SARS-CoV-2 and wastewater: What does it mean for non-human primates?

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Abstract
In most of our lifetimes, we have not faced a global pandemic such as the novel coronavirus disease 2019. The world has changed as a result. However, it is not only humans who are affected by a pandemic of this scale. Our closest relatives, the non-human primates (NHPs) who encounter researchers, sanctuary/zoo employees, and tourists, are also potentially at risk of contracting the virus from humans due to similar genetic susceptibility. “Anthropozoonosis”—the transmission of diseases from humans to other species—has occurred historically, resulting in infection of NHPs with human pathogens that have led to disastrous outbreaks. Recent studies have assessed the susceptibility of NHPs and predict that catarhine primates and some lemurs are potentially highly susceptible to infection by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. There is accumulating evidence that a new factor to consider with the spread of the virus is fecal-oral transmission. The virus has been detected in the watersheds of countries with underdeveloped infrastructure where raw sewage enters the environment directly without processing. This may expose NHPs, and other animals, to SARS-CoV-2 through wastewater contact. Here, we address these concerns and discuss recent evidence. Overall, we suggest that the risk of transmission of SARS-CoV-2 via wastewater is low. Nonetheless, tracking of viral RNA in wastewater does provide a unique testing approach to help protect NHPs at zoos and wildlife sanctuaries. A One Health approach going forward is perhaps the best way to protect these animals from a novel virus, the same way that we would protect ourselves.

KEYWORDS
COVID-19, coronavirus, disease transmission, monkeys, synanthropic primates, zoonosis

1 | COMMENTARY

The emergence of the novel coronavirus disease 2019 (COVID-19) in Wuhan, China caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), ushered in a new pandemic era as it swept the world in 2020, and continues to plague 2021. Initially believed to spread by fomite contact, the virus was later classified as airborne (Tang et al., 2021). Currently, viral RNA can be detected not just in nasal and saliva secretions, but also in feces and wastewater (Tang et al., 2021). As such, susceptible mammalian species, including...
many non-human primates (NHPs), might also be vulnerable to COVID-19. Many people have access to safe clean drinking water as a result of modern sanitation in developed nations. Yet, this is not the case in many countries with poor sewage treatment infrastructure. In these areas, there is some concern that NHPs may be impacted by the discharge of wastewater into natural water systems that could carry SARS-CoV-2 and other human pathogens. Here, we discuss how recent findings suggest that exposure of primates to the SARS-CoV-2 virus from contaminated watersheds and from exposed sewage is low. Rather, the highest risk of transmission of SARS-CoV-2 still appears to be from direct proximity to humans. For example, a recent anthropozoonosis event was reported for captive NHPs in January 2021, when eight gorillas at the San Diego Zoo Safari Park began showing symptoms of COVID-19 and then tested positive for SARS-CoV-2 RNA (Gibbons, 2021). We believe a One-Health approach presents the best strategy for protecting NHP species from anthropogenic transmission of SARS-CoV-2, which is especially important where the most vulnerable species live near humans. We further suggest that wastewater tracking of SARS-CoV-2 RNA in sewage offers an early-detection approach to inform risk assessment for these species.

The susceptibility of a species to a virus is a crucial factor for determining the risk of cross-species transmission. There have been numerous previous cases of human viruses being transmitted to NHP species. For example, NHP species have been infected with viruses causing yellow fever and respiratory syndromes that have caused mass mortality (Goldberg et al., 2008; Holzmann et al., 2010; Kaur et al., 2008; König et al., 2008; Palacios et al., 2011; Patrono et al., 2018). These examples illustrate how "anthropozoonoses" are a growing concern for the conservation of NHPs. The spillover of viruses between humans and NHP populations will occur at higher frequencies as suitable habitat decreases and there is increased contact between humans and wild populations of NHPs. As novel human pathogens take hold in human populations there is a need to better understand the transmission routes and factors involved in anthropozoonoses that could endanger other species—an essential process for the creation of procedures and guidelines that will protect NHPs. Importantly, spillover events may also have future consequences since NHPs represent a potential deadly zoonotic reservoir for a variety of human pathogens (Devaux et al., 2019; Kebede et al., 2020). Therefore, the reduction of inter-species transmission promotes a healthier community for both NHPs and humans.

### 2 | NHPS ARE SUSCEPTIBLE TO CORONAVIRUSES AND POTENTIALLY ANTHROPOZOOONOTIC TRANSMISSION OF SARS-COV-2 FROM HUMANS

An outbreak stemming from an anthropozoonosis was suspected at Tai National Park in Côte d’Ivoire between late December 2016 and early January 2017, when the resident chimpanzees showed symptoms of a mild respiratory syndrome (Patrono et al., 2018). The culprit turned out to be a human Betacoronavirus strain (HCOV O43) that was transmitted to the chimpanzees from humans. This study is especially relevant today, amid our global pandemic caused by another Betacoronavirus, SARS-CoV-2. After it was identified that the SARS-CoV-2 virus targets the angiotensin converting enzyme 2 (ACE2) as a receptor for entry into host cells, various research groups have looked at sequence similarity in the ACE2 protein among different species to predict susceptibility to SARS-CoV-2. For example, we used this approach to predict that several feline species, but not all carnivore species, are likely susceptible to the virus (Mathavarajah & Dellaire, 2020) and to examine the potential susceptibility of marine mammals to COVID-19 (Mathavarajah et al., 2021). A similar approach has also been applied by others to predict which NHP species are potentially susceptible to the SARS-CoV-2. For example, in two similar studies, catarrhine primates (African and Asian apes and monkeys) and some strepsirrhines (specifically, some lemurs) were predicted to be highly susceptible to the virus (Melin et al., 2020, 2021), although missense variants within populations may alter susceptibility for some individuals. For example, a common missense variant is present in West African/Caribbean green monkey (Chlorocebus sabaeus) populations and it is predicted to reduce the affinity of SARS-CoV-2 binding to ACE2, thus affecting susceptibility (Schmitt et al., 2020). A better understanding of the missense variants in wild primate populations will help us model the susceptibility and infection dynamics of SARS-CoV-2. In potentially better news, platyrhines (monkeys in the Americas) and many strepsirrhines carry ACE2 receptors with mutations that reduce SARS-CoV-2 binding, suggesting that species from these clades have lower susceptibility relative to other primates (Melin et al., 2020, 2021). However, as a group primates have higher predicted risk than many other mammals (Damas et al., 2020). Taken together, it appears that catarrhines have uniformly high risk for contracting SARS-CoV-2 and are a priority for protective efforts, especially as many species are threatened or endangered.

Infection models for NHPs studying COVID-19 have strongly supported some of the predictions for SARS-CoV-2 virus infection, including in the African green monkey (Chlorocebus aethiops), rhesus monkey (Macaca mulatta), and crab-eating macaque (Macaca fascicularis) (Blair et al., 2020; Lu et al., 2020; Yu et al., 2020). Although the spectrum of disease varied in terms of symptoms, infected African green monkeys showed rapid clinical deterioration (Blair et al., 2020). In addition, age seemed to be a role in disease presentation. As in humans, older rhesus monkeys (M. mulatta) showed more severe disease (Yu et al., 2020). Furthermore, the common marmoset (Callithrix jaccus), a platyrhine monkey predicted by Melin et al. (2020) to have low susceptibility, was challenged with the virus but did not show clear clinical symptoms, and the virus could not be identified in the tissues of necropsied animals (Lu et al., 2020). However, it is important to note that mild lesions with a minor amount of infiltrating inflammatory cells could be observed in the lung, liver, and spleen in the marmoset. Therefore, a severe immune response and viral RNA were absent in C. jaccus.
tissue, and this is in stark contrast to the response patterns seen in the macaque species examined. The differences in susceptibility likely contribute to the drastically differing pathologies observed when the different primates were exposed to the virus. These findings have spurred important discussion as to whether primatologists should continue field work in 2020 (Reid, 2020; Trivedy, 2020).

3 | WASTEWATER AS A MECHANISM FOR ANTHROPOGENIC TRANSMISSION OF INFECTIOUS MICROBES

There are different mechanisms for water procurement by primates and water taken directly from natural water systems is one option to maintain hydration. However, NHPs may not be able distinguish between water sources that are relatively pure and those that are heavily contaminated by humans. In many developing nations, sewage is directly discharged into natural waterways that animals may frequently access (Chauhan & Pirta, 2010). There are many factors that contribute to the risk this poses, including the relative titre of the pathogen, its survivability in the environment, and the potential susceptibility of the host animal (Bivins et al., 2020; Devaux et al., 2019; Köndgen et al., 2008; Oude Munnink et al., 2021). However, the extent to which anthropozaonoses result from human sewage has not been determined, with only a handful of studies describing occurrences (Debenham et al., 2015; Hermosilla et al., 2016; Olanyi et al., 2020; Ryan & Caccio, 2013; Sutherland et al., 2011).

NHPs have been documented to be affected by human sewage when they are in close proximity. For example, the chimpanzees at Gombe National Park are exposed to sewage that has likely precipitated recurring Cryptosporidium infections, which may have contributed to ongoing population decline (Parsons et al., 2015). Currently, the connection between our sewage disposal (untreated in many countries) and the extent to which it contributes to the emergence of infectious agents in NHP populations is unknown (Ferronato & Torretta, 2019). We thus highlight the importance of studying water-related factors involved in the cross-species transmission of infectious agents from humans to NHPs moving forward (Lonsdorf et al., 2018).

4 | SARS-COV-2 IN SEWAGE AND NATURAL WATER WAYS IS AN UNLIKELY MECHANISM FOR SARS-COV-2 TRANSMISSION TO NHPS

COVID-19 is a respiratory disease and symptoms include a dry cough, tiredness, fever, and in severe cases, pneumonia. However, there is accumulating evidence that SARS-CoV-2 infects multiple organs in patients, including the gastrointestinal tract in one-third of cases (Lamers et al., 2020). The shedding of the virus and the presence of viable virus in the feces of infected individuals, is the consequence of the virus’ capacity to infect the human gut resulting in a potential source of viral transmission (Xiao et al., 2020). In addition, viable virus has also been found in the urine of a human patient infected with SARS-CoV-2 (Sun et al., 2020) and it was demonstrated that SARS-CoV-2 virus isolated from both the urine and feces of a single patient was capable of infecting cells in culture. In contrast, Zang et al. (2020) found that while SARS-CoV-2 replicates in the small intestine, they were unable to recover viable virus from feces and the virus was inactivated by human colonic fluid. A review of the literature assessing viral cultures from human samples identified nine studies that indicated infectious SARS-CoV-2 in feces (Jefferson et al., 2020). And yet, even with these contradictory results, the potential for human waste to carry infectious particles of SARS-CoV-2 by a fecal-oral route of transmission appears to be possible at least under favorable conditions.

Under the assumption that infective virus can be transmitted through feces, one may posit that sewage becomes a potential route for virus transmission. SARS-CoV-2 stability in environmental conditions has been debated and was initially poorly understood with limited experimental evidence (Chin et al., 2020; Shutler et al., 2020). SARS-CoV-2 RNA has now been detected around the world in wastewater, including in France, Japan, and Italy (Hata et al., 2021; La Rosa et al., 2020; Wurtzer et al., 2020). Viral RNA can be detected in both the Lombo river of Italy, and Quinto river of Ecuador at different locations in the watershed (Guerrero-Latorre et al., 2020; Rinoldi et al., 2020). In Ecuador, the detected SARS-CoV-2 RNA was thought to be derived from the untreated sewage that was directly discharged into the river. Most primate range regions coincide with areas having densely populated, poverty-stricken municipalities around the world (Estrada et al., 2017). For such communities, public health issues and access to health care far outweigh concerns over wastewater management. For these reasons, there may be a concern regarding the potential transmission of SARS-CoV-2 through sewage.

However, an infectious form of the virus has not been detected in wastewater (both treated and untreated) or natural water ways as of time of writing of this commentary (Maal-Bared et al., 2020). Perhaps the most compelling evidence that wastewater transmission of SARS-CoV-2 is unlikely in "real-world" conditions is the recent work by Bivins et al. (2020), who report the survivability of viable virus in tap water and wastewater for the first time. They found a 90% reduction in viable SARS-CoV-2 in 1.5 days (wastewater) and 1.7 days (tap water) at room temperature (Bivins et al., 2020). These findings suggest that the potential for SARS-CoV-2 to persist in sewage is likely low under most environmental conditions. In addition, the dilution factor for when sewage enters larger rivers and lakes will reduce the risk of infection even further from raw sewage. These factors likely contribute to why infective SARS-CoV-2 virus has not been isolated from sewage contaminated waters. Although a theoretical possibility, these new studies together indicate that the overall risk of NHPs being exposed to viable SARS-CoV-2 in sewage or natural water systems is low.
5 | SYNANTHROPIC NHPS MAY HAVE A HIGH RISK OF CONTRACTING SARS-COV-2

While the potential for sewage and sewage contamination in natural waters is not a likely source for entry of SARS-CoV-2 into NHP populations in the wild, sewage in densely populated areas with limited sanitation is still a concern for transmission when fresh feces/sewage or faulty plumbing is present. The SARS-CoV-1 Amoy Gardens outbreak was a result of plumbing failure leading to virus-laden air causing transmission in the building (McKinney et al., 2006). Importantly, a study also revealed that SARS-CoV-2 transmission through sewage was responsible for an outbreak in a low-income community in Guangzhou, China (Yuan et al., 2020). In addition, street-sewage puddles and sewage-pipe surfaces tested positive for SARS-CoV-2 in the community (although cell-culturable virus was not isolated from the samples). Direct contact between NHPs and fresh sewage within the theoretical 1.5-day limit for viability could be a real threat, particularly for those populations living in proximity to dense human settlements with poor sanitation and large NHP populations such as in India (Southwick & Lindburg, 1986). In this transmission scenario, the concern focuses on synanthropic NHPs that live in proximity to, or within human habitats. These primates that have adapted to become our neighbors are likely to be especially vulnerable during the pandemic. A vulnerability arising not only from direct contact with humans, but also but from the inability to judge clean from contaminated water. The use of raw wastewater for irrigation of crops, which occurs in countries with large NHP populations including China and India, further compounds the problem and may place primate communities at risk (Thebo et al., 2017).

Many synanthropic primate species coexist with humans in densely populated areas of low-income. Consequently, they are in the crosshairs of poor sanitation and hygiene which may lead to viral transmission through the fecal-oral route. The behaviour of primates in these communities supports the notion. A study on the rhesus monkey and gray langur (Semnopithecus entellus) populations in Shimla, India observed that 68% of individuals from both species drink from polluted water sources originating from drainage channels around the city (Chauhan & Pirta, 2010). In contrast, <4% of the monkeys sought out water from nearby lakes and rivers. With this in mind, the susceptibility of these two synanthropic species merits discussion. Rhesus monkeys are not only predicted to be susceptible to the virus but have also been shown to develop symptoms that resemble those of humans with COVID-19 following infection (Qin et al., 2005). Although in vivo data from gray langur monkeys infected with the virus is currently nonexistent, and sequence data for the gray langur genome is unavailable, as a member of the infraorder Catarrhini, gray langurs are predicted to be highly susceptible to the virus (Melin et al., 2020). They also live in tight social groups, like most catarrhines. Accordingly, infection events would likely spread quickly through the population.

Although the NHPs of Shimla are one example, there are likely many cities around the world in similar predicaments. In addition, the discussion of viral transmission highlights one of many possible negative outcomes of synanthropic monkey adaptation to human urbanization which is largely driven by deforestation and habitat loss (Dobson et al., 2020; Faust et al., 2018; Gibb et al., 2020; Redding et al., 2019). The shift from obtaining water from primarily dietary sources, water ways, and tree-holes, to urban run-off and sewage poses many health risks. Yuan et al. (2020) suggest that routine inspection and plumbing development and maintenance are key factors in preventing these transmission events from fresh feces. Surveillance of these synanthropic populations will also be important going forward to identify pathogen presence and to then take swift action in affected habitats (Figure 1). Thus by monitoring waterways, we foster a scenario that reduces risk to both humans and these coexisting NHPs.

6 | MONITORING WASTEWATER TO PROTECT NHPS

Given the risk to primates situated near human settlements, we pose a timely question: should surveillance of NHPs for SARS-CoV-2 be implemented? The identification of virus spillover will help prevent or at least mitigate future outbreaks in NHP populations from SARS-CoV-2. When African green monkeys were administered the related SARS-CoV virus, the viral RNA was detected in the feces of animals (McAuliffe et al., 2004). However, more testing is required to better understand if feces can be used as a convenient way of sampling for qPCR detection of the SARS-CoV-2 virus. It is also unknown if it will be applicable for other NHP species. Utilizing known resources during surveillance such as the “International Union for Conservation of Nature’s best-practice guidelines for health monitoring and disease control in great-ape populations” is pertinent for such pandemic-related work. Routinely testing feces for traces of the virus will allow for early detection of the virus to protect these animals.

SARS-CoV-2 RNA does persist for a relatively long period of time in wastewater and provides an opportunity for using viral RNA as a surveillance system. For example, it can be used to determine hotspots within communities for clusters of positive cases, providing an early warning to the health system ahead of intensive care hospitalizations and allowing time for public health measures such as lockdowns and quarantine (Ahmed et al., 2020). By the same principle, detection of SARS-CoV-2 in wastewater not only warns us of the risk posed by sewage in a locality but the relative levels of viral RNA can be used to determine areas that may require public outreach or restricted access to prevent community members encountering susceptible mammalian species.

For captive NHPs the potential for exposure to wastewater is likely low. However, the potential for transmission through human-animal contact has been demonstrated to be high. There are already accounts of zoo animals contracting the virus. In the Bronx Zoo, four tigers and three lions contracted the virus (McAuloose et al., 2020; Wang et al., 2020). Astonishingly, two separate strains of the virus infected these animals indicating that there were at least two separate virus spillover events at the zoo (McAuloose et al., 2020).
Additionally, as aforementioned, a recent event involving captive gorillas at the San Diego Zoo Safari Park found NHPs that not only tested positive for SARS-CoV-2 but showed symptoms (Gibbons, 2021). Therefore, attentive and thoughtful practices are required for NHPs at zoos and sanctuaries to protect them from the public and contaminated water sources (Figure 1).

Through viral RNA monitoring of captive populations, similar to monitoring efforts described for wild primates, we would be able to warn the public and restrict access to NHP sanctuaries and zoos where the number of positive cases is high (Figure 1). Countries with constrained health systems infrastructures, testing systems, and personal protective equipment, can utilize wastewater surveillance as a practical option (Kavanagh et al., 2020; Martínez-Alvarez et al., 2020). Similarly, waste-water/feces collected from sanctuaries and zoos can be routinely tested to see if employees or volunteers carry the virus. This approach was recently shown to be efficient and viable at the University of North Carolina at Chapel Hill (Kavanagh et al., 2020). The One Health approach through wastewater monitoring is needed to protect NHPs (Figure 1). Catarrhines have been predicted and shown to be susceptible to the SARS-CoV-2 virus. Sanctuaries and zoos have adapted to the COVID-19 pandemic by implementing measures to restrict contact between the public and captive NHPs. However, a supplement to these measures is the incorporation of wastewater testing at zoos and sanctuaries for early detection of the virus. Different primate groups (captive, wild, and synanthropic) could be affected by the virus; in terms of relative risk: red = high risk and green = low risk. These approaches also prevent the possibility of NHPs becoming viral reservoirs in the event of a spillover, which ultimately then affects the health of our communities. There are also other approaches, which have been outlined, that we can implement to protect these species and monitor them going forward during the pandemic. NHP, non-human primate.
Carolina, where a wastewater surveillance system was pilots to determine asymptomatic COVID-19 cases on campus (Gibas et al., 2021). Using this approach, the research group was able to detect infected individuals within dorms of 150-200 people and allowed for early action to mitigate the spread of the virus. In cases like Yosemite park, where sewage recently tested positive for SARS-CoV-2 despite no positive cases of COVID-19 being reported in the area, the implementation of similar wastewater monitoring systems can be useful (Fox, 2020). Since a large proportion of the population is asymptomatic to the virus, there is always a risk that individuals may contribute to inadvertently spreading the virus to other animals. In the case of the captive gorillas that tested positive for SARS-CoV-2, the zoo took precautionary measures to protect the animals, but an asymptomatic worker was still responsible for the spillover event (Gibbons, 2021). Therefore, routinely testing wastewater provides insights which can be used in conservation approaches at zoos and wildlife sanctuaries during the pandemic.

7 | MANAGING PANDEMIC RISKS TO NHPS THROUGH VACCINATION PROGRAMS

Vaccination of captive primates and/or wild primates that can be safely handled, might be a valuable approach toward protecting them (Figure 1). The vaccination of NHPS has been an approach for preventing disease in the past. Rhesus monkeys on Cayo Santiago were treated with a Tetanus vaccine (excluding infants and 6 adult monkeys) that led to a 94% decline in Tetanus-related mortality after 2 years (Kessler et al., 1988). The technologies for targeting RNA viruses by vaccination are also available for NHPS. Oral vaccination was demonstrated to be feasible for chimpanzees after a vaccine was designed for the Ebola virus using the Rabies vaccine platform (Walsh et al., 2017). Administration of the vaccine led to a successful response as vaccinated chimpanzees generated Ebola virus targeting antibodies exponentially throughout the trial. Promisingly, vaccination did not cause any serious health complications in chimpanzees (Walsh et al., 2017). Therefore, it may be possible to utilize a similar approach with SARS-CoV-2, especially with NHPS species that are being used to develop a vaccine that is safe and effective in humans. A reassuring insight from biomedical research is that rhesus monkeys which were previously infected by SARS-CoV-2, were able to fight off the virus when it was administered a second time (Chandrashekar et al., 2020; Deng et al., 2020). It appears that the rhesus monkeys could form protective immunity toward the virus, and this is a good sign for the potential vaccination approach for SARS-CoV-2 to protect NHPS.

8 | A ONE-HEALTH APPROACH TO PROTECTING ECOSYSTEMS, INCLUDING PRIMATES, DURING A PANDEMIC

The complexities of managing the risk of current, emerging, and future diseases are vast, diverse and intertwined (Mackenzie et al., 2013). Here, we have briefly discussed the potential for wastewater monitoring and vaccination as two small tools for informing, monitoring, and preventing risk of SARS-CoV-2 in wildlife, with a focus on NHPS. We recognize that this is a small piece of the puzzle. Ideally, these—and many other—tools would be considered in the scale and scope of a comprehensive, multidisciplinary initiative and strategically employed. The benefits and need for this type of “One Health” approach that considers communicable zoonotic diseases in multidisciplinary landscapes (spanning sanitation, preventative and diagnostic medicine, surveillance, education, foodways and livestock management, human-animal contact points, reforestation initiatives, tracking and ending illegal wildlife movement, and so on) versus activities through a single-sector lens, has been highlighted by many recent studies (Mackenzie et al., 2013; Roberts, 2019; Sherman, 2010; Sleeman et al., 2017). Such collaborative, coordinated, and sustainable solutions are likely to be far more successful than small measures taken in isolation at achieving optimal outcomes for the health of the ecosystem, including the humans and NHPS living within it. Our aim has been a call for attention to the risk to NHPS of human diseases, and to suggest wastewater management as a viable tool to add to a One Health collaborative initiative.

9 | CONCLUSIONS

Our closest relatives, the NHPS, are an important part of the culture, religion, and livelihoods of many societies. Their importance in biomedical research has also been highlighted repeatedly, including during the past year, with COVID-19 research and discovery. Importantly, these organisms also provide invaluable insights into our own evolution, biology, and behaviour. However, the unfortunate reality is that these species will take a backseat to public health concerns in underdeveloped countries. While the risk of transmission through wastewater is low, we have discussed how wastewater can be utilized to protect these animals through routine monitoring for pandemic hotspots (Figure 1). There is a need to better strategize and work with communities living with or near NHPS to protect these animals from a novel pandemic-type virus. Human health is intricately linked to these animals. More specifically, susceptible NHPS exposed to the virus present threats of secondary anthrozoonoses events. A recent example of this occurring is with minks during the COVID-19 pandemic, where they have now become a reservoir for COVID-19 (Oude Munnink et al., 2021). Perhaps the best written reason for protecting NHPS comes from the writer of Spillover, David Quammen, “People and gorillas, horses and duikers and pigs, monkeys and chimps and bats and viruses: We’re all in this together” (Quammen, 2012).

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Sabateeshan Mathavarajah: conceptualization (lead); investigation (lead); visualization (lead); writing original draft (lead); writing review & editing (lead). Graham Dellaire: conceptualization (supporting); funding acquisition (lead); investigation (supporting); project administration (lead); supervision (lead); writing original draft (supporting); writing review & editing (supporting).

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