Coordinated surveillance is essential to monitor and mitigate the evolutionary impacts SARS-CoV-2 spillover and circulation in animal hosts

Suresh V. Kuchipudi[1,2*], Cedric Tan[3,4], Lucy van Dorp[3], Maureen Lichtveld[5]
Bradley Pickering[6,7,8], Jeff Bowman[9], Samira Mubareka[10,11]and Francois Balloux[3*]

[1] Center for Infectious Disease Dynamics, and the Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA

[2]Animal Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA, USA

[3]UCL Genetics Institute, University College London, London, UK

[4]Francis Crick Institute, London, UK

[5]Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA.

[6]National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada

[7]Department of Veterinary Microbiology and Preventative Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

[8]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Manitoba, Canada

[9]Wildlife Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Peterborough, Ontario, Canada

[10]Sunnybrook Research Institute, Toronto, Ontario, Canada

[11]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

*Corresponding author

Abstract SARS-CoV-2 lineages circulating in animal reservoirs may broaden the evolutionary potential of the virus and increase the risk of novel variants emerging. There is an urgent need for more- comprehensive surveillance of SARS-CoV-2 circulating in non-human hosts.

Main Text

Transmission of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) from humans to other animal hosts – known as zooanthroponotic transmission – continues worldwide and has been described for multiple companion, captive and wild animal species[1-3]. Indeed, evidence indicates that SARS-CoV-2 is a generalist pathogen that can infect at least one non-human animal species from nearly every mammalian Order (Fig. 1). Abundant, sustained and protracted circulation of SARS-CoV-2 in the human population is likely to promote the risk of establishment of secondary animal reservoirs for otherwise extinct variants and tangential paths of viral evolution. In addition, the circulation of SARS-CoV-2 in animals poses the risk of virus transmission back to humans, complicating variant-specific vaccination strategies. Cases of animal-to-human transmission of SARS-CoV-2 have been documented, involving infected mink in multiple countries, white-tailed deer (*Odocoileus virginianus*) in North America[4], pet hamsters in Hong Kong[5], and cats in Thailand[6]. Multi-host transmission of SARS-CoV-2 has resulted in novel SARS-CoV-2 clades[4], and variants with enhanced binding affinity to the angiotensin-converting enzyme 2 (ACE2) receptor[7], which is exploited by the virus for cell entry. Here, we describe these risks in more detail, and argue that enhanced surveillance of SARS-CoV-2 presence in non-human species and of cross-species transmission is an essential element of the ongoing global response to this new virus.

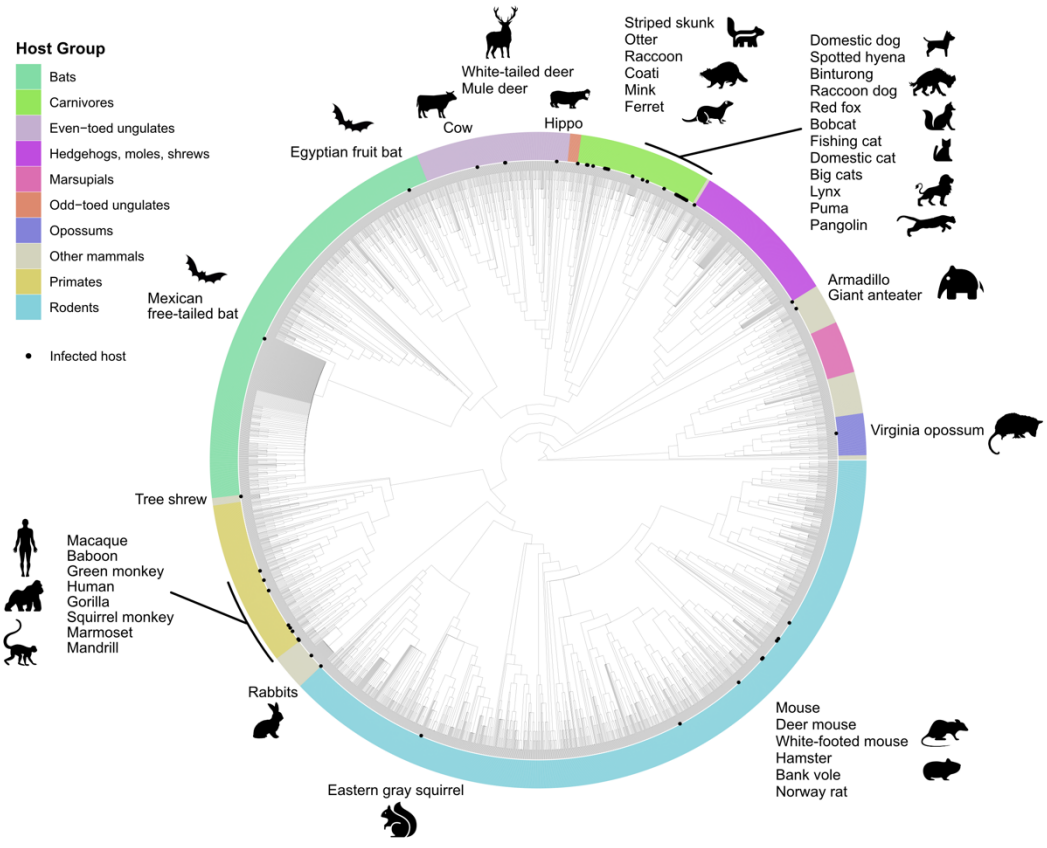

Fig. 1: The breadth of hosts known to be susceptible to SARS-CoV-2 as of April 2022. *Consensus maximum likelihood tree of mammals (4,098 species; 100 bootstraps).*

The risks of spillover into animals: persistence, host adaptation and recombination

A reservoir is an epidemiologically connected animal population or environmental niche where a pathogen such as SARS-CoV-2 can be maintained and transmitted to humans[9]. White-tailed deer potentially form one such reservoir for SARS-CoV-2 and have been suggested to perpetuate lineages that may be extinct in humans. Additionally, a cluster of infections detected in white-tailed deer from Ontario, Canada, assigned to PANGO lineage B.1.641, has likely been circulating outside the human population in wildlife since 2020 or early 2021[4]. The persistence of SARS-CoV-2 in zoonotic reservoirs is of particular concern since it facilitates host-specific adaptation and potential recombination with other viral lineages or species, threatening the health of wildlife, domestic animals and people.

Putative host-adaptive mutations in SARS-CoV-2 following spillover events into farmed mink and wild white-tailed deer have been identified[10]. While the functional role of these mutations has not yet been determined, one deer-specific mutation was found in the PLpro domain of non-structural protein 3 (Nsp3). Nsp3 is known to interfere with host innate immunity disrupting the type I interferon pathway by direct cleavage of host IRF3[11]. This is relevant to the key question of whether SARS-CoV-2 may become more pathogenic in humans after acquiring animal-specific adaptations. A common evolutionary trade-off when a virus adapts to novel hosts is reduced virulence in the original host. This feature has been known since the late 19th century and led to the development of the first attenuated vaccines, in which a viral strain is passaged in animal hosts. The long-term success of the SARS-CoV-2 shift to new animal hosts can be independent of reduced impact on human health if transmission to and from humans is not required for the survival of lineages adapting to their secondary non-human animal host[12]. If a secondary reservoir for SARS-CoV-2 can persist without the need for constant reseeding through repeated spillovers from humans, animal-adapted viral lineages may adapt and diverge from strains in circulation in humans, irrespective of their transmissibility in the former[12].

Another concern regarding the circulation of SARS-CoV-2 in animals is the potential for recombination with other lineages of SARS-CoV-2 or other animal-borne viruses that may allow them to emerge as novel zoonotic viruses. A key feature of coronavirus replication is the occurrence of homologous RNA recombination[13,14]. Coronaviruses can easily jump between non-human animal species or between animals and humans[15]. Recombination events followed by host-specific adaptation have contributed to the emergence of several new coronaviruses that have successfully jumped into novel host species[14], resulting in established diseases such as bovine coronavirus disease, canine coronavirus disease, and the two coronaviruses (HCoVs) endemic in the human population, HCoV-OC43 and HCoV-229E. Recombination involving the spike gene amino-terminal and receptor-binding domains and some accessory proteins between clades of SARS-CoV-related coronaviruses circulating in bats are also believed to have contributed to the emergence of SARS-CoV[16].

While there is no evidence to date for recombination between SARS-CoV-2 and any of the four HCoVs, it remains possible that SARS-CoV-2 may recombine with coronaviruses circulating in animals. Such recombinants could lead to the secondary introduction of novel variants with increased cross-transmissibility or virulence in humans. Furthermore, SARS-CoV-2 variants emerging from recombination in an animal host could be antigenically different, largely escaping protection afforded by SARS-CoV-2 vaccines and antibody-based therapeutics targeting spike

antigens. This possibility is underscored by an excess of recombination in the spike gene[17]. Indeed, evidence of recombination events occurring between endemic coronaviruses and other clade A betacoronaviruses exists for the Dromedary camel, the reservoir of MERS-CoV[18]. Here, the endemic camel virus HKU23 (DcCoV-HKU23) was found to have genomic segments of a rabbit virus RbCoV-HKU14 in the hemagglutinin esterase gene as well as homolog fragments of a rodent betacoronavirus in the spike protein[18].

What can surveillance of SARS-CoV-2 in humans and animals teach us about pandemic emergence?

From a macroecological perspective, humans are just a single node in a complex network of host species that pathogens continually jump between. Although there is some evidence that features associated with the virus, host and environment contribute to the cross-species transmission of pathogens, the drivers of host jumps are not fully understood[19]. The ongoing animal multi-host transmission of SARS-CoV-2 necessitates a focus on the drivers of inter-species viral spillover, maintenance, and adaptation. Analysis of the ecological and genetic features of both host and virus through complex modelling approaches such as machine learning may yield valuable insights into the drivers of SARS-CoV-2 exchange (direct or indirect) across vertebrate hosts. More broadly, these modeling approaches allow for rapid triaging of resource-intensive surveillance efforts and *in vitro* and *in vivo* characterization of pathogens predicted *in silico* to be zoonotic. Most importantly, understanding the ecological and molecular drivers of virus transmission among hosts may eventually allow us to pre-empt the emergence of novel zoonotic diseases. However, such complex modelling approaches require large datasets with comprehensive metadata including host health status and immunological profiles, and these are currently not available. Such datasets can only be generated through coordinated surveillance efforts in both humans and animals.

Coordination of multi-pillar disease surveillance - challenges and opportunities

Given the risks associated with secondary spillovers into zoonotic reservoirs, there is an urgent need to implement integrated surveillance programs to coordinate the monitoring of SARS-CoV-2 in humans, the environment, and domestic and wild animals. However, there are several challenges to implementing integrated disease surveillance. In most countries where surveillance systems exist, separate agencies manage surveillance in humans, animals, and the environment, often with no inter-sectoral communication or coordination. In high-income countries, disease surveillance systems in humans and animals are governed by entirely different sets of regulations, policies, standards, and funding sources, making inter-sectoral cooperation challenging. In contrast, in most low- and middle-income countries (LMICs), resource limitations and poor infrastructure are significant barriers. Regional and national governments and international agencies should implement policies ensuring inter-sectoral cooperation and providing dedicated, long-term and international funding for sustained and coordinated pathogen surveillance.

Additionally, many livestock producers lack the financial means or support to test their animals for target pathogens outside of funded surveillance programs, and may fear economic losses and a threat to their livelihoods if diseases are detected. Also, there are few mandated wildlife surveillance programs other than for specific pathogens such as rabies and chronic wasting disease. Therefore, comprehensive education and compensation programs will be essential to improve overall knowledge and response to zoonotic diseases in livestock, including transmission routes,

symptoms, economic impacts, and potential health risks. Such initiatives require support from the government, coordinated partnerships, and collaboration with all stakeholders in food animal production and the large corporate and private sectors.

Many in the disease surveillance field argue that a lack of precise guidelines for data sharing and usage is the primary reason for the mistrust between agencies and absence of inter-agency cooperation in disease surveillance[20]. In addition, data collection standards are very different among agencies, making data integration almost impossible. A potential solution is the development of data repositories, universal guidelines for data collection, analysis and sharing, interoperable data standards, and protocols among agencies. The utility of data repositories is further underscored by their potential use for viral phenotyping and assessment of risk determinants such as host cell receptor binding, viral replication, transmission, immune escape, and antiviral resistance. This information transforms genomic data into health intelligence, particularly when coupled with key ecological, behavioral and epidemiological elements such as R_0 (a measure of transmissibility). Standardization around these risk elements is also critical to knowledge synthesis and decision-making.

Testing all animals everywhere is not practical, feasible, or necessary to gain health intelligence for mitigation and control. Instead, a rational approach to targeted surveillance, coupled with laboratory experiments to validate zoonotic potential, is essential. To design this, we can incorporate computational models to predict and shortlist high-risk host species and locations. Molecular simulations have extensively been used to model ACE2-RBD interactions to predict the susceptibility of different species to wild-type SARS-CoV-2 and its variants[2]. Additionally, ecological models have been developed to predict locations where frequent contact between humans and animals, and between animals, increase transmission risk[21,22]. Such models do not generally include but can benefit from, incorporating existing knowledge of animal behavior and ecology, requiring the input of expertise from wildlife ecology and animal husbandry. Also, linkages with human and environmental (wastewater) data enable geotemporal prioritization based on areas of high viral activity among humans and risk of animal exposure. Finally, key epidemiological parameters can inform the prioritization of surveillance by focusing on high-risk transboundary and human-animal interfaces (e.g., urban wildlife and peri-domestic animals), as well as endangered animals, such as some species of primates at risk of high mortality.

A robust One Health surveillance system for infectious diseases requires four key programmatic pillars to be in place (Fig. 2). Surveillance of environmental samples, mainly involving wastewater, has previously been used to monitor antimicrobial resistance[23] and is becoming established for monitoring COVID-19[24]. Such surveillance can act as an early detection system; for example, identifying poliovirus in UK and New York sewage in 2022[25]. There has also been massive genomic surveillance for SARS-CoV-2, with over 15 million human-derived genomes for SARS-CoV-2 strains deposited on the public repository GISAID (current to April 2023)[26]. However, surveillance programs in humans are largely limited to patients seeking healthcare and often target specific subsets of human pathogens, precluding the ability to detect novel infectious agents. A SARS-CoV-2 surveillance scheme relying on untargeted RNA metagenomic sequencing would additionally inform on the incidence and prevalence of other known zoonotic pathogens such as filoviruses or highly pathogenic avian influenza (HPAI) viruses, such as H5N1 viruses, and facilitate early identification of potentially zoonotic pathogens yet to be characterized. Expanding this pillar to include routine surveillance of apparently healthy individuals and individuals with

severe febrile respiratory syndromes of unknown etiology may help us better understand the epidemiology of known or novel infectious agents circulating in the population and their potential outbreak risk.

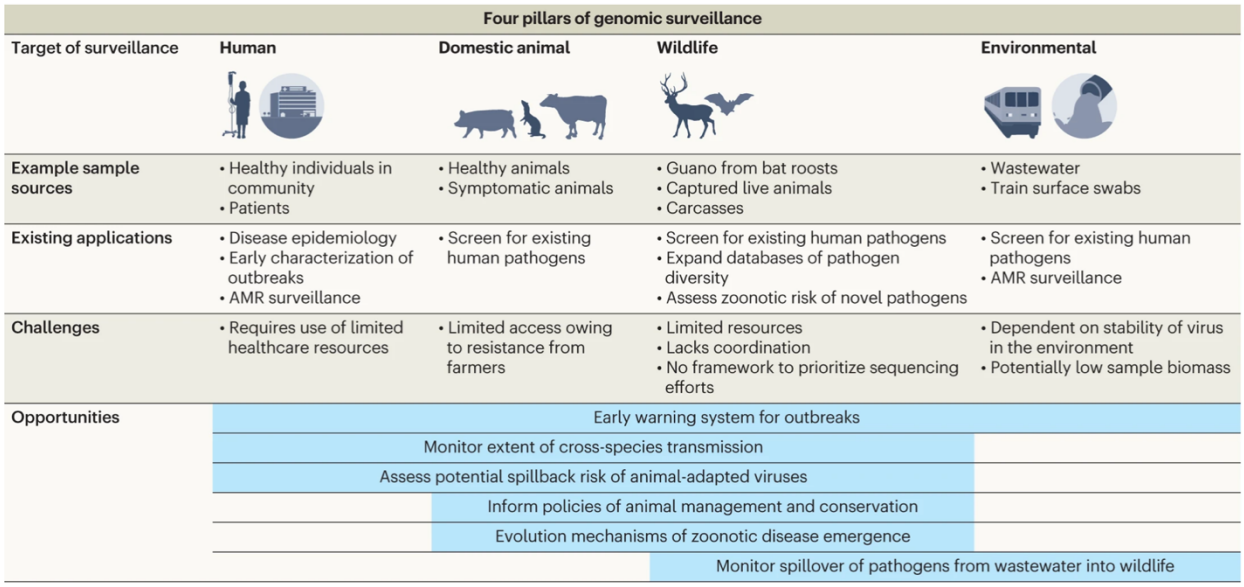

Fig. 2: Four pillars of genomic surveillance. *Coordinated SARS-CoV-2 genomic surveillance in humans, the environment, and domestic and wild animals is critical to inform policy development towards an early warning system for detecting outbreaks in humans and animals. AMR, antimicrobial resistance.*

There is a bigger gap in surveillance among the other two pillars: domestic animals and wildlife, despite the far-reaching impacts of animal reservoirs that we have described, for both SARS-CoV-2 and other pathogens at risk of zoonotic emergence. It is crucial that we build on and integrate existing surveillance networks and bolster surveillance pillars that are less established. Such integration requires global adoption of standardized frameworks such as the Tripartite Zoonotics Guide[27] and the recently released Quadripartite One Health Joint Plan of Action[28], engagement of stakeholders and linkages with other One Health preparedness and response activities. Emphasis should be placed on the prioritization of pathogens and populations, biocontrol strategies, early warning systems and development of decision-support tools and triggers for action. Clear guidance provides the basis for sustainable and versatile surveillance programs which require strong, harmonized support at local, national, regional, and global levels.

A key lesson we should all learn from the COVID-19 pandemic is that investing in infectious disease surveillance and risk prevention strategies can minimize the devastating global economic and social damages of disease outbreaks. Most importantly, only with the global coordination of existing surveillance efforts can we better manage the aftermath of the current pandemic and predict and thwart future ones.

References

1 Meekins, D. A., Gaudreault, N. N. & Richt, J. A. Natural and Experimental SARS-CoV-2 Infection in Domestic and Wild Animals. *Viruses* **13**, 1993, doi:10.3390/v13101993 (2021).

2 Damas, J. *et al.* Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. *Proc Natl Acad Sci U S A* **117**, 22311-22322, doi:10.1073/pnas.2010146117 (2020).

3 Oreshkova, N. *et al.* SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. *Euro Surveill* **25**, doi:10.2807/1560-7917.ES.2020.25.23.2001005 (2020).

4 Pickering, B. *et al.* Divergent SARS-CoV-2 variant emerges in white-tailed deer with deer-to-human transmission. *Nature Microbiology*, doi:10.1038/s41564-022-01268-9 (2022).

5 Yen, H.-L. *et al.* Transmission of SARS-CoV-2 delta variant (AY.127) from pet hamsters to humans, leading to onward human-to-human transmission: a case study. *The Lancet* **399**, 1070-1078, doi:10.1016/S0140-6736(22)00326-9 (2022).

6 Sila, T. *et al.* Suspected Cat-to-Human Transmission of SARS-CoV-2, Thailand, July–September 2021. *Emerging Infectious Disease journal* **28**, 1485, doi:10.3201/eid2807.212605 (2022).

7 Su, C. *et al.* Molecular Basis of Mink ACE2 Binding to SARS-CoV-2 and Its Mink-Derived Variants. *J Virol* **96**, e0081422, doi:10.1128/jvi.00814-22 (2022).

8 Upham, N. S., Esselstyn, J. A. & Jetz, W. Inferring the mammal tree: Species-level sets of phylogenies for questions in ecology, evolution, and conservation. *PLoS Biol* **17**, e3000494, doi:10.1371/journal.pbio.3000494 (2019).

9 Haydon, D. T., Cleaveland, S., Taylor, L. H. & Laurenson, M. K. Identifying reservoirs of infection: a conceptual and practical challenge. *Emerg Infect Dis* **8**, 1468-1473, doi:10.3201/eid0812.010317 (2002).

10 Tan, C. C. S. *et al.* Transmission of SARS-CoV-2 from humans to animals and potential host adaptation. *Nature Communications* **13**, 2988, doi:10.1038/s41467-022-30698-6 (2022).

11 Moustaqil, M. *et al.* SARS-CoV-2 proteases PLpro and 3CLpro cleave IRF3 and critical modulators of inflammatory pathways (NLRP12 and TAB1): implications for disease presentation across species. *Emerg Microbes Infect* **10**, 178-195, doi:10.1080/22221751.2020.1870414 (2021).

12 Longdon, B., Brockhurst, M. A., Russell, C. A., Welch, J. J. & Jiggins, F. M. The evolution and genetics of virus host shifts. *PLoS Pathog* **10**, e1004395, doi:10.1371/journal.ppat.1004395 (2014).

13 Zhang, X. W., Yap, Y. L. & Danchin, A. Testing the hypothesis of a recombinant origin of the SARS-associated coronavirus. *Arch Virol* **150**, 1-20, doi:10.1007/s00705-004-0413-9 (2005).

14 Graham, R. L. & Baric, R. S. Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. *J Virol* **84**, 3134-3146, doi:10.1128/JVI.01394-09 (2010).

15 Ruiz-Aravena, M. *et al.* Ecology, evolution and spillover of coronaviruses from bats. *Nat Rev Microbiol* **20**, 299-314, doi:10.1038/s41579-021-00652-2 (2022).

16 Hu, B. *et al.* Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog* **13**, e1006698, doi:10.1371/journal.ppat.1006698 (2017).
17 Turakhia, Y. *et al.* Pandemic-scale phylogenomics reveals the SARS-CoV-2 recombination landscape. *Nature* **609**, 994-997, doi:10.1038/s41586-022-05189-9 (2022).
18 So, R. T. Y. *et al.* Diversity of Dromedary Camel Coronavirus HKU23 in African Camels Revealed Multiple Recombination Events among Closely Related Betacoronaviruses of the Subgenus Embecovirus. *J Virol* **93**, doi:10.1128/JVI.01236-19 (2019).
19 Shaw, L. P. *et al.* The phylogenetic range of bacterial and viral pathogens of vertebrates. *Mol Ecol* **29**, 3361-3379, doi:10.1111/mec.15463 (2020).
20 van Panhuis, W. G. *et al.* A systematic review of barriers to data sharing in public health. *BMC Public Health* **14**, 1144, doi:10.1186/1471-2458-14-1144 (2014).
21 Eby, P. *et al.* Pathogen spillover driven by rapid changes in bat ecology. *Nature* **613**, 340-344, doi:10.1038/s41586-022-05506-2 (2023).
22 Gibb, R. *et al.* Zoonotic host diversity increases in human-dominated ecosystems. *Nature* **584**, 398-402, doi:10.1038/s41586-020-2562-8 (2020).
23 Chau, K. K. *et al.* Systematic review of wastewater surveillance of antimicrobial resistance in human populations. *Environ Int* **162**, 107171, doi:10.1016/j.envint.2022.107171 (2022).
24 Farkas, K., Hillary, L. S., Malham, S. K., McDonald, J. E. & Jones, D. L. Wastewater and public health: the potential of wastewater surveillance for monitoring COVID-19. *Curr Opin Environ Sci Health* **17**, 14-20, doi:10.1016/j.coesh.2020.06.001 (2020).
25 Ryerson, A. B. *et al.* Wastewater Testing and Detection of Poliovirus Type 2 Genetically Linked to Virus Isolated from a Paralytic Polio Case - New York, March 9-October 11, 2022. *MMWR Morb Mortal Wkly Rep* **71**, 1418-1424, doi:10.15585/mmwr.mm7144e2 (2022).
26 Elbe, S. & Buckland-Merrett, G. Data, disease and diplomacy: GISAID's innovative contribution to global health. *Glob Chall* **1**, 33-46, doi:10.1002/gch2.1018 (2017).
27 WHO. *The Tripartite Zoonoses Guide*, <https://www.who.int/initiatives/tripartite-zoonosis-guide> (2019).
28 WHO. *One health joint plan of action*, <https://www.who.int/publications/i/item/9789240059139> (2022).

Contributions

S.V.K and F.B. conceived the commentary and wrote the first draft. C.T created the figures. S.M, L.v.D, M.L, B.P, J.B and C.T conceptualized the ideas with S.VK and F.B. All authors edited the manuscript into its final form.

Competing interests: Authors declare that they have no competing interests