A one health perspective on a recent outbreak of monkeypox

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Abstract

The monkeypox virus, or mpox virus, is a highly communicable orthopoxvirus that was first observed in the mid-twentieth century. The virus causes monkeypox, a zoonotic disease characterized by pox lesions and swollen lymph nodes. While mpox is endemic in parts of Africa, it is not typically seen outside the continent. It was recently detected in the United States in May 2022, with rapid escalation in cases both domestically and internationally. The outbreak was declared an international public health emergency in July 2022 and a domestic public health emergency the following month.

Since the transmission of this zoonotic disease can be impacted by humans, animals, and the environment, a One Health approach to surveillance, treatment, prevention, and education is imperative for public health. More specifically, a successful One Health approach to any zoonotic disease outbreak must incorporate human, animal, and environmental health experts. Depending on the nature of the disease, the focus may initially be on one aspect of this triad. In the case of the recent outbreak, the emphasis was primarily on human health when only a small number of cases were reported. As the caseload grew from single digits to hundreds of new cases diagnosed daily, it became apparent that extensive human health investigations should include veterinary, environmental, wildlife, and public policy assessments. The purpose of this report was to discuss and apply a One Health approach in retrospect to address and mitigate the impact of the 2022 monkeypox outbreak in the United States.

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Chapter 1: Introduction to Monkeypox Virus

Classification and Genomics

Taxonomy and Family

The monkeypox (mpox) virus is composed of a double-stranded deoxyribonucleic acid (DNA) genome and is classified under the realm *Varidnaviria*, in the kingdom *Bamfordvirae,* under the apply named class of *Pokkesviricetes*. Further classification is under the order *Chitovirales*, the genus *Orthopoxvirus*, and family *Poxviridae* (Titanji et al., 2022). The *Poxviridae* family includes variola various, which causes smallpox; vaccinia virus, which is used in the smallpox vaccine; and various other more species-specific viruses, including buffalopox, horsepox, rabbitpox, skunkpox, and volepox viruses. The *Poxviridae* family also includes cowpox, which was used as the basis of the original smallpox vaccine development by Edward Jenner in 1796 (World Health Organization [WHO], 2010).

Previously, smallpox was considered the most significant orthopoxvirus in terms of public health. It was declared globally eradicated in 1980 by the World Health Organization, after the last naturally occurring smallpox case was seen in 1977 in Somalia (WHO, 2010). As a result of the successful eradication campaign, routine vaccination ceased in the United States in 1972, and vaccinations ceased in various countries prior to 1980 as well. Following the events of September 2001, vaccination in the U.S. military for smallpox resumed due to governmental concerns about bioterrorism (Grabenstein et al., 2003). However, due to the general cessation of routine vaccine programs, overall human immunity to smallpox, mpox and other orthopox viruses has waned substantially in recent years.

Morphology

The mpox virus is a linear DNA virus, approximately 200-250 nanometers in size. It consists of approximately 200 kilobase pairs, packed tightly with approximately 200 genes (Xiang et al., 2022). The morphology is a rectangular enveloped virus with a lipoprotein outer membrane and a dumbbell appearance at the core (Parker et al., 2013).

Genome

Overall, the mpox virus genome is not well-characterized, with little known about many of its major proteins (Forni et al., 2022). Available data indicates the mpox virus has a 197 kilobase linear DNA genome (Kumar et al., 2022). It is structured in the same way as other orthopoxviruses with 6379-bp inverted terminal repeats on variable ends, with 90% and 96.3% of its genome identically sequenced as other orthopox viruses and the human variola virus, respectively (Shchelkunov et al., 2002). However, a comparison of the genetic differences indicates that the mpox virus is a distinct species and evolved separately than variola, from a similar virus or the same orthopoxvirus ancestor (Kumar et al., 2022). Additionally, the evaluation of genomics across various cases shows clear evidence of viral mutation, genomic flexibility and adaptive changes observed since its discovery (Isidro et al., 2022).

Nomenclature

In late 2022, the WHO proposed a name change from ‘monkeypox’ to ‘mpox’, to reduce stigma and racist commentary surrounding the disease (WHO, 2022d). Per the most recent revision of the International Classification of Diseases (ICD), the disease may be referenced by either term for the next year, after which ‘mpox’ will be the preferred nomenclature. This change was devised to reduce prejudice related to the virus’s African origins and allow patients to seek medical treatment with less discrimination.

History

There is now preliminary evidence that mpox is an ancient disease that dates to the time of Egyptian mummies (Farahat et al., 2022). Museum specimens have presented with vesicular skin eruptions, indicative of smallpox, mpox, other orthopoxviruses. Previous investigations have identified smallpox virions on some ancient specimens, but further research would be needed to determine if all the cases with lesions have been correctly attributed to variola. Evaluation of antique museum holdings has produced evidence of mpox in multiple specimens of African squirrels, some dating back to 1899 (Farahat et al., 2022). It is presumed that the disease has existed in Sub-Saharan Africa for several thousand years. Due to its similarity to smallpox, vaccinia, and other orthopox viruses, the identification of mpox virus as a unique virus was delayed because of unreliable diagnostics.

The first confirmed mpox case in humans was in 1970 in a nine-month-old infant in the Democratic Republic of the Congo (Reynolds et al., 2019). At the time, smallpox had been eradicated in that area, so a sample was sent to the WHO Smallpox Reference Centre in Moscow for diagnostic evaluation. Monkeypox cases may have been circulating in this location prior to smallpox eradication in that area, and most likely, symptomatic individuals were incorporated into smallpox statistics. Six other human cases were confirmed via serological identification in nearby Liberia, Nigeria, and Sierra Leone within the following year (Xiang et al., 2022).

The first published report of mpox infection in nonhuman primates occurred in 1958, in a shipment of *Macaca fascicularis* (cynomolgus monkeys) received at a Danish polio research facility (Khodakevich et al., 1986). This virus was identified at the State Serum Institute in Copenhagen as a novel orthopoxvirus and was termed ‘monkeypox’. In 1959, an American facility reported a mpox outbreak in both *M. fascicularis* and *M. mulatta* (rhesus monkeys) (Von

Magnus et al., 1959; Khodakevich et al., 1986). This initial diagnosis was followed by clinically confirmed outbreaks in at least seven other international captive primate colonies over the next decade (Arita et al., 1968). Subsequent cases have been noted in dozens of additional species across the animal kingdom (Centers for Disease Control [CDC], 2023).

Distinctive Clades

There are various methodologies for differentiating endemic reservoirs of this virus. Historically, the clades were divided into the Central African or Congo basin group and the West African group (Kumar et al., 2022). There is an approximately 0.5% genomic sequence difference between the two originating locations (Xiang et al., 2022). Central African has been identified as Clade I, and West African has been identified as Clade II. More recently, the West African Clade has been subdivided as Clades IIa and IIb, or as Clades II and III (CDC, 2023).

The Central African clade, or Clade I, is generally considered endemic to Cameroon, Central African Republic, Democratic Republic of the Congo, Gabon, Republic of the Congo, and South Sudan (Kumar et al., 2022). The strain is considered more virulent, with an estimated fatality rate of up to 10%. Frequently, the fatalities are associated with patients who have human immunodeficiency virus (HIV) and/or other mitigating health factors (Yinka-Ogunleye et al., 2019).

The West African clade, or Clade II, is considered endemic to Benin, Cameroon, Cote d'Ivoire, Liberia, Nigeria, and Sierra Leone. Clade II is considered less virulent, with a lower fatality rate, up to 4% (Singhal et al., 2022). It is subdivided into two clades, either Clade IIa and Clade IIb, or Clades II and III. Genomic evaluation indicates that many recent cases that have occurred in the United States, Nigeria, and Europe are linked to the Clade IIb, or Clade III, viral strain (Kumar et al., 2022; Singhal et al., 2022).

While the primary differentiation between clades has been a combination of geography and virulence, it has also been noted that Clade I is generally transmitted as animal-to-human, while Clade II has been more frequently transmitted via human-to-human transmission. Much research about the various genomic differences between clades is still ongoing, but there is evidence to support that mutations within the last few years have likely contributed to a virus that is more effectively transmitted human-to-human and less successfully maintained in a rodent reservoir (Gigante et al., 2022).

Endemic Countries

Monkeypox is considered endemic to multiple countries in the central African continent: Benin, Cameroon, Central African Republic (CAR), Cote d'Ivoire, the Democratic Republic of the Congo (DRC), Gabon, Liberia, Nigeria, Sierra Leone, and South Sudan. These areas have consistently maintained naturally occurring animal reservoirs since the initial cases were confirmed. Reservoirs are the animals, plants, or environmental components where a virus naturally propagates and is maintained. Various native African rodents, non-human primates, and other species have been shown to carry the virus since it was initially diagnosed.

Overall, minimal resources have been devoted to mpox research in endemic countries, so not much research has been performed to confirm animal transmission cycles or potential animal reservoirs. International agencies monitored mpox cases in the DRC closely from 1970 until approximately 1985, with dwindling attention given to it until approximately 2001. Since 2001, case numbers have increased in DRC, CAR, Cameroon, South Sudan, Sierra Leone, Liberia, and Nigeria (Reynolds et al., 2019). Some of this increase may be attributed to the improved testing and disease surveillance available at various times during the last few decades.

While Ghana was the originating country for rodents that were implicated in a 2003 U.S. outbreak, the first documented cases in humans in Ghana were not recorded until May 2022 (Xiang et al., 2022). This is attributed to the under-reporting of cases and lack of systemic monitoring in that region of Africa, as well as the possibility that asymptomatic human carriers of the virus may exist. Serological assessment of a population (n = 185) in central Cameroon revealed a statistically significant number of people presenting with orthopoxvirus antibodies. While the serotesting was not specific to any particular orthopoxvirus, these cases were probably attributed to mpox virus due to the consumption of bushmeat amongst locals as well as proximity to a primate sanctuary, as both are known risk factors for potential exposure (Guagliardo et al., 2020).

Previous Outbreaks

Globally, the size of outbreaks has increased since the 1970s as well as the geographic spread (Bunge et al., 2022). These increases are primarily attributed to three main factors: 1) higher occurrence of international travel, 2) decreases in smallpox immunity following secession of vaccination programs, and 3) improvements in frequency and accuracy of testing methodologies (Corning, 2014). Before 2022, cases outside of Africa had previously been reported in the U.S., United Kingdom, Israel, and Singapore (Bunge et al., 2022). Sporadic cases from international travel occurred in the U.S. and Europe, but they were overall limited to the traveler and/or their immediate contacts, with minimal exposure cases beyond the originally infected patient (Adalja et al., 2022).

In the United States, the first and previously considered largest outbreak occurred in 2003 and was attributed to imported Gambian pouched rats, rope squirrels and dormice (Cohen, 2022). These infected animals were shipped to various secondary animal distributors, and some were

subsequently housed with prairie dogs. This resulted in a greater number of infected animals across multiple states, and 47 confirmed or suspected human cases (Adler et al., 2022). All human cases were a result of animal-to-human contact, with no evidence of human-to-human transmission during that outbreak.

From 2017 to 2020, Nigeria also experienced a large outbreak of the virus, affecting approximately 200 individuals, with many cases noted near major traffic convergence points, suggesting transmission across a wide geographic area. Cases were much higher in males than females, primarily attributed to the increased likelihood of exposure when hunting in forests or performing agricultural work (Nguyen et al., 2021). No human-to-human cases were reported in this outbreak as well.

In 2021, the U.S. reported two cases in individuals who had recently returned from Nigeria, albeit several months apart, to Texas and Maryland, respectively (WHO, 2021a; WHO, 2021b). No secondary transmissions were diagnosed following the initial infections, and no additional primary cases were noted from Nigerian travels in any other countries at that time. It is speculated that this limited viral transmission may be partially attributed to pandemic COVID-19 protocols that were in place on airplanes and other public venues at the time (WHO, 2021b).

Current Outbreak

In May 2022, mpox cases were detected in the United States at a low incidence. The first two cases in the United States occurred on May 4, 2022, and were reported in males in Massachusetts and New York City that had recently returned from international travel (Minhaj et al., 2022). Both patients presented with perigenital, perianal, and/or oral lesions that were initially diagnosed as a sexually transmitted disease and not identified as the mpox virus. The patients did not receive accurate diagnoses until May 17 and 19, 2022, respectively, following

information sent out by the United Kingdom Health Security Agency (UKHSA) in response to seven cases that occurred from May 7-16, 2022 (Minhaj et al., 2022). Several cases were noted in travelers returning from Nigeria and/or men who have sex with men. Case reports began to emerge from other countries as well, with rapid escalation into the summer and caseloads peaking at approximately 500-600 cases in July and August 2022 (CDC, 2022a). Cases were noted with increased incidence in both endemic and non-endemic countries, with at least 110 countries affected by late 2022 (WHO, 2022c). A substantial decrease was noted in late 2022, but the daily case rate in the first quarter of 2023 was still measurable. The current international case number is approximately 86,500 confirmed cases, with a total death count of 111 cases as of March 16, 2023. Of those, 30,262 cases and 38 deaths occurred in the United States (CDC, 2023).

One item of concern about the current outbreak is the genetic changes that were noted in the mpox viral genome compared to previous cases. Early research to identify the variant of mpox showed that while the virus was more closely associated with Clade II, it had enough notable differences to merit a new designation of Clade IIb or Clade III (Kumar et al., 2022). Multiple 2022 outbreak cases showed very similar characteristics to one another, indicating a single origin, but varied from samples taken prior to 2021 from Clade II by 46 single nucleotide substitutions, approximately half of which were missense mutations (Forni et al., 2022; Gigante et al., 2022). Evaluation of later cases in the outbreak showed additional nucleotide substitutions and missense mutations, which are considered indicative of the virus's adaptive abilities and/or interactions with the human host proteins. While much additional research is needed, these preliminary analyses show notable adaptive abilities of the mpox virus that could increase the potential for future outbreaks.

In previous outbreaks of mpox in humans, nearly all cases could be traced back to international travel or exposure via exotic pets, and in some cases, the 2022 outbreak did follow a similar pattern. For example, in Italy from May-June 2022, approximately 37% of cases were linked to recent international travel, with 25 out of 86 cases presumably originating from the Canary Islands (Guzzetta et al., 2022). Additionally, the first cases in the United States and the United Kingdom in May 2022 were associated with travel to Africa (Minhaj et al., 2022).

However, in many instances, the 2022 outbreak in the U.S. did not follow previous transmission dynamics, with many cases spread because of male-to-male sexual contact (Adalja et al., 2022). Before the 2022 outbreak, the human-to-human viral transmission rate of mpox virus was reported to be low, and the secondary transmission rate from animal-infected human sources was approximated at 8% (Adler et al., 2022). During the 2022 outbreak, transmissions have been nearly all attributed as human-to-human, with little evidence of animal-to-human transmission. This notable change compared to historic transmission combined with its known zoonotic nature points to how a One Health approach is imperative to managing, treating, and preventing mpox due to its ability to affect animals, humans, and the environment. Chapters 2-4 will focus specifically on the impacts of animals, humans, and the environment on the transmission of the mpox virus.

Chapter 2: Animal Health Impact

The monkeypox (mpox) virus is considered first and foremost an animal disease, with ample documentation showing its zoonotic potential and ability to transmit across multiple species. Over fifty mammalian species are known to carry the mpox virus, including non-human primates, dogs, small insectivores (such as hedgehogs and shrews), and rodents, such as squirrels, chinchillas, and prairie dogs. There are multiple species of rodents, livestock, and carnivores that are known to carry other orthopox viruses, including cowpox, camelpox and wild vaccinia-like viruses (Centers for Disease Control [CDC], 2023).

Animal Transmission Cycle

Less is known about mpox viral transmission amongst animals compared to human-to-human or animal-to-human transmission. Both the 2003 U.S. outbreak as well as controlled laboratory testing indicate that *Cynomys ludovicianus* (North American prairie dog) can successfully transmit the virus between animals of the same species (Hutson, 2011). The prairie dog's incubation period is considered narrower than interhuman transmission, with peak shedding at 9-13 days post-infection (Hutson, 2011).

The incubation period and clinical symptoms of animal infection are similar to humans, with animals generally displaying symptoms one to two weeks post exposure (AVMA, 2022). While there may be variability between species, all symptomatic animals appear to display the characteristic pox-like lesions, fever, and swollen lymph nodes. Additionally, nonhuman primates and ground hogs have displayed conjunctivitis, nasal discharge, inappetence, and cough symptoms. While diagnosis in potentially infected animals can be performed using similar tests that are used in humans, there are currently no approved treatments or vaccines for mpox in animals at this time.

Known Animal Reservoirs

Overall, there is limited data available regarding African wild animal reservoirs. The first documented case of a wild animal seropositive for the mpox virus was a symptomatic rope squirrel, captured locally to a human case in the Democratic Republic of the Congo (DRC) in 1985 (Reynolds et al., 2019). Diagnostic work performed by Russian and American World Health Organization Centers on skin lesion and organ samples from the diseased squirrel (*Funisciurus anerythrus*) confirmed the presence of mpox virus (Breman, 2000). Large-scale examinations in the DRC and surrounding areas over the years resulted in a very limited number of actively infected wild animals being discovered.

In 2012, the virus was detected in a juvenile *Cercocebus atys* (sooty mangabey) from Cote d'Ivoire (Reynolds et al., 2019). Several additional African animals, including two rope squirrels, a Gambian rat, and a shrew also showed evidence of mpox virus infection (Cohen, 2022). African squirrels have been the most frequent species to demonstrate viral antibodies. Multiple species of wild African squirrels, rats, and mice have demonstrated exposure via positive serological titers or polymerase chain reaction (PCR) arrays (Falendysz et al., 2015; Guagliardo et al., 2020). While the virus was named monkeypox after the initial cases noted in captive non-human primate colonies, data indicates the most prevalent and widespread reservoirs are actually various species of squirrel (Forni et al., 2022).

Monkeypox differs from many zoonoses in that it generally does not result in high levels of animal mortality in the wild (Reynolds et al., 2019). Many animals are asymptomatic, which increases the risk of potential human exposure, via either contact or consumption. The virus is known to infect multiple species of non-human primates, including gorillas (*Gorilla gorilla*), chimpanzees (*Pan troglodytes*), the Asian gibbon (*Hylobates lar*), South American squirrel

monkeys (*Saimiri sciureus*), African owl-faced monkeys (*Cercopithecus hamlyni*), mangabeys (*Cercocebus atys*) and South American marmosets (*Callithrix jacchus*). Other species are likely affected, based on the large number of identified carriers but expanded testing would be required for confirmation.

Multiple species of rodents, including Gambian pouched rats *(Cricetomys gambianus)*, various species of squirrels (primarily of the *Funisciurus* and *Heliosciurus* genus), multiple strains of marmots (genus *Marmota*), rufous-nosed rats (*Oenomys hypoxanthus*) and African dormice (multiple species under the genus *Graphiurus*) have been seropositive for mpox virus (Bonilla-Aldana et al., 2022). There is also confirmed evidence of persistent infection in African dormice (Hutson, 2015). Generally, nearly all species that may be potential carriers of the mpox virus are rodents or primates. Two cases of human-to-dog transmission were documented during the 2022 U.S. outbreak, but there is overall limited evidence to indicate that dogs are a highly likely species to substantially contribute to transmission (Seang et al., 2022).

Potential Carriers

At this point, no officially known animal reservoir exists outside of Africa. However, during the 2003 outbreak, accurate sale and transfer records could not be located for several potentially exposed animals (Cohen, 2022). It is unlikely any potential reservoirs would have lived over the past two decades, but due to large numbers of native prairie dog and squirrel varieties, the possibility does exist of transmission between generations of these animals. Additionally, the original species responsible for the outbreak, the Gambian pouched rat, is considered an invasive species in Grassy Key in Florida (Falendysz et al., 2015).

Many species are known to carry various orthopoxviruses, including common household pets, such as guinea pigs, gerbils, and hamsters. Domestic cats, cows, camels, and wildlife

frequently found on perimeters of populated areas, such as bats, foxes, coyotes, badgers, skunks, voles, and raccoons, are known to carry various orthopoxviruses and may be susceptible to the mpox virus (CDC, 2023; Khodakevich et al., 1986).

Thus far, no evidence is available to indicate that birds, reptiles, or amphibians can become infected by or transmit the mpox virus. Because of this lack of evidence, as well as the wide variety and range of these species, it is considered doubtful that they are carriers (CDC, 2023). There is also limited evidence to indicate that goats, sheep, or pigs can be infected with mpox virus, since it has not been confirmed they can carry orthopoxviruses. As a result of the 2022 outbreak, many scientists have begun researching more facets of the mpox virus, including a study initiated at Pennsylvania State University evaluating whether cattle or pigs can transmit mpox virus (Culbertson, 2022).

Animal Models

The most common large animal laboratory model was generated via intravenous injection of Zaire 1979 MPXV into the *Macaca fascicularis* (crab-eating macaque; cynomolgus monkey) model (Mucker et al., 2022). While this infection route does not mimic the human route of exposure, the method reliably produced fever, detectable viremia levels, distinctive pox lesions, and ultimately, mortality. *Macaca mulatta* (rhesus macaque) display comparable symptoms, although mortality and lesion onset are slightly delayed. Overall, both laboratory species produce a reliable disease course when administered $\geq 10^6$ plaque-forming units (PFU), as well as respond reliably to vaccines and antivirals under these conditions (Parker et al., 2013).

In the cynomolgus monkey model, the disease course begins with lymphadenopathy and fever within 3-4 days post-injection, followed by detectable viremia and vesiculopustular rash from Day 4 onward (Parker et al., 2013). This is followed by body weight loss, inappetence,

lethargy, and lesion counts of >1000 over subsequent days. Ultimately, the animals are generally euthanized between Days 9-15 (Mucker et al., 2022).

While many of the natural reservoirs of the mpox virus are rodents, the affected species are generally not used for large scale medical research due to lack of commercial availability. Many strains of standard, adult laboratory mice (*Mus musculus)*, rats (*Rattus norvegicus)*, and rabbits (*Oryctolagus cuniculus*) with functional immune systems have overall low susceptibility to mpox infection (Reynolds et al., 2019). However, newborn laboratory rabbits and rats are highly susceptible to mpox virus, as well as various strains of immunocompromised mice. One commonly used mouse model is the CAST/EiJ (*Mus musculus castaneus*) strain, which can be infected via intranasal or intraperitoneal inoculation (Reynolds et al., 2019). The CAST/EiJ mouse strain is a wild-derived, inbred strain developed from one of the three major mouse subspecies (*Mus musculus castaneus*) and is commonly used in genetic resource due to its notable genetic differences from standard laboratory mice (*Mus musculus*).

Ultimately, most mpox research is performed in cynomolgus monkeys (*Macaca fascicularis*) that have been infected via aerosol exposure. Ongoing preclinical research for treatments and vaccines frequently utilizes mice, rats and cynomolgus monkeys as the primary research models. The amount of preclinical and clinical research has increased because of the 2022 outbreak. This increase in resources will allow future implementation of One Health-based methods for predicting, preventing, and managing future mpox outbreaks.

Chapter 3: Human Health Impact

The monkeypox (mpox) virus is phylogenetically similar to smallpox virus and other orthopoxviruses, with the viruses exhibiting cross-reactivity and cross-protection (Titanji et al., 2022). Therefore, previous smallpox vaccination campaigns and post-infection immunity offer theoretical protection against mpox. Since smallpox has been considered eradicated for four decades, overall global human immunity is at its lowest point in human history. Smallpox differs from mpox in that it is strictly a human disease, with no animal reservoir, and has a 10-fold higher transmission efficiency in humans than mpox (Reynolds et al., 2019). While mpox is considered less virulent, it is still a highly communicable virus and considered the most significant of the orthopoxviruses in terms of human health concerns.

Human Transmission Cycle

The mpox virus may be transmitted to humans via human or animal exposure, via contact with lesions or body fluids, inhaled droplet transmission, or fomites (Kumar et al., 2022). Monkeypox DNA has been detected in human whole blood, plasma, semen, urine, feces, rectal swab, nasopharyngeal swab, skin/lesion sample, and saliva (Jiang et al., 2022). While human-to-human transmission is less prevalent than human-to-animal transmission in Africa, instances of up to six sequential human-to-human transmissions have been laboratory-documented (Hutson, 2011). Additionally, Clade III has demonstrated a higher incidence of human-to-human transmissions internationally than previously seen in endemic African areas.

Disease modeling based on cases in the Democratic Republic of the Congo (DRC) from 1980-1984 indicated that, in the absence of a reliable animal reservoir, the mpox virus lacked the necessary virulence to become as prevalent as smallpox, even as immunity from smallpox vaccination continued to wane (Fine et al., 1988). In the 2017-2020 Nigerian outbreak, many of

the cases were reported in people too young to have received a smallpox vaccine, indicating that the decades-old vaccinations still offered cross-protectivity against mpox (Nguyen et al., 2021).

Research performed in the first few decades after discovery in the DRC indicated that humans may be more susceptible to transmission in disturbed agricultural, or peridomestic, areas where squirrels and other terrestrial rodents may inhabit (Reynolds et al., 2019). Later estimations using ecologic niche models at different scales showed greater incidence of mpox within the rainforest in Sub-Saharan Africa (at the continental scale) and highly forested areas (at the local scale) (Reynolds et al., 2019). Several years later, positive cases were noted in areas predicted by the results of this modeling.

In endemic areas, people may be exposed while hunting, preparing, or consuming bushmeat from contact with asymptomatic carcasses (Reynolds et al., 2019). Gambian pouched rats, considered responsible for the 2003 U.S. outbreak, can shed the virus in oral or nasal secretions, even in the absence of visible skin lesions (Falendysz et al., 2015). Since these animals are frequently hunted for food in their native Sub-Saharan Africa, the potential for disease transmission in asymptomatic animals to humans is a concern.

Data on the human incubation period varies, with onset within as few as one day or as many as 30 days post-exposure reported, although generally it is considered to be 7-10 days (World Health Organization [WHO], 2022b). First symptoms generally include lymphadenopathy or small lesions, followed within 1-4 days of exposure by larger rash and/or fever. Disease progression is generally self-limiting, with symptoms resolving within 2-3 weeks of onset. The most common symptoms consist of rash, fever and chills, adenopathy, headache, and muscle pains. Rarely, infected individuals may develop seizures or encephalitis. The current epidemic shows a greater proportion of patients with rashes in the genital or perianal areas than

previous outbreaks, and since lesions in the anal or genital region may initially present as herpes or syphilis, there is potential for initial misdiagnosis (Kumar et al., 2022).

Diagnosis

Testing methodology for mpox virus is based on clinical presentation and nucleic acid amplification testing, such as loop-mediated isothermal amplification, polymerase chain reaction (PCR), or recombinase polymerase amplification (Breman, 2000; Kumar et al., 2022). Serological antibody detection cannot be used to confirm the mpox virus but can be useful for determination of past infections or to help corroborate findings when other testing is inconclusive. Pock lesion samples may be evaluated histologically to differentiate from herpes or similar lesion-based conditions. Tissue samples may also be evaluated for infection with immunohistochemistry or electron microscopy. Finally, chick chorioallantoic membranes (CAM) has also historically been used for confirmatory evaluation of orthopoxvirus (Breman, 2000; Kumar et al., 2022). Following inoculation with a viral sample, the CAM can be evaluated for size and pattern of pock formation, as well as time of pock onset, and this can aid in diagnosis of various orthopoxvirus, including mpox (Von Magnus et al., 1959).

Unfortunately, molecular testing may not be practical in rural areas, remote villages, or other poorly accessible African communities. As discussed later, one aspect of an impactful One Health strategy is improving accuracy, rapidity, and prevalence of diagnostics. Access to effective and efficient diagnostic assays has been limited due to the previously low incidence, particularly in more developed countries. Overall testing for mpox virus has been limited in many areas, so enhanced testing in endemic regions may result in artificially increased viral rates. This shortfall in the sensitivity of readily available, in-clinic diagnostics can result in a delay in administering appropriate treatment and implementing quarantine procedures.

Additional and expanded diagnostic methodologies are crucial for the control and treatment of the mpox virus infections.

Treatment

Currently there are no standard FDA-licensed treatments for human mpox in the United States. Two orally bioavailable antiviral drugs, brincidofovir and tecovirimat, produced as Tembexa® and Tpoxx®, respectively, have been approved in the U. S. for the treatment of smallpox (Gessain et al., 2022). The U.S. maintains supplies of both products as part of the Strategic National Stockpile (SNS). While these antivirals are not fully approved in the U.S. to treat mpox, tecovirimat has been conditionally accepted under a compassionate use Investigational New Drug (IND) protocol. Cidofovir, a prodrug of brincidofovir, has been shown to have *in vitro* efficacy against mpox (Siegrist et al., 2022).

Multiple preclinical and clinical trials are ongoing for various antivirals and monoclonal antibodies. A synthetic precursor of tecovirimat, NIOCH-14, has shown efficacy in animal models and is currently in Phase I trials (Delaune et al., 2020). Additional preclinical and clinical testing for tecovirimat and brincidofovir specifically for efficacy as mpox treatment is ongoing. In the U. S., a Phase III clinical trial, nicknamed STOMP (Study of Tecovirimat for Human Monkeypox Virus) was initiated in September 2022 (*STOMP*, 2023).

Prevention

The primary methodology of prevention for mpox is vaccination. While ideally the vaccination would be contemporaneous and virus-specific, in this instance, people vaccinated for smallpox prior to 1970 have existing immunity and a 5-fold lower risk of contracting mpox now (Delaune et al., 2020). Currently, the existing vaccines for mpox were all repurposed from previously approved smallpox inoculations. There are currently two vaccines that can be used to

prevent pox: Jynneos® (MVABN) and ACAM2000® (Gessain et al., 2022). Jynneos® is a live, attenuated third generation modified vaccinia Ankara vaccine produced by Bavarian Nordic that has been FDA-approved for both smallpox and mpox. ACAM2000® is a live, attenuated vaccinia-based second-generation vaccine produced by Emergent BioSolutions that was given an expanded access Investigational New Drug (EA-IND) protocol to allow it to be used for mpox.

One of the issues related to vaccination is that the current available supply at the time of the outbreak start was very limited. This has led to governmental efforts to prioritize distribution to high-risk populations as well as modify existing vaccine protocols to provide protection over a larger number portion of the population. For example, the official recommendation for the Jynneos® vaccine is a subcutaneous injection followed by a booster approximately four weeks later (Centers for Disease Control [CDC], 2022b). However, the protocol was modified in some instances to allow for a larger pool of people to be vaccinated, by removing the booster dose entirely or reducing it to an intradermal injection at $1/6^{th}$ of the original dosage (Wolff Sagy et al., 2022)

There have been limited opportunities to test efficacy of either mpox vaccine, so during the 2022 outbreak, various vaccinated groups were closely monitored, or their data was used for retrospective analysis. In July 2022, a group of approximately 2,000 men in the Tel Aviv, Israel area were given a single subcutaneous dose of Jynneos® and monitored for exposure and/or viral onset. In a retrospective evaluation of the data, the vaccine efficacy rate was established at 86%, with a 95% confidence interval (Wolff Sagy et al., 2022). This rate would likely have been increased if patients had been dosed with the standard two-dose protocol, pointing to the vaccine as highly effective in preventing viral spread. Similarly high efficacy rates were seen in an evaluation of people who were given one or two doses of Jynneos®, compared with unvaccinated

individuals, from July 31 to October 1, 2022. Monkeypox cases were reduced 7- or 10-fold in men who were given one or two doses of Jynneos® vaccine, respectively, compared to unvaccinated men during the same time frame (CDC, 2022b).

Another significant component of the governmental efforts to prioritize vaccination was based on the lessons learned during the 1980s acquired immune deficiency syndrome (AIDS) crisis. Since the primary target population during the 2022 outbreak was men who have sex with men, there was a significant push by the government, queer activism groups, and AIDS advocacy organizations to prioritize people who are considered high-risk. In the 1980s, the delay in diagnostic, preventative, and proactive measures in the United States led to years of AIDS-related challenges for these marginalized communities.

Another vaccination tactic has been the 'ring vaccination' strategy, which provides vaccines to close contacts of a person who has tested positive for the mpox virus. By focusing vaccine efforts on high-risk populations (men who have sex with men and people who are proximal to affected persons), epidemiologists were able to slow the spread of human-to-human transmission.

One concern is the percentage of population that are contraindicated for the currently approved vaccinations (Yu et al., 2019). This includes immunocompromised individuals, as well as those that have comorbidities. The ACAM2000® vaccine is not recommended for people with human immunodeficiency virus (HIV), but the Jynneos® vaccine is considered safe for patients who are otherwise healthy despite being HIV positive (HIV, 2022). However, since HIV positive patients may be more likely to be exposed to mpox, appropriate vaccination is generally recommended by the Centers for Disease Control (CDC) when feasible. Nearly half of the American patients diagnosed with mpox in 2022 were also HIV positive; similar numbers were

seen internationally as well (HIV, 2022). The challenges of comorbidities, potential viral mutations, and changes in the affected demographics further substantiate the need for a One Health approach to preventing and managing this zoonotic originating disease such as seen in the 2022 mpox outbreak.

Chapter 4: Environmental Health Impact

The environment has a significant impact in many ways on the geographical spread of zoonotic disease, as well as maintenance of disease reservoirs in endemic areas. For a disease such as monkeypox (mpox), where transmission can be through contact or consumption, this increases the potential for human exposure in the natural environment. Increasing urbanization of previously agricultural or natural lands further intensifies these risk factors.

Monkeypox can impact both the natural and built environment. Exposures can occur at the interconnection between urbanized and agricultural areas, where reservoir species live on the peripheral areas near humans. This potential at the human-to-animal interface is currently only an issue in West and Central Africa, where endemic reservoirs are present. Higher risk populations are those that rely on bushmeat for sustenance, hunt and trap animals for resale, and/or are in forestry or agricultural positions. Unfortunately, this also tends to place people of lower socioeconomic status at higher risk for zoonotic exposure (World Health Organization [WHO], 2022a). These same populations are also less likely to seek medical help from larger clinics or hospitals or report cases to any authorities who keep statistical information on mpox.

The wildlife trade is attributed as a key nexus of previous outbreaks, although it is not implicated in the 2022 outbreak. This industry can increase potential human exposure during capture, transport, wholesale/retail housing, and ultimate sale to the public. Potential animal escape during this process can lead to invasive species inhabiting new areas, such as the reservoir of Gambian pouched rats that has become established in Florida (Falendysz et al., 2015). As mpox spreads throughout the globe, there is potential for future endemic area development and more likelihood for higher socioeconomic areas to be more affected. The current epidemic has impacted a large number of people outside of Africa than in previously less-affected populations.

This trend has caused mpox to have a greater impact on the built environment than in previous outbreaks.

Viral spread can be monitored in the urban and suburban environment, and this is by wastewater-based surveillance (WBS) (Tiwari et al., 2023). This allows scientists to monitor emerging pathogens along with existing known viral, pharmaceutical, and chemical contaminants. In some instances, WBS can allow for more robust community surveillance than clinical testing, due to costs of individual evaluations, the stigma associated with mpox, asymptomatic individuals not seeking medical help, etc. By initiating WBS in a community, epidemiologists may monitor changing pathogen levels without individual diagnostics. There are limitations for WBS that may be attributed to viral shedding from previously vaccinated individuals, limited information on quantity of viral load that may be shed during infection, and the amount of time needed to develop a robust method for evaluation for a specific pathogen (Tiwari et al., 2023).

Another aspect of environmental health that impacts zoonotic diseases is climate change (Lacetera, 2018). The globally raised temperatures can increase the potential endemic range of reservoir species and lengthen the seasonal exposure period. Furthermore, the increased environmental stress induced by temperature fluctuations, drought, and human encroachment on habitat space can cause stress on reservoir species. The stressors may raise diseased animals' probability of transmission to other animals and humans (Lacetera, 2018). Since interactions at the human-animal-environmental interface will only continue to increase over time, it is important to consider all three factors of the One Health triad when evaluating, implementing, and managing mpox or similar outbreaks.

Chapter 5: One Health Approach to Monkeypox

One Health is a transdisciplinary, collaborative approach that considers the interconnection between animal, environmental, and human health when attempting address societal issues, such as monkeypox (mpox) (Corning, 2014). A truly effective One Health program to address the mpox virus should involve: 1) communication at the local, national and international level, 2) collaboration between veterinary, human and environmental health professionals, and 3) coordination of educational, preventative, diagnostic and health management resources. Further assessment of the animal-to-animal and animal-to-human transmission opportunities is needed as part of a solid One Health approach to mitigate the threat of mpox.

One Health Implications of Monkeypox

A One Health approach toward the mpox virus should incorporate strategies for endemic areas, where animals and people may routinely carry the virus, as well as the locations of current outbreaks. Transmission must be monitored to determine whether cases are human- or animal-derived, and how far transmission can travel from patient zero, or the original animal nexus. Additionally, areas where outbreaks have higher potential should be monitored closely for animal or human cases. Both high-level and community-based efforts will be imperative to address potential for exposure in endemic areas.

There should also be a focus on captive animals, from those in the laboratory to animals used for breeding, food sources, or companionship. Data indicates that close husbandry conditions during animal importation, transport, and resale are more favorable for infection and transmission and can increase the risk of transcontinental spread. One successful response to the 2003 U.S. outbreak was to ban importation of African rodents (Reynolds et al., 2019). No

notable outbreaks have been declared due to African rodents since that time, and the importation ban has not been lifted as of this time.

Historical One Health Response

Historically, most One Health initiatives have been responsive to threats, pandemics, and outbreaks, rather than proactive in anticipation of major zoonotic incidents. Some of this trend is attributed to the large number of potential zoonotic disease risks, as over 80% of the Centers for Disease Control's (CDC) high priority 'Class A' bioterrorism agents or diseases are zoonotic diseases (Corning, 2014). An increasing number of emerging infectious diseases are considered zoonotic in nature, similar to mpox. During previous major zoonotic outbreaks, joint teams compiled by the World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), and World Organisation for Animal Health (WOAH/OIE) were deployed to assist with crisis management on the ground (Nuttall et al., 2005).

Current One Health Response

The current One Health response to mpox has been informed by historical outbreaks and the ongoing experience with the 2019 COVID-19 pandemic associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In October 2022, the Quadripartite (comprised of the WHO, FAO, WOAH/OIE, and United Nations Environment Programme) established the One Health Joint Plan of Action (OH JPA). This five-year, six-pronged plan focuses on various One Health issues, including combating emerging zoonotic epidemics and managing endemic zoonoses (WHO, 2022e). Parts of this plan have been informed by lessons learned from past epidemics and the current COVID-19 and mpox outbreaks.

In addition to international multi-organizational responses, there have been significant national responses in the Americas and Europe. National laboratories in developed countries

have generally been responsible for providing support at the state or local level since experience with mpox is limited overall. During the 2022 mpox outbreak, national medical experts have been responsible for development and refinement of existing methodologies, as well as deploying vaccines prophylactically to target populations.

Changes for Future One Health Response

Effective cross-sectoral collaboration requires many elements to be combined across a large scale. Key supporting elements, including strong governance, equitably distributed resources, involvement of all relevant stakeholders, guidance on implementation across the sectors, and strong health organizations within each country, are key to developing an international approach to deal with the mpox virus (Nuttall et al., 2005). The countries or regions involved must be willing to coordinate research and diagnostic probing, share collected data and risk assessment parameters, and cooperate to compile appropriate disease control programs. Effective handling of outbreaks requires coordination at the local, national, and international level. It is key that future efforts enable effective liaisons between epidemiologists doing theoretical work and on-the-ground laboratory workers in human and animal health.

Conclusions

Moving forward, the goal is to continue the trend of lower case and transmission rates in the current mpox outbreak seen in the first quarter of 2023. The rates have lowered through a combination of education, vaccination, prevention, and close monitoring of vulnerable populations. This reduction of cases is a positive step toward showing why global One Health application is imperative for successful management of emerging zoonotic diseases, including mpox. While additional research and development is needed, many researchers and ongoing data trials are exploring effective diagnostic, prevention, and treatment strategies for mpox. This

report has outlined the history of mpox, the 2022 outbreak, and the potential effects on animal, human, and environmental health. It has also highlighted the importance of ensuring that future mpox exploration and treatment continue to encompass a One Health approach. In the words of the Wildlife Conservation Society's Manhattan Principles, established in 2004, 'We are in an era of "One World, One Health" and we must devise adaptive, forward-looking, and multidisciplinary solutions to the challenges that undoubtedly lie ahead' (Nuttall et al., 2005).

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