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REVIEW

Recent advances in the diagnosis monkeypox: implications for public health

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ABSTRACT

Introduction: Monkeypox virus is a zoonotic double-stranded DNA poxvirus in the genus *Orthopoxvirus*, family *Poxviridae*. Until recently, monkeypox was found primarily in Central and West Africa, where the virus had split into Congo Basin and West African clades.

Areas Covered: On 6 May 2022, monkeypox was detected in the United Kingdom and the virus has now been detected in every continent except Antarctica. The current outbreak represents the first documentation of widespread community transmission outside of Africa.

Expert Opinion: On 23 July 2022, the World Health Organization declared monkeypox a public health emergency of international concern and issued a series of guidance and recommendations for governments, health professionals and the public. This manuscript reviews what is known about monkeypox virus, with a focus on recent diagnostics and epidemiologic advances, and explores how recent advances in our understanding of the virus will be used to combat the expanding outbreak.

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1. Introduction

Monkeypox virus was first detected in 1958 during an outbreak in an animal facility in Copenhagen, Denmark [1]. The virus was determined to be the causal agent in two outbreaks of pox infection in cynomolgus monkeys that had recently been received from Singapore at the Statens Serum Institut in Denmark [2]. These outbreaks occurred several weeks after the monkeys had been received (51 and 62 days after arrival, respectively) and only a small percentage of the exposed animals showed signs of illness [3,4].

Twelve years later, the first case of monkeypox in humans was detected in the Democratic Republic of the Congo. On 1 September 1970, a nine-month-old child suspected of having smallpox was admitted to Basankusu Hospital, Equatorial Provide, Democratic Republic of the Congo [5]. The boy developed a fever on 22 August 1970, and developed a rash two days later. The lesions, which lasted approximately two weeks, were noted to be hemorrhagic with a centrifugal distribution resembling smallpox [6]. Crusts were collected from the patient and samples were sent to the World Health Organization Smallpox Reference Center in Moscow, and monkeypox virus was isolated [3].

During the scabbing phase, the child developed otitis and mastoiditis as well as enlarged cervical lymph nodes that were subsequently incised and drained. The boy recovered from monkeypox and was about to be discharged from the hospital, but on 23 October 1970, he contracted measles and died six days later [5].

Until the child's presentation, it was thought that only two poxviruses could cause generalized infection accompanied by skin manifestations in humans: smallpox virus and the agent causing cowpox [7]. Between October 1970 and May 1971, however, six more cases of human monkeypox infection were detected in Nigeria, Liberia, and Sierra Leone [8]. Four of the cases were confirmed by virus isolation and two were diagnosed on the basis of epidemiological and serological investigations [9]. Surveillance performed by the World Health Organization between 1981 and 1986 in Democratic Republic of the Congo 338 confirmed cases and 33 deaths, with a case fatality rate of nearly 10% in that cohort, placing it between variola major (case fatality rate = 30%) and variola minor (case fatality rate = 1%) [10].

It is now known that there are two distinct types of monkeypox: Congo Basin clade and the milder West African clade, which is driving the current worldwide outbreak [11,12]. Using prairie dog test animals, Hutson and colleagues found that respiratory transmission of the Congo Basin clade was slightly greater than West African clade (16.7% and 0% respectively) [13]. In humans, the latter was initially associated with milder disease, no reported mortality, and rarely associated with person to person transmission [14]. By contrast, the Congo Basin clade was associated with up to 10% mortality and appeared to transmit more easily between humans [8].

Human monkeypox infection was first recognized outside of Africa in 2003, when an outbreak occurred in the United States after importation of infected rodents from west Africa [15]. Forty-seven confirmed and probable cases were reported across six states, including Missouri, Ohio, Kansas, Indiana, Wisconsin, and Illinois, after having contact with pet prairie dogs (Cynomys species) [16]. The virus was introduced into the United States through a shipment to Texas from Ghana of 800 small animals that included African giant pouched rats,

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Article highlights

- The first case of monkeypox in humans was detected in 1970 in the Democratic Republic of the Congo.
- Monkeypox is often mistaken for other viral infections, including varicella, variola, and measles
- Monkeypox symptoms range from systemic illness (fever, headache, myalgias, lethargy) to a maculopapular rash that may initially appear as pimples or blisters on the face, oral or genital mucosa, or the hands, feet, and chest, and may evolve into vesicles that eventually scab.
- Testing capacity for monkeypox is rapidly expanding; the preferred method of diagnosis involves nucleic acid amplification from a direct specimen.
- Processing and testing of monkeypox specimens should be performed in facilities that have recently vaccinated personnel, preferably within the past three years.

squirrels, brush-tailed porcupines, dormice, and striped mice [17]. More than a dozen of the imported animals were infected with monkeypox virus; some of the infected animals were housed near prairie dogs at the facilities of an Illinois animal vendor and these prairie dogs were sold as pets before they developed signs of infection [18].

Interestingly, the 2003 outbreak in the United States was associated with less severe disease than prior accounts. An experimental infection with representative central African and North American strains compared virulence in a ground squirrel model of the disease [19]. The virus isolated from the United States, which phylogenetically is a member of the West African monkeypox virus clade, was less virulent than central African virus strains [20].

Monkeypox is now endemic in some parts of Central and Western Africa [21]. Various animal reservoirs have been identified for monkeypox virus, including squirrels, anteaters, Gambian pouched rats, prairie dogs, shrews, dormice, and non-human primates [22–25]. Given the widespread transmission of monkeypox outside of Africa in 2022, there are now concerns that monkeypox may become endemic on other continents. Clinicians, researchers, scientists, and other healthcare personnel should be aware of telltale symptoms as well as recent advances in molecular diagnostics and the implications for public health.

2. Clinical manifestations

Monkeypox has a heterogenous presentation in humans, ranging from systemic symptoms (fever, headache, myalgias, chills, lethargy) to a macular or papular rash that may initially appear as pimples or blisters on the face, oral or genital mucosa, or the hands, feet, and chest. Vesicles, pustules, and scabs have also been observed; pitted scars and areas of lighter or darker skin may remain after scabs have fallen off. A person is no longer contagious once all scabs have fallen off.

An evaluation of 282 patients with human monkeypox in Zaire between 1980 and 1985 found that lymphadenopathy, occurring in the early stage of the illness, was the most important sign differentiating human monkeypox from smallpox and chickenpox [26]. Those who had been vaccinated against vaccinia differed significantly from those in unvaccinated subjects: skin lesions similar to chickenpox occurred in 31% of vaccinated and 18% of unvaccinated patients [27]. There were no deaths occurred among vaccinated patients; however, in unvaccinated patients the fatality rate was 11% and was higher among the youngest children (15%) [26].

A review of the 2003 outbreak in the United States found that patients with complex exposures such as animal bites or scratches were more likely than patients with noninvasive exposures to have experienced signs of systemic illness (49.1% vs. 16.7%; P = .041) and to have been hospitalized (68.8% vs. 10.3%) [28]. Bites and scratches were less likely to result in fever and associated with shorter incubation periods (13 days for noninvasive exposures versus 9 days for invasive exposures) [28].

A retrospective, observational study between 2018 and 2019 of seven monkeypox cases in the United Kingdom found prolonged monkeypox virus DNA detection in upper respiratory tract swabs; one patient had a monkeypox virus PCR-positive deep tissue abscess and five patients spent more than 3 weeks in isolation due to prolonged PCR positivity [29]. However, the role of respiratory transmission has been fully elucidated. Monkeypox has been consistently associated with vesiculopustular skin and mucosal lesions, gastrointestinal symptoms, and abnormal hematologic or hepatic laboratory findings [28,30,31].

The 2022 monkeypox outbreak is unique [32,33]. It is due to the less transmissible and less virulent west African clade, however, the symptoms remain highly variable [34]. In a study of approximately one thousand cases across thirty countries, the most prevalent symptom was fever (in 54.3% of cases) followed by inguinal lymphadenopathy (45.7%) and exanthem (40%), and severe illness requiring hospitalization was uncommon [35]. Both genital and anal lesions were reported in 31% of cases [36]. Risk factors included being a young male, having sex with other men, engaging in condomless sex, and prior sexually transmitted infections, including syphilis [35].

The emerging data leaves many questions unanswered. Why, for example, has the less transmissible west African clade spread so quickly? What are the major animal vectors? How often does respiratory transmission occur? These answers to these questions will rely heavily on enhanced surveillance and widespread use of molecular diagnostics.

3. Diagnosis

Monkeypox is commonly confused with varicella in countries where these infections are endemic; both may be characterized by fever, swollen lymph nodes, and a well-circumscribed, umbilicated rash [37–39]. However, monkeypox lesions (macule, papule, vesicle, or scab) are typically in the same stage of development in distinct anatomic locations and are more frequently associated with lymphadenopathy [40].

Several nucleic acid amplification tests have been developed to detect monkeypox [41–43]. A real-time polymerase chain reaction (PCR) assays targeting different orthopoxvirus genes, including DNA polymerase (E9L) and envelope protein (B6R), demonstrated 100% specificity for monkeypox, suggesting that

two discrete viral gene targets used together could provide a reliable and sensitive method for rapid diagnosis [44].

Additional real-time PCR assays based on TaqMan probe technology have been reported, targeting both monkeypox clades as well as a generic monkeypox assay [45]. However, access remains limited. Recently, a self-contained cartridge (Cepheid GeneXpert) was created to provide an alternative to traditional PCR detection methods [46]. The GeneXpert assay displayed high sensitivity, specificity, negative predictive value, and positive predictive value in suspected specimens regardless of the type of specimen collected (crust versus vesicular swab) [46]. However, costs associated with the system and its cartridges appear to present a barrier to more widespread use. For now, monkeypox testing remains highly centralized in laboratories with appropriately trained personnel.

Diagnostic testing for orthopoxviruses, including monkeypox, is available at Laboratory Response Network laboratories, which are found in the United States and at other sites around the world [23,47–50]. Nucleic acid amplification testing of skin samples is the preferred method of diagnosis, although in some cases the diagnosis may be inferred based on the patient's history of present illness and physical examination. PCR blood tests are usually non-diagnostic because monkeypox virus remains in blood only a short time. However, monkeypox DNA in lesion material is stable for a long period of time if is stored in a relatively dark, cool environment [51,52].

Processing and testing of monkeypox skin lesions should be performed in facilities that have recently vaccinated personnel, preferably within the past three years. For laboratories with appropriate personnel, specimens may be handled in Biosafety Level 2 (BSL-2) facilities. Culture-based testing for monkeypox should not be routinely performed clinical or diagnostic laboratories [53–55].

Alternatively, tests indicating the presence of Orthopoxvirus in suspected sample can be sufficiently diagnostic; these methods include immunohistochemical staining for orthopoxvirus antigens, visualization on electron microscopy, as well as serum studies for anti-orthopoxvirus IgM (indicating recent exposure) [51]. However, these tests are rarely used in clinical practice [56,57].

Until recently, monkeypox testing in the United States was limited to a handful of government-sponsored laboratories [58]. The United States CDC has developed and received clearance for a non-variola Orthopoxvirus real-time PCR diagnostic test to detect monkeypox, but prior approval and bureaucratic hurdles prevented widespread use of the assay [53]. On 22 June 2022, the United States Department of Health and Human Services announced that five commercial laboratories had been granted authorization to offer monkeypox tests. On 11 July 2022, Mayo Clinic Laboratories joined Labcorp in offering testing, bringing the United States diagnostic capacity up to 30,000 tests per week.

4. Public health implications

The world is better equipped to respond to a monkeypox outbreak than it was two decades ago. On 24 May 2003, the Wisconsin Division of Public Health was notified of a three-year-old girl hospitalized in central Wisconsin with cellulitis and fever after a bite from a prairie dog on May 13 [59]. The animal became ill on May 13, died one week later, and an enlarged submandibular lymph node was submitted for bacterial culture. On 2 June 2003, the Wisconsin Division of Public Health was notified of a poxvirus in a skin lesion from the mother of the three-year-old girl, who developed symptoms on May 26 [59]. Two days later, on June 4, orthopoxvirus was visualized by negative-stain electron microscopy of cell-culture supernatants. On June 9, polymerase chain reaction analyses of tissue- and virus-culture supernatants from the mother were positive for monkeypox-virus DNA signatures [59].

This diagnostic delay was not an isolated event. However, some medical centers were more prepared than others [60]. On 7 June 2003, three Illinois residents with a febrile rash syndrome presented to a community hospital [61]. Staff immediately reported the cases to the Illinois Department of Public Health, and infection control was quickly implemented, despite the absence of preexisting policies for monkeypox and uncertainty regarding best practices for the prevention of person-to-person transmission [62]. It was noted that participation in bioterrorism exercises facilitated best practices infection-control protocols during a time of diagnostic uncertainty [62].

The scenario is different now and our capacity for testing is greatly expanding. Between 17 May 2002 and 30 June 2022, the United States Laboratory Response Network tested more than two thousand specimens from patients with suspected monkeypox; of these, 36% were positive [53]. These laboratories continue to expand, increasing capacity to eight thousand tests per week. With greater access to testing, public health leaders must target high-risk groups with evidence-based messaging. It is important to inform relevant networks that postexposure prophylaxis with the use of the vaccinia vaccine (ACAM2000) or the smallpox and monkeypox vaccine (JYNNEOS) is recommended after high-risk exposures and can also be considered for intermediate-risk exposures [36].

A recent observational analysis of 54 confirmed cases of monkeypox in England found frequent anogenital symptoms, suggesting transmissibility through local inoculation during close skin-to-skin or mucosal contact [63]. As the monkeypox outbreak expands, it is clear that more resources will be necessary, both to support patients and their doctors, and to inform infection control policies and prevention strategies. Current guidance indicates that persons infected with monkeypox should remain in isolation until all skin lesions have resolved and a fresh layer of skin has grown [36]. It will be important to reevaluate this recommendation as more epidemiologic data becomes available.

As the outbreak expands, the monkeypox genome will require close monitoring [47,64]. Orthopoxviruses adapt to their environment due to an ability to evolve through gene loss rather than by progressive mutation as seen in SARS-CoV -2 [65]. Monkeypox virus will undoubtedly adapt to its human host, and its evolution should be kept under tight surveillance for signs that it is become more transmissible, less responsive to vaccines, or more deadly [66].

5. Expert opinion

On 6 July 2022, the first commercial laboratory in the United States began accepting specimens for non-variola Orthopoxvirus testing

based on clinician orders [53]. This marks an infection point in the current outbreak that will leverage provider-laboratory relationships while eliminating the need for prior public health approval. This will ultimately enhance containment efforts.

Increased detection will augment our understanding of the epidemiology of the current outbreak, and will help categorize risk factors for severe disease while ensuring that high-risk patients are identified, isolated, and treated. Moreover, it will improve our approach to close contacts. A prospective national cohort evaluating ring vaccination as post-exposure prophylaxis for monkeypox is ongoing with modified vaccinia Ankara (MVA), an attenuated vaccinia virus that cannot achieve complete replication in mammalian cells [67]. MVA has shown protection in primate models challenged with lethal doses of monkeypox virus, but the durability of protection may rely on the immune status of the host [68]. Our approach to exposure will certainly evolve.

Monkeypox infections have increased steadily since the 1980s. This has been attributed to a variety of factors, including: mutations that enhance transmissibility, ecological changes, changes in host behavior, and waning immunity from smallpox vaccination, which ended in the United States in 1972, as well as advances in molecular diagnostics and greater awareness of the disease. Nonetheless, the situation now is unexpected. There are clusters of cases occurring with human-to-human transmission in non-endemic settings as well as a surge of cases in certain networks of primarily gay, bisexual, and other men who have sex with men [69,70].

If monkeypox becomes endemic, as it is poised to do, we will need to develop therapeutic and prophylactic strategies to limit the burden of disease. At the moment, there are no approved treatments for monkeypox [29]. Rather, we are relying on drugs, such as vaccinia immune globulin, tecovirimat, and cidofovir, that were initially developed for smallpox [71,72]. All are available in the United States Strategic National stockpile [36]. Randomized, controlled trials of monkeypox therapeutics are urgently needed.

There is also no monkeypox vaccine for children under age 18 [73,74]. In the years ahead, we will need better data to expand access and to appropriately discuss the risks and benefits of monkeypox vaccination, especially for low- and moderate-risk groups. Continually raiding the Strategic National Stockpile is not a plan.

The monkeypox outbreak of 2022 has laid bare an uncomfortable truth: we must strengthen epidemiological surveillance systems and enhance our ability to respond to a novel or uncommon pathogen. This begins with testing capacity. For now, it may seem like monkeypox is a self-limiting condition that targets patients with specific risk factors. Once it becomes endemic, however, the virus becomes a threat to us all. As with the early phase of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, diagnostic assays have been difficult to access for both patients and providers. This must change. As with the COVID-19 pandemic, the monkeypox outbreak highlights global inequalities in access to the most basic medical care [75]. We must do better.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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