

Monkeypox virus, together with other poxviruses, is considered one of the largest and most complex viruses (Ferreira Barreto-Vieira and Monika Barth 2015). They are brick-like shaped particles with a size ranging from 220 nm to 450 nm in length and 140 nm to 260 nm in width (Jahrling et al. 2007, pp. 215–240); therefore, MPXV is large enough to be discerned by light microscope, with its ultrastructure resolvable via electron microscopy. However, higher magnification provided by electron microscopy is needed to resolve ultrastructure (Moss and Damon 2013). The orthopox virion consists of four major elements—core, lateral bodies, outer membrane, and the outer lipoprotein envelope. The central core contains the viral

Table 2.1 Classification of orthopoxviruses

Family	*Poxviridae*
Subfamily	*Chordopoxvirinae*
Genus	*Orthopoxvirus*
Species	*Camelpox virus*
	Cowpox virus
	Ectromelia virus
	Monkeypox virus
	Raccoonpox virus
	Skunkpox virus
	Taterapox virus
	Vaccinia virus
	Variola virus
	Volepox virus

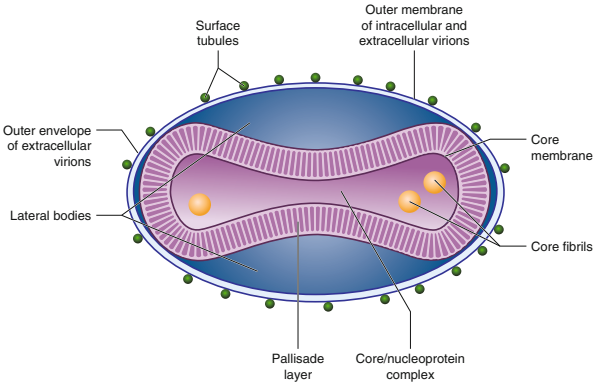

Fig. 2.1 Schematic representation of a poxvirus particle. Adapted from Principles of Molecular Virology, 6th Edition (p. 46), by Alan J. Cann, 2016, UK: Elsevier. Copyright 2016 by Elsevier. Adapted with permission

double-stranded DNA (dsDNA) and core fibrils, and it is surrounded by a tightly arranged layer of rod-shaped structures known as palisade layer. The central core, palisade layer, and the lateral bodies are enclosed together by the outer membrane that is composed of many surface tubules (Fig. 2.1). Spontaneously released virions often have the outer lipoprotein envelope, while virions released by cellular disruption lack this envelope (Appleyard et al. 1971; Ladnyi et al. 1988). A mature virion contains at least 80 viral proteins (Resch et al. 2007).

2.3.2 Genome

The monkeypox genome (Fig. 2.2) is a large (197 kbp) single linear molecule of dsDNA, which is among the largest of all viral genomes (Moss and Damon 2013). Each end of the genome contains identical but oppositely oriented terminal reads with a size of about 6 kbp (Shchelkunov et al. 2002) with a set of short tandem repeats (Wittek and Moss 1980) and terminal hairpin loops (Baroudy et al. 1982). The genome consists of about 190 nonoverlapping open reading frames (>180 bp long) containing 60 or more amino acid residues. Of these, four are present within the inverted terminal repetition (Seet et al. 2003; Shchelkunov et al. 2002). The guanine and cytosine content of MPXV DNA is low, about 31.1% (Shchelkunov et al. 2001). Two distinct genetic clades of MPXV have been characterized including the West African (WA) and the Central African (CA) clade (Likos et al. 2005).

Sequencing of the whole genome of many OPVs has revealed a high degree of homology in the genes located centrally, and high variability in the genes located terminally on both sides of the genome. Conserved OPV genes are mostly involved in essential viral functions like replication and virion assembly (Seet et al. 2003), and the variable OPV terminal reads are likely to contribute to the virulence of different

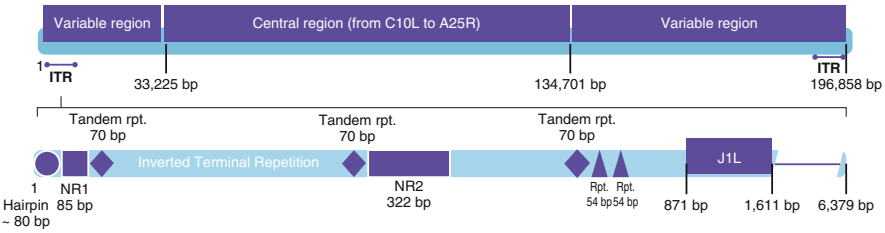

Fig. 2.2 Schematic genome representation of Zaire-96-I-16 (MPXV-ZAI) strain isolated during the 1996 outbreak of monkeypox in Zaire. The whole genome consists of 196,858 bp with the central genomic region comprising of 101,476 bp (Shchelkunov et al. 2001). Both terminal variables end (right end longer than the left one) include a 6379 bp terminal inverted repetition (ITR) (Shchelkunov et al. 2002) with approximately 80 bp long hairpin loop (Chen et al. 2005a), 70 bp or 54 bp short tandem repeats and unique ITR sequences NR 1 and NR 2 and the coding region (Shchelkunov et al. 2002). Adapted from "Human monkeypox and smallpox viruses: a genomic comparison" by Shchelkunov et al. (2001), FEBS Letters, 509, pp. 66–70. Copyright by John Wiley & Sons, Inc. and from "Analysis of the Monkeypox Virus Genome" by Shchelkunov et al. (2002), Virology, 297, pp. 172–194. Copyright by Elsevier. Adapted with permission

OPVs (Afonso et al. 2002; Chen et al. 2005b; Goebel et al. 1990; Tulman et al. 2006). Many terminal genes contribute to immune evasion by interfering with signaling, presentation, and recognition of antigens and apoptosis (Barry et al. 2004; Seet et al. 2003).

2.3.3 Replication Cycle

The replication cycle (Fig. 2.3) of poxviruses, unlike most DNA viruses, occurs in the cytoplasm of the host cell (Buller and Palumbo 1991). Poxviruses enter cells by a multistep process consisting of attachment, hemifusion, and core entry that can occur at the plasma membrane or after endocytosis (Moss 2016) The exact mechanism used by poxviruses to enter cells depends on its infectious form—mature virion (MV) with single outer membrane or extracellular enveloped virion (EV) which has an additional membrane with a different protein composition. For EV form, the external EV-specific membrane is discarded exposing the underlying MV membrane, which then fuses with the cell. Although MV is more abundant, EV is specialized for cell-to-cell spread largely by its long, mobile, projections that are formed by actin polymerization which adhere to the cell surface (Moss 2016; Moss and Damon 2013).

The mature virion undergoes the first uncoating during its entry, and once in the cytoplasm, the virus releases prepackaged viral proteins and enzymatic factors that disable cell defenses and stimulate expression of early genes. This is followed by a synthesis of early messenger RNA (mRNA) by viral DNA-dependent RNA polymerase. Translation of early mRNA facilitates the second uncoating process, DNA replication, and production of intermediate transcription factors. In the following stage, intermediate mRNA is transcribed and translated to induce the expression of

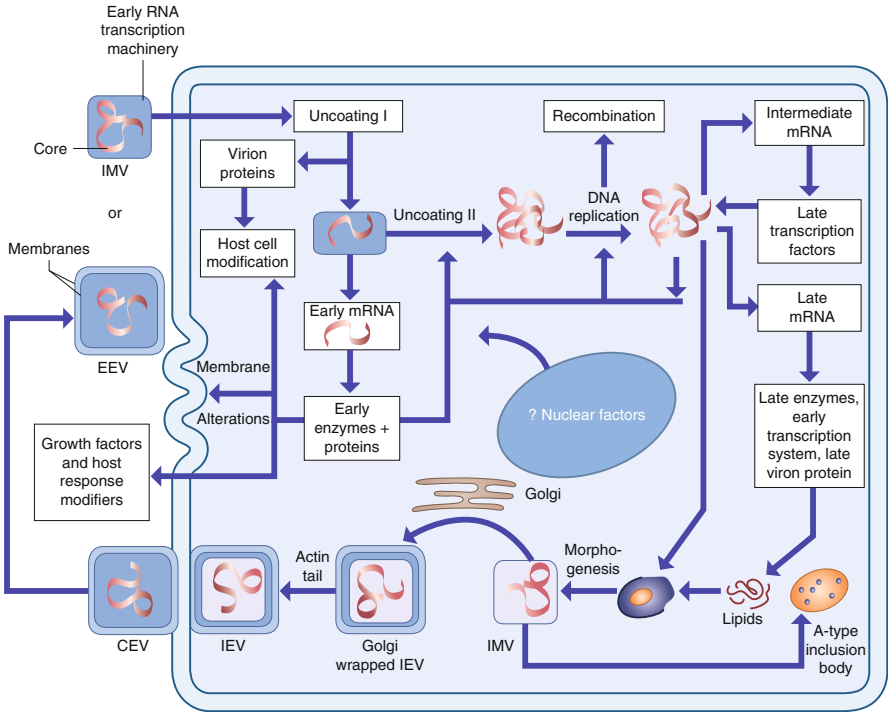

Fig. 2.3 Schematic representation of a poxvirus life cycle (Bray and Buller 2004). After the virion binds and fuses with the host cell membrane, the viral core is released into the cytoplasm of the host cell. Enzymes and factors carried within the core initiate transcription. Most virions stay in the cytoplasm as intracellular mature virions (IMVs) and end up encased within the protein matrix of scabs. The rest of the virions acquire an additional envelope (intracellular enveloped virions, IEVs) and are moved and attached to the host cell membrane. These cell surface-associated enveloped virions (CEVs) are responsible for the cell-to-cell spread of the virus, whereas extracellular enveloped virions (EEVs) can participate in systemic spread of the virus. Virus- and host-encoded proteins on the surface of CEV and EEV protect them against complement activation. Reprinted from "Looking Back at Smallpox" by Bray and Buller (2004), 38, pp. 882–889. Public domain

late mRNAs and its translation into structural proteins and non-structural proteins (enzymes and early transcription factors). The translated proteins get assembled alongside DNA concatemers formed during the early stage of replication and get packed into immature virions that develop into intracellular mature virions (IMVs). IMVs lack an outer membrane and are infectious only when they are released by cell disruption. IMV particles which did not end up encased within the protein matrix of cytoplasm become the intracellular enveloped virions (IEVs) by acquiring a second membrane (Bray and Buller 2004; Hiller and Weber 1985; Roberts and Smith 2008). Those migrate to the inner cell membrane with the help of microtubules and fuse with it, forming cell-associated virions (CEVs), which trigger actin polymerization and formation of filaments that help CEVs to leave the cell. The CEVs which have left the cell are called extracellular enveloped virions (EEVs) (Roberts and Smith 2008).

Both intracellular and extracellular virions play an important role in the pathogenesis. The intracellular virions (IMV and IEV) and CEVs are responsible for the spread of the virus from cell to cell, while EEVs are important for the systemic spread of the virus within the infected organism (Pauli et al. 2010).

2.4 Clinical Profile

Most people infected with MPXV are symptomatic, but subclinical (asymptomatic) infection can occur (Jezek et al. 1986, 1987c). It was suggested that subclinical infections could constitute up to 30% of all monkeypox infections (Jezek and Fenner 1988). Limited information is available regarding the incubation period of MPXV in humans, although recent analysis suggests 5–13 days (Nolen et al. 2016). The longest documented incubation period was roughly 17 days (Breman et al. 1980). However, a maximum incubation period of 21 days has been assumed for extra caution. The incubation period, disease presentation, severity, and duration can also be influenced by the route of infection. For example, infection via bites can result in a shorter incubation period, absence of a distinct febrile stage, and more severe illness than non-invasive exposures (Reynolds et al. 2006). The clinical presentation of monkeypox closely resembles that of smallpox, although it is clinically less severe. The major difference distinguishing monkeypox from smallpox is the occurrence of lymph node enlargement.

The monkeypox disease in humans can be divided into two periods, the prodrome and the rash period. The prodrome is defined by fever, headache, chills and/or sweats, sore throat, muscle ache, lack of energy, and lymphadenopathy (Nalca et al. 2005). The rash period usually manifests 1–3 days after the onset of fever and lymphadenopathy, and is characterized by a few to several thousand lesions (Jezek et al. 1987d). The lesions appear simultaneously and evolve at about the same rate. The lesions progress from macules to papules, vesicles, pustules, and finally to crusts. Their distribution is mainly peripheral but can cover the whole body during a severe illness (Fig. 2.4). Depending on the severity of the illness, it takes about 2–3 weeks for the lesions to dry and desquamate (Ladnyi et al. 1988). Patients vaccinated against smallpox with vaccinia vaccine have significantly less lesions than non-vaccinated (Jezek and Fenner 1988).

Patients often experience gastrointestinal symptoms such as nausea, vomiting, diarrhea, and loss of appetite. Oral and alimentary tract lesions can be apparent. Skin perturbation from the rash can lead to secondary bacterial infection (common) and dehydration. Ocular infections with MPXV and secondary bacterial infections can also occur and often render the patient's eye swollen, red, sensitive to light, and can lead to loss of vision. The respiratory tract can also be affected; patients can present with coughing, difficulty breathing, or bronchopneumonia. Other complications include encephalitis and sepsis (Reynolds et al. 2017). A case fatality ranges between 1 and 11% in unvaccinated patients (Jezek et al. 1987d; WHO 1997), and

Fig. 2.4 Typical clinical presentation of human monkeypox in a 7-year-old female child, Sankuru District, Democratic Republic of Congo. Reprinted from "Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo" by Anne W. Rimoin et al. 2010, PNAS, 107(37), pp. 16262–16267. Copyright by Proceedings of the National Academy of Sciences of the United States of America. Reprinted with permission

is generally higher in cases infected with the CA clade of the virus than with the WA clade.

Monkeypox can clinically resemble various rash illnesses which need to be considered during differential diagnosis. This includes smallpox (eradicated in nature), measles, bacterial skin infections, scabies, syphilis, medication-associated allergies, and chickenpox. The latter, chickenpox, also known as varicella (caused by varicella-zoster virus, VZV), is most commonly confused with monkeypox (up to 50% of cases in some outbreaks) (Jezek et al. 1988b; Meyer et al. 2002) because of the similarities in the clinical presentation of the two diseases. Unlike the varicella lesions, the lesions of monkeypox appear simultaneously (varicella lesions appear gradually) and they concentrate on the face, arms, and legs but can cover a whole body (varicella lesions appear mainly on the trunk of the body) (Heymann et al. 1998). Monkeypox lesions are hard, deep, and well-circumscribed, while varicella lesions are superficial with irregular borders (McCollum and Damon 2014). Furthermore, chickenpox has a shorter and milder prodrome and clinical course, lymphadenopathy is infrequent, and death is extremely rare (Breman 2000; Jezek et al. 1988b). However, it was recently reported that a large proportion of varicella patients in the DRC presented with non-typical varicella rash and clinical signs and symptoms (Leung et al. 2019). Coinfections with both MPXV and VZV have been reported several times (Hutin et al. 2001; Meyer et al. 2002; Morier 2014; Rimoin et al. 2007). The role of the VZV in MPXV epidemiology is not clear.

2.5 Pathobiology

The disease pathobiology is only partially described and is predominantly based on animal studies. Black-tailed prairie dogs (*Cynomys ludovicianus*) have been shown to mimic the human disease better than other models, experiencing a prolonged incubation period and development of skin rash (Hutson et al. 2009).

A model of MPXV pathogenesis is depicted in Fig. 2.5. MPXV is first detected at the local site of infection (through respiratory, percutaneous, or per mucosal exposures) and is associated with an intense inflammatory response characterized by cell necrosis, phagocytosis, vasculitis, and local replication of MPXV (Cho and Wenner 1973). This is followed by the virus multiplication occurring in the regional lymphatics and then in the bloodstream leading to transient primary viremia. Following this, the virus multiplies in the spleen, liver, bone marrow, and other reticuloendothelial organs (Moss and Damon 2013) but it can also be detected in other organs like the small intestines (Hutson et al. 2015). After this, a secondary viremia period ensues, followed by the seeding of other organs leading to clinical signs of disease including characteristic disseminated cutaneous lesions. Monkeypox antibodies can be detected at the same time or shortly after the cutaneous lesion presentation.

In the prairie dog model, day 12 post-infection seems to be a pivotal time associated with unexpected deaths, uniform antibody production, and peak virus levels. Furthermore, this was also the only point at which viable virus was recovered from blood samples (Hutson et al. 2015).

Histopathological changes, both intracellular and in tissues, attributable to viral infection exhibit around day 6 in affected organs (Hutson et al. 2015). Cytoplasmic inclusion bodies are a typical intracellular histopathological feature of orthopoxvirus infections. Two morphologies manifest, A-type inclusion bodies, where virions are clustered within an intracytoplasmic structure, and B-type inclusions (Guarneri bodies), which are perinuclear and contain the viroplasm and maturing viral particles (Moss and Damon 2013). Some tissues also show prominent histopathological

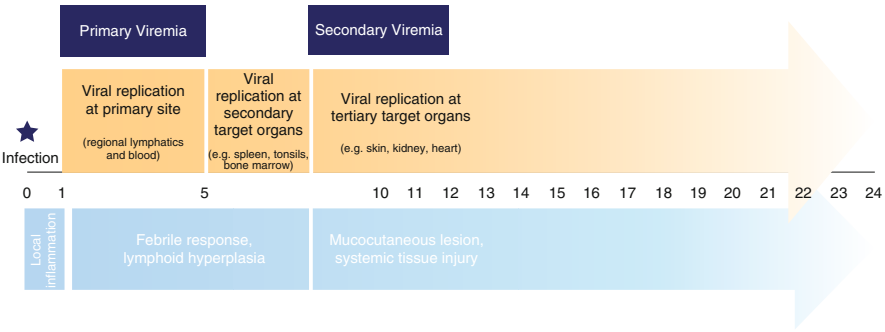

Fig. 2.5 Model of monkeypox virus pathogenesis. Adapted from "Comparison of Monkeypox Virus Clade Kinetics and Pathology within the Prairie Dog Animal Model Using a Serial Sacrifice Study Design" by Hutson et al. (2015), BioMed Research International, Volume 2015, Article ID 965710, 19 pages. Copyright Christina L. Hutson et al. Adapted under the Creative Commons Attribution License

changes at this stage of infection. Spleen samples typically exhibit neutrophil infiltration within the red pulp and increased apoptotic or necrotic cells. Conversely, the liver only tends to show the minimal trend of increased apoptotic cells even though these tissues generally have similarly high loads of virus in infected animals (Hutson et al. 2015).

While the two clades of MPXV, the CA and the WA clade, show similar onset and range of clinical symptoms in the prairie dog animal model, they show certain pathological differences. Generally, the CA MPXV spreads more rapidly, accumulates to greater levels in tissues, and causes greater morbidity in animals compared to the WA MPXV (Hutson et al. 2010, 2015).

2.6 Epidemiology

2.6.1 Prevalence and Incidence

Surveillance activities for monkeypox are not well established, and limited information is available on the prevalence and incidence of the disease. To identify the prevalence of MPXV infection of humans, serological studies of persons without vaccination scars were carried out in the DRC, Republic of Congo, Ivory Coast, and Sierra Leone in 1981. Of all 10,300 sera samples, 15.4% tested positive for orthopoxvirus of which 0.71% tested positive for MPXV. Later follow-up showed that some samples were taken from vaccinated individuals making the results inconclusive (Jezek and Fenner 1988).

The average annual cumulative incidence for inhabitants living in forested areas of the northern DRC between 1981 and 1986 was 1.58 per 10,000 population (Jezek and Fenner 1988). A study in the Sankuru Province (DRC) between November 2005 and November 2007 showed an average annual cumulative incidence of 5.53 per 10,000 (2.18–14.42). This study suggested a 20-fold increase in monkeypox in the same health zone from the 1980s (Rimoin et al. 2010). The most recent analysis of data from the DRC between 2010 and 2015 identified an average annual cumulative incidence of 0.13 cases per 10,000 inhabitants (Mandja et al. 2019).

Seasonal patterns of infections are undetermined: data between 1970 and 1980 suggested January–March (Breman et al. 1980); data between 1981 and 1986 (the 6-year cumulative) suggested June–August (Jezek and Fenner 1988); data between 2000 and 2009 suggested July–September; while between 2010 and 2015 suggested January–March (Mandja et al. 2019).

2.6.2 Sex and Age

Human MPXV infections have been reported to affect all age groups. Between 1980 and 1986, however, 52% were between the ages of 0 and 4 and 37% were between

the ages of 5 and 9. This age pattern may reflect the decrease in the collective immunity after the cessation of the smallpox vaccination. During the same period, there were more males (58%) than females (42%) among the primary cases, and more females (57%) than males (43%) among the secondary cases (Jezek and Fenner 1988). This pattern was likely caused by the social roles linked with gender (e.g., males are more often exposed to animals and females to a sick family member) (Jezek and Fenner 1988; Quiner et al. 2017). More recent data from Nigeria between September 2017 and September 2018 showed that persons with confirmed MPXV infection were between 2 days and 50 years (median 29 years) and majority were males (69%) (Yinka-Ogunleye et al. 2019). This shift towards older age might reflect the further decrease in immunity against OPVs.

2.6.3 Geographical Distribution

Monkeypox most commonly occurs in areas covered by rainforest in Central and West Africa. This type of habitat has been identified as suitable for the transmission of the virus by ecological niche models built based on the association of reported cases and potential risk factors including environmental conditions (e.g., location, temperature, precipitation, vegetation indexes from satellite imagery, etc.) (Ellis et al. 2012; Nakazawa et al. 2015).

Analysis of historical data showed that most monkeypox cases are reported in small villages in tropical rain forests which are, however, not closely surrounded by high forest on all sides. A common situation is that they consist of groups of houses along roads through the forests surrounded by agricultural areas (consisting of gardens and secondary forest) and primary rain forest close by. Each of the three zones (settlement, an agricultural area, and forest) has a characteristic fauna (Khodakevich et al. 1987a, b). Monkeypox cases, however, have also been recorded in urban areas of Africa (Yinka-Ogunleye et al. 2019).

Cases of confirmed human monkeypox in Africa were reported from the DRC, Republic of Congo, Cameroon, Central African Republic, Nigeria, Liberia, Ivory Coast, Gabon, Sierra Leone. Additionally, monkeypox has been imported to Benin (Beninese infected in Nigeria) (Breman et al. 1980) and to South Sudan (movement of people from DRC) (Nakazawa et al. 2013). The virus was also exported outside of the African continent to the USA in 2003 via infected animals from Ghana (Reed et al. 2004). Finally, multiple infected travelers from Nigeria were confirmed in the UK (Vaughan et al. 2018) and Israel (Erez et al. 2019) in 2018 and Singapore in 2019 (Fig. 2.6).

After the first human case of monkeypox was described in 1970, a total of 59 cases of monkeypox have been confirmed in West and Central Africa till 1980 (Jezek et al. 1987b). After the declaration of smallpox eradication in 1980, monkeypox was designated as the most important orthopoxvirus infection in humans in the post-smallpox eradication era resulting in establishing enhanced monkeypox surveillance by the World Health Organization (WHO) in the DRC between 1981 and

Fig. 2.6 Countries which reported confirmed cases of monkeypox in humans

1986 (WHO 1980). During this time, 338 confirmed monkeypox cases were identified in the DRC, and much of the current knowledge on monkeypox was obtained during this time. The total number of confirmed monkeypox cases in West and Central Africa between 1970 and 1986 was 404 (Jezek and Fenner 1988). The number of reported cases has dramatically decreased after the intensified surveillance was discontinued (Table 2.2).

Since its discovery, there have been several prominent MPX outbreaks. A prolonged, relatively large outbreak of 511 suspected cases was reported in DRC in 1996–1997 (WHO 1997) but a substantial proportion might have been chickenpox cases (WHO 1997). The longest chain of transmission was recorded in the Republic of Congo in 2003, accounting for seven viral transmission generations (Learned et al. 2005). In South Sudan in 2005, monkeypox was thought to have expanded outside of its traditional ecology when it was recorded in a dry savannah environment for the first time (Formenty et al. 2010) but it was likely an importation from the DRC (Nakazawa et al. 2015).

An increase in monkeypox geographical range and number of cases has been observed in recent years. The DRC has reported more than 1000 suspected cases per year since 2005 (Durski et al. 2018). Outbreaks were reported in Sierra Leone (2014), Liberia (2017), and Nigeria (2017) after 40 years since the first and only occurrence. The most recent outbreak in Nigeria in 2017 was the biggest outbreak of the West African clade ever documented. The number of monkeypox cases is likely underestimated due to limited specific surveillance and laboratory capacity in forested areas of West and Central Africa.

2.6.4 Host Species

Monkeypox is a zoonotic disease for which the natural reservoir that maintains the virus in nature is not known. Many animal species have been identified as animals that are susceptible to the virus, mainly rodents and non-human primates, listed in Table 2.3 (Reynolds et al. 2019a). Non-human primates are generally accepted as incidental hosts with no critical role in the maintenance of the virus in nature due to the low OPV seroprevalence in these animals. Squirrels (*Funisciurus* spp.), giant pouched rats (*Cricetomys* spp.), and African dormouse (*Graphiurus* spp.) and possibly other forest rodents are considered to be the most likely reservoir hosts based on evidence obtained from multiple fields and laboratory investigations (Doty et al. 2017). The virus has only been isolated twice from a wild animal, a rope squirrel (*Funisciurus anerythrus*) in the DRC (Khodakevich et al. 1986) and a sooty mangabey (*Cercocebus atys*) in Ivory Coast (Radonić et al. 2014).

Table 2.2 Number of suspected and confirmed human cases of monkeypox between 1970 and 2019

Country	Year	Total number of cases (confirmed cases)	References
Democratic Republic of the Congo	1970–1986	386 (386)	Jezek and Fenner (1988)
	1997	511 (?)	WHO (1997)
	After 1997	Not fully enumerable	–
Central African Republic	1984	6 (6)	Khodakevich et al. (1985)
	2001	4 (4)	CDC (2015a)
	2010	2 (2)	Berthet et al. (2011)
	2015–2016	12 (4)	Kalthan et al. (2016)
	2016	26 (3)	WHO (2016)
	2017	? (3)	WHO (2017b)
	2018–2019	34 (25)	WHO (2019b)
	2018	6 (6)	Besombes et al. (2019)
Cameroon	1979	2 (1)	Eozenou (1980)
	1989	1 (1)	Tchokoteu et al. (1991)
	2018	7 (1)	Sadeuh-Mba et al. (2019)
Nigeria	1971	2 (2)	Breman et al. (1980)
	2017–2018	276 (122)	Yinka-Ogunleye et al. (2019)
Ivory Coast	1971	1 (1)	Breman et al. (1980)
	1981	1 (1)	Merouze and Lesoin (1983)
Liberia	1970	3 (3)	Lourie et al. (1972)
	1970	1 (1)	Lourie et al. (1972)
	2016–2017	16 (2)	WHO (2018)
Sierra Leone	1970	1 (1)	Lourie et al. (1972)
	2014	1 (1)	Reynolds et al. (2019b)
	2017	1 (1)	Ye et al. (2019)
Gabon	1987	4 (1)	Meyer et al. (1991)
	1991	?	Heymann et al. (1998)
Benin	1978	1 (1)	Breman et al. (1980)
Republic of Congo	2003	12 (3)	Learned et al. (2005)
	2009	10 (2)	Reynolds et al. (2013)
	2017	88 (7)	Doshi et al. (2018)
	2019	9 (2)	WHO (2019b)

(continued)

Table 2.2 (continued)

Country	Year	Total number of cases (confirmed cases)	References
South Sudan	2005	49 (10)	Formenty et al. (2010)
USA	2003	72 (37)	Reed et al. (2004)
UK	2018	1 (1)	Vaughan et al. (2018)
	2018	2 (2)	Vaughan et al. (2018)
Israel	2018	1 (1)	Erez et al. (2019)
Singapore	2019	1 (1)	WHO (2019a)

2.6.5 Transmission

Monkeypox virus can be transmitted both from animal to human (primary transmission) and from human to human (secondary transmission). The virus can enter the body through broken skin (even if not visible), mucous membranes (eyes and mouth), and the respiratory tract. Primary animal to human transmission results from direct contact with body fluids, lesion material, or respiratory droplets (the latter being the least efficient) of infected animals (Hutson et al. 2011, 2013). Viral shedding via urine and feces has also been documented and may represent another exposure source (Hutson et al. 2015). Secondary human-to-human transmission is associated with direct contact with body fluids and lesion material of infected persons. Respiratory transmission also occurs through direct contact with large respiratory droplets after prolonged face-to-face contact. Transmission can also occur through virus-contaminated objects, such as bedding and clothing (Formenty et al. 2010; Nolen et al. 2015). Transmission of the virus from infected pregnant women to the fetus has been described. Limited information is available on the impact of human MPXV infection on pregnancy outcomes with vertical transmission; however, case studies of miscarriage and fetal death exist (Mbala et al. 2017). Patients are infectious from the onset of the illness (fever), and the lesions contain infectious virus through all stages until the crusts separate and a fresh layer of skin forms. This can take up to 4 weeks.

During 1980–1986, up to 70% of human infections were caused by primary transmission from animals. The main presumptive risk factor for primary transmission is close contact with infected animals when hunting (Quiner et al. 2017). Secondary transmissions were more common in persons without a history of smallpox vaccination and those living in the same household. Among household contacts of monkeypox cases in the DRC, there was an observed attack rate of 1.3% for individuals vaccinated against smallpox versus 9.3% for unvaccinated individuals, and 11.7% for age group 0–4 years (7 times higher) (Jezek et al. 1988a). A more recent study showed an attack rate within households to be 50% (Nolen et al. 2016).

Models which used data from 1981 to 1986 calculated the human-to-human transmission reproductive rate (R_0) to be 0.8 predicting that the disease would not be able to sustain human infections without repeated zoonotic introductions (Fine et al. 1988; Jezek et al. 1987a). However, these older models may no longer provide

Table 2.3 Animal species susceptible to monkeypox virus infection

Order	Family	Species	References
Didelphimorphia	*Didelphidae*	*Monodelphis domestica*; *Didelphis marsupialis*	Hutson et al. (2007)
Eulipotyphla	*Erinaceidae*	*Atelerix* spp.	Hutson et al. (2007)
Lagomorpha	*Leporidae*	*Oryctolagus cuniculus*	Marennikova and Seluhina (1976)
Macroscelidea	*Macroscelididae*	*Petrodromus tetradactylus*	Doty et al. (2017) and Hutin et al. (2001)
Pilosa	*Myrmecophagidae*	*Myrmecophaga tridactyla*	Peters (1966)
Rodentia	*Chinchillidae*	*Chinchilla lanigera*	Hutson et al. (2007)
	Cricetidae	*Sigmodon hispidus*	Reynolds et al. (2019a)
	Dipodidae	*Jaculus* spp.	Hutson et al. (2007)
	Gliridae	*Graphiurus* spp.	Doty et al. (2017), Earl et al. (2015), and Hutson et al. (2007)
	Muridae	*Mus musculus*; *Mastomys natalensis*; *Oenomys hypoxanthus*; *Rattus norvegicus*	Americo et al. (2010), Doty et al. (2017), Earl et al. (2015), Reynolds et al. (2012), and Reynolds et al. (2019a)
	Nesomyidae	*Cricetomys* spp.	Doty et al. (2017) and Hutson et al. (2007)
	Sciuridae	*Cynomys ludovicanus*; *Funisciurus anerythrus*; *F. isabella*; *F. lemniscatus*; *F. congicus*; *Heliosciurus gambianus*; *H. rufobrachium*; *Protexerus strangeri*; *Marmota monax*; *M. bobak*; *Spermophilus tridecemlineatus*; *Sciurus vulgaris*; *Xerus* sp.	Doty et al. (2017), Falendysz et al. (2014), Hutin et al. (2001), Hutson et al. (2007), Jezek and Fenner (1988), Khodakevich et al. (1986), Marennikova et al. (1989), Reynolds et al. (2010), and Sbrana et al. (2007)
	Hystricidae	*Atherurus africanus*	Jezek and Fenner (1988)
Primates	*Callitrichidae*	*Callithrix jacchus*	Peters (1966)
	Cercopithecidae	*Cercocebus galeritus*; *C. atys*; *Macaca irus*; *M. mulatta*; *M. fascicularis philippinensis*; *Cercopithecus petaurista*; *C. ascanius*; *C. mona*; *C. neglectus*; *C. pogonias*; *C. aethiops*; *C. nictitans*; *C. hamlyni*; *Semnopithecus* spp.; *Colobus badius*	Arita et al. (1972), Arita and Henderson (1968), Breman et al. (1977a), Breman et al. (1977b), Gispen et al. (1976), Jezek and Fenner (1988), Peters (1966), Radonić et al. (2014), and Sauer et al. (1960)

(continued)

Table 2.3 (continued)

Order	Family	Species	References
	Hominidae	*Gorilla* sp.; *Pan troglodytes*; *Pongo pygmaeus*	Arita et al. (1972), Arita and Henderson (1968), and Peters (1966)
	Hylobatidae	*Hylobates lar*	Peters (1966)
	Cebidae	*Saimiri sciureus*	Peters (1966)
	Lorisidae	*Perodicticus potto*	Jezek and Fenner (1988)
Carnivora	*Procyonidae*	*Nasua nasua*	Hutson et al. (2007)
	Felidae	*Felis* spp.	Jezek and Fenner (1988) and Khodakevich et al. (1987b)
Artiodactyla	*Suidae*	*Sus scrofa*	Hutin et al. (2001)

an accurate representation of the epidemic potential of the virus. This may be due to changes within human or zoonotic populations, including the spread of HIV/AIDS, altered access to health care facilities, altered population age structure of the population, ecologic disturbance, and others (Antia et al. 2003). Nonetheless, a more recent model did not suggest any changes in monkeypox transmissibility (Blumberg and Lloyd-Smith 2013) but acknowledges that more surveillance data is required for a reliable assessment of changes in transmissibility of monkeypox (Blumberg et al. 2014).

2.6.6 Genetic Characterization of MPXV

Two genetic clades of MPXV have been characterized, including the WA and the CA clade. The two clades are geographically separated and have defined epidemiological and clinical differences. The WA clade demonstrates a case fatality of between 0 and 6%, and limited human-to-human transmission has been documented (Breman et al. 1980; Yinka-Ogunleye et al. 2019). In comparison, the CA clade mortality can be as high as 11% (Jezek et al. 1987d), and up to 17% in children (Breman et al. 1980). Human-to-human transmission up to six sequential events (seven when including the primary transmission from animal to human) has been observed (Learned et al. 2005). The WA clade has been reported in Nigeria, Liberia, Ivory Coast and Sierra Leone, while the CA clade in Gabon, Cameroon, the Republic of Congo, and the DRC (Chen et al. 2005b; Jezek et al. 1987d; Likos et al. 2005; Sbrana et al. 2007).

Sustainability maps for the MPXV transmission produced by using ecological niche modeling suggested the Cameroon Highlands as a break in the distribution of suitable environmental conditions for the MPXV transmission. This partition of the MPXV geographic range coincides with the WA and CA clades (Ellis et al. 2012). This theory was supported by the analysis of many genomic sequences from MPXV

isolates covering the known geographic distribution of MPXV. However, it is not clear whether the presence of a river (Cross or Sanaga River), change in elevation, or change in the dominant vegetation cover is involved in the genetic differentiation of MPXV (Nakazawa et al. 2015).

2.7 Laboratory Diagnosis

Historically, poxviruses used to be diagnosed based on their biological properties through virus isolation assays. The morphology of viral pocks produced on a chicken embryo chorioallantoic membrane or the reproductive ceiling temperature in a cell culture allowed identification of particular poxviruses. However, these methods are laborious, time-consuming (because they require virus isolation and propagation), and are restricted to well-equipped laboratories (Jezek and Fenner 1988; Lewis-Jones 2004). Similarly, negative-stain electron microscopy was widely used for the diagnosis of viruses before the development of molecular techniques, but given the similar morphological characteristics of OPVs, the differentiation of species within the genera is not possible (Ferreira Barreto-Vieira and Monika Barth 2015; Kurth and Nitsche 2007). Lesion material is the most suitable specimen for the abovementioned techniques.

Confirmation of the MPXV infection is best done by polymerase chain reaction (PCR) as it is the only method which can differentiate between the orthopoxvirus species. The large central genomic region is highly conserved among OPV isolates which explains the significant degree of cross-reactivity in various tests, whereas terminal regions are much more variable which makes them ideal targets for PCR-based techniques. Genes often targeted for monkeypox diagnosis are hemagglutinin (Ropp et al. 1995), the acidophilic-type inclusion body gene (Meyer et al. 1997), the *crmB* gene (Loparev et al. 2001) envelope protein gene (*B6R*) (Li et al. 2006), *B7R* gene (Shchelkunov et al. 2011), and the tumor necrosis factor binding protein gene (Davi et al. 2019).

The most suitable specimen is lesion material—biopsy, roof, fluid, or crust depending on the rash stage. The timing and duration of viremia are variable, and results are often inconclusive. Therefore, the collection of blood is not recommended for diagnostic purposes.

Protein-based methods detecting different antigens from clinical samples were developed (Czerny et al. 1989; Hughes et al. 2014; Johann and Czerny 1993; Stern et al. 2016b) but they are less sensitive than PCR and do not permit differentiation of OPV (Pauli et al. 2010). Nevertheless, protein-based methods are usually robust and well-adaptable for field use. There were two systems developed for detection of orthopoxviruses: Tetracore Orthopox BioThreat® (Townsend et al. 2013) and ABICAP immunofiltration system (Stern et al. 2016a).

When no virologic specimen is available, serologic diagnostic methods are very useful for retrospective analysis. The most commonly used serologic test for poxvirus diagnosis (not specific to MPXV) is antibody-capture enzyme-linked

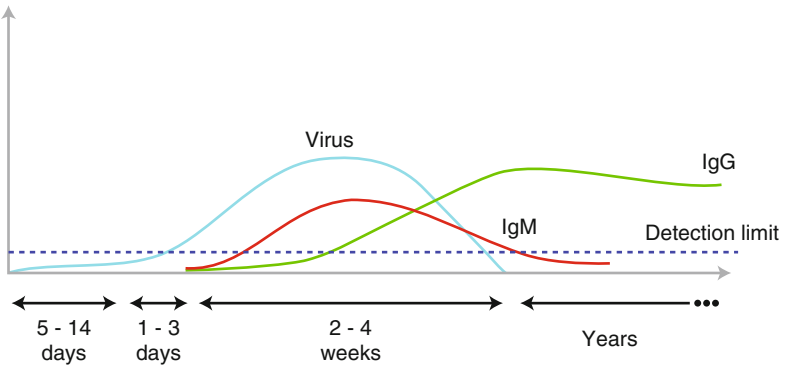

Fig. 2.7 Schematic representation of the relevant diagnostic markers. Virus: present in the blood from the end of the incubation period, through a febrile stage and the beginning of rash stage; in the oral mucosa from lesions which typically appear as the febrile stage is ending and the rash is beginning; in the lesions throughout all rash stages. IgM: appears soon after the rash onset and rises for about 2 weeks before declining and disappearing within a year. IgG: appears soon after the rash onset, rises for about 6 weeks, and lasts for decades

immunosorbent assay. The kinetics of an antibody response varies from person to person and can be dependent on smallpox vaccination history. The optimal time for collecting serum for IgM detection is between 4 and 56 days post-rash onset. This antibody response typically rises during the first 2 weeks of rash illness before eventually waning within a year. IgG titers will rise as antibody production switches from the acute to memory phase. IgG appears soon after rash onset, rises for up to 2 months and antibodies may remain observable for a lifetime. Serum collection for IgG detection should occur 2 weeks or more after rash onset (Karem et al. 2005).

While there are numerous diagnostic tests for clinically relevant infectious diseases, there are no commercially available laboratory assays for monkeypox, including on-site diagnostic tests (Stern et al. 2016a). Routine MPXV specimen preparation, pathological and molecular diagnostic tests should be conducted in BSL-2 facilities with BSL-2 work practices, while culturing MPXV specimens should be carried out in BSL-3 facility (CDC 2015b; Jezek and Fenner 1988; Tian and Zheng 2014) (Fig. 2.7).

2.8 Control Measures

2.8.1 Prevention

Orthopoxviruses induce cross-reactive antibodies that protect against infection from other orthopoxvirus species. Live vaccinia virus vaccine (first generation), which was used during the smallpox eradication program, was estimated to be 85% effective against monkeypox infection (Fine et al. 1988). The vaccination was ceased after smallpox eradication was declared in 1980, causing the proportion of

the unvaccinated population to rise. This first generation of vaccinia vaccine can cause serious adverse events and is contradicted in pregnant women, immunocompromised people, and people with a history of eczema (Lane et al. 1970).

Improved manufacturing procedures allowed the development of the second, third, and fourth generation vaccinia vaccines with reduced side effects and simplified administration. They were developed to be used in the case of the natural or deliberate reemergence of smallpox. The major challenge is that no new developments can be evaluated against naturally occurring smallpox. One example of the second-generation vaccines is ACAM2000, a live attenuated vaccinia vaccine administered by bifurcated needle (like the first generation), only approved in the USA. LC16m8 is an attenuated replication-competent third-generation vaccinia vaccine, immunogenic after a single dose with a good safety profile licensed in Japan (Kenner et al. 2006). Another third-generation vaccine is modified vaccinia Ankara (MVA) requiring a two-dose administration by injection which was approved in the European Union (marketed as IMVANEX) and Canada (marketed as IMVAMUNE) for smallpox (Overton et al. 2018). MVA is also approved by the US Food and Drug Administration (marketed as JYNNEOS) for prevention of smallpox and monkeypox in adults determined to be at high risk for the infection. This makes MVA the first approved vaccinia vaccine for monkeypox, although its approval is based on survival data obtained in lethal MPXV challenge studies in non-human primates (BavarianNordic 2019). MVA's effectiveness, immunogenicity, and safety are also being evaluated in healthcare personnel at risk of monkeypox infection in the DRC (Petersen et al. 2019). The fourth generation of vaccinia vaccines (gene-based and protein-based) is still in development phase (Buchman et al. 2010; Hooper et al. 2004).

For the general public, there is no vaccinia vaccine available, but vaccine stockpiles are maintained by several countries and WHO (WHO 2017a). There has been no formal study on post-exposure use of vaccinia vaccine for monkeypox infections, but it has been used for this purpose in the cases of imported monkeypox to UK (Vaughan et al. 2018) and Singapore (WHO 2019a).

Given the lack of approved vaccines for monkeypox, the only prevention of this disease involves education for health workers (Bass et al. 2013) and education of the population at risk on the dangers of contact with sick or dead animals which could carry the virus (Jezek and Fenner 1988). The awareness-raising should mainly focus on how to recognize the disease and how people can protect themselves from the infection.

2.8.2 Treatment

To date, there is no approved treatment for MPXV infections. Therefore, treatment is symptomatic and supportive. However, several investigational antivirals demonstrate activity against MPXV in vitro and animal model systems (Yu and Raj 2019). These include cidofovir (Andrei and Snoeck 2010), brincidofovir (Lanier et al. 2010), and tecovirimat (Berhanu et al. 2015; Yang et al. 2005), but none was

evaluated in a clinical trial. Tecovirimat is approved by the US Food and Drug Administration for the treatment of smallpox.

The mechanism of action of cidofovir is through the inhibition of viral DNA polymerase. The same is true for brincidofovir, which is a modified cidofovir, lacking nephrotoxicity and being orally available. Instead, tecovirimat targets a specific viral product blocking the release of intracellular virus from the cell.

2.9 Zoonotic and Transboundary Threat

Monkeypox has been, until recently, considered a rare zoonotic disease. Nevertheless, we have seen an increase in the number of reported cases and expansion in the geographical range in the last few years (Sklenovska and Van Ranst 2018). This is probably caused by a myriad of factors like the reduced immunity since the cessation of smallpox vaccination, better means of diagnosis and stronger surveillance systems, and other environmental and social factors whose scope is not fully understood. Climate change and deforestation might be increasing the risk for contact between humans and infected animals, but also the displacement of populations or necessity might drive people into the bush looking for potentially infected meat.

Currently, monkeypox is a public health concern in various countries of Central and West Africa, with a seemingly increasing trend which cannot be explained solely by improvements in surveillance (Mandja et al. 2019). MPXV was exported outside of the African continent for the first time to the USA in 2003 through the infected African rodents. This was followed by reports of 4 independent infected travelers from Nigeria to UK (2), Israel (1), and Singapore (1) in 2018 and 2019, of which one involved a secondary transmission to a health worker. These examples illustrate how globalization, animal trade, and travel increase the transboundary threat of monkeypox.

The threat of monkeypox would be expected to increase in the following cases: an increase in virulence (both naturally (Blumberg and Lloyd-Smith 2013; Shchelkunov et al. 2005) or via genetic engineering (Jackson et al. 2001)), the virus spilling into more widely distributed taxa (Reynolds et al. 2012) or introduction in other continents (Rimoin et al. 2010). That is why MPXV belongs to the "biosafety level 3" category, the "high threat" biodefense category in the EU (Tian and Zheng 2014) and why it is on the list of select agents in the USA (FSAP 2017).

2.10 Conclusion and Prospects

Monkeypox virus is an emerging pathogen causing a disease of epidemic potential about which much is still unknown. Health workers are often not aware of the existence of monkeypox and its characteristics, laboratory capacity in the affected countries is limited, and there is no systematic surveillance mechanism to report

monkeypox, leaving significant gaps in our understanding of the disease epidemiology and burden.

At the same time, cases of monkeypox in humans have been increasing, which is probably driven by a combination of environmental and anthropogenic factors. Climate change, deforestation, and war, among others, result in more frequent contact of people with infected wildlife. Additionally, vaccination against smallpox with vaccinia vaccine was ceased in 1980, which is still causing an increasingly growing proportion of the population to become vulnerable to MPXV and other orthopoxviruses.

The recent approval of the MVA vaccine for monkeypox prevention by the US Food and Drug Administration is a significant milestone, but no monkeypox-specific treatment options are approved, and clinical guidelines do not exist. Symptomatic and supportive treatment is currently the only care a patient can receive; however, experimental evidence of the efficacy of several compounds against MPXV infection seems promising.

Considering the perceived public health importance of monkeypox in affected countries on the one hand, and the lack of understanding and means to prevent and control it on the other, it is clear that monkeypox needs to receive more attention. Awareness-raising, surveillance strengthening, and diagnostic capacity building are some of the most important activities to improve the detection, treatment, and limit further spread of the virus. Furthermore, research activities to generate knowledge and guide further improvement in prevention and control of monkeypox are needed. This includes clinical trials to further test modern vaccinia vaccines and antivirals for monkeypox.

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