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Factors affecting the likelihood of monkeypox's emergence and spread in the post-smallpox era[☆]

Mary G Reynolds, Darin S Carroll and Kevin L Karem

In 1980, the World Health Assembly announced that smallpox had been successfully eradicated as a disease of humans. The disease clinically and immunologically most similar to smallpox is monkeypox, a zoonosis endemic to moist forested regions in West and Central Africa. Smallpox vaccine provided protection against both infections. Monkeypox virus is a less efficient human pathogen than the agent of smallpox, but absent smallpox and the population-wide immunity engendered during eradication efforts, could monkeypox now gain a foothold in human communities? We discuss possible ecologic and epidemiologic limitations that could impede monkeypox's emergence as a significant pathogen of humans, and evaluate whether genetic constraints are sufficient to diminish monkeypox virus' capacity for enhanced specificity as a parasite of humans.

Address

US Centers for Disease Control and Prevention, Poxvirus and Rabies Branch, 1600 Clifton Rd. NE, Atlanta, GA 30333, USA

Corresponding author: Reynolds, Mary G (Nzr6@cdc.gov)

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Background

The history of vaccination begins with the use of an animal virus to immunize humans against smallpox [1]. It ends with this same practice. By the close of 1979, the concerted application of vaccinia virus-based vaccine in at-risk populations had effectively interrupted the spread of smallpox, resulting in the eradication of naturally occurring disease throughout the world. This was possible because of antigenic similarities between vaccinia and variola (the agent of smallpox) viruses, and the fact that Variola is human-specific, leaving no potential for zoonotic reservoirs.

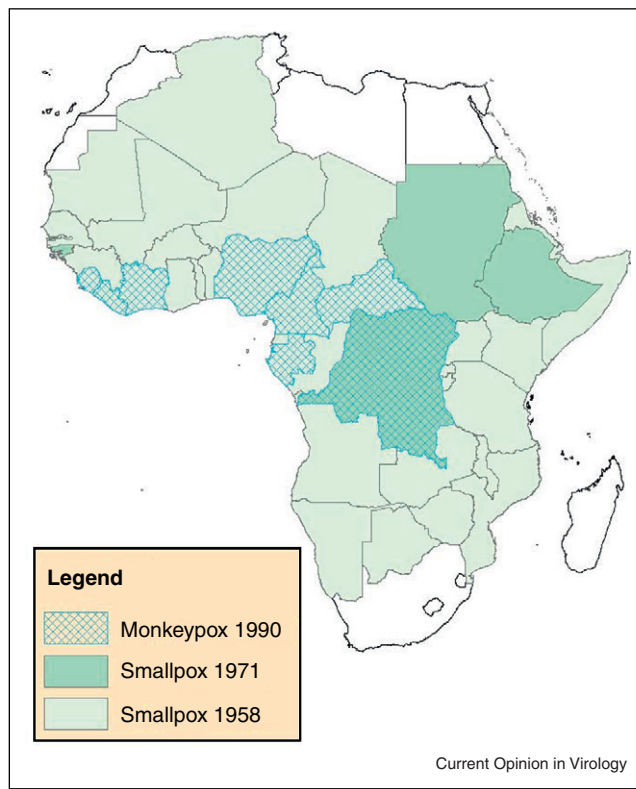
[☆] *Disclaimer:* The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the funding agency.

Vaccinia and variola are *Orthopoxviruses*. Orthopoxviruses encompass an array of pathogens that elicit serologic cross-reactivity, among which only one, variola, is an exclusive parasite of humans. Several zoonotic Orthopoxviruses — including vaccinia virus, cowpox virus, and monkeypox virus — can infect humans opportunistically (in the event of an encounter between a virus-infected animal and a susceptible individual), but none manifest variola's capacity for relatively efficient inter-human spread, with the possible exception of monkeypox.

Initial observations of a 'smallpox-like' illness caused by infection with monkeypox virus rather than variola were made in 1970, during the final stages of smallpox eradication [2]. The discovery occurred during time of intensified effort to verify that smallpox had been eliminated from regions of West and Central Africa that had been deemed 'smallpox-free'. The two diseases, smallpox and monkeypox, share a distinctive clinical presentation and almost certainly existed historically in sympatry across what is recognized now as the endemic range for monkeypox (Figure 1). But, in the absence of laboratory testing to specify the etiologic agent responsible for the condition, it is likely that most Orthopoxvirus-associated 'smallpox-like' illnesses were assumed to be smallpox; smallpox being broadly distributed and extremely well known.

It is now more than 30 years since the WHO recommended cessation of routine smallpox vaccination. Very few individuals born since eradication have received smallpox vaccination, and among those over 30 years of age who did receive vaccination, immunity is waning. This increasing deficit of human immunity raises the specter of whether, under these conditions, monkeypox might emerge as a more significant human pathogen, perhaps even 'replacing' smallpox. Indeed recent reports of increasing monkeypox incidence in the Democratic Republic of the Congo (DRC) [3[•]], as well as sporadic occurrences in neighboring countries imply that this may be a possibility [4–6]. But before concluding that smallpox eradication and the cessation of vaccination have opened an ecologic, or immunologic, niche for monkeypox to exploit, it seems reasonable to address the following questions. First, during the era before smallpox eradication, was the level of immunity in human populations — engendered either by smallpox vaccination or by the circulation of smallpox — responsible in some way for suppressing the emergence and spread of monkeypox? Or, are there in fact particular characteristics intrinsic to

Figure 1



Map depicting the distribution of the smallpox in Africa 1954–1958, by country, before the inception of global eradication efforts ('Smallpox 1958', light green), and during the latter stages of eradication, at the time human monkeypox was discovered ('Smallpox 1971', dark green) [12]. Countries reporting at least one case of human monkeypox through 1990 are depicted with cross-hatching. Image courtesy of Benjamin Monroe, CDC.

monkeypox (ecologic requirements, genetic determinants, among others) that have served to establish fundamental limits on the virus' capacity to emerge and spread beyond its current geographic confines? And if so, are there mechanisms or opportunities that could allow monkeypox to overcome these limitations?

Immunologic niche

Smallpox (*variola major*) is associated with higher fatality rates than monkeypox, but the clinical presentation of monkeypox is difficult to distinguish from discrete, ordinary smallpox (Figure 2), and smallpox vaccine is protective against both. A debate continues as to the duration of immunity provided by smallpox vaccine in the absence of periodic boosting [7–9], but it is inarguable that lifelong protection from re-infection was a lasting indemnity for having survived smallpox [10]. Presumably, a smallpox survivor would also possess life-long protection against infection with monkeypox virus (and vice versa), thus an individual infected with one virus would be permanently removed from the pool of susceptible hosts for the other.

In contrast, smallpox vaccination provides only limited-term protection from infection with either monkeypox or variola. Could smallpox circulation in monkeypox endemic regions of Africa then have been sufficient to impede the spread of human monkeypox? What level of vaccination would have been required to achieve the same effect?

During a 40-year period from 1919 to 1958 an estimated 122 600 cases of smallpox were reported by the Colonial authorities in DRC (Belgian Congo), on average ~3065 cases per year [11]. During that same period, roughly 78 million vaccinations and re-vaccinations were administered, the vast majority after 1945. In the 7 years immediately before the inception of the concerted vaccination programs, 14 000 total cases of smallpox were reported (from a population of ~7.7 million persons [11]). In time, vaccination had a clear impact on smallpox and ultimately led to eradication, and collaterally on potential human monkeypox infections. However, in the absence of data describing the incidence of monkeypox human infections before and during much of the eradication era, it is difficult to determine the role of smallpox population (herd) immunity for vaccine induced immunity in the incidence of monkeypox human disease. Despite the absence of quality control of vaccines before the 1960s, the combination of smallpox and vaccine-derived immunity would provide protection against monkeypox infection. Since vaccination rates exceeded smallpox case rates in central Africa during this period, it is easy to imagine a converse relationship between smallpox vaccination and human monkeypox incidence during this period and immediately following eradication of smallpox.

Between 1967 and 1971, at the height of the smallpox eradication efforts, an estimated 15 236 000 doses of vaccine were provided to 21 countries in West and Central Africa for purposes of vaccination or re-vaccination against smallpox [12]. The sheer numbers of immunizations doubtless had an impact on the incidence, and possibly the geographic distribution of not just smallpox, but of other Orthopoxvirus-associated human infections including monkeypox. No human monkeypox cases have been reported from West Africa since 1981 [13], though evidence points to the fact that monkeypox virus still circulates enzootically [14,15]. And in the years immediately following smallpox eradication (1985–95), reports of monkeypox from the Congo Basin declined measurably [16].

It is difficult to assess what the ecologic impact of variola might have been on monkeypox virus over earlier time-periods, when the viruses were circulating independent of the influence of vaccine. As a solely human pathogen, variola's ability to persist in populations is vulnerable to immunologically driven interruptions in human-to-human transmission chains. By contrast, monkeypox is

Figure 2



(a) Legs and feet of monkeypox patient (photo courtesy of J. Harvey). (b) Legs and feet of smallpox patient at similar stage of rash (pustular) (photo courtesy of J. Nobel, CDC).

a sylvatic zoonosis and human infections are incidental and probably of little consequence to the overall persistence of the virus in nature. The current endemic distribution of monkeypox is in all likelihood governed by the distribution of its principal host(s). However, incidence of human infection is also dependent on cross protective immunity stemming from the vaccination campaign and previous exposure to variola or other Orthopoxviruses. Monkeypox virus is, however, capable of infecting a broad range of hosts, and spillover into a new permissive host with a more cosmopolitan distribution could — in theory — contribute to the virus' emerging as a threat to humans.

Ecologic context of monkeypox

Broad host range zoonotic agents have been highlighted as being more likely to be emerging or re-emerging human pathogens. Over 50% of zoonotic viruses with 3 or more types of non-human hosts have been classified as emerging agents [17]. Monkeypox virus can infect an array of mammalian taxa including Sciurid, Glirid and Nesomyid rodents (*Cynomys* sp., *Funisciurus* sp., *Graphiurus* sp., *Cricetomys* sp.), marsupials (*Monodelphis domestica*, *Delphius marsupialis*), and primates (*Callithrix jacchus*, *Homo sapiens*) [18,19*]. In each of the examples provided, infections occurred without experimental induction by humans, but for most, human intervention was responsible for bringing the species in question into proximity with the virus. Monkeypox virus has only been isolated once from an animal captured in its natural environment — in 1986

monkeypox virus was isolated from the carcass of a *Funisciurus* squirrel found in Equateur Province of DRC [20]. The host range of naturally occurring (sylvatic) monkeypox remains undefined, but given its capacity to infect many different types of animals, it is likely to exceed the 3-host threshold.

Large mammals, gazelles and primates, have been singled out as potentially important sources of human infection in Central Africa [21], but the consistency of associations between rodent hosts and viruses across the *Orthopoxvirus* clade suggest that a rodent reservoir (or reservoirs) would be more likely for monkeypox [1,22] (Table 1). The perpetuation of acute viral infections in small populations is often theorized to necessitate either virus persistence or latency in the host (which is not characteristic of Orthopoxvirus infections) or high host turnover [23], which again points toward a rodent reservoir. Rodent fauna, such as squirrels (*Funisciurus* sp., *Heliosciurus* sp.) and *Cricetomys*, that are known to be susceptible to monkeypox virus infection, and that exploit food sources and refuges in areas adjacent to forest margins and human communities in DRC, are perhaps the most likely reservoirs and agents of virus transmission to humans [24,25]. Virus spillover into a more widely distributed sister taxa could raise concerns about the spread of disease beyond Africa.

In artificial settings, the common European squirrel *Sciurus vulgaris* has proven to be sensitive to infection with monkeypox virus [26] and the North American Sciurid

Table 1

Examples of (non-primate) mammalian species that are susceptible to infection with monkeypox virus, and their suitability as vectors of infection to humans.

| Order | Family | Species | Circumstance of infection | Potential as significant monkeypox vector to humans ^a |
|-----------------|--------------------------|---|---|---|
| Rodentia | Sciuridae | <i>Sciurus vulgaris</i> | Experimental infection ^b | Not a significant vector of directly transmitted zoonotic pathogens |
| | | <i>Funisciurus</i> spp. | Natural infection (sylvatic); experimental infection ^b | Range restricted |
| | | <i>Heliosciurus</i> spp. | Captive animal; experimental infection ^b | Range restricted |
| | | <i>Marmota monax</i> | Captive animal | Not a significant vector of directly transmitted zoonotic pathogens; range restricted |
| | | <i>Cynomys ludovicianus</i> | Captive animal; experimental infection ^b | Not a significant vector of directly transmitted zoonotic pathogens; range restricted |
| | Gliridae | <i>Graphiurus</i> spp. | Captive animal; experimental infection ^b | Range restricted |
| | Nesomyidae | <i>Cricetomys</i> spp. | Captive animal | Range restricted ^d |
| | Dipodidae | <i>Jaculus</i> sp. | Captive animal | Range restricted |
| Muridae | <i>Rattus norvegicus</i> | Experimental infection ^b (1–3-day-old animals) ^c | Only very young animals are susceptible to infection | |
| | <i>Mus musculus</i> | Experimental infection ^b (8–15-day-old animals) ^c | Only very young animals are susceptible to infection | |
| | | | | |
| Langomorpha | Leporidae | <i>Oryctolagus cuniculus</i> | Experimental infection ^b (10-day-old animals) ^c | Only very young animals are susceptible to infection |
| Didelphimorphia | Didelphidae | <i>Monodelphis domestica</i> | Captive animal | Range restricted |
| | | <i>Didelphis marsupialis</i> | Captive animal | Range restricted |
| Erinaceomorpha | Erinaceidae | <i>Atlerix</i> spp. | Captive animal | Range restricted |

^a Refers to the species' potential to serve as significant monkeypox vector to humans outside of the current areas of endemic monkeypox disease in Africa.

^b Routes of experimental infection include abrasion, foot pad inoculation, intranasal introduction of virus [26,27,53,54].

^c In these experiments, adult animals refractory to infection [27].

^d A colony of introduced *Cricetomys* sp. has been described in the State of Florida, USA [55].

rodent *Cynomys ludovicianus* has proven to be not only susceptible to infection but also capable of transmitting infection to humans [14,19^{*}]. The more common commensal rodents, *Rattus* spp. and *Mus* spp. are not considered to be especially susceptible to monkeypox virus infection, although monkeypox virus can be propagated in several inbred strains of mice and in immature animals [27,28].

In the absence of virus spillover and perpetuation in a readily susceptible, broadly distributed animal host, the spread of monkeypox beyond its areas of current endemicity in Africa would be dependent on human-to-human transmission which prompts the question of whether the inter-human transmission of monkeypox is sufficiently robust for this to occur?

Inter-human transmission potential

Whether monkeypox virus can exploit humans as a viable maintenance host will inevitably depend on the virus' capacity for sustained inter-human transmission. Epidemiologic modeling studies performed in the 1980s led to the conclusion that it would be highly improbable for monkeypox to become established in human populations

owing to the virus' intrinsic lack of transmissibility [29^{*},30]. The stochastic models used in these studies incorporated numerical estimates for contact and transmission rate variables that were derived from directly observed data [31]. Observations collected from 1980 to 1984 in DRC showed that people living in communities at risk for monkeypox had on average 10.7 close contacts (with 50% being high-risk household contacts), that secondary attack rates were approximately 6.7 times higher for unvaccinated contacts than vaccinated contacts, and that approximately 70% of the population had been vaccinated. Assuming these conditions, only 2% of model simulations resulted in a 3rd-generation virus transmission event, and no iterations resulted perpetuation beyond the 6th generation of spread. And even assuming 'worst case scenario' conditions — whereby vaccine-derived immunity in the starting population was 0% — the resultant number of cases per simulation increased by approximately a factor of 4, but still no simulations resulted in indefinite, sustained virus transmission [30]; the R_0 never achieved ≥ 1 .

The basic reproductive rate of an infection, R_0 , describes the inherent transmissibility of an infection within a

population which has no prior immunity [32], effectively however the value is subject to influence by population demographics, contact patterns, and heterogeneities of susceptibility among individuals. Employing a straightforward calculation of the number of new cases generated by a single monkeypox infection [29^{*}], the R_0 of the modeled scenario above could pass the threshold of 1 by simply augmenting of the total number of close contacts from 10.7 to 13.7. Alternatively, increasing the proportion of contacts that are high-risk household contacts from 50% to 80% achieves the same outcome. Thus, within this framework (which assumes an absence of vaccine-derived immunity), fairly minor shifts in the epidemiologic context of monkeypox could tip the balance in favor of sustained spread even in the absence of other ecologic or evolutionary modifications.

Obtaining modern estimates of secondary contact rates and knowledge of human contact patterns in monkeypox endemic areas will be important for assessing the epidemiologic potential of monkeypox for sustained inter-human transmission in contemporary at-risk communities. Regardless, however, of the current reproductive rate of monkeypox in human populations, probabilistic arguments suggest that a zoonotic pathogen with an R_0 near to one (such as monkeypox) retains a greater potential to evolve to a state of higher transmissibility as transmission chains lengthen and as the number of primary introductions increases [33]. Under this scenario, evolutionary advancements could accrue in stepwise fashion through individual character state changes, provided each step were to confer an incremental advantage in transmissibility (fitness) [33]. For example, an initial (hypothetical) virus mutation that enhances seeding and proliferation of virus in the epithelium of the human throat, followed by a second mutation that potentiates irritation and coughing, could provide a theoretical fitness advantage at each step; whereas reversing the steps would likely not.

Zoonotic pathogens of intermediate transmissibility to humans such as monkeypox may be well positioned to derive selective advantage (for heightened transmissibility) from minor gains in host specialization. But, would increasing the inter-human transmission potential of monkeypox necessarily require increased specialization for humans and, if so, would that in turn necessarily lead toward recapitulation of a pathogen with the virulence and characteristics of variola?

Evolutionary constraints

Though monkeypox and (discrete ordinary) smallpox would be difficult to distinguish from one another in a clinical setting, there are subtle clues that point toward one illness as opposed to the other. Lymphadenopathy, for example, is a prominent feature of monkeypox [34,35] yet was nearly absent in smallpox patients. Nodal

swelling has been described with smallpox [36,37], but the underlying process for this — localized edema — is distinct from the process of lymphoid hyperplasia (lymphocyte proliferation) observed in non-human primates infected with monkeypox virus [38,39]. Other functional differences affecting immune evasion and manipulation of the host immune system are predicted based on genome-level comparisons between variola and monkeypox viruses.

A core set of 90 conserved genes has been proposed as the ‘minimum essential genome’ of all Chordopoxviruses (the subfamily that encompasses those poxviruses that parasitize vertebrate animals) [40]. This set accounts for only ~50% of the haploid gene content of variola virus [41^{*}]. A typical Orthopoxvirus such as variola or monkeypox will have, in addition, genes associated with host specificity, immunomodulation and subcellular trafficking (for example), as well as a complement of open reading frames (orfs) with unknown function, regions of non-coding sequence, and long inverted terminal repeats (ITRs). Fluctuations in gene content — gene gain, gene loss — can provide opportunities for Orthopoxvirus adaptation to alternative hosts [42]. In fact, broad-scale evaluation of Orthopoxviruses genomes suggests that it is not uncommon for genes that have been acquired or lost to be those associated with host-specific properties [40,42].

In general, monkeypox virus genomes have, or have retained, considerably more DNA content than variola. A comparison of the Zaire-96 strain of monkeypox [41^{*},43] and the Kuwait-1967 strain of variola captures trends present across a broader sampling of each species: here, the monkeypox genome includes 4 additional genes and is ~11 000 nucleotides longer than the variola genome; it has ~10.5× longer ITRs, and extra coding sequences within the ITRs (whereas variola has none) [41^{*}]. Variola unquestionably has one of the most significantly size-restricted genomes of all the *Orthopoxviruses*, yet it is not a trimmed-down version of monkeypox. Variola has (depending on the analysis) up to 9 defined coding sequences that monkeypox viruses do not have, or of which monkeypox viruses have only retained fragments [44^{*}]. In contrast, monkeypox has ~16 defined orfs not present in variola [44^{*},45] (Table 2).

Several of the loci found in variola that are missing or truncated in monkeypox are hypothesized to play a role in immune evasion and virulence. For example, the variola genome harbors a virulence-associated gene (C3L) that expresses an inhibitor of complement enzymes. The ortholog of this gene (D14L) is either missing or expressed as a truncated (but functional) protein in monkeypox viruses [45,46^{*}]. The question of how pivotal the protein is to establishing robust Orthopoxvirus infections in humans is still the subject of investigation, but the

Table 2

Loci with known function in variola that are missing or truncated in monkeypox.^{a,b}

| Locus | Function | Variola | Monkeypox (Central African) |
|-------|---------------------------------|---|---|
| C3L | Inhibitor of complement enzymes | Co-factor in cleavage of C3b and C4b; C3 and C5 decay-accelerating activity | Truncated version of protein; C3b and C4b cleavage activity (lower efficiency than variola protein); no decay-accelerating activity |
| C10L | IL- β antagonist protein | C-terminal domain of protein binds host IL- β receptor blocking IL- β -mediated cellular activation pathways | Truncated version of protein lacking C-terminal domain |
| K3L | eIF-2 α protein | Mimic of host cell translation initiation factor, mimic protein binds to the host's INF-induced inhibitor of translation, thereby allowing translation to continue in infected cells | Gene absent or fragmented |
| E3L | INF-resistance protein | N-terminal domain of protein binds Z-DNA and may influence the expression of immune response genes; C-terminal domain of protein binds dsRNA and inhibits Type I interferon-mediated host cell activation | Truncated version of protein with only the C-terminal, dsRNA-binding domain |
| A49R | Phosphotransferase | No predicted role in virulence | Gene absent |

^a This is not intended to constitute an exhaustive list. Adapted from [44*].

^b On the basis of genome comparisons between the Bangladesh-75 strain of variola and a strain of monkeypox isolated from a Congolese patient in 1996 (ZAI-96) [45,56].

smallpox protein is presumed to modulate a critical feature of the host innate immune system early during infection [45,46*,47,48]. (Experimental attempts to demonstrate the functional importance of this locus to other orthopoxvirus virulence phenotypes — either by adding the locus to deficient genetic backgrounds or by ablating the function from virulent background — have generated inconsistent results [49,50].)

If the gene complement of monkeypox is lacking certain essential coding sequences related to host specialization, monkeypox virus' larger genome size and unique orfs could theoretically provide enough genetic plasticity to overcome the limitation. For instance, deficiencies in certain variola-specific functions could be met through alternative pathways — that is, functional pathways for immune evasion or inhibition that differ from variola's, yet ultimately impact the same target within the host.

A scan of the genome indicates that monkeypox viruses are deficient with respect to full-length orthologs for the two prominent loci in variola that influence interferon-resistance (E3L, K3L) [44*,45]. Yet, host-expression microarrays generated following infection of primary human monocytes with monkeypox virus unambiguously demonstrated diminution of interferon-associated host gene expression [51]. Thus, although monkeypox virus lacks full-length orthologs for these variola genes associated with interferon resistance, suppression of host interferon-induced gene expression is still achieved. This particular phenomenon, though not fully characterized, provides one example of a virus phenotype, common to both variola and monkeypox, that is manifest through

non-equivalent processes. The inhibition of host interleukin-1 beta (IL1- β) may be another.

Variola expresses a full-length IL1- β antagonist protein (C10L ortholog) that binds at its C-terminal end to host IL1- β receptors effectively preventing or diminishing host cell activation by the cytokine [52*]. Only the N-terminal portion of the protein is expressed by monkeypox virus [45] suggesting that the monkeypox protein would not demonstrate IL1- β receptor binding capacity. However, some Central African strains of monkeypox appear to possess the capacity to interfere with host cell activation by IL1- β . These variants of monkeypox virus putatively express a protein (B15R) that binds directly to IL-1 β , rather than to its host cell receptor [44*,46*,52*]. If borne out by functional studies, this could constitute an alternative means — not found in variola — of achieving the same host immune-modulatory effect.

Conceivably, further adaptation of monkeypox virus to humans, if it happens at all, could arrive through gene gain, or through nucleotide changes and optimization of these non-equivalent, redundant pathways (convergent evolution).

Conclusion

If the question initially posed was 'What is the intrinsic potential of monkeypox to fill the void left by the eradication of smallpox?', we conclude here with a mixed assessment. The scope of human immunity generated by eradication-era vaccinations unquestionably had an impact on the prevalence and distribution of both monkeypox and smallpox. But only smallpox was eradicable

through the human vaccination program. The immunologic picture appears favorable for the resurgence of monkeypox in disease endemic areas — owing to increasing population-level vulnerability — but several factors inherent to the genetic makeup and ecology of monkeypox virus would seem to diminish the probability that this disease will spread to a significant degree outside the moist tropical forests of West and Central Africa.

The 2003 outbreak of monkeypox in the United States, which began with importation of infected animals from West Africa, provided a stark example of how spillover and propagation in a permissive animal could, at least temporarily, expand the range of monkeypox. Yet the most plausible animal taxa for monkeypox virus propagation and spread (Sciurid rodents, for example) are likely to be inefficient transmitters of infection to humans. Conversely, taxa more frequently implicated in transmission of zoonotic diseases to humans (*Mus* and *Rattus*) are not particularly susceptible to infection with monkeypox virus. It is arguable that for emergence to occur, gains in transmission efficiency and in the capability of monkeypox virus to exploit humans as hosts would be required. The path to achieving these gains (and an $R_0 > 1$ in human populations) could involve relatively minor changes to the epidemiology of the disease (e.g. increasing the number of high-risk contacts by ~20%) or evolutionary modifications that enhance infection success and specificity in humans hosts. But, in the immediate future, neither path is likely to lead to the recapitulation of a pathogen with the same virulence properties as smallpox.

In the meantime, monkeypox will continue to be a significant public health concern for people living in endemic areas. Waning immunity, inadequate housing and health infrastructure, and the lack of alternatives to bush meat consumption all likely contribute to increasing the concern that monkeypox may re-emerge in Central Africa. This in turn contributes to fears about export of the virus to neighboring countries. Appropriate and effective interventions are urgently needed to prevent ongoing human infections. By focusing on disease prevention efforts in areas already affected by monkeypox, we may ultimately diminish the probability that monkeypox will be a future threat in other environments.

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