

Assessing the risks of SARS-CoV-2 in wildlife

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Abstract

The novel coronavirus SARS-CoV-2 likely emerged from a wildlife source with transmission to humans followed by rapid geographic spread throughout the globe and dramatic impacts on both human health and global economies. Since the onset of the pandemic, there have been several instances of human-to-animal transmission involving companion, farmed and zoo animals, with the clear potential for spread into free-living wildlife. The establishment of reservoirs of infection in wild animals would create significant challenges to infection control in humans and could pose a threat to the welfare and conservation status of wildlife. Herein, we discuss the potential for exposure, maintenance and onward transmission of SARS-CoV-2 in an initial selection of wild and feral species (bats, canids, felids, mustelids, great apes). Targeted surveillance and dynamic risk assessment are important tools for the early detection of infection in wildlife and a means of collating and synthesising emerging information in a rapidly changing situation. Such efforts should be integrated with public health information to provide insights into the potential role of wild mammals in the continuing epidemiology of SARS-CoV-2. This approach should also be adopted to address the wider need to proactively assess threats to human and animal health from other diseases that may emerge from wildlife.

Keywords: SARS-CoV-2, Covid-19, wildlife, host-switching, reservoirs, risk assessment, surveillance

Introduction

It is estimated that there have been over 64 million cases of human infection with Covid-19 globally, with over 1.4 million deaths [1] and widespread community transmission in many countries. The pandemic appears to have originated from a seafood market selling live wild animals in Wuhan, China [2]. Evidence suggests that the causative coronavirus (SARS-CoV-2; previously called 2019-nCoV) emerged from wildlife, although the species responsible remains a 'missing link' and if identified would improve our knowledge of the disease and the mechanism for the initial host switch to humans. Since the onset of the pandemic, human-to-animal transmission (zoonanthroponosis) has occurred on many occasions, in multiple countries, and involving several species, although there is no evidence of SARS-CoV-2 infection in any free-living wildlife population. Nevertheless, this may be only a matter of time, since many coronaviruses have a broad host range [3] with the clear possibility that a reverse zoonoses spillback event from humans could lead to the establishment of a reservoir of infection in wild mammals [4].

Controlling the transmission of pathogens from wild animals to humans or domestic animals is extremely challenging, and hence the emergence of a reservoir of SARS-CoV-2 infection in wildlife could seriously hamper effective disease control in the human population. Infection in free-living wildlife would also have substantial practical implications for management, research, rehabilitation and conservation activities [5] and could generate negative public opinion towards some species leading to persecution and disengagement from conservation initiatives. There is potential for both direct and indirect adverse effects on wildlife with implications for animal welfare, conservation and global species diversity [6]. These concerns are reflected in emerging guidance on how those who work directly with wildlife can reduce risks of SARS-CoV-2 transmission to wild mammals [5,7]. However, there is also a need for risk reduction measures to be extended to others in the wider community who may have contact with wildlife, for example where wild animals are harvested and traded for food [8]. Such measures are an important first line of defence, but in the face of widespread infection in the human population there is a need to also plan for the implications of SARS-CoV-2 establishing in free-living wildlife. This will require an assessment of the potential role of wildlife populations in the epidemiology of infection, and in particular identification of those species and the circumstances most likely to amplify to reservoirs of infection, so that surveillance, preventative measures and contingency plans can be developed and targeted appropriately. Based on the available evidence, the potential role of wildlife in the persistence, spread, and possible re-emergence of SARS-CoV-2 is discussed below, and the case for targeted disease surveillance and the dynamic assessment of risks to the health of humans and wildlife are considered.

Wildlife origins of SARS-CoV-2

SARS-CoV-2 is a betacoronavirus (β -CoV), closely related to SARS-CoV and MERS-CoV which have also caused serious outbreaks of disease in human populations. All are thought to have originated in bats [9], with evidence of intermediate or bridge hosts being responsible for virus transmission to humans [10,11,12]. Palm civets (*Paguma larvata*) were identified as the initial proximal source of SARS (Severe Acute Respiratory Syndrome) in humans [13], and dromedary camels (*Camelus dromedarius*) are a reservoir and source of MERS (Middle Eastern Respiratory Syndrome) in humans [14,15]. Although SARS-CoV-2 may have its ancestral origins in bats, its closest genetic ancestor (RATG13) being a β -CoV isolated from the intermediate horseshoe bat (*Rhinolophus affinis*) [11], the proximal cause of infection in humans has yet to be identified. Malayan pangolins (*Manis javanica*) have been the subject of some speculation on the basis of infection with a closely related coronavirus in animals seized in southern China [16]. Sequence analysis of the spike glycoprotein (S) of SARS-CoV-2 and related coronaviruses suggest a series of recombination events between bat and pangolin coronaviruses, eventually leading to the emergence of this novel coronavirus [17]. However, raccoon dogs (*Nyctereutes procyonoides*), which were identified as possible intermediate hosts for the SARS pandemic of 2002-2003 [18], have also been suggested as candidate intermediate hosts for SARS-CoV-2 as experimental infection resulted in intense viral shedding [19]. Both pangolins and raccoon dogs have been found in wildlife markets in Southern China, along with many other wild mammals (some wild caught and others bred in captivity) and domesticated species [20,21]. Reports of

spillover of SARS-CoV-2 from humans to companion, captive and farmed animals (see below) provide additional insights into other species that may have facilitated the virus jump from wildlife to humans.

Host susceptibility

Information on the susceptibility of animal hosts to SARS-CoV-2 is emerging rapidly. There are several strands of evidence that can be used to infer the susceptibility of potential wild animal hosts, including predictions based on the characteristics of the host receptor to which the virus binds in order to infect cells, the demonstration of experimental infection of cell lines or of individual animals, and the confirmation of naturally acquired infection. The existence in wildlife hosts of recent progenitors of SARS-CoV-2 or coronaviruses with nucleotide similarity across all genes may also be useful in inferring susceptibility.

Analysis of the angiotensin converting enzyme 2 (ACE2) protein, the functional receptor for the spike protein of SARS-CoV-2 in a broad range of vertebrates, predicts susceptibility to infection in many mammal species [22,23]. Experimental studies using cell lines modified to express ACE2 have also demonstrated potential for SARS-CoV-2 infection in a wide variety of hosts including bats, rodents, cetaceans, carnivores and primates [24,25]. However, all such predictions are subject to substantial uncertainty and neither close phylogenetic relationships nor similarity in ACE2 protein sequences can be considered confirmatory for predicting susceptibility to SARS-CoV-2 infection [26], so this information needs to be considered alongside other evidence. Despite extensive *in silico* structural analysis and *in vitro* virus binding assessments being performed against ACE2 in numerous species, further information is required on the levels and locations (nasal cavity, trachea, lungs and gastro-intestinal tract) of ACE2 expression in different mammals to inform assessments of host susceptibility to SARS-CoV-2 infection.

Results from studies of experimental infection are available for a small number of mammal species, with more information emerging daily. Such studies demonstrate susceptibility to SARS-CoV-2, albeit with varying levels of viral replication and shedding, in domestic cats (*Felis catus*) and dogs (*Canis lupus familiaris*), ferrets (*Mustela putorius furo*), Syrian hamsters (*Mesocricetus auratus*), rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*M. fascicularis*), African green monkeys (*Chlorocebus* sp.), Chinese tree shrews (*Tupaia belangeri chinensis*), common marmosets (*Callithrix jacchus*), Egyptian fruit bats (*Rousettus aegyptiacus*), racoon dogs (*Nyctereutes procyonoides*) and laboratory rabbits (*Oryctolagus cuniculus*) [19,27,28,29,30,31,32,33,34,35]. However, experimental studies are models of disease dynamics and do not precisely mimic the dynamics of infection and onward transmission under natural conditions. In particular, experimental studies frequently overestimate susceptibility, as they often seek to maximise the likelihood of infection through the use of large volumes, high titre inocula and/or direct instillation to target sites. This may explain the contrasting observations of effective transmission to in-contact ferrets following experimental infection [35] and the apparent absence of infection in pet ferrets despite close contact with infected humans in the same household [36]. Dogs appear less susceptible to experimental infection than cats and ferrets as they exhibit low levels of viral replication [32]. Two studies report that pigs (*Sus domesticus*) were not able to be infected experimentally [32,35] despite predictions based on the binding capacity of porcine ACE2 to SARS-CoV-2 spike proteins suggesting the opposite [25,37].

Naturally acquired infections of SARS-CoV-2 have only been demonstrated in pet dogs and cats in domestic settings, in tigers (*Panthera tigris*), lions (*Panthera leo*) and a puma (*Puma concolor*) in zoological collections, and in farmed American mink (*Neovison vison*) [38,39,40,41]. All such cases of natural infection have been linked to initial transmission from humans to the animals in their care. There have also been recorded cases of infection in what are described as 'stray' cats [39,42] although the extent to which these animals were truly free-living and their levels of contact with humans are unclear.

Persistence and spread in wildlife

Although many viruses can switch species into new host populations, onward transmission and persistence are not assured, affected as they are by many inter-related factors [43]. Host susceptibility, behaviour and demography must align with pathogen characteristics to result in a host switching event. In these optimal

conditions, viral adaptation and intra-species transmission occurs at the population level, for both the host and viral variant selection. Hence, in order to determine the most likely species of wild mammal and circumstances whereby they might play a role in the epidemiology of SARS-CoV-2, we need to look beyond the evidence for susceptibility to infection alone.

Evidence suggests that all cases of natural infection of SARS-CoV-2 detected in non-human animals have been linked to transmission from infected humans to domestic or captive animals in their care. Although infection has been detected in 'stray' cats sampled in the areas surrounding infected mink farms in the Netherlands [39], the absence of genetic sequence data and no evidence of close contact with the mink, means that transmission from the local human population or the environment cannot be discounted. Also, the extent to which the cats in question were truly free-living is unclear. Clearly, there are far fewer opportunities for transmission from humans to free-living wildlife than to domestic animals and farmed wildlife. However, there are a range of activities involving direct human-animal contact which may pose significant risks, such as wildlife rehabilitation, field research, practical conservation work and some wildlife-related tourism. Also, indirect transmission might occur where there are opportunities for human contamination of the environment (e.g. faeces in wastewater), supplemental food (deployed for wildlife watching, hunting or pest control purposes) or fomites (e.g. surfaces of traps used for hunting or pest control and which may be visited by animals that are not subsequently killed). There will also be situations where host switching through reverse zoonoses from infected humans to domestic or farmed animals creates a potential pathway for pathogen transmission to wildlife (see below).

Evidence to inform whether SARS-CoV-2 infection is likely to be maintained in wild animal populations is extremely scant. The animal reservoir responsible for the initial spillover into humans remains unknown, although phylogenetic analyses suggest that the virus lineage giving rise to SARS-CoV-2 may have been circulating in bats for at least several decades [44]. Experimental studies provide some evidence for intra-species transmission via direct contact amongst racoon dogs [19], cats [32], ferrets [35,45] and Egyptian fruit bats [35]. For experimentally infected cats and ferrets, there is also evidence for airborne virus transmission [32,45,46]. Rabbits exposed to high experimental doses subsequently excreted infectious virus from the nose and throat, although at levels that suggested relatively low risks of onward transmission compared to other species [30].

Sequencing of viral genomes has shown that natural transmission has taken place amongst farmed mink following initial introductions by infected workers [38,39]. Transmission amongst separately housed mink suggests that the infection may have been spread by fomites, respiratory droplets or contaminated dust from bedding [39]. Mink farms provide the only source of evidence for maintenance of naturally acquired infection in an animal population, transmission of infection between animal species (i.e. potential spread from mink to stray cats), and spillback to humans. The housing of mink at unnaturally high densities and the spatial structure of farms may facilitate spread and persistence of the virus in these captive populations. Indeed, another respiratory virus, pandemic influenza A H1N1/09 of 'swine-origin', spread around the world in humans and then infected several farmed animal species including mink [47].

Given the scale and widespread distribution of infection in the global human population, the current role of wildlife in the epidemiology of SARS-CoV-2 is likely to be negligible. However, this could change over time, with the significance of a reservoir of infection in wild mammals potentially increasing as community transmission in human populations is reduced in the face of effective control measures. In such situations, the implications of spillback from a reservoir of infection in wildlife populations would be more significant. It is also possible that the circulation of SARS-CoV-2 in a wild animal population might lead to the evolution of mutations as the virus adapts to new species, with potential implications for onward transmission and control in human populations.

Potential wildlife reservoirs

Clearly, it is not possible to assess the risks of SARS-CoV-2 in all wild mammal species, but given the available evidence, a priority list of species groups for initial consideration can be assembled. Below we

discuss the potential for exposure, maintenance and onward transmission of SARS-CoV-2 in an initial selection of wild and feral species, which on the basis of evidence could be considered of particular relevance.

Bats

Many of the known coronaviruses appear to have a bat origin [48], with over 200 identified in bats, and representing over a third of the sequenced bat virome [49]. These include several SARS-related coronaviruses detected in rhinolophid bats from China, Slovenia, Bulgaria, Italy and Japan [50,51,52,53], and in *Hipposideros* and *Chaerophon* species from Ghana, Kenya and Nigeria [54]. The closest known genetic ancestor of SARS-CoV-2 (RATG13) was isolated from a rhinolophid bat in southern China [11]. The wide diversity of coronaviruses found in bats, suggests high potential for viral evolution in these hosts, and raises the potential for recombination of SARS-CoV-2 with other viruses [55].

In addition to their role in the emergence of novel coronaviruses in humans, bats are at risk of infection with SARS-CoV-2 from humans. A preliminary analysis indicates that about 40 species of North American bats might be susceptible to SARS-CoV-2 infection [56]. Although recorded instances of transmission of viruses from humans to bats are rare and onward spread has not been recorded [56], systematic surveillance has been lacking and so cases may have gone unreported. Opportunities for pathogen transmission between bats and people may occur via the actions of bat carers, veterinarians, consultant ecologists, conservation and research workers, or through inadvertent contact between bats and humans arising from deforestation, mining, ecotourism and food production [57]. Humans may also come into contact with bat faeces where roosts and hibernation sites occur in occupied buildings.

The high population density at which many bat species roost and high population sizes are likely to facilitate transmission and persistence of a novel pathogen such as SARS-CoV-2. On the other hand, the existence of related viruses in many bat populations may confer a level of immunity to SARS-CoV-2 and so reduce the likelihood that it will persist. Coronaviruses in bats typically have a narrow host range but there is genetic evidence of many host switching events, and that this process contributes to coronavirus evolution [58,59].

Should SARS-CoV-2 enter a previously uninfected bat population due to transmission from humans the impacts would be highly uncertain, and given their nocturnal and often cryptic behaviour, population-level effects would be unlikely to be detected in many situations. But the indirect consequences of detecting infection in a bat population could be substantial as it could precipitate an erosion of the perceived biodiversity value of bats, resulting in loss of current protections and ill-conceived interventions. Indeed, even the perception (with no evidence) that bats could be involved in the transmission of SARS-CoV-2 might be sufficient to have an adverse impact, as indicated by anecdotal reports of the killing of bats in several countries (India, Cuba, Peru, Indonesia and Rwanda) in misguided attempts to control Covid-19 infection in humans [60]. These events are concerning for bat conservation given that so many species are of threatened or unknown status [61].

People working with bats are generally familiar with procedures and guidelines to minimise the risk of bat-to-human transmission of pathogens, however in the current context IUCN guidance has been published for reducing the risk of SARS-CoV-2 transmission from humans-to-bats in field research [62] and in bat rescue and rehabilitation centres [63]. A recent rapid qualitative assessment of the risks of SARS-CoV-2 becoming established in Australian bat populations concluded that the risk was low, but that uncertainty was high at least in part because of the absence of data on the frequency and context of human-bat interactions [64]. Other sources of uncertainty were unknown susceptibility to infection and the capacity for subsequent viral shedding in bats.

Surveillance for SARS-CoV-2 in bats could be targeted at animals coming into very close proximity with humans, such as those undergoing rehabilitation prior to release, those maintained in captivity or captured for research purposes. Priority bat populations for surveillance could include those that roost in buildings or public spaces (e.g. urban parks) where inadvertent contact with humans is more likely to occur. There is some evidence that SARS related coronaviruses are more strongly associated with bat species in the Old-

World suborder Yinpterochiroptera and those in the genus *Rhinolopus* in particular [56] which would suggest these groups could be prioritised. However available surveillance and sampling data is heavily biased by specific research activities, both geographically and by species, and representative sampling has not been undertaken.

Felids

Observations from experimental and natural SARS-CoV-2 infections in animals clearly suggest relatively high susceptibility amongst felids (Felidae family). Both wild and domestic felids are amongst the most epidemiologically relevant animal hosts of SARS-CoV-2 because human-to-feline transmission has been sporadically recorded both in pet cats [40,42,65] and captive wild animals such as tigers and lions [41,66]. Moreover, there is experimental evidence for transmission amongst cats both via direct contact [67] and from airborne virus [32]. SARS-CoV-2 antibodies have been detected in 'stray' cats in Wuhan during the Covid-19 outbreak consistent with human-to-cat transmission occurring outside the domestic setting [42], although possible cross-reactions with other coronaviruses need to be fully assessed. Also, the observation of both SARS-CoV-2 antibodies and RNA in 'stray' cats in the vicinity of an infected mink farm in the Netherlands raised the possibility of inter-species transmission [39]. In addition, cats may theoretically be exposed to infection through interactions with their prey such as rabbits, and even bats, which they typically encounter when young or moribund animals fall from their roosts.

Domestic cats are the most abundant felids, reaching densities in excess of 2000 animals km² in urban areas [68], and their proximity to human beings, mobility and social interactions provide ample opportunities for inter-species pathogen transmission. Although 'stray' and truly feral domestic cats typically have less contact with humans, they may nevertheless be exposed to human-derived infection via fomites in residential areas and farm environments for example. Social interactions amongst colony-living cats may be conducive to intra-specific transmission, although there is no evidence for SARS-CoV-2 maintenance within cat populations, nor for transmission from infected cats to humans. Nevertheless, on the basis of available evidence, surveillance for SARS-CoV-2 in felids could usefully prioritise free-living domestic cat populations, particularly where they are abundant in urban environments or in the vicinity of other sources of infection such as mink farms. In contrast, wild felid species tend to be more solitary, are far less abundant and seldom come into contact with humans and urban environments, so would not be expected to contribute to virus maintenance. However, rare and endangered species could be at risk of exposure to infected people involved in research and conservation programmes. Domestic cats naturally infected with SARS-CoV-2 have often been reported as asymptomatic or showing only mild clinical signs, although some instances of more serious disease have also been reported [40,65,67], whereas only mild respiratory signs accompanied infection in captive tigers and lions [41,66]. It is therefore unclear whether infection could have notable impacts on wild felid populations.

Canids

There are several instances of SARS-CoV-2 infection in domestic dogs living in households with COVID-19 positive human residents [69,70]. Nasal swabs collected from two asymptomatic pet dogs living in close proximity with COVID-19 positive owners tested positive for SARS-CoV-2 RNA and antibodies, with genetic sequencing confirming human-to-animal transmission [69]. However, experimental infections of five three-month old dogs only demonstrated limited seroconversion and low levels of viral excretion, suggesting low susceptibility [32]. This apparent difference in susceptibility may relate to age or breed of dog, or simply reflect the much larger number of dogs that must have been exposed to infected humans in the domestic setting.

As clinical signs in domestic dogs appear generally mild with only limited viral shedding, the sum of evidence to date suggests that transmission amongst dogs and to other species is unlikely. However, this may not necessarily hold true for wild canids. For example, raccoon dogs have been shown to be susceptible to

experimental infection and onward transmission of SARS-CoV-2 [19]. The susceptibility of other wild canids such as foxes, and jackals is unknown, although analysis of the ACE2 receptor predicts that red foxes (*Vulpes vulpes*) would be susceptible [71]. Onward transmission amongst free-living canids would be most likely where they reach high densities such as in breeding colonies or in urban feral dog populations. Although raccoon dogs do not aggregate in large numbers, they have a high reproductive rate and densities of 0.2 to 1.1 km² [72,73] are sufficient for the effective transmission of some zoonotic viruses (e.g. rabies virus). However, it is the combined density of raccoon dogs and other wild carnivores, which appears to pose the greatest risk of rabies virus maintenance [74]. Hence, it is possible that if other sympatric carnivores are not particularly susceptible then maintenance of SARS-CoV-2 in wild raccoon dogs may be less likely.

Perhaps the greatest opportunities for close interactions between humans and wild canids are in relation to feral and community-owned dogs, in which case risks of transmission would likely be highest during dog management programmes, which are undertaken in many urban areas in low-income countries for rabies control. Similar opportunities may arise where wild and domestic canids are traded in markets or kept at high densities in breeding facilities. In China raccoon dogs are farmed for their fur and so akin to the situation in mink farms in Europe and the United States (see below), there is potential for spillover of SARS-CoV-2 from infected workers to captive animals, followed by onward spread and spillback to humans [19]. Thus any surveillance for SARS-CoV-2 in canids might most usefully be targeted at urban areas with feral dogs, and at breeding facilities and markets.

Mustelids

There have been many cases of SARS-CoV-2 in farmed mink with infections reported from Europe and the USA [75]. Transmission among mink and spillback to humans have been confirmed [38,39], including the emergence of a new variant of SARS-CoV-2 in communities adjacent to mink farms in North Jutland, Denmark [76]. Escaped mink and the presence of other free-living mammals (e.g. feral cats and scavenging wild carnivores) in the vicinity of mink farms could provide potential routes for onward inter-species transmission, perhaps via fomites and airborne virus from contaminated bedding and dust [39]. Mink farms in Canada for example have been identified as sources of Aleutian disease (a parvovirus) in local wild mink populations [77]. Such relationships may arise because of escaped animals establishing in the locality or because wild animals are able to access facilities where sources of food and the scent of con-specifics may act as attractants. Surveillance of wild mustelids (and other carnivores) in the vicinity of mink farms would therefore be an effective means of targeting wildlife at a relatively high risk of exposure to SARS-CoV-2. Ferrets are also farmed (for the pet trade and medical research), and in some parts of the world (e.g. UK, Spain) are used for hunting rabbits, both of which could provide potential opportunities for reverse zoonotic transmission and subsequent spread of SARS-CoV-2.

It has been suggested that mink could be a true reservoir species [38], although population density and the frequency of contact in the wild are far lower than in captivity. Studies of SARS-Cov-2 outbreaks in farmed mink have revealed evidence of rapid virus evolution, together with onward transmission to humans [38,76]. Rapid mutation of the virus and the potential emergence of host-adapted variants are probably less likely in lower density wild populations, but cannot be ruled out as indicated by the identification of amino acid polymorphisms that might influence function of the spike protein in low numbers of experimentally infected ferrets [78].

Scavenging and predation on other potentially susceptible mammals could provide opportunities for spillover into wild mustelid populations, although in most species their social organisation is likely to mitigate onward spread. Wild mustelids generally occur at relatively low densities, and with few exceptions are largely solitary, with contact amongst adults being typically confined to the breeding season, thus limiting opportunities for virus transmission and persistence. One notable exception is the European badger (*Meles meles*) which lives in social groups of varying size across its wide geographic range and reaches high densities in some locations (up to 38/km² recorded in southern England; [79]). In some places badgers thrive in agricultural

landscapes and in others they have adapted to urban environments, both of which can bring them into conflict with people and may cause them to become the subject of management interventions (e.g. trapping for culling, vaccination or relocation). Such circumstances provide opportunities for virus transmission in both directions, but may also be useful for surveillance purposes.

Great Apes

Captive and wild great apes (Hominidae) are highly susceptible to viral pathogens of humans [80,81] with respiratory infections of human origin [82,83] causing disease that poses a significant and growing threat to the conservation of wild populations across Sub-Saharan Africa [80]. Furthermore, the characteristics of ACE2 predict high susceptibility to SARS-CoV-2 amongst primates, particularly Old-World species [22] and this is borne out by the results of experimental studies [28,31,33].

Risks of exposure of primates to SARS-CoV-2 could arise wherever they have direct or indirect interactions with humans from local communities, which is not uncommon as they can become highly habituated to human activity. Interactions with humans also become more likely where deforestation opens up habitats to human access and displaces primate populations. Cross-species transmission may be facilitated wherever wild primates are captured and traded, rehabilitated in sanctuaries, approached closely for the purposes of tourism, or are the subject of field research and conservation management. The potential for transmission from humans to great apes is of particular concern because most of their important conservation areas are surrounded by densely populated human settlements where interactions with people may be inevitable, but where local community transmission of SARS-CoV-2 is not well documented. Also, although tourism provides much needed income for these conservation projects, visitors also represent a risk of introducing SARS-CoV-2 from affected human populations elsewhere.

Several documented outbreaks of respiratory disease in great apes have been linked to spillover from infected humans. These include outbreaks in chimpanzees (*Pan troglodytes*) in Uganda caused by two distinct negative-strand RNA viruses of human origin [80], human respiratory syncytial virus (HRSV) infection in western lowland gorillas (*Gorilla gorilla gorilla*) in central Africa [84], and influenza A and parainfluenza 1, 2 and 3 in mountain gorillas (*Gorilla beringei beringei*) in Rwanda [81]. Human-derived infections can have a devastating impact on endangered primate populations. Respiratory diseases are the second most common cause of morbidity and mortality among human-habituated mountain gorillas in the Virungas [81] and the second most common infectious disease amongst chimpanzees in the Centre de Rehabilitation de Primates de Lwiro (CRPL), Democratic Republic of Congo [85].

Hence, there is ample evidence for the vulnerability of endangered great ape populations to SARS-CoV-2 transmission from humans, and onward intra-species spread would be facilitated by their highly social behavior, but the potential impacts on their health are less predictable. Infection in great apes could be accompanied by high levels of mortality, might manifest as a mild or asymptomatic respiratory infection or could reflect the age and health-related variation in outcomes observed among humans. However, given past experience of human-derived respiratory infections and the precarious conservation status of the last wild great ape populations, the potential for adverse health and population impacts in great apes should be considered high and risks managed accordingly. This might involve strict health surveillance of tourists, researchers and conservation workers who may come into close contact with primates, accompanied by improved hygiene and sanitation, use of protective equipment and safe distancing, with quarantine measures where management interventions require moving animals.

Surveillance and dynamic risk assessment

The sections above describe some of the potential pathways for human to wildlife transmission and onward spread of SARS-CoV-2 in wild mammals that may be particularly susceptible to infection. However, there are many evidence gaps which limit our ability to accurately assess these risks, although new information is constantly emerging. Targeted surveillance and dynamic risk assessment provide two vitally important tools for efficiently gathering and synthesising information in this rapidly changing situation. Furthermore,

integration of these approaches permits them to inform one another, with the outcome of risk assessments directing surveillance activities and the latter providing crucial data to underpin the assessment of risks. This creates a dynamic iterative process whereby the assessment of risk is continually updated by the incorporation of emerging empirical evidence.

Pathogen surveillance in wildlife populations is challenging owing to the difficulties in undertaking representative and unbiased sampling, the practicalities of obtaining samples from free-living animals, determining the most cost-effective sampling design and the limitations of diagnostic test performance. The choice of surveillance approach should be strongly influenced by the primary purpose, which may be early detection of infection, demonstration of absence of infection, determination of presence or mapping the distribution of infection. The activities undertaken to suit a particular purpose will depend on various factors including the expected severity of disease (i.e. can mortality or morbidity be useful indicators of infection), the distribution and abundance of the host species, the availability of financial resources and of facilities for sample collection, transport, storage and testing. Programs for general surveillance to investigate unexplained mortality events in wildlife are not present in many countries and where they are they may have limited sensitivity to detect early stage SARS-CoV-2 infection and the early incursion of infection into a population when prevalence may be low.

In the first instance, opportunistic sampling of wild mammals through existing opportunities such as wildlife rehabilitation centres, veterinary hospitals, wildlife management and field research programs is likely to provide a cost-effective approach to sampling. Moving towards a more refined approach involving active targeted surveillance may be warranted where evidence from risk assessments indicates particular species or populations are at relatively higher risk. The species groups listed above could be considered as candidates for opportunistic sampling in the first instance, and for risk assessment to determine the case for more targeted surveillance.

A variety of types of diagnostic test and sampling approach could be applied to surveillance activities. Serological sampling can be applied to large numbers of individual animals, either directly by active surveillance or using sera collected for other purposes. Appropriate test validation, including determination of diagnostic sensitivity and specificity, for the species in which the test is to be applied is required to allow appropriate interpretation of results. Serology has the limitation of detecting only historical infection with limited temporal accuracy but this can make it the method of choice for screening at the population level. Testing individual animals for SARS-CoV-2 infection by PCR (polymerase chain reaction) provides confirmation of current infection status and the same sample material can be used for viral culture. This approach also allows the sequencing of genetic material which provides highly valuable phylogenetic information on the relatedness of viral lineages and allows the inference of transmission pathways. Environmental sampling has the potential to detect recent infection in large groups of individuals and has proven useful in surveillance for SARS-CoV-2 in humans via testing sewage and wastewater [86]. Similar approaches could be considered for wild animal populations, particularly if the geography of water catchments is suitable [87].

Risk assessment approaches have been usefully employed to identify likely pathways for disease incursions into wildlife populations [88]. There is an urgent need to develop frameworks for assessment of the risk of SARS-CoV-2 becoming established in wild mammal populations and onward transmission to humans. The dynamics that need to be considered in such risk assessments include three broad components: the probability of infection in a wildlife population from a human source; the probability of establishment in the wildlife population and the probability of spillback of infection to humans (Figure 1). This is clearly a non-linear (dynamic) process but combining these three probabilities constitutes the over-arching risk of infection in wildlife impacting on human health. It will also inform the potential for impacts on wild populations themselves. Each of the components of the risk assessment will be informed by data on several inter-related processes that require careful consideration. It is highly likely that the data will be deficient for many, however such a framework will identify the data required to populate a risk assessment and consequently the emerging

information from experimental studies and surveillance can be input to the assessment in a dynamic iterative process.

The potential for exposure of wildlife to an infection that is widespread in the human population and has already spilled over into companion and captive animals must be considered significant, but the likelihood of exposure, and subsequent infection, will be greatest where wild animals are sympatric with humans or are subject to close interactions through their management (e.g. veterinary intervention, conservation, research, hunting, pest control etc.). Such circumstances are also likely to provide opportunities for spillback from infected wildlife. Direct contact with infected hosts may however not be necessary for exposure to SARS-CoV-2, as studies have demonstrated the potential for coronaviruses to remain infectious for several hours on some surfaces [89] and in human faeces [90]. One recent study suggested that the contamination of aquatic systems with faeces from infected humans could provide a potential route for spillover into wild mammals such as raccoons and bats [87]. Information on the demography and social behavior of candidate wild hosts will be critical determinants of the potential for spillover to lead to maintenance and onward spread. Host abundance will be an important consideration although simple relationships between density and infection dynamics may be confounded by host behavior and indirect transmission routes [91]. Similarly, aggregations such as colonies of roosting or hibernating bats and the social groups of some social carnivores and primates might be expected to enhance transmission, although social structure in wild mammal populations can also limit epidemic spread [92]. Hence, the ecological characteristics of potential host populations will need to be carefully considered during the assessment of risk. Figure 1 provides a non-exhaustive list of the types of information that would be informative for such a risk assessment.

There is an absence of quantitative information on the likelihood of the various processes required for infection, maintenance and onward transmission of SARS-CoV-2 in wildlife. In such situations a qualitative assessment can be adopted, using the current body of existing knowledge and, if necessary, expert opinion (approaches endorsed by the World Organisation for Animal Health [88]). A semi-quantitative risk assessment may be appropriate if the aim is to rank the risks across different wildlife species or interventions [93]. Most risk assessments undertaken for wildlife health (due to emergence of a pathogen or for translocations) are qualitative because accurate quantitative data on many of the requirements identified in Figure 1 are often unavailable [94,95]. Nevertheless, where some quantitative information is available, it can be incorporated within a qualitative assessment.

For both qualitative and quantitative approaches, and especially due to the many data gaps and deficiencies in our understanding of the epidemiology of SARS-CoV-2, it is essential that the uncertainty is captured within the framework and communicated. This could include uncertainty relating to not only the quality of the data used to assess the risk (e.g. published literature vs expert opinion, the biological relevance of experimental studies), but also model uncertainty as many of the pathways of SARS-CoV-2 transmission between humans and wildlife may be speculative. Such uncertainty is often reduced as further information is collated and it is therefore important that such a risk framework is readily adaptable to the inclusion of new data. The value of such a framework clearly extends more widely than the assessment of SARS-CoV-2, to other emerging zoonotic diseases that could have a significant impact on human and wildlife health.

The inter-species infection dynamics of SARS-CoV-2 is highly complex compared to previous zoonotic CoVs due in part to its broad host range [75]. Another reason is that none of the previous zoonotic coronaviruses have achieved the same rapid, efficient, and large scale spread within the human population sufficient to support widespread spillover and cross species jumping. Consequently, molecular epidemiological surveillance will be required across this host range, using viral isolates obtained from different animal species and identifying the genetic changes and novel adaptations linked to cross-species transmission [17].

Conclusions

Although the dynamics of the SARS-CoV-2 pandemic are being driven by human-to-human transmission with no evidence that domestic or wild animals are playing an important role, this may not remain the case. The establishment of a reservoir of infection in a wild animal population would pose a significant risk to public

health if it had potential to spillback into communities where the burden of infection had been reduced through control measures. Furthermore, sustained transmission in a wild host population would provide an opportunity for evolutionary adaptation of the virus, which could potentially influence transmission dynamics and the effectiveness of diagnostics and vaccines, although host-adaptations might equally limit risks of transmission to other species. These processes are of particular relevance to coronaviruses as their ability to undergo genetic recombination combined with a relatively high mutation rate facilitates their rapid adaptation to new ecological and host niches [96,97]. This is illustrated by the recent identification of a SARS-CoV-2 variant originating in farmed mink with subsequent detection in the local human population [98], although the epidemiological implications of this phenomenon are as yet unclear.

Identifying the wildlife reservoir of SARS-CoV-2 before the virus spilled over into the human population will fill an important gap in our current knowledge of host switching events. Understanding the pathogen transmission chain and why viruses jump from one species to another remains a paradox and a 'black box' in our knowledge of the evolution of SARS-CoV-2. Studies demonstrating the susceptibility of wildlife species as either maintenance hosts or reservoirs of SARS-CoV-2 are in progress and these data will corroborate our understanding of virus-host interactions and assist in identifying the 'missing link' between wildlife species and infection in humans. Subsequent adaptation of the virus has accelerated human-to-human transmission resulting in the largest pandemic in modern times. Furthering our understanding of the mechanisms that support a virus host switching event and how the virus adapts to a new host will inform the development of strategies to help prevent future pandemics.

The SARS-CoV-2 pandemic serves as a powerful demonstration of the links between the health of wildlife, domestic/farmed animals and humans, and the importance of disease surveillance and risk assessment at the many interfaces. The emergence of this virus from an unknown wildlife source has had a significant impact on the global human population, and spillback to wildlife may carry risks of enhanced mortality in some wild species with implications for conservation. Hence, it is important to take a One Health approach to investigation of the potential role of wild mammals in the continuing epidemiology of SARS-CoV-2. This should include integrated programmes of targeted surveillance and the dynamic assessment of risks to animal and human health. Importantly, this need extends beyond the current pandemic, and speaks to the wider requirement for a proactive approach to assessing the dangers of diseases emerging from wildlife [8] and the implications for vaccine pipelines [99]. Hence we reiterate the call by Olival *et al.* [56] for the development of an adaptive framework for surveillance and risk assessment of other coronaviruses in wildlife, domestic animals and human populations at high risk of exposure, so that in the future we may be better prepared to prevent and control their potential impacts on human and animal health.

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Figure 1. System dynamics for the establishment of SARS-CoV-2 in humans, environment and wildlife showing example data requirements for a risk assessment and the types of surveillance methodologies that could be used to detect and monitor infection.