Responses of Professor Thomas Gillespie, Emory University, to Questions for the Record

Subcommittee on Oversight and Investigations
Committee on Natural Resources
U.S. House of Representatives

1. How does illegal poaching and smuggling of monkeys undermine efforts to control and prevent disease outbreaks?

Wild mammals are the primary source of emerging viral pathogens of concern to humans and virus richness scales with wild mammal richness (Johnson et al. 2020). The highest mammalian diversity occurs in tropical forested areas, such as the natural range of the long-tailed macaque (*Macaca fascicularis*) (Jones et al. 2008; Olival et al. 2017). Furthermore, the close phylogenetic relationship between humans and nonhuman primates ensures that many pathogens occurring naturally in wild primates have minimal biological barriers to clear to expand their host range to humans (Gillespie et al. 2008; Calvagnic-Spencer et al. 2012).

Consequently, wild primates have long been monitored for zoonotic diseases such as yellow fever, malaria, and schistosomiasis; however, the urgency of this surveillance intensified dramatically following the global HIV/AIDS pandemic, which was definitively linked to the zoonotic transmission of SIV-1 from chimpanzees (Gao et al., 1999; Keele et al., 2006). Additionally, related retroviruses (e.g., simian foamy viruses) and filoviruses (e.g., Ebola and Marburg viruses) are frequently transmitted between wild primates and humans, particularly through the hunting and butchering of these animals (Leroy et al., 2004; Wolfe et al., 2005). While HIV/AIDS and Ebola are perhaps the most well-known examples, they represent only a fraction of the diverse array of viral, bacterial, fungal, and parasitic pathogens that can be transmitted from nonhuman primates to humans (Gillespie et al. 2008; Strahan et al. 2024).

The capture and smuggling of wild monkeys have the potential to lead to novel human exposures to pathogens throughout the timeline from capture in the wild (exposure of primate trappers), through transport (exposure of local and international traders and transportation and government employees), to laboratory (exposure of researchers and caretakers) (Karesh et al. 2005). Further, as many pathogens can be spread through various bodily fluids, inappropriate disposal of such biohazardous materials could lead to environmental exposure to the human population, companion animals, livestock, and wildlife. Further, stress and poor handling conditions implicit in the illegal capture and smuggling of wild monkeys can also compromise the health of these animals, making them more susceptible to disease and exacerbating the public health risk (Vicente-Santos et al. 2023). Considering these risks, tremendous effort should be made to ensure that primates entering the United States are not of wild origin.

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2. What are the implications for our research on vaccines and other medications if wild monkeys are passed off as captive bred when they are not?

The expectation of purpose-bred/captive-bred monkeys for use in sophisticated and expensive experiments is that the animals will have been raised in controlled environments that guarantee that veterinary care and pathogen screening have been applied from birth and the animal's health history, pedigree, and genetic definition are available to researchers. Recent scientific publications have raised concerns that critical research and toxicology studies are being impacted by the presence of unexpected viruses, bacteria, and parasites in macaques being used to test the safety and efficacy of drugs and treatments (Johnson et al., 2022; Powell et al., 2024).

The introduction of wild-caught monkeys into US facilities also increases the risk for zoonotic disease transmission to laboratory personnel and their families. Purpose-bred monkeys are expected to be free of dangerous pathogens that can spill over into humans or other animals. A recent increase in the number of monkeys imported into the US infected with tuberculosis, simian retrovirus and herpes B-- a zoonotic virus that is prevalent is wild monkeys, but should not be present in captive-bred monkeys-- and the transmission of the deadly herpes B virus to laboratory workers in Asia is a strong indication that wild-caught monkeys are circulating in the supply chain (CDC, 2021; Wang et al., 2021).

Further, many of the pathogens that naturally infect wild primates present asymptomatically (Gillespie et al. 2008; Calvignac-Spencer et al. 2012). Thus, without extensive pathogen screening, infected wild monkeys passed off as purpose-bred could easily be enrolled in biomedical research (Grimm 2022). This is particularly detrimental for studies evaluating vaccine efficacy and safety, as the immune response of wild-caught monkeys is not representative of the broader population of captive-bred monkeys or humans due to previous or ongoing infections. Even asymptomatic infections in these monkeys would produce divergent immune response compared to captive bred monkeys, invalidating study results (Conroy 2023). For example, Simian T-lymphotropic virus type 1 (STLV-1), a retrovirus commonly found in wild primates in Africa and Asia (Ishikawa et al. 1987; Sintasath et al. 2009), triggers cells to release high levels of cytokines, proteins that regulate the immune response (Gardner et al. 2004). Thus, use of wild STLV-1-infected monkeys would compromise studies examining immune response and could lead to inaccurate conclusions about the effectiveness and safety of vaccines and medications.

Simian foamy virus (SFV) is another retrovirus that occurs at high prevalence in Asian monkeys including long-tailed macaques (Gardner et al. 2004; Jones-Engel et al. 2007). SFV infection can significantly alter cell membrane structure and function, leading to cell fusion and other cytopathic effects, particularly in in vitro cell cultures, making it difficult to maintain cultures of cell lines from infected monkeys (Welsch et al. 2007; Couteaudier et al. 2022). Further, as an enveloped virus, SFV acquires its viral envelope by budding from the host cell membrane, which can disrupt cell membrane integrity within the host (Welsch et al. 2007). Consequently, use of wild SFV-infected monkeys and tissues derived from such monkeys would compromise any studies examining infectious disease mechanisms involving viruses replicating inside such impacted cells.

Beyond viruses, parasitic worms and protozoa are abundant in wild primates (Gillespie 2006; Gillespie et al. 2008) and can suppress immune response to viral infection due to balanced antagonism between the components of the immune system that deal with extracellular parasites (type II helper T lymphocytes) vs. intracellular viral infections (type I helper T lymphocytes) (Desai et al. 2021). Consequently, use of wild parasite-infected monkeys would have the potential to compromise vaccine trials for viral pathogens or any studies examining infectious disease mechanisms involving viruses (Wait et al. 2020; Whitehead et al. 2022).

These examples demonstrate the significant public health risks inherent in using wild primates in biomedical research and the strong potential of this practice to exacerbate health crises rather than alleviating them.

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3. The CDC requires procedures and measures to prevent diseases from being introduced into the US through imported monkeys. What are some examples of diseases that could still spill over- or have already spilled over- into people from imported nonhuman primates and how would they get past protections currently in place? Has there been evidence of spillover into people from imported nonhuman primates?

The requirement for imported non-human primates (NHPs) to undergo a minimum 31-day quarantine, mandated by the CDC, is based on the time needed to complete three consecutive tuberculin skin tests (TSTs) at two-week intervals. This duration also exceeds the incubation period for filoviruses (i.e., Ebola and Marburg viruses) and many other high-consequence pathogens that have previously been imported along with wild primates to biomedical facilities in Europe and the United States (Petts et al. 2021). However, other than tuberculosis, CDC does not currently require screening tests to be performed in apparently healthy non-human primates. If importers choose to screen apparently healthy animals for zoonotic infections during the quarantine period, positive results must be reported to CDC within 24 hours (CDC 2022; 2023). Consequently, many asymptomatically carried and / or latent infections my go undetected. For example, multiple cases of melioidosis have been diagnosed in macaques imported from Cambodia (CDC, 2022). Melioidosis is a potentially fatal disease caused by the Tier 1 Select Agent *Burkholderia pseudomallei*, which is endemic to much of the geographical range of long-tailed macaques. Importantly, Taetzsch et al. 2022, note that, "the incubation period of melioidosis is highly variable, not well defined in animals, and can exceed 31 d. One report described a rhesus macaque that developed melioidosis 10 y after importation into the US. In an unpublished case from 2015, *B. pseudomallei* was isolated from a liver abscess found at necropsy in an NHP that was euthanized due to persistent lethargy and dehydration almost a year after importation and release from CDC-mandated quarantine. After the case reported here, 5 additional macaques imported in separate shipments from Cambodia developed abscesses from which *B. pseudomallei* was isolated several months after importation and, in 4 of the cases, after release from CDC-mandated quarantine. These cases illustrate the impracticality of holding imported NHPs in quarantine beyond the incubation period for melioidosis. In addition, serology is not a useful screening tool in animals from endemic regions due to cross-reactivity and poor correlation with active infection or development of clinical signs."

Equally troubling, CDC data indicate that culture-confirmed tuberculosis in imported non-human primates was undetected from 2013-2020 but has increased since the COVID pandemic and consequent cessation of monkey imports from China (CDC, 2023). Both *Burkholderia* sp. and *Tuberculosis* sp. can present asymptomatically in macaques and false negatives to approved diagnostic tests for both pathogens are not uncommon (CDC, 2022; 2023). Therefore, it is not surprising that multiple cases of TB were reported to CDC in monkeys up to two years post-quarantine (Yee et al. 2021). TB species isolated and reported to CDC from non-human primates, including *M. fascicularis*, included *M. bovis*, *M. caprae*, *M. orygis*, and *M. tuberculosis* (CDC, 2023). Both tuberculosis and *Burkholderia* are capable of infecting and causing disease in a broad range of mammalian hosts including humans, domesticated animals, and livestock and environmental conditions in the southern US could promote establishment of *Burkholderia* (Portacci et al. 2017; Hall et al. 2015; CDC, 2023).

Further, a recent increase in the number of monkeys imported into the US infected with tuberculosis, simian retrovirus and herpes B-- a zoonotic virus that is prevalent is wild monkeys, but should not be present in captive-bred monkeys-- and the transmission of the deadly herpes B virus to laboratory workers in Asia is a strong indication that wild-caught monkeys are circulating in the supply chain (CDC, 2021; Wang et al., 2021). To date, there have been 50 documented cases of herpes B virus infection in humans, with 21 deaths (Hu et

al. 2022). Most of these infections were caused by direct contact with macaques (i.e., bites, scratches, or contact with monkey tissue or fluids) (Hu et al. 2022). These are just the tip of the iceberg in terms of zoonotic threats to the American public. Most future emerging infectious diseases remain to be discovered, and the tropical forest habitat of the long-tailed macaques is a known hotspot (Jones et al. 2008; Calvignac-Spencer et al., 2012; Gillespie et al. 2021).

Considering these risks, tremendous effort should be made to ensure that primates entering the United States are not of wild origin. In addition, CDC should implement broad and rigorous pathogen screening protocols for non-human primates imported into the United States. Vigilance, comprehensive screening, and strict adherence to quarantine and safety measures will be essential for mitigating the risks of disease spillover. These efforts will help safeguard public health and ensure that potential pathogens are effectively managed before they can impact both human populations and local ecosystems.

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4. Many of us have not had to deal with Tuberculosis in our lifetimes. Can you please remind us what that entails for people who contract it?

For the average American today, this question would likely evoke the image of a pale and fragile figure from a century ago coughing blood into a handkerchief, but tuberculosis (TB) is not a disease of the past. This bacterial infection, primarily caused by *Mycobacterium tuberculosis*, is deadliest among all infectious diseases today, killing more people than malaria and HIV/AIDS combined (WHO 2023). Globally, almost 10 million people fall ill each year and 1.5 million succumb to TB (WHO 2023).

TB is an airborne disease that spreads easily as people cough or talk in proximity to others. Consequently, 25% of people globally are infected with TB (Houben & Dodd 2016). Although the vast majority have asymptomatic, latent TB; five to 10% will develop disease at some point without treatment (Menzies et al. 2018; Vynnycky et al. 2020). Initial symptoms can resemble a common cold, making TB difficult to detect. As the illness progresses, the victim develops a persistent cough producing blood or sputum, chest pain, fever, night sweats, weight loss, and loss of appetite (WHO 2023). If untreated, TB can cause severe lung damage and other systemic effects, respiratory failure, and death (WHO 2023).

Successful treatment of TB is challenging, involving daily use of five drugs whose side effects include nausea, rashes, and jaundice for four months (or longer depending on severity and drug sensitivities) (WHO 2023). To further complicate the process, multi-drug resistance develops in 20% of previously treated cases of TB and drug-resistant TB requires extensive treatment (> two years) with only a 50% survival rate (Pai and Memish 2016). Treatment of

DR-TB is also very expensive because of the high cost of second-line TB drugs (Pai and Memish 2016).

Laboratory-acquired tuberculosis (TB) is a serious occupational hazard for laboratory workers, especially those who test for TB. TB is caused by the bacteria Mycobacterium tuberculosis, which is primarily spread through the air by inhaling infectious aerosols. Laboratory workers can be exposed to these aerosols when handling liquids that contain the bacteria.

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5. Has the illegal importation of long-tailed macaques into the US for animal research impacted the conservation status of wild monkey populations?

Long-tailed macaques are listed by the IUCN as Endangered (Ruppert et al. 2022), and have experienced an 80% decrease in their population size over the past 35 years (Koch Liston et al., 2024). Habitat degradation, coupled with pest control measures at the human interface (culling and sterilization), pose severe risks to the species (Valle 2024). Moreover, extensive trade and use in biomedical research, has exacerbated these declines (Hansen et al., 2022). Despite being perceived as overabundant in some areas, data on local populations are often anecdotal and inconsistent, hindering effective conservation efforts (Valle 2024). In some regions, macaque populations have declined by over 50% in just a decade (Hansen et al., 2022). This not only disrupts ecological balance but also threatens the survival of species that are already vulnerable due to habitat loss and other pressures (Estrada et al., 2017).

While more data is needed to determine the extent to which illegal capture for biomedical research contributes to the sharp decline of wild long-tailed macaques, it is undoubtedly a significant factor. Given the regulatory and ethical constraints surrounding research on endangered species, it is in the best interest of stakeholders within the US biomedical industry and funding agencies, including the National Institutes of Health, to invest substantially in effective conservation programs for this endangered primate species. Such efforts will not only support the species but also align with ethical research practices.

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