Spillback in the Anthropocene: the risk of human-to-wildlife pathogen transmission for conservation and public health

Anna C. Fagre*[1], Lily E. Cohen[2], Evan A. Eskew[3], Max Farrell[4], Emma Glennon[5], Maxwell B. Joseph[6], Hannah K. Frank[7], Sadie Ryan[8, 9,10], Colin J Carlson[11,12], Gregory F Albery*[13]

*Corresponding authors: anna.fagre@gmail.com; gfalbery@gmail.com

1: Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO
2: Icahn School of Medicine at Mount Sinai, New York, NY 10029
3: Department of Biology, Pacific Lutheran University, Tacoma, WA, 98447 USA
4: Department of Ecology & Evolutionary Biology, University of Toronto
5: Disease Dynamics Unit, Department of Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES, UK
6: Earth Lab, University of Colorado Boulder, Boulder, CO 80309
7: Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, 70118 USA
8: Quantitative Disease Ecology and Conservation (QDEC) Lab Group, Department of Geography, University of Florida, Gainesville, FL, 32610 USA
9: Emerging Pathogens Institute, University of Florida, Gainesville, FL, 32610 USA
10: School of Life Sciences, University of KwaZulu-Natal, Durban, 4041, South Africa
11: Center for Global Health Science and Security, Georgetown University Medical Center, Washington, DC, 20057 USA
12: Department of Microbiology and Immunology, Georgetown University Medical Center, Washington, DC, 20057 USA
13: Department of Biology, Georgetown University, Washington, DC, 20057 USA

Abstract

The SARS-CoV-2 pandemic has led to increased concern over transmission of pathogens from humans to animals (“spillback”) and its potential to threaten conservation and public health. To assess this threat, we reviewed published evidence of spillback events, including instances where spillback could threaten conservation and human health. We identified 97 verified examples of spillback, involving a wide range of pathogens; however, infected hosts were mostly non-human primates or large, long-lived captive animals. Relatively few spillback events resulted in morbidity and mortality, and very few led to maintenance of a human pathogen in a new reservoir or subsequent “secondary spillover” back into humans. Together, these results imply that spillback represents an apparently minor threat to conservation and public health, particularly relative to other anthropogenic stressors like land use and climate change. Lastly, we outline how researchers can collect experimental and observational evidence that will expand our capacity for spillback risk assessment.

Introduction

Multi-host pathogens are becoming a dominant feature of the Anthropocene. Driven by deforestation, land use conversion, and climate change, pathogens are spilling over from animals into human populations at an increasing rate, presenting a significant threat to public health (Jones *et al.* 2013; Plowright *et al.* 2015; Woolhouse & Brierley 2018). Recently, concerns have been raised about the transmission of pathogens from humans back into wild animals (Edwards & Santini 2020; Oreshkova *et al.* 2020; Shi *et al.* 2020; Wang *et al.* 2020; Prince *et al.* 2021). This process, known as "(zoo)anthroponosis", "reverse zoonosis", or "spillback", could pose a problem for wildlife conservation and public health efforts in the near future.

Concerns about spillback have grown throughout the ongoing COVID-19 pandemic (Briggs, Helen 2020; Edwards & Santini 2020; Gorman 2020; Olival *et al.* 2020; Santini & Edwards 2020; Prince *et al.* 2021). SARS-CoV-2 transmission into animals appears to be relatively common: the virus has been transmitted to household cats and dogs, and to big cats and gorillas in zoos, and — perhaps most notably — has established epizootic transmission in mink farms on multiple continents (Garigliany *et al.* 2020; Molenaar *et al.* 2020; Munnink *et al.* 2020b; OIE-World Organisation for Animal Health 2020; Oreshkova *et al.* 2020; Patterson *et al.* 2020; Sailleau *et al.* 2020; Segalés *et al.* 2020; Sit *et al.* 2020; Gibbons, Ann 2021) and, only recently, spilled back into wild mink in Spain (Aguiló-Gisbert *et al.* 2021). Further, because SARS-CoV-2 likely originated in horseshoe bats (genus *Rhinolophus*), some fear that the virus might become established in bat populations outside of Asia and form a novel reservoir, complicating efforts to prevent future resurgence in humans (Olival *et al.* 2020; Zhou *et al.* 2020; Banerjee *et al.* 2021; Hedman *et al.* 2021). Additionally, if SARS-CoV-2 infection can cause clinical disease in some bats, introduction of the virus might further threaten bat species that have already been devastated by white-nose syndrome (Olival *et al.* 2020; Cook *et al.* 2021). So far, these potential risks have led to far-reaching policy decisions including widespread moratoria on bat research (Aizenman 2020; Donahue 2020), as well as broader discussions about the prohibition of wildlife farming for high-risk species like mink, which have been subject to some of the largest disease-related cullings in recent memory (Dobson *et al.* 2020).

While spillback-related research has progressed quickly in the context of the SARS-CoV-2 pandemic (Bosco-Lauth *et al.* 2020; Frank *et al.* 2020; Munnink *et al.* 2020b; Olival *et al.* 2020; Schlottau *et al.* 2020; Shi *et al.* 2020), little is understood about the overall frequency of broader spillback events and their underlying drivers. There is some documented evidence for spillback of human pathogens into wildlife populations (e.g. (Osterhaus *et al.* 2000; Nizeyi *et al.* 2001; Goldberg *et al.* 2007; Kaur *et al.* 2008; Rwego *et al.* 2008; Obanda *et al.* 2013; Terzian *et al.* 2018; Britton *et al.* 2019; Favoretto *et al.* 2019), but there has been little critical analysis concerning the magnitude of the threat, limiting our understanding of its realised and potential impacts on conservation and public health (Ryan & Walsh 2011), particularly compared to our advancing understanding of its inverse (i.e., the more classical animal-to-human spillover, or zoonosis).

This research gap leaves three intertwined questions unanswered. First, on a fundamental level, it is unclear to what degree spillover and spillback are symmetrical processes: do the same filters act when a pathogen makes its way from a human into an animal and *vice versa* (Plowright *et al.* 2017), or are some filters direction-specific? Second, uncertainty exists surrounding which pathogens are most likely to undergo spillback and into which animal hosts, making it difficult to assess how the rising tide of infectious diseases in humans will affect free-ranging and captive or habituated wildlife. Finally, it remains to be seen how great a threat spillback poses relative to other anthropogenic impacts on biodiversity like habitat destruction and urbanisation (McKinney *et al.* 2010; Barlow *et al.* 2016). The unknown magnitude of this risk leaves us unable to weigh spillback prevention efforts relative to other

health and conservation priorities — particularly where such efforts might directly compete for funding with other priorities, or otherwise compromise ongoing animal disease research efforts (e.g. white-nose surveillance in bat populations). These questions can be addressed using a comprehensive assessment of previous spillback evidence informed by known susceptibility, exposure, and sampling processes.

Here, we critically assess the evidence for spillback-related processes and the inferential underpinnings of spillback studies. First, we propose a conceptual framework for two pathways of spillback with meaningful differences in both likelihood and impact (Figure 1). We then discuss documented examples of human pathogens transmitted to free-ranging, captive, or habituated wildlife, and we use these data to highlight common trends in each of the two spillback pathways. We also interpret spillback's conservation threat relative to other anthropogenic activities, and assess the evidence for secondary spillovers of a zoonotic pathogen from a newly established maintenance reservoir. Finally, we propose a hierarchy of evidence by which researchers can assess spillback risk for human pathogens in the future (Figure 2), whether they be emerging (e.g. SARS-CoV-2) or well-established (e.g. influenza).

The two spillback pathways

Narratives surrounding spillback usually focus on one of two negative outcomes (or "**Pathways**"), which we outline in Figure 1. In **Pathway 1** (Figure 1A-C), because some human pathogens can cause morbidity or mortality in animals (Kaur *et al.* 2008), spillback events run the risk of inflicting increased burdens of disease on animal populations, raising conservation concerns. Problematically, because pathogens can exhibit extreme virulence in host species that are distantly related to their original hosts (Farrell & Davies 2019), prediction of disease severity in immunologically naive hosts remains difficult. As such, if humans expose a vulnerable animal population to their pathogens, the conservation impacts could be severe — similar to threats posed by infectious diseases that spread from domesticated animals to endangered wildlife (Pedersen *et al.* 2007).

The second major concern for spillback, and one that has been frequently discussed in reference to SARS-CoV-2, is the potential for "secondary spillover" from animals back into human populations, raising concern for public health, as illustrated in **Pathway 2** (Figure 1D-E) (Edwards & Santini 2020; Gorman 2020; Santini & Edwards 2020). In situations where spillback occurs through infection of a novel competent host species, the new host may be able to maintain enzootic circulation of the pathogen, sourcing additional cross-species transmission events back into human populations. In this case, the wild host may represent a significant barrier to the control and elimination of the disease, as is illustrated by bovine tuberculosis in badgers in the United Kingdom (Donnelly *et al.* 2003) and Guinea worm in feral dog populations (Callaway 2016). This pathway is particularly problematic because it demands implementation of a whole new suite of measures to reduce or prevent subsequent epidemics in humans. Medical countermeasures and non-pharmaceutical interventions would be insufficient to prevent recurring outbreaks, and authorities would have to also monitor and prevent pathogen reintroduction using One Health strategies such as surveillance, vaccination, or population management of the new reservoir. Evidence for **Pathway 2** requires demonstrating that a pathogen has established itself in a vertebrate population following human introduction and subsequently re-emerged into human populations. As yet, it is unclear how likely this phenomenon is. Importantly, pathogens causing high mortality in novel hosts (fulfilling **Pathway 1**) are less likely to be sustained, resulting in stuttering transmission (Blumberg & Lloyd-Smith 2013), so it is possible that these two pathways will trade off in terms of their probability (i.e., if a pathogen fulfils **Pathway 1** it may be less likely to fulfill **Pathway 2**).

How common is spillback?

To synthesize existing knowledge on spillback, we developed an evidence base from primary literature (Table S1). Noting hosts and pathogens involved, reported clinical signs, and potential onward transmission, we performed a comprehensive literature search to update and extend a recent review of 30 studies on zooanthroponoses (Messenger *et al.* 2014). We excluded studies of domesticated animal infections, experimental inoculation of animals with human pathogens, review papers and meta-analyses, and documents outlining management of wildlife populations, which represented 11 of 30 wildlife-related references in the previous study. To supplement these studies, we performed a search in PubMed and Web of Science using the following terms: *anthroponosis, zooanthroponosis, spillback, "reverse zoonosis",* and *human-to-animal disease transmission,* published between January 1, 1900 and March 1, 2021, and written in English. This search produced 693 records, reduced to 519 following removal of duplicates. We removed studies not written in English (n = 35), unrelated to disease transmission (n = 200), and not specifically discussing zooanthroponotic transmission between humans and wildlife (e.g. transmission from human to domestic animal or between animals) (n = 163). We also removed studies of spillback outside of vertebrate hosts, though we note that intriguing reports describe human pathogens in invertebrates like coral (Sutherland *et al.* 2011) and bivalves (Guyader *et al.* 2000). We retained 121 studies for initial review, and augmented the selection with articles referenced in these papers where relevant.

Inclusion and exclusion criteria for spillback references

When examining multi-host pathogens, there is a risk of inferring interspecific transmission where in fact this may not be the case — for example, where humans and a focal animal species both contract the pathogen from a shared source, rather than transmitting it in either direction through spillover or spillback (Hockings *et al.* 2020). Therefore, we used a stringent set of criteria to ensure that we only included references indicating human-to-animal transmission, and on the fine timescales required to infer recent transmission. For example, we did not include phylogenetic studies of accessioned genetic data showing a shared historical phylogenetic origin among humans and animals, because it is difficult to prove directionality with these data, and they operate on a prohibitively coarse timescale (Noël *et al.* 2005; Wevers *et al.* 2011; Villabruna *et al.* 2019).

We focused on pathogens with simple life cycles, rather than those requiring an arthropod vector or intermediate host for transmission. We eliminated these pathogens from scope preemptively, due to the difficulty of conclusively identifying human origins. Vector-borne pathogens are known to have wide host ranges (Olival *et al.* 2017) which, in conjunction with the mobile nature of arthropod vectors, allows them to easily establish sylvatic cycles, confounding conclusive identification of recent spillback. Nevertheless, we note that several verified examples of human-to-wildlife transmission of arboviruses have been demonstrated using macroecological evidence. For example, Asian lineage Zika virus (ZIKV) RNA was detected in Brazilian marmosets and free-ranging capuchin monkeys during the 2014-2015 outbreak in the Americas (Terzian *et al.* 2018; Favoretto *et al.* 2019). In this instance, spatiotemporal patterns were sufficient to determine that the pathogen had moved from human populations to wildlife populations via an arthropod vector (Pathway 1; Favoretto *et al.* 2019; Han *et al.* 2019). Notably, when reviewing the literature, we discovered no examples of fine-scale novel human-to-animal transmission of vector-borne pathogens, so we believe this exclusion criterion had minimal influence on our evidence base.

As a special case of excluding multi-host pathogens, we also exclude special cases of spillback at the lineage or genotype level. For example, because influenza A is cosmopolitan across wild birds and mammals, farmed poultry and swine, and humans, it should be rare that the virus species finds a new

host, but novel strains arising from recombination are constantly passing between species for the first time. Similarly, antimicrobial resistance (AMR) is particularly driven by livestock management practices (Van Boeckel *et al.* 2015), but also frequently evolves in humans (Mendelsohn *et al.* 2020), and AMR genes could easily spillback into wild or domesticated animals (Jobbins & Alexander 2015; McDougall *et al.* 2019). In cases like these, there may be separate relevance to conservation and public health. For example, during the COVID-19 pandemic, significant concern has been expressed that mink variants of SARS-CoV-2 may have evolved higher transmissibility, though data remain poorly resolved on this point (Lesté-Lasserre 2020). More broadly, if a zoonotic pathogen is eliminated by public health response measures, any changes that microbes evolve during a human-to-human epidemic (i.e. increased transmissibility) will not increase future epidemic risk unless human strains undergo genetic spillback into reservoirs. Many of the frameworks we discuss here are relevant to these cases, but we again exclude them as a subset of multi-host pathogen dynamics, and suggest independent work could explore these dynamics in greater depth.

For conceptual consistency, we also focused largely on non-serological pathogen detection methods, and excluded studies reporting only on serology of an individual or population. The use of serology for inference comes with a wide range of caveats that have been extensively discussed (Gilbert *et al.* 2013), and (most importantly) they are often most representative of *exposure to* a given pathogen rather than *infection with* said pathogen. This fact weakens the inferential value of serological evidence, and so we assess studies that only include serological assays separately, rather than including them in our evidence base (Table S1). The remaining studies comprise molecular pathogen detection, pathogen isolation, or diagnosis via microscopic examination.

Finally, we did not consider pathogens that infect synanthropic animals, rather than infecting humans themselves. Livestock and companion animals host a range of pathogens that have been known to infect wild animals — for example, domestic dogs play an important role transmitting rabies to native wildlife in Africa, creating a substantial problem for conservation of African canids (Lembo *et al.* 2008). Humans have facilitated the spread of several important pathogens in this and similar ways; the widespread amphibian fungus *Batrachochytrium dendrobatidis*, for example, would not likely have become a panzootic conservation threat without the human trade in amphibians as pets and food (Schloegel *et al.* 2009; Wombwell *et al.* 2016; O'Hanlon *et al.* 2018). We consider these cases as outside the remit of our study, as we are specifically interested in the processes that contribute to human-to-animal transmission of human pathogens themselves, rather than more broadly how humans drive the transmission of pathogens in animals.

The distribution of spillback risk across pathogens and hosts

With the novel research synthesized, we found 97 studies describing human-to-animal pathogen transmission. This list represents a substantial advance from the 19 studies retained from a previous review of zooanthroponoses (Messenger *et al.* 2014). As a whole, these studies suggest that spillback events themselves are well-documented and diverse, but the evidence for ecological and public health-related consequences of spillback is far sparser (Table S1).

A diverse set of pathogens appear in our dataset, with representatives from 8 bacterial genera, 10 viral families, 3 fungal classes, 6 protozoan genera, 5 helminth genera, and 1 parasitic mite. For example, documented pathogens include viruses (measles virus, herpesviruses, human respiratory syncytial virus, polio virus, hepadnaviruses related to hepatitis B virus, adenoviruses, noroviruses, rotaviruses, influenza viruses, and human metapneumovirus), bacteria (*Mycobacterium tuberculosis*, bacteria in family *Enterobacteriaceae, Staphylococcus, Streptococcus,* and *Helicobacter*), and eukaryotic parasites (*Cryptosporidium*, *Giardia*, scabies, and various nematodes and helminths) (see Table S1 for references). These pathogens are transmitted via a range of modes, including aerosol and droplet

transmission (tuberculosis, influenza), direct contact (scabies, *Candida* fungus), and faecal-oral transmission (helminths, rotavirus, and *Giardia*). Spillback can therefore emerge from a range of close encounter scenarios involving either humans or their waste.

The selection of 104 host species uncovered by our review was phylogenetically skewed, particularly relative to the wide range of pathogens involved. Most notably, our literature search reaffirms that spillback is common to non-human primate (hereafter, "primate") populations (Nizeyi *et al.* 2001; Goldberg *et al.* 2007; Kaur *et al.* 2008; Palacios *et al.* 2011), which comprised 57/97 (58.8%) of our studies. Phylogenetic distance is a strong predictor of an animal's capacity to transmit pathogens to humans (Olival *et al.* 2017; Albery *et al.* 2021), and our literature review supports the bidirectional nature of this relationship. That is, because they are our closest living relatives, primates are both more likely to act as sources for pathogens that can infect humans, and to become infected with pathogens of humans (Pedersen & Davies 2009).

Human pathogens were also reported in a diverse range of non-primate hosts, including carnivores (e.g. *Giardia intestinalis* protozoa in African wild dogs, influenza A virus in cheetahs, and rotavirus in Japanese raccoon dogs); birds (e.g. *Campylobacter* bacteria in kiwis and penguins, *Candida albicans* fungus in crows and hawks); and bats (e.g. Cryptosporidium protozoa in flying foxes or Staphylococcal infections in Egyptian fruit bats or Nathusius' pipistrelle). Over a quarter (15/44; 34.1%) of non-primate records involved *Mycobacterium tuberculosis* infecting elephants, a number of different ungulates (tapirs, addax, babirusa pig, black rhinoceros, bongo, eland, Hanuman langur, lesser kudu, mountain reedbuck, nyala, Rocky Mountain goat, sable antelope, Scimitar horned oryx, warthog, and waterbuck), captive birds (mealy parrot, green-winged macaws, and African grey parrot), mesocarnivores (banded mongoose and suricate), and a rodent (beaver) (Table S1).

Although it is thus clear that spillback *can* happen in a wide range of hosts, the non-primate species involved were largely long-lived, charismatic, and from captive populations, which are generally subject to elevated study effort and increased exposure to human pathogens. Specifically, our dataset contained an abundance of elephants, large carnivores in zoos, and long-lived birds. There was a notable absence of rodents (only 7 rodent species out of >2000 known to science) and ungulates (only 18 of 327 extant species), and fast-lived animals in general. There were only 6 species of bat, despite the fact that bats comprise ~20% of mammal species and (because they are important zoonotic hosts) have been heavily sampled for pathogens (Li *et al.* 2010; Ge *et al.* 2012; Barr *et al.* 2015; Banskar *et al.* 2016; Wu *et al.* 2016). Although life history (e.g. reproduction, survival, sex ratio, etc.) is an important predictor of zoonotic risk (Gibb *et al.* 2020; Mollentze & Streicker 2020; Albery & Becker 2021), it seems unlikely that these charismatic, long-lived species are truly more susceptible to human-infecting pathogens; instead, it is possible that these species are known to host human pathogens because they more frequently live alongside humans and are therefore more often exposed, or because they have simply been more intensively monitored and sampled (Albery *et al.* 2021). This skew confirms that the observation of human pathogens in animal populations is heavily dependent on the attention that humans are paying to those populations, and future analyses of spillback may have to deal with the fact that exposure and observation are heavily confounded in this way. Furthermore, this bias implies that there could be a hidden burden of human-origin pathogens spread across small, fast-lived animals, just as there is a hidden diversity of zoonotic pathogens lying unsampled (Carlson *et al.* 2019), which could have unforeseen consequences for the perceived risk landscape of secondary spillover (see below). Overall, data suggest that human pathogens from diverse taxonomic groups and transmission modes pose a spillback risk, and primates are disproportionately well-represented as hosts. Beyond primates, spillback incidents are widely distributed, but the collected evidence base is heavily skewed towards large, slow-lived animals. These findings confirm that an animal's spillback risk is strongly influenced by two of the main rules governing an animal's risk as a zoonotic reservoir: evolutionary relatedness and spatial proximity to humans (Olival *et al.* 2017; Albery *et al.* 2020). That is, an animal is more at risk of

infection with human pathogens if it is more closely related and therefore more susceptible (i.e. primates), or if it is in closer proximity and therefore more exposed (i.e. captive or zoo animals). Moreover, these findings demonstrate a widely-observed sampling bias that emerges again from human proximity: animals under stricter surveillance have more often been observed with human pathogens, demonstrating that spillback inference is vulnerable to well-documented sampling biases (Olival *et al.* 2017; Mollentze & Streicker 2020; Albery *et al.* 2021). These risk factors, while unsurprising, make a promising argument that spillback and spillover are governed by qualitatively similar, roughly symmetrical processes.

However, demonstrating that spillback has happened widely does not prove that it poses a threat: infection in one species rarely produces the same clinical outcome in others, and thus, human pathogens may not necessarily cause morbidity and mortality in their new animal hosts. For example, Reston virus is nonpathogenic in humans yet causes mortality in macaques (Demetria *et al.* 2018); conversely, herpesvirus B is apathogenic in macaques but fatal in humans (Engel *et al.* 2002).

Pathway 1: The conservation threat of spillback in context

Using our database, we evaluated the evidence that spillback can cause morbidity and mortality in new hosts, and thus have a potential cost to conservation. The majority of documented spillback events (61/97, or 62.9%) resulted directly in observed morbidity or mortality in the naive animal population (Pathway 1, Figure 1A-C). This number confirms that human pathogens can have meaningful and observable health impacts across a wide range of hosts (Table S1), and therefore might pose a non-negligible conservation threat. Of these, a substantial 50 reports (82.0%) described captive populations (e.g. zoo, rehabilitation center, owned animals, etc.) or habituated primates subject to ecotourism (Table 1, Table S1). These scenarios involve animals in close proximity to humans, with regular visits from or observations by humans. As well as driving higher rates of spillback and observation thereof (see above), this consistent monitoring likely allows ready identification of unhealthy or dead animals compared to wild or unmonitored populations.

Many of the references concerning spillback in primates (24/57, 42.1%) reported on infection in habituated primate populations maintained in national parks or natural areas. These are nominally wild populations, many of which are of conservation concern; however, most are closely monitored with longitudinal sampling and safeguarding (Dunay et al. 2018). Their proximity to park rangers and tourists likely increases the risk of zooanthroponosis, but the option of veterinary care or post-mortem examination in instances of morbidity and mortality also allows for close health monitoring. Moreover, ecotourism provides vital support to the ongoing conservation of these species (Ryan & Walsh 2011). Habituation therefore presents a substantially increased risk of spillback, but this risk may be alleviated by increased disease surveillance and veterinary attention, and the disease-related costs could be countered by the financial benefits of tourism.

Strikingly, only 11/61 (18%) of reports of spillback-associated health impacts involved free-ranging animals: 5 concerned *Mycobacterium tuberculosis* in elephants or mesocarnivores; 3 described seals or skunks infected with influenza A virus; 1 described rotavirus in Southeast Asian mesocarnivores; 1 described a free-living hedgehog with *Streptococcus pyogenes*; and 1 described *Candida albicans* in animals presented to rehabilitation centers. There were also 3 comparative studies (4.9%) describing infection in both captive/habituated and free-ranging populations, including two describing multi-species mortality events caused by *M. tuberculosis* and a diagnostic work-up evaluating resistant *Staphylococcus aureus* isolates from zoos and wildlife rehabilitation centers (Table S1). There are two potential explanations for the relatively low frequency of documented health impacts in free-ranging animals: underreporting of spillback events, or low virulence of pathogens in more distant relatives (i.e.,

non-primates). Underreporting may be due to morbidity and mortality events going undetected in the wild, and to logistical difficulties conducting health assessments in free-ranging populations. It is difficult to identify empirically which of these processes is responsible, particularly given the low number of relevant studies. This difficulty with extricating exposure, sampling bias, and clinical impacts accentuates the difficulty of assessing spillback's conservation threat, encouraging the use of alternative lines of evidence.

Paradoxically, one line of evidence that we can interpret to understand the conservation risk of spillback is the absence of evidence itself — particularly emerging from long-term ecological studies and zoo populations. Worldwide, dozens of wild animal populations have been studied seasonally or year-round by human researchers, each over the course of several decades, and many of these studies include a disease component (Clutton-Brock & Sheldon 2010; Hayes & Schradin 2017). These populations are generally well-understood and occasionally individuals are known by name; as such, oddly behaving, sick, or dying individuals will be noticed and sampled, with infectious disease considered as a potential cause. For example, an avian pox epidemic in Britain was quickly detected in a long-term study of great tits (*Parus major*) using standard non-disease-focussed sampling procedures (Lachish *et al.* 2012). Similarly, it is worth noting that when a disease-related conservation crisis arises, researchers do think of spillback fairly readily: for example, when >60% of the global population of Saiga antelope (*Saiga tatarica tatarica*) died off in Kazakhstan in 2015, human pathogens were specifically considered and rejected as a potential cause (Kock *et al.* 2018). Nevertheless, despite their close proximity to humans and many decades of high-intensity observations, we found no incidental records of spillback occurring into long-term study populations like these. It is possible that individual animals are regularly infected with human pathogens and suffer disease without being detected; however, this possibility itself undermines the premise that spillback presents a substantial conservation risk, because it implies limited clinical significance of these pathogens, particularly due to onward intraspecific transmission: if animals suffer mortality from human pathogens, the infections are more likely to be detected. This is a strong indication that human pathogens have been relatively unlikely to cause morbidity or mortality sufficient to threaten otherwise successful populations.

Similarly, despite the common assertion that captive animals are commonly kept in high-risk environments for disease, there are surprisingly few reports of human pathogens in zoos or wildlife rehabilitation centers — only 23 examples emerging from the millions of zoo animals that exist under extreme scrutiny by veterinary professionals (Table S1). We interpret this to demonstrate that either spillback events (and associated health impacts) happen relatively infrequently, or that they most often occur through events that are unlikely to arise within zoos, such as faecal-oral transmission or violent interactions (Box 1). Alternatively, concerns about public perception of such reports may result in limited disclosure of disease transmission in zoos and other similar establishments.

What do we know about the conservation status of the spillback-affected animals in our database and their relationships with infectious disease? Of the 56 species for which spillback occurred in free-ranging or habituated populations, 13 (23.2%) were classified as either endangered or critically endangered by the IUCN, and 6 (12.5%) of these had infectious disease listed as a salient threat. Of those 6, the African wild dog is the only non-primate species threatened by infectious diseases present as a result of human encroachment (Woodroffe & Sillero-Zubiri 2020); however, the chief reason for this status is rabies and other canine-associated diseases, rather than human-infecting pathogens specifically. The other 5 species for which infectious disease is listed as a threat (all primates) have other activities listed as more impactful than infectious disease to their population, predominantly associated with land use change (Humle *et al.* 2016; Plumptre *et al.* 2016; Greer *et al.* 2018; Maisels *et al.* 2018; Hickey *et al.* 2020; Wallis *et al.* 2020). While the IUCN Red List threat assessment suggests that the most significant threat to most of these species is habitat loss resulting from land use change,

the threat of infectious disease can interact with other anthropogenic stressors (Heard *et al.* 2013), and so should not be discounted.

Climate and land use change have well-appreciated links with wildlife disease, and are generally thought to be among the foremost drivers of disease emergence in the anthropocene (Jones *et al.* 2008; Gibb *et al.* 2020; Carlson *et al.* 2021). Habitat loss results in resource competition and nutritional stress, suppressing individuals' immune systems and rendering them prone to opportunistic infections while simultaneously driving greater pathogen exposure rates through increased interspecific contact (with humans and domestic animals). Small, fragmented populations that are subject to a range of anthropogenic threats may be further stressed and vulnerable to the introduction of novel human pathogens in a way that current surveillance operations have been unable to identify, and thus spillback could provide the "final nail in the coffin" in some circumstances. Therefore, surveillance for human pathogens should not be disregarded as an important priority for threatened animal populations.

Alternatively, spillback's effect could be overshadowed by other, more immediate threats. In areas where wild lands are actively being degraded for resource extraction, spillback risk to the native wildlife could pale in comparison to habitat destruction — for example if the population as a whole is unlikely to survive the destruction of a vital habitat, such that exposure to human pathogens is unlikely to affect them further. As a parallel, inbreeding is occasionally thought to be an important determinant of endangered species' declines, but inbreeding may not contribute to extinctions in cases when population declines begin before inbreeding has a chance to substantially reduce population viability (Lande 1998). Monitoring how human pathogens threaten endangered populations that are under other concurrent existential threats, and asking whether spillback's conservation impact is exacerbated or inhibited by the impact of other anthropogenic forces, will be an important component of future investigations into the threat of spillback.

Pathway 2: The surprising scarcity of spillback-generated maintenance reservoirs

In contrast to **Pathway 1**, documented instances of **Pathway 2** (Figure 1D-E) are infrequent. In fact, we were unable to identify a verified example of novel maintenance of a human pathogen in any of our 97 studies. There were multiple examples of pathogens infecting populations or individuals that appeared healthy, and therefore more likely to survive long enough to transmit the pathogen further (Table S1); however, all of these findings were the results of cross-sectional surveillance and thus could have been produced by a recent spillback event rather than extended maintenance and transmission within the population. Other examples of human-to-animal transmission resulting in a novel maintenance population are marred by uncertainty or caveats, and some have been refuted: for example, although humans are thought to be the main source of leprosy (*Mycobacterium leprae*) for wild animals, leprosy was recently confirmed in several disparate populations of chimpanzees with no history of prolonged contact with humans, implying the existence of an unknown animal or environmental reservoir (Hockings *et al.* 2020). This example demonstrates the inferential difficulties facing spillback researchers, which we further detail below. Regardless, this absence of evidence accentuates that future spillback-related studies should aim to assess whether or not the focal pathogen is being maintained, or whether it is the result of recent human-to-animal transmission.

Moreover, despite our targeted search, the only documented report of "secondary spillover" from a novel host back into humans (Figure 1E) was of SARS-CoV-2 from farmed mink into farm employees (Munnink et al., 2020). If wild and farmed mink represent a widely susceptible potential reservoir, they could make it very difficult to eliminate the pathogen (as outlined above). Perhaps the best example of this phenomenon is of feral dogs (*Canis familiaris*) and dracunculiasis ("Guinea worm"), caused by the nematode *Dracunculus medinensis* in sub-saharan Africa. Although the disease is nearing elimination, recent re-emergence events have been traced to feral dog populations (Eberhard *et al.* 2014;

McDonald *et al.* 2020), severely complicating efforts to eliminate the disease using public health measures (Hopkins *et al.* 2019). Note that we do not count this example as a documented secondary spillover because, while Guinea worm provides an excellent example of the difficulties created by animal reservoirs in elimination settings, the spillback story is complicated by the fact that it is unclear whether dogs' infections were sourced by humans recently (making them a novel host), or whether they are a long-standing maintenance reservoir that have played a role in *D. medinensis* ecology for a considerable time (McDonald *et al.* 2020).

The surprising scarcity of evidence for Pathway 2 suggests that secondary spillover is not currently a widely-supported threat to health security. The dynamics observed with SARS-CoV-2 in mink are apparently relatively unique; concerns about secondary spillover, or about the preservation of human-adapted viral lineages in novel wildlife reservoirs, are mostly speculative. In cases like a multinational epidemic (e.g., the Ebola virus epidemic in West Africa) or a pandemic (e.g., SARS-CoV-2), our data suggest that secondary spillover likely represents a minor risk compared to the challenges of outbreak response and possibility of unsuccessful containment and elimination in humans themselves.

Challenges to observing spillback events

Why is secondary spillover (and Pathway 2 more broadly) so rare? We suggest that this can probably be explained by the complexity of interspecific transmission, and the number of steps required for these events both to occur and to be observed. Interspecific pathogen transmission is well-appreciated as a multi-stage process, involving interspecific encounters, exposure to a novel pathogen, pathogen invasion, replication, and (possibly) transmission onwards (Alexander *et al.* 2018; Becker *et al.* 2019). Each of these processes requires the pathogen to pass a series of filters, such that the probability of progression decreases with each step (Plowright *et al.* 2017). It is relatively simple for **Pathway 1** to manifest empirically: spillback takes place (exposure and invasion) and the pathogen causes sickness, involving some degree of pathogen survival and replication within the new host. In contrast, for **Pathway 2** to manifest empirically, exposure and invasion must occur as in **Pathway 1**, and subsequently the pathogen must replicate to the point of onward transmission and attain an R0 of greater than 1 in the new animal population. This event is seemingly rare: only ~10% of zoonotic pathogens have achieved this status in humans (Woolhouse & Brierley 2018). Subsequently, the pathogen must then spill over *again* into human populations, following the exact same series of events but in the reverse direction, and all under the assumption that the pathogen does not cause rapid mortality (as it frequently will (Best & Kerr 2000; Rothenburg & Brennan 2020)), thereby diverting it towards **Pathway 1** (Fig 1C).

Assuming that spillback, maintenance, and secondary spillover into human populations *do* take place, our empirical inference must then contend with a series of sampling processes. For **Pathway 1**, the sampling is fairly simple: human observers notice sick or dying animals, and identify the aetiological agent as being human in origin (e.g. SARS-CoV-2 in tigers at Bronx zoo (McAloose et al., 2020)). This process is facilitated by the fact that, as mentioned above, most of these scenarios involve captive, domestic, or habituated animals that are under close observation and in close proximity to humans - the same characteristics that drive their spillback risk by elevating their rates of exposure to human pathogens. This sampling process presumably generates the bias towards large charismatic animals that we detail above. For **Pathway 2**, because novel maintenance reservoirs are perhaps more likely to become established if the pathogen does not cause extreme pathology or host die-offs, they may be inherently more difficult to detect — potentially requiring active sampling rather than passive observation. If prior knowledge of the wild animal's pathogen community is limited before the spillback event, it may be impossible to reconcile the directionality of the observed shift (e.g. it remains plausible that palm civets were spillback hosts for SARS-CoV in at least some circumstances (Tu *et al.* 2004;

Wang *et al.* 2005)). Moreover, if the maintenance population does source secondary spillovers, the new human infections must then be traced back to the animal population of origin, which must then be verified as a novel host that was infected by a human (rather than a natural reservoir of a known multi-host pathogen).

A perceived absence of secondary spillover events could also emerge from the bias towards surveillance of charismatic slow-lived animals that we outline above. Fast-lived animals are thought to host and source a disproportionate number of zoonoses as a result of their high abundance, lower immune investment, and proximity to humans (Albery & Becker 2021); therefore, it is possible that instances of spillback in free-ranging animal populations are underreported due to sampling bias and underrepresentation of fast-lived animal species like rodents, and the use of pseudoabsences for unsampled species (Albery & Becker 2021). Similarly, we found almost exclusively positive reports of pathogens: only two studies reported negative findings for human pathogens in free-ranging or habituated animal populations (Bonnedahl *et al.* 2005; Benavides *et al.* 2012). Although these studies were not included in our assessment and in the statistics we report (because they did not report an instance of spillback), confirming the *absence* of human pathogens in wild populations will provide an important baseline for future spillback monitoring purposes, and for countering the observation bias evident in our dataset.

Due to the complexity of the processes involved and the limited relevant evidence, our ability to answer the question “how much of a threat might spillback pose to free-ranging wildlife populations?” is currently severely limited. Nevertheless, it is vital that we attempt to answer this question as pathogens continue to emerge in human populations at an accelerated rate, with some reaching a very high prevalence in human populations (e.g. SARS-CoV-2). The problem involves weighing spillback’s low probability but potentially great consequences: failing to appreciate the threat of spillback could, in the worst case, result in species extinctions or the successful establishment of a pathogen in a novel maintenance reservoir that then acts as an important source of zoonotic infections for the foreseeable future. Conversely, overreacting to this probability risks overenthusiastically diverting scarce funds from outbreak response into spillback prevention, or limiting zoonotic disease research unnecessarily. Reducing this possibility requires an evidence-based risk assessment that currently eludes us.

How to infer and understand spillback potential

Given the difficulty of characterising high-risk hosts and pathogens with the available evidence, researchers will likely have to continue relying on case-by-case assessment of spillback risk for a focal pathogen (Figure 2). Such understanding will come from three main sources: 1) laboratory experiments demonstrating host-pathogen compatibility (e.g. *in vivo* infections); 2) host susceptibility demonstrated by incidental transmission in captive or managed animal populations; and 3) historically documented transmission events in free-ranging animal populations. All of these scenarios possess strengths and limitations in their ability to inform the complete ecological narrative, which we discuss here. To assess spillback risk, researchers should apply a combination of the three approaches, and in time the evidence base may build to the point that it is possible to carry out meta-analyses and to build predictive models for spillback in the same way as others have for spillover (Olival *et al.* 2017; see below).

1) Laboratory experiments. Because zoonotic pathogens by definition possess the ability to infect more than one host, researchers often ask whether a particular vertebrate could host the pathogen using *in vivo* and *in vitro* infection experiments. For example, in an effort to characterize potential sylvatic spillback reservoirs for SARS-CoV-2 in the last year, researchers have successfully infected deer mice, tree shrews, ferrets, rabbits, bushy-tailed woodrats, striped skunks, Egyptian fruit bats, and

raccoon dogs (Fagre *et al.* 2020; Freuling *et al.* 2020; Griffin *et al.* 2020; Mykytyn *et al.* 2020; Schlottau *et al.* 2020; Shi *et al.* 2020; Zhao *et al.* 2020; Bosco-Lauth *et al.* 2021). Equally importantly, researchers have identified wildlife species that are more difficult to infect (big brown bats, cottontail rabbits, fox squirrels, Wyoming ground squirrels, black-tailed prairie dogs, house mice, and raccoons) (Hall *et al.* 2020; Bosco-Lauth *et al.* 2021). These findings can establish new model animal systems, interrogate the cellular and molecular underpinnings of susceptibility, and provide clues about a given host's possible reservoir status.

Successfully infecting an animal in the lab is critical, incontrovertible evidence that it *could* become infected in the wild through incidental spillback events. However, these approaches may offer limited information about the total risk of spillback in the wild for several reasons. First, they do not take into account the ecological nuances of contact rates or transmission routes between susceptible hosts: that is, while they approximate interspecific *compatibility*, they ignore the role of *opportunity* in determining patterns of infection in nature. Further, experimental studies often use very high infectious doses or exposure techniques like direct inoculation (Gerdts *et al.* 2007), which are extremely unlikely to be recapitulated in the wild. Laboratory animal populations may also differ immunologically from their wild counterparts in ways that could affect their propensity to host and transmit diseases. For example, recent work has shown that the immune systems and microbiota of laboratory house mice (*Mus musculus*) change considerably when they are (re)introduced to wild environments, altering their resistance to pathogens (Leung *et al.* 2018; Bär *et al.* 2020). As such, mice could be interpreted as a spillback risk for a given pathogen based on results in laboratory populations, while wild counterparts are in fact minimally susceptible and therefore less vulnerable to spillback. This argument could apply to a range of laboratory-maintained animals. Similarly, a recent deworming study in wild *Peromyscus* mice found that helminth coinfection reduces the prevalence of Sin Nombre Virus (Sweeny *et al.* 2020); lab-reared *Peromyscus* do not host helminths, whereas wild animals are nearly all infected, and so their susceptibility in the wild is in fact lower than would be expected based on laboratory experiments. Finally, experimental approaches are limited to hosts that can be housed and maintained (which excludes many large animals and species of conservation concern), and pathogens that can be cultured and administered (which eliminates several species of anaerobic bacteria, metazoan parasites with complex life cycles, and more).

2) Incidental captive infections. Incidental spillback events are relatively commonly documented in zoos and captive environments, when an animal falls ill with a human pathogen contracted from a handler or visitor (Michalak *et al.* 1998; Wang *et al.* 2020). Such events comprised 52/97(53.6%) of our spillback records, and 12 (12.3%) reports reflected pathogen detection in captive healthy animals. While such transmission events have resulted in some morbidity or mortality (Oh *et al.* 2002; Crossley *et al.* 2012; McAloose *et al.* 2020; Munnink *et al.* 2020a) as well as onward transmission of the pathogen (McClure & Keeling 1971; Li *et al.* 2014; Oreshkova *et al.* 2020), these scenarios still do not accurately recapitulate the ecological conditions affecting free-ranging populations, for several reasons. For example, direct contact rates between humans and captive animals likely far surpass those in the wild, increasing the probability that spillback will occur in the first place. Post-exposure, captive animals may also be immunosuppressed compared to their free-ranging counterparts due to inadequate housing conditions (Fischer & Romero 2019; Seeber *et al.* 2020), which could lower their resistance and paint an exaggerated picture of their susceptibility. Further, following successful initial infection, confined housing may result in artificially high contact and transmission rates, so that their ability to transmit and maintain the pathogen is similarly exaggerated compared to wild animals. As such, opportunity, compatibility, and reservoir competence are all potentially exaggerated in captive contexts, likely inflating perceived spillback probability in the wild for both Pathway 1 and 2. These incidents are useful in that they provide a more realistic view of infection dynamics than laboratory experiments, but they fall short of being truly representative of spillback risk for these reasons.

3) Incidental wild infections. Because laboratory and captive sources do not accurately reflect the conditions experienced by wild populations, documented historical transmission events in free-ranging or habituated animal populations in the wild are the most biologically relevant standard for spillback evidence, comprising 45/97 (46.4%) of our evidence base. Unfortunately, these examples come with their own set of inferential difficulties. Most importantly, directionality is difficult to determine in observational contexts in the absence of molecular analysis and epidemiological tracing (Nizeyi *et al.* 2001; Palacios *et al.* 2011). Verifying that humans were responsible for transmitting a pathogen to an animal (rather than e.g. both receiving the pathogen from the same source) requires a combination of well-understood transmission mechanisms, verified human-animal contact, known local prevalence in both humans and animals, and time-structured phylodynamic sampling of both populations. At macroscopic scales, time-structuring or geographic expansions are occasionally sufficient to assume zooanthroponosis (see vector-borne examples above). For example, newly emergent strains of Influenza A (H1N1) were detected in striped skunks (*Mephitis mephitis*) at the height of flu season, which was deemed highly unlikely unless humans were sourcing the disease (Britton *et al.* 2019). Similarly, Zika virus was discovered in New World primates following introduction of the virus to the Americas (Favoretto *et al.* 2019), implying that humans were responsible for transporting it.

Directionality becomes difficult to determine in observational scenarios when a pathogen achieves a prevalence threshold and is maintained in both human and animal populations. In the case of influenza A viruses (IAV), transmission from humans to swine has been documented, and is even considered responsible for a majority of viral diversity in farmed swine (Zhou et al., 1999), but directionality is difficult to infer without molecular tracing. While the decreased cost associated with next-generation sequencing has accelerated these efforts, simultaneous circulation of IAVs in migratory birds, farmed swine, and human populations complicates analysis of spatiotemporal patterns and directionality (Lam *et al.* 2012; Roche *et al.* 2014). Similarly, *Mycobacterium tuberculosis* complex (MTC) bacteria have been shown to transmit from humans to cattle (Fritsche *et al.* 2004), primates (Coscolla *et al.* 2013), and elephants (Zachariah *et al.* 2017). Although they are ancestrally human pathogens, in many regions where MTC is both endemic and enzootic, epidemiological tracing becomes nearly impossible. A final obstacle comes in cases where the pathogen exhibits latent transmission through the environment, such that the lack of direct human-animal contact makes causality and directionality even harder to infer. In this case, tracking down contaminated areas or environmental sources of pathogens is important and can provide clues.

In summary, our evidence for spillback is based on several lines of inquiry (Figure 2), none of which are alone sufficient to understand spillback risk to wildlife in natural settings. While laboratory experiments and captive infection reports are indicative of host-pathogen compatibility, they occur in potentially non-representative (high-susceptibility and high-exposure) environments that may be minimally relevant to the risk of spillback *in situ*. Meanwhile, observational reports of spillback in wild populations are fraught with inferential difficulties inherent to ecological systems. Further, a lack of directed sampling for human pathogens in wild populations has produced an evidence base that is biased towards large, captive animals. Unfortunately, when observational reports are received, it is too late from a conservation and public health perspective: all they can tell us currently is whether or how often spillback has happened in the past. Therefore, when researchers are concerned about a specific pathogen (e.g., SARS-CoV-2), experiments are necessary to properly anticipate spillback risk.

Ideally, to overcome the shortcomings that we have outlined, we suggest that more experiments aimed at identifying spillback risks be carried out in semi-natural settings that more closely approximate the conditions experienced by wild populations. Such studies are already conducted in some contexts: for example, researchers verified the SARS-CoV-2 spillback risk of *Rousettus aegyptiacus* bats by inoculating individuals and then observing as the virus was transmitted to co-housed animals (Schlottau *et al.* 2020). Emulation of a natural environment and stressors faced in nature is difficult, and answering

these questions (particularly without breaching ethical animal husbandry procedures) will require collaboration between virologists, immunologists, veterinarians, and field ecologists. Future investigations could also aim to surveil areas of suspected high spillback risk, across a wider range of hosts, to increase the observability of incidental wild infections when they do occur. Ultimately, the more evidence that we can accumulate, the easier it will be to build advanced models of spillback risk for use in prediction and prevention efforts.

The future of spillback research: zoonotic prediction in reverse

A crucial step to understanding spillback, and the logical end point of a sufficiently bolstered evidence base, is the construction of sophisticated models of cross-species transmission to predict spillback risk. Using analogous models applied to other infectious disease processes, researchers have made enormous strides towards understanding host-pathogen ecology, allowing reservoir host identification (Babayan *et al.* 2018; Albery *et al.* 2020), zoonotic risk prediction (Mollentze & Streicker 2020), pinpointing of geographic sampling gaps (Han *et al.* 2016; Olival *et al.* 2017), and more. Although model results are not always well-integrated into pandemic prevention efforts (Holmes *et al.* 2018; Carlson 2020), they are crucial for understanding broad-scale patterns of zoonotic spillover — and the same will be true of spillback. As it stands, researchers have just produced a model-based prediction of spillback risk for the most intensively-studied pathogen in recent years (SARS-CoV-2 (Fischhoff *et al.* 2021)), and given enough data on broad-scale spillback trends, we may be able to do the same more broadly in the near future.

In principle, host-pathogen models should be applicable to spillback, just as they are to zoonotic spillover. Interspecific pathogen transmission is a two-way street, and humans can be conceptualised as “just another host” for a multi-host pathogen. For example, as outlined above, species that are closely related to humans (i.e., primates) are both more likely to source zoonoses and to be potential recipients of human pathogens (Heldstab *et al.* 1981; Engel *et al.* 2002; Terzian *et al.* 2018). As such, it stands to reason that larger datasets on spillback (and large-scale analyses of host and pathogen traits) will allow us to quantify the relative contribution of processes underlying spillback in the service of prediction. However, doing so will require subtle changes in analytical framing. For example, models are often framed as “predicting the *original* reservoir host(s)” (Babayan *et al.* 2018; Becker *et al.* 2020; Brierley & Fowler 2020), rather than “predicting *potential future* reservoir hosts.” This is also often true of laboratory infection studies (Botten *et al.* 2000; Richter *et al.* 2004; Cogswell-Hawkinson *et al.* 2012; Jones *et al.* 2019), although work on SARS-CoV-2 is a notable exception given the concern over spillback (Fagre *et al.* 2020; Griffin *et al.* 2020; Hall *et al.* 2020; Schlottau *et al.* 2020; Fischhoff *et al.* 2021). Because of the different ways that we obtain and interpret human and animal infection data, it remains a near-total unknown whether these approaches are equally valid, whether spillover and spillback are symmetrical processes, and whether the answers to these questions depend heavily on the host and pathogen of interest (Box 1); nevertheless, this symmetry is sometimes implicitly assumed to be the case.

Important caveats must be considered when constructing models of spillback. First, while spillover and spillback plausibly follow a similar set of rules, these rules do not necessarily act symmetrically on transmission to and from humans (see Box 1). Researchers may be able to construct models of spillback risk based on spillover-related processes and then to test their predictions with spillback datasets, or *vice versa*, in order to verify whether these approaches are valid (Fischhoff *et al.* 2021). Second, while it is likely universally useful to understand and predict how pathogens transmit from animals to humans, it is possible that a smaller subset of spillback-related frontiers will be worthy of (urgent) consideration or investment in mitigation. For example, we are increasingly able to understand the genomic basis of zoonotic potential (Mollentze & Streicker 2020), but the time and financial

investment required to predict the genomic basis of infection could feasibly be equally expensive for every other host species, such that the cost of developing a predictive framework for genomic prediction for every animal species could be prohibitive. Alternatively, the same genomic signatures may be generally applicable to predicting a pathogen's spillover and spillback potential (e.g. if they are indicative of wide host range), rendering the investment worthwhile. Regardless, identification of at-risk species or groups could lead to researchers prioritising high-risk groups for further identification, leading to a better understanding of the general drivers of infection in both humans and animals. Although the experimental infections of a range of animals with SARS-CoV-2 were partially motivated by concern about spillback risk (Bosco-Lauth *et al.* 2020; Fagre *et al.* 2020; Olival *et al.* 2020; Schlottau *et al.* 2020; Shi *et al.* 2020), their findings are likely to eventually inform the underlying physiological and ecological mechanisms responsible for mediating susceptibility. For example, the knowledge that brown bats are relatively resistant to infection with SARS-CoV-2 may lead to further investigations into the immune mechanisms responsible for viral clearance (Hall *et al.* 2020).

Ultimately, integrating the results of statistical models to predict spillback as well as spillover, and more accurately delineating the similarities and differences between the two processes, will draw necessary attention to the perception of multi-host pathogens as part of a complex metapopulation of hosts (Viana *et al.* 2014; Frutos *et al.* 2021). Researchers working on infectious disease at the human-animal interface could benefit from the renewed interest in spillback engendered by the SARS-CoV-2 pandemic, building on the established knowledge in this review. This fuller understanding of spillback and related processes will reveal at-risk hosts and pathogens, informing fundamental biological understanding of the symmetry of interspecific pathogen transmission, and improving human and animal health in the coming century. This kind of understanding is only likely to become more important: ongoing increases in human population density, epidemic and pandemic risk, human-wildlife contact, and anthropogenic stressors on animal health are likely to make spillback a more common phenomenon over the coming decades.

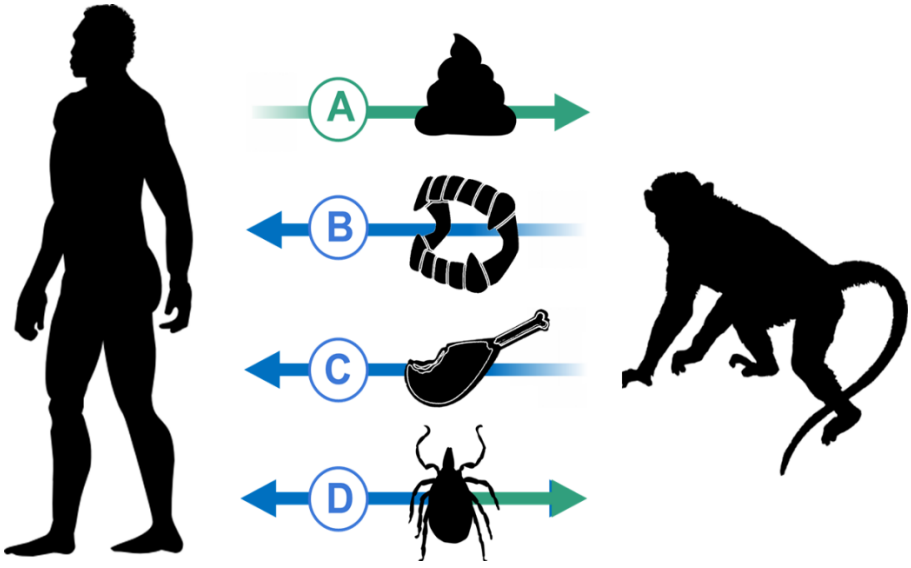
Declaration of Interests

Authors declare no competing interests.

Box 1. Two sides of different coins: symmetry in spillover and spillback

Many studies that investigate animals' susceptibility to human pathogens rest on the implicit assumption that interspecific pathogen transmission is symmetrical — i.e., that pathogens go through the same series of hurdles in transmitting from humans to pathogens as they do in the reverse direction. One of the greatest unknowns concerning spillback is its symmetry with spillover: that is, do the same processes govern transmission from humans to animals as those governing animal-to-human transmission? In reality, a great many processes could create asymmetry in this relationship. For example, host immune cells often use cell surface proteins such as glycans to identify self from non-self; when one species encounters a virus that has just budded off another species' cells, its immune response may be able to more easily identify the glycans of the other species, and the propensity to identify other species' glycans may not be equally effective in both directions. Several mechanisms act on humans specifically — most notably hygiene. Humans wash themselves, use bednets and readily employ other non-pharmaceutical interventions that result in a lower incidence and transmission of infectious disease, both among humans and to animals. Due to their better nutritional state, humans may be more resistant to pathogens than wild animals are, which will further reduce the transmission from humans to animals.

We suggest that contact events between humans and wildlife are more likely to occur in certain directions according to the pathogen's transmission mode. For example, humans may be less likely to inhabit areas that involve concentrated animal waste, whereas a great many animal species are subjected to human sewage or runoff, exposing them more readily to human pathogens (A). Similarly, it is unlikely that humans will bite wild animals, but relatively more likely that the opposite will happen; as a result humans are regularly exposed to rabies, but the reverse is not true (B). Very few animals eat humans, but many humans eat wild animals, which provides a well-established spillover route for food-borne pathogens from animals to humans (C). However, there are some transmission modes that are likely to be more-or-less symmetrical — most notably vector-borne transmission, provided the arthropod does not have narrow host feeding preferences and feeds on both humans and non-human vertebrates (D). Animal silhouettes are from phylopic.org.



Figures and Tables

Figure 1. Pathways detailing the two general scenarios in which spillback is problematic. We separate these scenarios into concerns for 1) conservation and 2) public health. In **Pathway 1**, spillover into humans (A) results in successful zoonotic establishment; humans, which are competent transmitters of the pathogen, then transmit it onto a new animal population in an instance of "spillback" (B). The novel host species in question is not a competent reservoir. Instead, morbidity or mortality caused by the pathogen endangers the population, presenting a conservation risk (C). In **Pathway 2**, the new animal species instead proves a competent reservoir (D), and maintains the pathogen within the population. This population presents a novel spillover risk, potentially creating numerous novel infections in humans that threaten human public health (E). This maintenance population may *also* suffer substantial morbidity and mortality, creating concerns for conservation (C). Silhouettes are taken from phylopic.org.

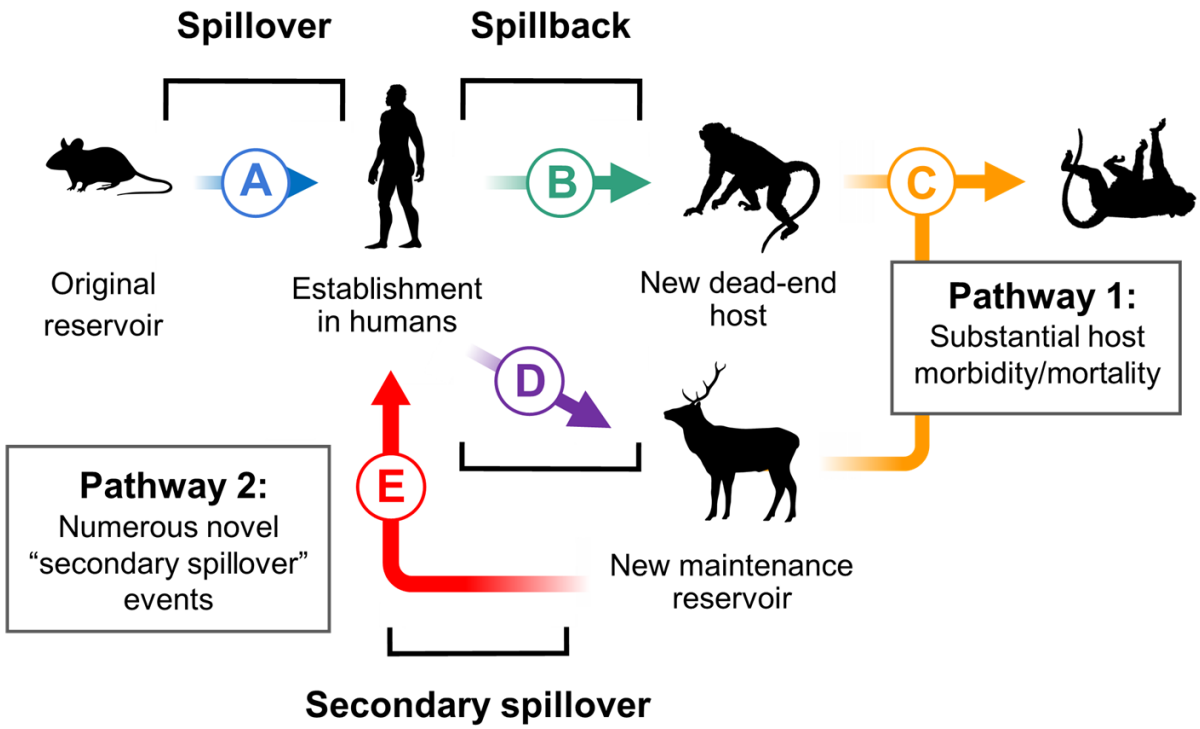


Figure 2. Options for investigating and understanding human-to-wildlife pathogen spillback. Although there is currently a limited evidence base on spillback (1-3), further investigation in the future may allow the formation of spillback datasets and the training of predictive models (4).

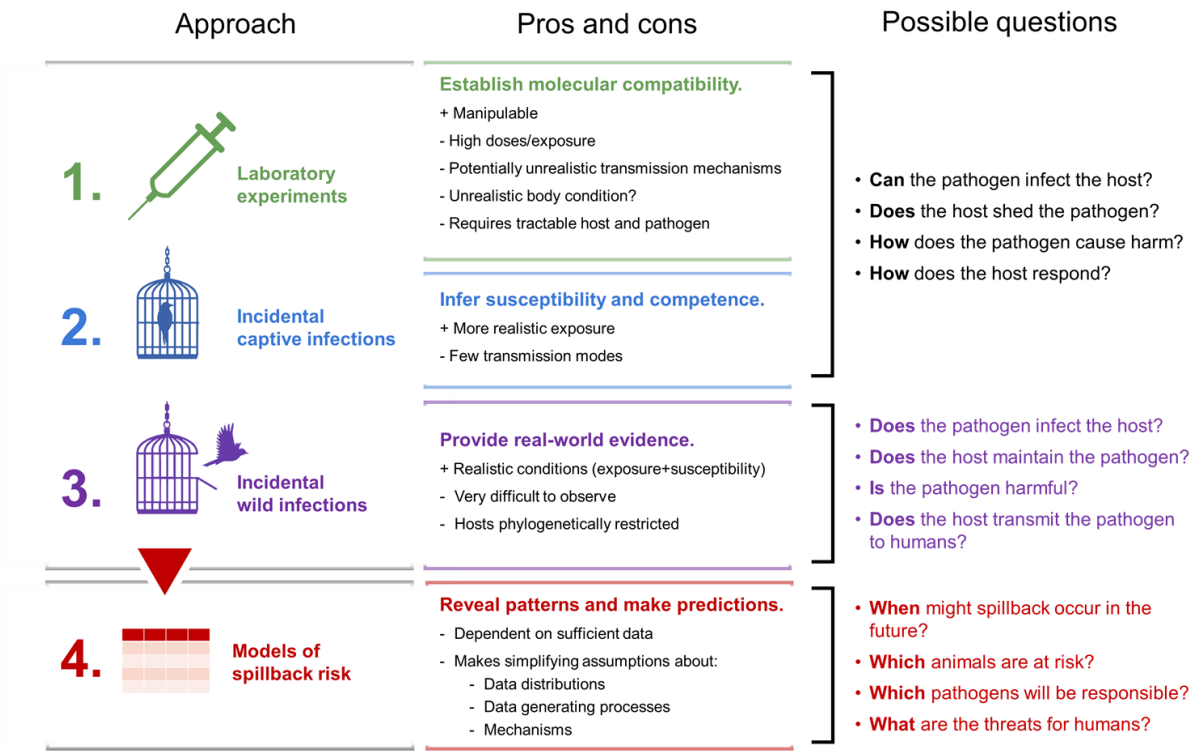


Figure 3. Spillback of SARS-CoV-2 into animal populations has been documented in domestic animals (dogs and cats), farmed wildlife (mink), and captive wildlife (lions, tigers, and a snow leopard in zoos). Movement of SARS-CoV-2 from infected animal populations back into human populations would first require establishment of the virus in the animal population (enzootic potential). Secondary spillover (from animals back into human populations) has only been documented in employees working with infected farmed minked. Silhouettes are taken from phylopic.org.

Interface	Spillback risk: *Human-animal contact frequency*	Enzootic potential: *Intraspecific contact frequency*	Mitigation effort required to prevent secondary spillover	Evidence from SARS-CoV-2	
Companion animals	HIGH	LOW	LOW	Domestic cats, dogs, and ferrets	Halfmann et al 2020
Intensively managed or farmed animals (domestic or wild)	MEDIUM	HIGH	HIGH	Farmed mink	Oreshkova et al 2020
Captive wildlife	MEDIUM	MEDIUM	LOW	Lions, tigers, snow leopard, & gorillas in zoos	McAloose et al 2020
Synanthropic wildlife	MEDIUM	MEDIUM	MEDIUM	Escaped mink	Shriner et al 2021
Novel wildlife reservoirs	LOW	MEDIUM	HIGH	Free-ranging mink	Aguiló-Gisbert et al 2021

Table 1. Studies reviewed are summarized (Table 1a), and further stratified based on primate-only studies (Table 1b) and non-primate studies (Table 1c). Study counts for Tables 1b and 1c do not equal the total count in Table 1a because four studies described pathogens present in both primate and non-primate populations, and thus, were counted in both Tables 1b and 1c.

A. All studies describing spillback (n = 97)

	Captive	Free-ranging	Habituated (NHP)	Comparative	TOTAL
Healthy (or not discussed)	7	10	14	5	36
Morbidity ± mortality	37	11	10	3	61
Total	44	21	24	8	97

B. Studies describing spillback in primates (n = 57)

	Captive	Free-ranging	Habituated	Comparative	TOTAL
Healthy (or not discussed)	4	1	14	2	21
Morbidity ± mortality	23	0	10	3	36
Total	27	1	24	5	57

C. Studies describing spillback in non-primates (n = 44)

	Captive	Free-ranging	Comparative	TOTAL
Healthy (or not discussed)	3	9	3	15
Morbidity ± mortality	16	11	2	29
Total	19	20	5	44

References

Aguiló-Gisbert, J., Padilla-Blanco, M., Lizana, V., Maiques, E., Muñoz Baquero, M., Chillida-Martínez, E., Cardells, J., & Rubio-Guerri, C. (2021). First description of natural SARS-CoV-2 infection in two wild American minks (*Neovison vison*). *Preprints*, 2021030647.

Aizenman, N. (2020). Why The U.S. Government Stopped Funding A Research Project On Bats And Coronaviruses. *Natl. Public Radio*.

Albery, G.F. & Becker, D.J. (2021). Fast-lived Hosts and Zoonotic Risk. *Trends Parasitol.*, 37, 117–129.

Albery, G.F., Carlson, C.J., Cohen, L.E., Eskew, E.A., Gibb, R., Ryan, S.J., *et al.* (2021). Urban-adapted mammal species have more known pathogens. *bioRxiv*.

Albery, G.F., Eskew, E.A., Ross, N. & Olival, K.J. (2020). Predicting the global mammalian viral sharing network using phylogeography. *Nat. Commun.*, 11, 2260.

Alexander, K.A., Carlson, C.J., Lewis, B.L., Getz, W.M., Marathe, M.V., Eubank, S.G., *et al.* (2018). The ecology of pathogen spillover and disease emergence at the human-wildlife-environment interface. In: *The connections between ecology and infectious disease*. Springer, pp. 267–298.

Babayan, S.A., Orton, R.J. & Streicker, D.G. (2018). Predicting reservoir hosts and arthropod vectors from evolutionary signatures in RNA virus genomes. *Science*, 362, 577–580.

Banerjee, A., Mossman, K. & Baker, M.L. (2021). Zooanthroponotic potential of SARS-CoV-2 and implications of reintroduction into human populations. *Cell Host Microbe*, 29, 160–164.

Banskar, S., Bhute, S.S., Suryavanshi, M.V., Punekar, S. & Shouche, Y.S. (2016). Microbiome analysis reveals the abundance of bacterial pathogens in Rousettus leschenaultii guano. *Sci. Rep.*, 6, 1–13.

Bär, J., Leung, J.M., Hansen, C., Hall, A.R., Conour, L. & Graham, A.L. (2020). Strong effects of lab-to-field environmental transitions on the bacterial intestinal microbiota of Mus musculus are modulated by Trichuris muris infection. *FEMS Microbiol. Ecol.*

Barlow, J., Lennox, G.D., Ferreira, J., Berenguer, E., Lees, A.C., Mac Nally, R., *et al.* (2016). Anthropogenic disturbance in tropical forests can double biodiversity loss from deforestation. *Nature*, 535, 144–147.

Barr, J., Smith, C., Smith, I., de Jong, C., Todd, S., Melville, D., *et al.* (2015). Isolation of multiple novel paramyxoviruses from pteropid bat urine. *J. Gen. Virol.*, 96, 24–29.

Becker, D., Albery, G.F., Sjodin, A.R., Poisot, T., Dallas, T., Eskew, E.A., *et al.* (2020). Predicting wildlife hosts of betacoronaviruses for SARS-CoV-2 sampling prioritization. *bioRxiv*.

Becker, D.J., Washburne, A.D., Faust, C.L., Pulliam, J.R., Mordecai, E.A., Lloyd-Smith, J.O., *et al.* (2019). *Dynamic and integrative approaches to understanding pathogen spillover*. The Royal Society.

Benavides, J.A., Godreuil, S., Bodenham, R., Ratiarison, S., Devos, C., Petretto, M.-O., *et al.* (2012). No evidence for transmission of antibiotic-resistant Escherichia coli strains from humans to wild western lowland gorillas in Lopé National Park, Gabon. *Appl. Environ. Microbiol.*, 78, 4281–4287.

Best, S.M. & Kerr, P.J. (2000). Coevolution of host and virus: the pathogenesis of virulent and attenuated strains of myxoma virus in resistant and susceptible European rabbits. *Virology*, 267, 36–48.

Blumberg, S. & Lloyd-Smith, J.O. (2013). Inference of R0 and Transmission Heterogeneity from the Size Distribution of Stuttering Chains. *PLOS Comput. Biol.*, 9, e1002993.

Bonnedahl, J., Broman, T., Waldenström, J., Palmgren, H., Niskanen, T. & Olsen, B. (2005). In Search of Human-associated Bacterial Pathogens in Antarctic Wildlife: Report from Six Penguin Colonies Regularly Visited by Tourists. *AMBIO J. Hum. Environ.*, 34, 430–432.

Bosco-Lauth, A., Root, J.J., Porter, S., Walker, A., Guilbert, L., Hawvermale, D., *et al.* (2021). Survey of peridomestic mammal susceptibility to SARS-CoV-2 infection. *bioRxiv*, 2021.01.21.427629.

Bosco-Lauth, A.M., Hartwig, A.E., Porter, S.M., Gordy, P.W., Nehring, M., Byas, A.D., *et al.* (2020). Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis,

transmission, and response to reexposure in cats. *Proc. Natl. Acad. Sci.*, 117, 26382–26388.
Botten, J., Mirowsky, K., Kusewitt, D., Bharadwaj, M., Yee, J., Ricci, R., *et al.* (2000). Experimental infection model for Sin Nombre hantavirus in the deer mouse (Peromyscus maniculatus). *Proc. Natl. Acad. Sci.*, 97, 10578–10583.
Brierley, L. & Fowler, A. (2020). Predicting the animal hosts of coronaviruses from compositional biases of spike protein and whole genome sequences through machine learning. *bioRxiv*, 2020.11.02.350439.
Briggs, Helen. (2020). Coronavirus: Spain orders culling of almost 100,000 mink. *Br. Broadcast. Corp.*
Britton, A.P., Trapp, M., Sabaiduc, S., Hsiao, W., Joseph, T. & Schwantje, H. (2019). Probable reverse zoonosis of influenza A (H1N1) pdm 09 in a striped skunk (Mephitis mephitis). *Zoonoses Public Health*, 66, 422–427.
Callaway, E. (2016). Dogs thwart effort to eradicate Guinea worm. *Nat. News*, 529, 10.
Carlson, C.J. (2020). From PREDICT to prevention, one pandemic later. *Lancet Microbe*, 1, e6–e7.
Carlson, C.J., Albery, G.F. & Phelan, A. (2021). Preparing international cooperation on pandemic prevention for the Anthropocene. *BMJ Glob. Health*, 6, e004254.
Carlson, C.J., Zipfel, C.M., Garnier, R. & Bansal, S. (2019). Global estimates of mammalian viral diversity accounting for host sharing. *Nat. Ecol. Evol.*, 3, 1070–1075.
Clutton-Brock, T. & Sheldon, B.C. (2010). Individuals and populations: the role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends Ecol. Evol.*, 25, 562–573.
Cogswell-Hawkinson, A., Bowen, R., James, S., Gardiner, D., Calisher, C.H., Adams, R., *et al.* (2012). Tacaribe Virus Causes Fatal Infection of An Ostensible Reservoir Host, the Jamaican Fruit Bat. *J. Virol.*, 86, 5791–5799.
Cook, J.D., Grant, E.H.C., Coleman, J.T.H., Sleeman, J.M. & Runge, M.C. (2021). Risks posed by SARS-CoV-2 to North American bats during winter fieldwork. *Conserv. Sci. Pract.*, n/a, e410.
Coscolla, M., Lewin, A., Metzger, S., Maetz-Rennsing, K., Calvignac-Spencer, S., Nitsche, A., *et al.* (2013). Novel Mycobacterium tuberculosis complex isolate from a wild chimpanzee. *Emerg. Infect. Dis.*, 19, 969.
Crossley, B., Hietala, S., Hunt, T., Benjamin, G., Martinez, M., Darnell, D., *et al.* (2012). Pandemic (H1N1) 2009 in captive cheetah. *Emerg. Infect. Dis.*, 18, 315.
Demetria, C., Smith, I., Tan, T., Villarico, D., Simon, E.M., Centeno, R., *et al.* (2018). Reemergence of Reston ebolavirus in Cynomolgus Monkeys, the Philippines, 2015. *Emerg. Infect. Dis.*, 24.
Dobson, A.P., Pimm, S.L., Hannah, L., Kaufman, L., Ahumada, J.A., Ando, A.W., *et al.* (2020). Ecology and economics for pandemic prevention. *Science*, 369, 379–381.
Donahue, M. (2020). U.S. advises suspending bat research over concerns coronavirus could infect North American species. *Wash. Post*.
Donnelly, C.A., Woodroffe, R., Cox, D.R., Bourne, J., Gettinby, G., Le Fevre, A.M., *et al.* (2003). Impact of localized badger culling on tuberculosis incidence in British cattle. *Nature*, 426, 834–837.
Eberhard, M.L., Ruiz-Tiben, E., Hopkins, D.R., Farrell, C., Toe, F., Weiss, A., *et al.* (2014). The peculiar epidemiology of dracunculiasis in Chad. *Am. J. Trop. Med. Hyg.*, 90, 61–70.
Edwards, S.J.L. & Santini, J.M. (2020). Anthroponotic risk of SARS-CoV-2, precautionary mitigation, and outbreak management. *Lancet Microbe*.
Engel, G.A., Jones-Engel, L., Schillaci, M.A., Suaryana, K.G., Putra, A., Fuentes, A., *et al.* (2002). Human exposure to herpesvirus B–seropositive macaques, Bali, Indonesia. *Emerg. Infect. Dis.*, 8, 789.
Fagre, A., Lewis, J., Eckley, M., Zhan, S., Rocha, S.M., Sexton, N.R., *et al.* (2020). SARS-CoV-2 infection, neuropathogenesis and transmission among deer mice: Implications for reverse zoonosis to New World rodents. *bioRxiv*, 2020.08.07.241810.
Farrell, M.J. & Davies, T.J. (2019). Disease mortality in domesticated animals is predicted by host evolutionary relationships. *Proc. Natl. Acad. Sci.*, 116, 7911–7915.
Favoretto, S.R., Araujo, D.B., Duarte, N.F., Oliveira, D.B., Nathalia, G., Mesquita, F., *et al.* (2019). Zika Virus in peridomestic neotropical primates, Northeast Brazil. *Ecohealth*, 16, 61–69.

Fischer, C.P. & Romero, L.M. (2019). Chronic captivity stress in wild animals is highly species-specific. *Conserv. Physiol.*, 7, coz093–coz093.
Fischhoff, I.R., Castellanos, A.A., Rodrigues, J.P.G.L.M., Varsani, A. & Han, B.A. (2021). Predicting the zoonotic capacity of mammal species for SARS-CoV-2. *bioRxiv*, 2021.02.18.431844.
Frank, H.K., Enard, D. & Boyd, S.D. (2020). Exceptional diversity and selection pressure on SARS-CoV and SARS-CoV-2 host receptor in bats compared to other mammals. *bioRxiv*, 2020.04.20.051656.
Freuling, C.M., Breithaupt, A., Mueller, T., Sehl, J., Balkema-Buschmann, A., Rissmann, M., *et al.* (2020). Susceptibility of raccoon dogs for experimental SARS-CoV-2 infection. *bioRxiv*.
Fritsche, A., Engel, R., Buhl, D. & Zellweger, J.P. (2004). Mycobacterium bovis tuberculosis: from animal to man and back. *Int. J. Tuberc. Lung Dis.*, 8, 903–904.
Frutos, R., Gavotte, L. & Devaux, C.A. (2021). Understanding the origin of COVID-19 requires to change the paradigm on zoonotic emergence from the spillover model to the viral circulation model. *Infect. Genet. Evol.*, 104812.
Garigliany, M., Van Laere, A.-S., Clercx, C., Giet, D., Escriou, N., Huon, C., *et al.* (2020). SARS-CoV-2 Natural Transmission from Human to Cat, Belgium, March 2020. *Emerg. Infect. Dis. J.*, 26.
Ge, X., Li, Y., Yang, X., Zhang, H., Zhou, P., Zhang, Y., *et al.* (2012). Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China. *J. Virol.*, 86, 4620–4630.
Gerdts, V., Littel-van den Hurk, S. van D., Griebel, P.J. & Babiuk, L.A. (2007). Use of animal models in the development of human vaccines. *Future Microbiol.*, 2, 667–675.
Gibb, R., Redding, D.W., Chin, K.Q., Donnelly, C.A., Blackburn, T.M., Newbold, T., *et al.* (2020). Zoonotic host diversity increases in human-dominated ecosystems. *Nature*, 584, 398–402.
Gibbons, Ann. (2021). Captive gorillas test positive for coronavirus. *Sci. Mag.*
Gilbert, A.T., Fooks, A.R., Hayman, D.T.S., Horton, D.L., Müller, T., Plowright, R., *et al.* (2013). Deciphering Serology to Understand the Ecology of Infectious Diseases in Wildlife. *EcoHealth*, 10, 298–313.
Goldberg, T.L., Gillespie, T.R., Rwego, I.B., Wheeler, E., Estoff, E.L. & Chapman, C.A. (2007). Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. *Biol. Conserv.*, 135, 511–517.
Gorman, J. (2020). Can humans give coronaviruses to bats, and other wildlife? *N. Y. Times*.
Greer, D., Hickey, J., Robbins, M., Stoinski, T., Kirsten Gilardi (University of California, D., Expertise), A.B. (Primate, *et al.* (2018). *IUCN Red List of Threatened Species: Gorilla beringei ssp. beringei*. *IUCN Red List Threat. Species*. Available at: https://www.iucnredlist.org/en. Last accessed 1 March 2021.
Griffin, B.D., Chan, M., Tailor, N., Mendoza, E.J., Leung, A., Warner, B.M., *et al.* (2020). North American deer mice are susceptible to SARS-CoV-2. *bioRxiv*, 2020.07.25.221291.
Guyader, F.L., Haugarreau, L., Miossec, L., Dubois, E. & Pommepuy, M. (2000). Three-Year Study To Assess Human Enteric Viruses in Shellfish. *Appl. Environ. Microbiol.*, 66, 3241–3248.
Hall, J.S., Knowles, S., Nashold, S.W., Ip, H.S., Leon, A.E., Rocke, T., *et al.* (2020). Experimental challenge of a North American bat species, big brown bat (Eptesicus fuscus), with SARS-CoV-2. *Transbound. Emerg. Dis.*
Han, B.A., Kramer, A.M. & Drake, J.M. (2016). Global Patterns of Zoonotic Disease in Mammals. *Trends Parasitol.*, 32, 565–577.
Han, B.A., Majumdar, S., Calmon, F.P., Glicksberg, B.S., Horesh, R., Kumar, A., *et al.* (2019). Confronting data sparsity to identify potential sources of Zika virus spillover infection among primates. *Epidemics*, 27, 59–65.
Hayes, L.D. & Schradin, C. (2017). Long-term field studies of mammals: what the short-term study cannot tell us. *J. Mammal.*
Heard, M.J., Smith, K.F., Ripp, K.J., Berger, M., Chen, J., Dittmeier, J., *et al.* (2013). The Threat of Disease Increases as Species Move Toward Extinction. *Conserv. Biol.*, 27, 1378–1388.

Hedman, H.D., Krawczyk, E., Helmy, Y.A., Zhang, L. & Varga, C. (2021). Host Diversity and Potential Transmission Pathways of SARS-CoV-2 at the Human-Animal Interface. *Pathogens*, 10, 180.
Heldstab, A., Rüedi, D., Sonnabend, W. & Deinhardt, F. (1981). Spontaneous generalized Herpesvirus hominis infection of a lowland gorilla (Gorilla gorilla gorilla). *J. Med. Primatol.*, 10, 129–135.
Hickey, J.R., Basabose, A., Gilardi, K.V., Greer, D., Nampindo, S., Robbins, M.M., *et al.* (2020). *Gorilla beringei ssp. beringei (amended version of 2018 assessment). The IUCN Red List of Threatened Species 2020: e.T39999A176396749.*
Hockings, K.J., Mubemba, B., Avanzi, C., Pleh, K., Düx, A., Bersacola, E., *et al.* (2020). Leprosy in wild chimpanzees. *bioRxiv*, 2020.11.10.374371.
Holmes, E.C., Rambaut, A. & Andersen, K.G. (2018). *Pandemics: spend on surveillance, not prediction*. Nature Publishing Group.
Hopkins, D.R., Weiss, A.J., Roy, S.L., Zingeser, J. & Guagliardo, S.A.J. (2019). Progress Toward Global Eradication of Dracunculiasis - January 2018-June 2019. *MMWR Morb. Mortal. Wkly. Rep.*, 68, 979–984.
Humle, T., Boesch, C., Campbell, G., Junker, J., Koops, K., Kuehl, H., *et al.* (2016). *Pan troglodytes ssp. verus (errata version published in 2016). The IUCN Red List of Threatened Species 2016: e.T15935A102327574.*
Jobbins, S.E. & Alexander, K.A. (2015). From whence they came—antibiotic-resistant Escherichia coli in African wildlife. *J. Wildl. Dis.*, 51, 811–820.
Jones, B.A., Grace, D., Kock, R., Alonso, S., Rushton, J., Said, M.Y., *et al.* (2013). Zoonosis emergence linked to agricultural intensification and environmental change. *Proc. Natl. Acad. Sci.*, 110, 8399–8404.
Jones, K., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., *et al.* (2008). Global trends in emerging infectious diseases | Nature. *Nature*, 451, 990–993.
Jones, M.E., Amman, B.R., Sealy, T.K., Uebelhoer, L.S., Schuh, A.J., Flietstra, T., *et al.* (2019). Clinical, histopathologic, and immunohistochemical characterization of experimental Marburg virus infection in a natural reservoir host, the Egyptian rousette bat (Rousettus aegyptiacus). *Viruses*, 11, 214.
Kaur, T., Singh, J., Tong, S., Humphrey, C., Clevenger, D., Tan, W., *et al.* (2008). Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees (Pan troglodytes) at Mahale Mountains National Park, Western Tanzania. *Am. J. Primatol. Off. J. Am. Soc. Primatol.*, 70, 755–765.
Kock, R.A., Orynbayev, M., Robinson, S., Zuther, S., Singh, N.J., Beauvais, W., *et al.* (2018). Saigas on the brink: Multidisciplinary analysis of the factors influencing mass mortality events. *Sci. Adv.*, 4, eaao2314.
Lachish, S., Lawson, B., Cunningham, A.A. & Sheldon, B.C. (2012). Epidemiology of the Emergent Disease Paridae pox in an Intensively Studied Wild Bird Population. *PLOS ONE*, 7, e38316.
Lam, T.T.-Y., Hon, C.-C., Lemey, P., Pybus, O.G., Shi, M., Tun, H.M., *et al.* (2012). Phylodynamics of H5N1 avian influenza virus in Indonesia. *Mol. Ecol.*, 21, 3062–3077.
Lande, R. (1998). Anthropogenic, ecological and genetic factors in extinction and conservation. *Popul. Ecol.*, 40, 259–269.
Lembo, T., Hampson, K., Haydon, D.T., Craft, M., Dobson, A., Dushoff, J., *et al.* (2008). Exploring reservoir dynamics: a case study of rabies in the Serengeti ecosystem. *J. Appl. Ecol.*, 45, 1246–1257.
Lesté-Lasserre, C. (2020). Pandemic dooms Danish mink—and mink research. *Science*, 370, 754.
Leung, J.M., Budischak, S.A., Chung The, H., Hansen, C., Bowcutt, R., Neill, R., *et al.* (2018). Rapid environmental effects on gut nematode susceptibility in rewilded mice. *PLoS Biol.*, 16, e2004108.
Li, D., Zhu, L., Cui, H., Ling, S., Fan, S., Yu, Z., *et al.* (2014). Influenza A(H1N1)pdm09 virus infection in giant pandas, China. *Emerg. Infect. Dis.*, 20, 480–483.
Li, L., Victoria, J.G., Wang, C., Jones, M., Fellers, G.M., Kunz, T.H., *et al.* (2010). Bat guano virome:

predominance of dietary viruses from insects and plants plus novel mammalian viruses. *J. Virol.*, 84, 6955–6965.
Maisels, F., Bergl, R.A. & Williamson, E.A. (2018). *Gorilla gorilla (amended version of 2016 assessment). The IUCN Red List of Threatened Species 2018: e.T9404A136250858.*
McAloose, D., Laverack, M., Wang, L., Killian, M.L., Caserta, L.C., Yuan, F., *et al.* (2020). From people to Panthera: Natural SARS-CoV-2 infection in tigers and lions at the Bronx Zoo. *bioRxiv*, 2020.07.22.213959.
McClure, H.M. & Keeling, M.E. (1971). Viral diseases noted in the Yerkes Primate Center colony. *Lab. Anim. Sci.*, 21, 1002–1010.
McDonald, R.A., Wilson-Aggarwal, J.K., Swan, G.J.F., Goodwin, C.E.D., Moundai, T., Sankara, D., *et al.* (2020). Ecology of domestic dogs Canis familiaris as an emerging reservoir of Guinea worm Dracunculus medinensis infection. *PLoS Negl. Trop. Dis.*, 14, e0008170.
McDougall, F., Boardman, W., Gillings, M. & Power, M. (2019). Bats as reservoirs of antibiotic resistance determinants: A survey of class 1 integrons in Grey-headed Flying Foxes (Pteropus poliocephalus). *Infect. Genet. Evol. J. Mol. Epidemiol. Evol. Genet. Infect. Dis.*, 70, 107–113.
McKinney, L.A., Kick, E.L. & Fulkerson, G.M. (2010). World system, anthropogenic, and ecological threats to bird and mammal species: a structural equation analysis of biodiversity loss. *Organ. Environ.*, 23, 3–31.
Mendelsohn, E., Ross, N., White, A., Whiting, K., Basaraba, C., Watson Madubuonwu, B., *et al.* (2020). Extracting novel antimicrobial emergence events from scientific literature and medical reports [version 1; peer review: 1 approved with reservations]. *F1000Research*, 9.
Messenger, A.M., Barnes, A.N. & Gray, G.C. (2014). Reverse zoonotic disease transmission (zooanthroponosis): a systematic review of seldom-documented human biological threats to animals. *PloS One*, 9, e89055.
Michalak, K., Austin, C., Diesel, S., Bacon, M.J., Zimmerman, P. & Maslow, J.N. (1998). Mycobacterium tuberculosis infection as a zoonotic disease: transmission between humans and elephants. *Emerg. Infect. Dis.*, 4, 283.
Molenaar, R.J., Vreman, S., Hakze-van der Honing, R.W., Zwart, R., de Rond, J., Weesendorp, E., *et al.* (2020). Clinical and pathological findings in SARS-CoV-2 disease outbreaks in farmed mink (Neovison vison). *Vet. Pathol.*, 57, 653–657.
Mollentze, N. & Streicker, D.G. (2020). Viral zoonotic risk is homogenous among taxonomic orders of mammalian and avian reservoir hosts. *Proc. Natl. Acad. Sci.*, 117, 9423.
Munnink, B.B.O., Sikkema, R.S., Nieuwenhuijse, D.F., Molenaar, R.J., Munger, E., Molenkamp, R., *et al.* (2020a). Jumping back and forth: anthropozoonotic and zoonotic transmission of SARS-CoV-2 on mink farms. *bioRxiv*, 2020.09.01.277152.
Munnink, B.B.O., Sikkema, R.S., Nieuwenhuijse, D.F., Molenaar, R.J., Munger, E., Molenkamp, R., *et al.* (2020b). Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science*.
Mykytyn, A.Z., Lamers, M.M., Okba, N.M.A., Breugem, T.I., Schipper, D., van den Doel, P.B., *et al.* (2020). Susceptibility of rabbits to SARS-CoV-2. *bioRxiv*, 2020.08.27.263988.
Nizeyi, J.B., Innocent, R.B., Erume, J., Kalema, G.R., Cranfield, M.R. & Graczyk, T.K. (2001). Campylobacteriosis, salmonellosis, and shigellosis in free-ranging human-habituated mountain gorillas of Uganda. *J. Wildl. Dis.*, 37, 239–244.
Noël, C., Dufernez, F., Gerbod, D., Edgcomb, V.P., Delgado-Viscogliosi, P., Ho, L.-C., *et al.* (2005). Molecular Phylogenies of Blastocystis Isolates from Different Hosts: Implications for Genetic Diversity, Identification of Species, and Zoonosis. *J. Clin. Microbiol.*, 43, 348–355.
Obanda, V., Poghon, J., Yongo, M., Mulei, I., Ngotho, M., Waititu, K., *et al.* (2013). First reported case of fatal tuberculosis in a wild African elephant with past human–wildlife contact. *Epidemiol. Infect.*, 141, 1476–1480.
Oh, P., Granich, R., Scott, J., Sun, B., Joseph, M., Stringfield, C., *et al.* (2002). Human exposure following Mycobacterium tuberculosis infection of multiple animal species in a metropolitan zoo.

Emerg. Infect. Dis., 8, 1290.
O'Hanlon, S.J., Rieux, A., Farrer, R.A., Rosa, G.M., Waldman, B., Bataille, A., *et al.* (2018). Recent Asian origin of chytrid fungi causing global amphibian declines. *Science*, 360, 621–627.
OIE-World Organisation for Animal Health. (2020). *Infection with SARS-CoV-2, Slovenia.* (Immediate notification (Final Report)).
Olival, K.J., Cryan, P.M., Amman, B.R., Baric, R.S., Blehert, D.S., Brook, C.E., *et al.* (2020). Possibility for reverse zoonotic transmission of SARS-CoV-2 to free-ranging wildlife: A case study of bats. *PLOS Pathog.*, 16, e1008758.
Olival, K.J., Hosseini, P.R., Zambrana-Torrelio, C., Ross, N., Bogich, T.L. & Daszak, P. (2017). Host and viral traits predict zoonotic spillover from mammals. *Nature*, 546, 646–650.
Oreshkova, N., Molenaar, R.J., Vreman, S., Harders, F., Oude Munnink, B.B., Hakze-van der Honing, R.W., *et al.* (2020). SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. *Euro Surveill. Bull. Eur. Sur Mal. Transm. Eur. Commun. Dis. Bull.*, 25.
Osterhaus, A., Rimmelzwaan, G.F., Martina, B.E.E., Bestebroer, T.M. & Fouchier, R.A.M. (2000). Influenza B virus in seals. *Science*, 288, 1051–1053.
Palacios, G., Lowenstine, L.J., Cranfield, M.R., Gilardi, K.V., Spelman, L., Lukasik-Braum, M., *et al.* (2011). Human metapneumovirus infection in wild mountain gorillas, Rwanda. *Emerg. Infect. Dis.*, 17, 711.
Patterson, E.I., Elia, G., Grassi, A., Giordano, A., Desario, C., Medardo, M., *et al.* (2020). Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. *Nat. Commun.*, 11, 1–5.
Pedersen, A.B. & Davies, T.J. (2009). Cross-species pathogen transmission and disease emergence in primates. *EcoHealth*, 6, 496–508.
Pedersen, A.B., Jones, K.E., Nunn, C.L. & Altizer, S. (2007). Infectious Diseases and Extinction Risk in Wild Mammals. *Conserv. Biol.*, 21, 1269–1279.
Plowright, R.K., Eby, P., Hudson, P.J., Smith, I.L., Westcott, D., Bryden, W.L., *et al.* (2015). Ecological dynamics of emerging bat virus spillover. *Proc. R. Soc. B Biol. Sci.*, 282, 20142124.
Plowright, R.K., Parrish, C.R., McCallum, H., Hudson, P.J., Ko, A.I., Graham, A.L., *et al.* (2017). Pathways to zoonotic spillover. *Nat. Rev. Microbiol.*, 15, 502–510.
Plumptre, A., Hart, J.A., Hicks, T.C., Nixon, S., Piel, A.K. & Pintea, L. (2016). *Pan troglodytes ssp. schweinfurthii (errata version published in 2016). The IUCN Red List of Threatened Species 2016: e.T15937A102329417.*
Prince, T., Smith, S.L., Radford, A.D., Solomon, T., Hughes, G.L. & Patterson, E.I. (2021). SARS-CoV-2 Infections in Animals: Reservoirs for Reverse Zoonosis and Models for Study. *Viruses*, 13, 494.
Richter, D., Klug, B., Spielman, A. & Matuschka, F.-R. (2004). Adaptation of diverse Lyme disease spirochetes in a natural rodent reservoir host. *Infect. Immun.*, 72, 2442–2444.
Roche, B., Drake, J.M., Brown, J., Stallknecht, D.E., Bedford, T. & Rohani, P. (2014). Adaptive evolution and environmental durability jointly structure phylodynamic patterns in avian influenza viruses. *PLoS Biol*, 12, e1001931.
Rothenburg, S. & Brennan, G. (2020). Species-specific host–virus interactions: implications for viral host range and virulence. *Trends Microbiol.*, 28, 46–56.
Rwego, I.B., ISABIRYE-BASUTA, G., Gillespie, T.R. & Goldberg, T.L. (2008). Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. *Conserv. Biol.*, 22, 1600–1607.
Ryan, S.J. & Walsh, P.D. (2011). Consequences of Non-Intervention for Infectious Disease in African Great Apes. *PLOS ONE*, 6, e29030.
Sailleau, C., Dumarest, M., Vanhomwegen, J., Delaplace, M., Caro, V., Kwasiborski, A., *et al.* (2020). First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. *Transbound. Emerg. Dis.*
Santini, J.M. & Edwards, S.J.L. (2020). Host range of SARS-CoV-2 and implications for public health. *Lancet Microbe*, 1, e141–e142.

Schloegel, L.M., Picco, A.M., Kilpatrick, A.M., Davies, A.J., Hyatt, A.D. & Daszak, P. (2009). Magnitude of the US trade in amphibians and presence of Batrachochytrium dendrobatidis and ranavirus infection in imported North American bullfrogs (Rana catesbeiana). *Biol. Conserv.*, 142, 1420–1426.
Schlottau, K., Rissmann, M., Graaf, A., Schön, J., Sehl, J., Wylezich, C., *et al.* (2020). SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *Lancet Microbe*.
Seeber, P.A., Morrison, T., Ortega, A., East, M.L., Greenwood, A.D. & Czirják, G.Á. (2020). Immune differences in captive and free-ranging zebras (Equus zebra and E. quagga). *Mamm. Biol.*, 1–10.
Segalés, J., Puig, M., Rodon, J., Avila-Nieto, C., Carrillo, J., Cantero, G., *et al.* (2020). Detection of SARS-CoV-2 in a cat owned by a COVID-19− affected patient in Spain. *Proc. Natl. Acad. Sci.*, 117, 24790–24793.
Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., *et al.* (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. *Science*, 368, 1016–1020.
Sit, T.H., Brackman, C.J., Ip, S.M., Tam, K.W., Law, P.Y., To, E.M., *et al.* (2020). Infection of dogs with SARS-CoV-2. *Nature*, 1–6.
Sutherland, K.P., Shaban, S., Joyner, J.L., Porter, J.W. & Lipp, E.K. (2011). Human Pathogen Shown to Cause Disease in the Threatened Eklhorn Coral Acropora palmata. *PLOS ONE*, 6, e23468.
Sweeny, A.R., Thomason, C.A., Carbajal, E.A., Hansen, C.B., Graham, A.L. & Pedersen, A.B. (2020). Experimental parasite community perturbation reveals associations between Sin Nombre virus and gastrointestinal nematodes in a rodent reservoir host. *bioRxiv*.
Terzian, A.C.B., Zini, N., Sacchetto, L., Rocha, R.F., Parra, M.C.P., Del Sarto, J.L., *et al.* (2018). Evidence of natural Zika virus infection in neotropical non-human primates in Brazil. *Sci. Rep.*, 8, 1–15.
Tu, C., Crameri, G., Kong, X., Chen, J., Sun, Y., Yu, M., *et al.* (2004). Antibodies to SARS coronavirus in civets. *Emerg. Infect. Dis.*, 10, 2244.
Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., *et al.* (2015). Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci.*, 112, 5649.
Viana, M., Mancy, R., Biek, R., Cleaveland, S., Cross, P.C., Lloyd-Smith, J.O., *et al.* (2014). Assembling evidence for identifying reservoirs of infection. *Trends Ecol. Evol.*, 29, 270–279.
Villabruna, N., Koopmans, M.P.G. & de Graaf, M. (2019). Animals as Reservoir for Human Norovirus. *Viruses*, 11.
Wallis, J., Benrabah, M.E., Pilot, M., Majolo, B. & Waters, S. (2020). *Macaca sylvanus. The IUCN Red List of Threatened Species 2020: e.T12561A50043570.*
Wang, L., Mitchell, P.K., Calle, P.P., Bartlett, S.L., McAloose, D., Killian, M.L., *et al.* (2020). Complete genome sequence of SARS-CoV-2 in a tiger from a US zoological collection. *Microbiol. Resour. Announc.*, 9.
Wang, M., Yan, M., Xu, H., Liang, W., Kan, B., Zheng, B., *et al.* (2005). SARS-CoV infection in a restaurant from palm civet. *Emerg. Infect. Dis.*, 11, 1860.
Wevers, D., Metzger, S., Babweteera, F., Bieberbach, M., Boesch, C., Cameron, K., *et al.* (2011). Novel adenoviruses in wild primates: a high level of genetic diversity and evidence of zoonotic transmissions. *J. Virol.*, 85, 10774–10784.
Wombwell, E.L., Garner, T.W.J., Cunningham, A.A., Quest, R., Pritchard, S., Rowcliffe, J.M., *et al.* (2016). Detection of Batrachochytrium dendrobatidis in Amphibians Imported into the UK for the Pet Trade. *EcoHealth*, 13, 456–466.
Woodroffe, R. & Sillero-Zubiri, C. (2020). *Lycaon pictus (amended version of 2012 assessment). The IUCN Red List of Threatened Species 2020: e.T12436A166502262*.
Woolhouse, M.E. & Brierley, L. (2018). Epidemiological characteristics of human-infective RNA viruses. *Sci. Data*, 5, 180017.
Wu, Z., Yang, L., Ren, X., He, G., Zhang, J., Yang, J., *et al.* (2016). Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging

infectious diseases. *ISME J.*, 10, 609–620.
Zachariah, A., Pandiyan, J., Madhavilatha, G.K., Mundayoor, S., Chandramohan, B., Sajesh, P.K., *et al.* (2017). Mycobacterium tuberculosis in wild Asian elephants, southern India. *Emerg. Infect. Dis.*, 23, 504.
Zhao, Y., Wang, J., Kuang, D., Xu, J., Yang, M., Ma, C., *et al.* (2020). Susceptibility of tree shrew to SARS-CoV-2 infection. *Sci. Rep.*, 10, 16007.
Zhou, N.N., Senne, D.A., Landgraf, J.S., Swenson, S.L., Erickson, G., Rossow, K., *et al.* (1999). Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. *J. Virol.*, 73, 8851–8856.
Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., *et al.* (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *nature*, 579, 270–273.