COVID-19: Animal Epidemiology and Zoonotic risk

Maurizio Ferri * and Shin Jie Yong **

* Corresponding author at: Via Nizza 11, 00198 – Roma, Tel.+39 06 8540347/3332653644; *email:* ferrim282@gmail.com.
** Department of Biological Sciences, School of Science and Technology, Sunway University, Bandar Sunway, Malaysia

Abstract

The COVID-19 pandemic represents one of the greatest public health crises in recent history that caused unprecedented and massive disruptions of social and economic life globally. It is widely acknowledged that bats are the animal reservoir of coronavirus 2 of the severe acute respiratory syndrome (SARS-CoV-2), the causative agent of the human coronavirus disease 2019 (COVID-19). It has also long been known that coronaviruses circulate among different animal species. However, much remain to be understood of the epidemiology, the presumed existence of intermediate animal species and current and potential animal routes of SARS-Cov-2 transmission to humans. The recent observational and experimental studies also highlight the role of domestic and farmed animals in the epidemiology of COVID-19. This raises concerns of the potential spread of infection among susceptible animal species, with the risk of evolving into panzootic, and the likely occurrence of anthropozoonoses or reverse zoonosis (from humans to animals), and again the reverse anthroponosis, with spill-back to humans (eg. recent human-mink-human transmission). As for other wildlife emerging pathogens, the animal-human spill-over of SARS-CoV-2 is linked to a closer interface with humans, with the resulting risk of a pandemic. This knowledge has meaningful implications for the design of effective wildlife animal surveillance (epidemic intelligence) targeting CoVs in animal reservoirs, and requires the mobilization of

different lines of expertise, notably veterinary epidemiologists and virologists, within a multi-disciplinary approach according to the One-Health principles.

Keywords

COVID-19; SARS-CoV-2; animal reservoir, epidemiology, anthroponotic risk, epidemic surveillance, One Health.

1. Introduction

The novel pneumonia infection, coronavirus disease 2019 (COVID-19), caused by coronavirus 2 of the severe acute respiratory syndrome (SARS-CoV-2), was first detected in China in December 2019. It later marched relentlessly across the world, causing a pandemic, as the World Health Organization (WHO) declared in March 2020. COVID-19 has caused unequaled and unprecedented disruptions of the social structure, hit the world economy, and overstretched the global health care systems beyond their actual capacity. It created a distinct line between before and after (the 'new normal'), both at the community and individual levels. As of 25 November 2020, there have been 59.204.902 confirmed cases of COVID-19 reported worldwide, with more than 1.3 million deaths (https://www.worldometers.info/coronavirus/). The US, Brazil, and India have the world's largest caseload. The latest risk assessment carried out by the European Center for Disease Prevention and Control (ECDC) finds that community transmission persists in most EU/EEA countries and the UK and EU candidate and potential candidate countries (ECDC, 2020a). The ECDC also reported a resurgence of cases or large localized outbreaks, mostly caused by the relaxation of non-pharmaceutical interventions, such as physical distancing in several countries. To exacerbate this unsettling backdrop, yet much more remains to be understood about this protean SARS-CoV-2 due to a plethora of known unknowns. Indeed, COVID-19 is a new infection with a relatively short period of occurrence,

and time is key to understanding how it will evolve. Despite the massive scientific research efforts, the medium and long-term effects are difficult to quantify due to the high levels of uncertainty encompassing both the veterinary and human clinical side. For the former to name a few: the original animal reservoir (Zhou, et al.., 2020), intermediate host (Zhang, et al., 2019), routes and dynamics of virus transmission to humans (ASM, 2020), predictors of reinfection (Ledford, 2020), and roles of food (EFSA, 2020a) and animals. Whilst from the latter, there is insufficient understanding of the impact of mutations on the pathogenicity of SARS-CoV-2 (Hangping et al., 2020), the potential long-term neurological disorder sequelae (Troyer, Kohn, & Hong, 2020), the effectiveness and safety profile of the current therapeutic regimes (Tobaiqy et al., 2020), and the immune responses to the virus and the duration of neutralizing antibodies (Ju et al., 2020). Moreover, medical literature is now turning its attention to the long-term health effects of COVID-19, substantiated by a myriad of lingering symptoms, including lung, heart, and nervous system impairment experienced by patients colloquially known as 'long haulers' (Honigsbaum & Krishnan, 2020). Nobody can predict the duration and depth of the health consequences of this post-viral syndrome, and the impact on the public health systems. Indeed, COVID-19 pandemic is producing outrageous spill-over effects encompassing a wide range of deeply intermingled social and biological phenomena by virtue of its syndemic nature (Horton, 2020).

2. The zoonotic origin of COVID-19 pandemic

As a necessary caveat to explain the origin of the COVID-19 pandemic, we have to recall that about 60% of all emerging human pathogens are zoonotic in origin (found in animals), with a whopping 72% of these originating in wildlife, and are increasing significantly over time (Jones

et al., 2008). The emerging zoonosis or the re-emergence of existing ones recognize the combined effects of several factors such as socio-economic, environmental, and ecological. Notably, the environmental and climate changes, mostly spurred on by human activities, have led to increased vector population and ecological disruption of wildlife and their natural habitats. All of these caused changes in the pathogen's niche, leading to their introduction into the human population. But, what is particularly worrying and poses a serious threat to the wild animal-human interface is the existence of an estimated 1.67 million unknown viruses that are infecting the animals. Many of these have the potential to cause the next animal-man spill-over (Harding, 2020). Thus, preventing the transfer of these pathogens from animals into humans and reducing the risk of related outbreaks, and the impact on human health and the global economy, represent a key challenge for any society. Although there is debate about its exact source and infection pathway, SARS-CoV-2 infection or COVID-19 is a zoonosis that originated from a wildlife host, which jumped to humans possibly via an intermediate animal host, and consequently spread by person-person transmission (Andersen et al., 2020). Laying on close links among the animal, environment, and human health, the COVID-19 control strategy requires the inter-disciplinary collaboration between human medical and veterinary professions and merging of the two health perspectives in a One Health approach (Katharina Stark, 2020). Moreover to accomplish the goal of a better understanding of complex socio-ecological systems, there is the necessity for a greater inclusion of social scientists and anthropologists (Lainé & Morand, 2020).

2. SARS-CoV-2: a novel coronavirus

SARS-CoV-2 belongs to the family *Coronaviridae*, subfamily *Coronavirinae,* genus *Betacoronavirus*, in the species *severe acute respiratory syndrome-related coronavirus*.

(Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020), CoVs are ecologically diverse (de Groot, et al., 2020). There are four subgroups of the coronaviruses family: alpha, beta, gamma, and delta-coronavirus. The alpha-coronaviruses include the canine coronavirus (CCoV), responsible for enteric and respiratory forms, and the feline coronavirus (FCoV), causing the feline infectious peritonitis (FIP). These coronaviruses are not associated with the current COVID-19 pandemic. Beta-coronaviruses also infect mammals and include SARS-CoV, responsible for the severe acute respiratory syndrome (SARS) in 2002 and 2003, and MERS-CoV, that caused the Middle East respiratory syndrome (MERS) endemic in Middle Eastern countries in 2012. Beta-coronaviruses also include low pathogenicity coronaviruses that are endemic in humans, such as HCoV (Human Coronavirus)-OC43 and HCoV-HKU1. In contrast, alpha-coronaviruses include HCoV-NL63 and HCoV-229E (Corman et al., 2018), which globally contribute to about one-third of the common cold infections in humans. In severe cases (Pene, et al., 2003), these four HCoV can cause life-threatening pneumonia and bronchiolitis, especially in the elderly, children, and immunocompromised patients (Gorse et al. , 2009). Gamma and delta-coronaviruses mostly infect birds, but some can infect mammals; for example, pigs can be infected by porcine deltacoronavirus (PDCoV). SARS-CoV-2, which is genetically related to SARS-CoV based on the 82% sequence identity (Chan et al., 2020) is a virus minute in size (65-125 nm in diameter), harbouring a linear single-stranded positive RNA (ribonucleic acid) genome of nearly 30k.

3. The evolutionary lines of coronavirus among animal species

It is noteworthy that coronavirus found in almost all species of domestic and wild animals recognize common ancestors. Recent work on the phylogenetic analysis has clarified the origins and the evolutionary lines of CoVs among animal species (Lorusso et al, 2020). For example,

canine and feline coronaviruses recognize a common ancestral virus, with some lineages derived from multiple recombination events with an unidentified genetic source; canine coronavirus CCoV-II is an ancestor of the pig transmissible gastroenteritis virus (TGEV); another canine coronavirus CRCoV most likely originates from the bovine coronavirus (BCoV), which is the direct ancestor of the human coronavirus HCoV-OC43; genomic sequences very similar to those of the porcine epidemic diarrhoea virus (PEDV) have also been found in bats and humans (HCoV-NL63) suggesting a common evolutionary precursor (Banerjee et al., 2019), the most recent swine acute diarrhoea syndrome coronavirus (SADS-CoV) HKU2, which was responsible for a large-scale outbreak of the fatal disease in pigs in China, is a most likely recent spillover from bats to pigs. From a study conducted in China in 2018, the analysis of the sequence of the HKU2 virus, causing a large-scale outbreak in four pig farms in China, suggested the correlation (sequence identity of 96-98%) to bat coronaviruses, mainly in bats of *Rhinolophus* spp., a known reservoir of SARS-related CoVs, demonstrating the inter-species transmission of CoVs (Zhou et al., 2018).

4. CoVs genetic diversity

CoVs are distinguished by their remarkable complexity and high genetic diversity. Generally, these attributes rely both on the phenomenon of recombination and the high rate of mutations. Indeed, the high frequency of viral recombination explains both the emergence of the SARS-CoV through multiple recombination events between different bat SARS-related coronaviruses (SARSr-CoVs), (Li et al., 2020) and the new SARS-CoV-2 as the product of recombination between a bat SARS-like CoVs and a coronavirus of unknown origin. (Ji et al., 2020). As for genetic variability, the gene encoding the spike (S) protein, present on the surface or envelope of the virus, shows significant variability. However, no genome changes have been identified

that are shown to affect virulence. Notably, a recent mutation-based evolutionary study of SARS-CoV-2 showed that, at least in this stage of the COVID-19 pandemic, the virus had accumulated only moderate genetic diversity, with an average pairwise difference of 9.6 single nucleotide polymorphisms (SNPs) between any two genomes (van Dorp et al., 2020).

4.1 CoVs recombination

Is well known that coronaviruses are prone to high recombination and adaptive events leading to the generation of new viral species, host shifts, and the emergence of new strains (Rehman, et al., 2020; Graham & and Baric, 2010). The phenomenon of recombination of beta-coronavirus, to which SARS-CoV-2 belongs, is linked to the gene encoding the S protein, which helps virus particles to penetrate the cells. The S protein is composed of two subunits, with S1 responsible for receptor binding, and S2 for membrane fusion (Wrapp et al., 2020). The receptor-binding domain (RBD) on the S protein's N-terminal is the key element for beta-coronavirus entering into host cells. Therefore, the structure and configuration of S protein determine the infectivity of the virus and its transmissibility in the host (Hulswit et al., 2016). Basically, S glycoprotein has the function of recognizing and locking the ACE2 receptor (angiotensin-conversion enzyme 2) expressed by the target cells of the respiratory system and other organs, and allowing the virus to penetrate inside the cell following the fusion of the viral lipoprotein with the cell membrane and then to replicate. The S protein is the major target for neutralizing antibodies that bind to the virus and render it non-infectious. The acquisition of new genetic traits and the greater efficiency of adaptation to the human cells allowed the SARS-Cov-2 virus to achieve a rapid and uncontrolled community spread. An explanation of this high plasticity in terms of gene content and recombination resides on the 'long genomes expanding the sequence space available for

adaptive mutation, and the S protein which can adapt with relative ease to exploit different cellular receptors' (Forni et al., 2017).

4.2 CoVs mutation

All replicating viruses, including coronaviruses, continuously accumulate genomic mutations that persist due to natural selections and contribute to the enhancement of viral replication and infection, as well as to quelling the host immune attack. Despite that RNA viruses, such as SARS-CoV-2, human immunodeficiency virus (HIV) and influenza, have a higher mutation rate than DNA viruses (Lauring & Andino, 2010) as they are copied inside the cell, sequencing data suggest that SARS-CoV-2 is changing its genome more slowly as it spreads, with an overall mutation rate 10-fold lower than a typical RNA virus. This was the case of the SARS virus, which during the early stages of the human-to-human transmission chain, picked up a mutation called deletion that might have slowed its spread (Muth et al., 2018). The SARS-CoV-2's genetic code has just under 30,000 nucleotides of RNA, or letters, that spell out at least 29 genes. Typically, it accumulates only two single-letter mutations per month in its genome, a rate of change about half that of influenza and one-quarter that of HIV. On a global scale, two SARS-CoV-2 viruses collected from anywhere in the world differ by an average of just 10 RNA letters out of 29,903 (Callaway, 2020). But why such a low mutation rate? Coronaviruses have the largest known genomes of animal RNA viruses (ranging from 26-32 kilobases (kb) (Gorbalenya et al., 2006). The large genome size and its expansion are made possible by proofreading enzymes that identify and correct errors that are introduced during genome replication. This mechanism is mediated by the non-structural protein 14 (nsp14) that cuts out mutated genes as the RNA dependent RNA polymerase (RdRp) (an enzyme that makes RNA

195 genes) synthesizes them. Despite the internal proofreading, various mutations have been
196 detected in SARS-CoV-2 genomes, and they are being used to track virus spread and evolution,
197 and control the pandemic (Rausch et al., 2020). The genomic mutation, leading to amino acid
198 changes in the surface protein, can significantly alter the viral function and/or interactions with
199 the host's neutralizing antibodies. Moreover, any mutation in the gene encoding S protein and
200 particularly in the RBD affects its infection and cross-species capability. These mutations are
201 being continuously reported (Pachetti et al., 2020). However, not all mutations are beneficial
202 for the virus's ability to spread or cause disease. A recent study assembled a dataset of 7,666
203 public genome and analysed the emergence of genomic diversity over time (van Dorp et al.,
204 2020). Due to extensive transmission, SARS-CoV-2 showed a genetic diversity in several
205 countries, which recapitulates its entire global diversity. Some genomes have remained largely
206 invariant to date, and others have already accumulated diversity. Notably, nearly 80% of the
207 detected recurrent nucleotide mutation are non-synonymous substitution, that alters the amino
208 acid sequence of the protein level, and suggest a possible ongoing adaptation of SARS-CoV-2 to
209 its novel human host. The massive sequencing efforts of the molecular epidemiology
210 contributed not only to estimating the global genetic variability and evolutionary rate of SARS-
211 CoV-2, with significant implications for disease progression, but also to the development of drug
212 and vaccine, notably associated to information on RBDs. Consequently, it is important to study
213 and characterize SARS-CoV-2 mutation not only to assess possible drug-resistance viral
214 phenotypes, but also to spot mutations that might correlate with different SARS-CoV-2
215 mortality rates. Moreover, what is interesting in terms of phylogenetic analysis and past
216 demography of SARS-CoV-2 is that the results obtained by multiple independent groups point to
217 all sequences sharing a relatively recent common ancestor towards the end of 2019, and supports

that the COVID-19 pandemic started sometimes around 6 October 2019-11 December 2019, a period when the animal-human spill- over occurred.

4.3 The G614 variant

A mutation increased in frequency in almost all sequenced samples of SARS-CoV-2 from people with COVID-19, occurred at the 614th amino-acid position of the S protein subunit 1, where the amino acid aspartate (D, in biochemical shorthand) is replaced by glycine (G) because of a copying fault that altered a single nucleotide. The so-called D614G mutation (the initial 'D' is now the 'G' variant), which was first spotted in viruses collected in China and Germany in late January has been shown to rapidly accumulating since its emergence (Korber et al., 2020**),** and is now the dominant SARS-CoV-2 lineage in Europe as well as in the United States, Canada and Australia, constituting more than 70% of the global circulating SARS-CoV-2 variants (Korber et al, 2020a). Despite conclusions on D614G mutation enhancing the viral spread are not reached yet (studies suggest, but not prove increased viral transmissibility), epidemiological data suggest that SARS-CoV-2 with G mutation transmit more efficiently (Zhang et.al., 2020) and is significantly more infectious (Korberet al , 2020a; Qianqian Li et al., 2020), but is not linked to severe clinical outcomes and increased fatality rate. A study carried out in the UK did not show a conclusive signal that patients infected with the 614G variant have higher COVID-19 mortality and clinical differences in people infected with either virus (Volz et al., 2020). However, one explanation of the increased infectivity is that the D614G mutation, by relaxing connections between the three smaller peptides, makes open conformations more likely and facilitates the binding to receptors on human cells, which might increase the chance of infection (Mansbach et al., 2020; Yurkovetskiy et al., 2020). Although the D614G mutation happens in the viral S

protein, it does not change the S protein's RBD, a region that neutralizing antibodies often target. This suggests that D614G does not stop the immune system's neutralizing antibodies from recognizing SARS-CoV-2. Indeed, a recent study has shown that sera from hamsters infected with the D614 variant contain antibodies that could neutralize the G614 variant (Plante et al., 2020).

5. SARS-CoV-2 and the role of bats and intermediate hosts

The genome sequence of SARS-CoV-2 has 79.6% identity to a SARS-CoV and 96.0% identity to a bat RaTG13 beta-coronavirus subgenus sarbecovirus (**Figure 1)**. This provides evidence that the virus is of bat origin and that bats are a natural host. SARS CoV-2 is most likely the result of a leap of the virus from bats to an intermediate host, and from this to humans (spillover), similarly to the transmission mechanism of SARS and MERS coronaviruses. Based on a molecular clock evaluation of spike and nucleocapsid genes of these viruses, the most current frequent ancestor of all genotypes of these viruses is dated to the 1950s. This clearly demonstrates that no way anyone in a Wuhan laboratory in 2019 should have affected the RNA code of a virus of the mid-1950s. This is confirmed by a recent analysis, which shows that SARS-CoV-2 is not a laboratory construct or a purposefully manipulated virus, thus demystifying the laboratory-based scenario behind the emergence of related SARS-CoV-like coronavirus (Andersen et al., 2020). A key factor for the infectivity and the high efficiency of person-to-person transmission is the binding affinity of the RBD with the cell entry receptor ACE2 of the host, similar to SARS-CoV, but with 10 to 20 times higher binding capacity (Wrapp et al., 2020). Structural and biochemical studies indicate that RBD in the S protein of SARS-CoV-2 shows a high affinity and binding capacity to ACE2 of different animal species such as ferrets, cats, and other species with high homology of receptors, and therefore increases

their susceptibility to infection (Shi et al.2020; Wan et al., 2020). This mechanism, supporting a likely circulation of coronavirus among animal species (inter-species transmission), with a possible animal-human passage, could have mediated the initial transmission of SARS-CoV-2 from bats to other mammals living in closer contact with humans. Subsequently, the acquisition of new genetic traits and the greater efficiency of adaptation to the human cells allowed the SARS-CoV-2 virus to achieve a rapid and uncontrolled community spread. This mechanism makes it necessary to implement appropriate up-to-date and integrated surveillance programs to detect genetic signals within eco-environmental hotspots with a close animal-human interface and epidemic or pandemic potential.

5.1 The role of bats as reservoirs of CoVs and other human lethal viruses

Bats are among the world's most diverse mammals. There are approximately 1,400 species of bats inhabiting every continent except Antarctica (Simmons & Cirranello, 2020). Bats contribute to the balanced ecosystem by serving as pollinators and do a phenomenal job of eating mosquitoes harbouring dangerous viruses such as malaria, yellow fever, chikungunya, Zika, and others. Unfortunately, alongside these advantages, bats of certain species are well recognized as reservoirs hosts of emerging viruses that can cross species barriers (i.e., spill-over) to infect humans and other domestic and wild mammals. Some of these viruses, such as Ebola, Nipah, Hendra, Marburg, and Rabies, are lethal for humans, but not for bats, although bats infected with rabies virus will eventually die of the disease (Wang & Anderson, 2019). Each of these viruses recognises an intermediate host before they jump to humans (e.g., gorilla and chimpanzee for Ebola, pigs for Nipah, horses for Hendra, and African green monkeys for Marburg). This poses a serious threat to human and animal health, in particular when human activities, such as deforestation, modern agricultural practices, hunting, and urbanization, disrupt their habitat

and create conditions for constant and repeated jumps of viruses from these natural hosts to humans. It is also plausible that physiological and environmental stressors caused by humans encroaching upon their habitat, could increase the probability of individuals becoming shedders of more viruses in saliva, urine, and faeces and thereby to infect other animals and humans (Plowright et al., 2015). This is also confirmed by findings of physiologically stressed condition due to white-nose syndrome, a disease caused by the fungal *P. destructans* (Davy et al., 2018) or other pressures (Plowright et al., 2008) that make bats more susceptible to viral infection, with worsening of disease outcomes, and/or increased viral shedding. The bats' protection against lethal viruses lies in its biological evolution, in particular in the adaptation to flight, which has modified their immune system and led to the development of protective cellular mechanisms (O'Shea et al., 2014; Zhang et al. 2013). Chinese researchers have speculated on the mechanism of DNA sensing (Xie et al. 2018). The metabolic demands of flight cause DNA damage and the release of self-DNA into the cytoplasm. In bats, like other mammals, these fragments are recognised as external invasions. However, the bat's evolution caused a loss of some genes encoding for the immune response, with the result of a weak response and less cellular damage. It is also known that some bats constantly activate an antiviral immune response called the interferon pathway (Brook et al., 2020). Therefore, while in most mammals, an over-activation of innate immune pro-inflammatory pathways causes harmful effects, bats can regulate the response against stimulatory sensing of cytosolic DNA caused by oxidative stress. This may explain their longevity (they live up to 40 years), and their ability to maintain a balance and coexistence with viruses. Also bats are natural reservoirs of coronaviruses, with which they have ancient and ancestral relationships. Indeed, bats potentially are progenitor host of all coronaviruses (Vijaykrishna et al., 2007) with a global

diversification through a process of within-host evolution and cross-taxonomic host switching (Olival et al., 2020). In Japan, for instance, a new betacoronavirus, subgenus sarbecovirus called Rc-0319 was recently identified from *Rhinolophus cornutus* endemic to Japan. While Rc-0319 belongs to the same evolutionary clade as SARS-CoV-2, the two are very distantly related with only 81.47% genetic identity. However, importantly, this study indicated that there are still uncharted relatives of SARS-CoV-2, even in places outside of China (Murakami et al. 2020).

5. 2 From bats to humans

It is now accepted that the wild fauna, probably bats, constitute the initial reservoir of the SARS-CoV-2. Likewise SARS and MERS viruses, with their jump from the bat to the palm civet and camel (intermediate hosts), respectively, and from these to humans, phylogenetic analysis, and epidemiological investigations have demonstrated that the animal-to-human spill-over most likely occurred for SARS-CoV-2. Genetic sequence data reveals that the closest known betacoronavirus to SARS-CoV-2 is the RaTG13 sarbecovirus (>96% homology) of *Rhinolophus affinis*, a horseshoe bat (Zhou et al., 2020). However, there is a substantial difference in the RBD between the two coronaviruses, which hints at the possible involvement of an intermediate host (Wrapp et al., 2020). Pangolin was strongly suspected as the intermediate host of SARS-CoV-2 at the beginning of the pandemic, but more recent data suggests otherwise, as discussed in the subsequent paragraph. Epidemiological and phylogenetic investigations of SARS-CoV and SARS-CoV-2 viruses have shown that human hunting, sale, and consumption of wild animals over long periods have created the conditions for the transmission of coronavirus from bats to humans directly (Suwannarong &. Schuler, 2016) or through proximity to other animals acting as intermediate hosts. It should also be noted that no animal coronaviruses similar enough to be the direct progenitor of SARS-CoV-2 have

been identified. Simultaneously, however, the diversity of coronaviruses in bats and other species is largely under-sampled (Crowley, et al., 2020). Regarding the place where the zoonotic spill-over may have occurred, human epidemiological data link a high percentage of first and second-generation cases of SARS-CoV-2 infections to the seafood market in Wuhan Province of China (WHO, 2020). Therefore, SARS-CoV-2 may have made the leap from animals to humans in the market (Gralinski & Menachery, 2020). However, some of the first confirmed cases of COVID-19 (i.e., 13 out of 41) had no connection to the market, suggesting a different source of infection (Huang, et al., 2020). In another group of 99 patients hospitalized for COVID-19 early in the outbreak in Wuhan, only 49% had prior exposure to the market (Chen et al., 2020). According to a Chinese news release, previous attempts to find SARS-CoV-2 isolates in animals in the Wuhan market have been unsuccessful (Global Times, 2020). Instead, SARS-CoV-2 isolates could only be detected in environmental surfaces such as doorknobs and floors (Zhang & Holmes, 2020). Henceforth, the sharp rise in COVID-19 cases linked to the Wuhan market may have been due to a human super-spreader event instead of actual zoonotic transmission (Yuen et al., 2020). As follows, since SARS-CoV-2 has an incubation time of approximately two weeks, the first zoonotic transfer most likely happened in November before its detection in December of 2019 (Coen, 2020). Another possible scenario is that the SARS-CoV-2 progenitor has jumped directly from bats to humans, even before cases are identified by the surveillance system (Wu et al., 2020). This progenitor could even be the RaTG13 sarbecovirus that, interestingly, can infect cells expressing the human ACE2 receptor (Shang et al., 2020). Inside the human host, the SARS-CoV-2 progenitor could have undergone rapid evolutionary adaptation that enabled the efficient replication in human cells. These adjustments would then have allowed SARS-CoV-2 to produce the number of cases large

enough to be detected by the surveillance system. This hypothesis could explain the strange phenomenon where SARS-CoV-2 genomes sampled early in the pandemic were relatively stable with low mutation rates (Chaw et al., 2020). In contrast, numerous genetic mutations and recombination were observed with SARS and MERS genomes when they first appear in the human population (Forni et al., 2017). Indeed, rapid evolutionary adaptations or changes in the viral genome is expected during host-switching (Longdon et al., 2014). These indicate that the SARS-CoV-2 evolutionary adaptation may have been completed before its discovery in Wuhan in December 2019. Others have even suggested the possibility of SARS-CoV-2 circulating silently in humans for years before its outbreak, probably initiated by a human super-spreader event, in the Wuhan market. Scientists have predicted that the 4% genetic differences between RaTG13 and SARS-CoV-2 represent about 50 years of evolutionary time gap. Indeed, a more recent phylogeny dating study discovered that the common ancestor of SARS-CoV-2 and RaTG13 sarbecovirus dated back to either 1948-1982 (Boni et al., 2020). This indicates that the lineage giving rise to SARS-CoV-2 have been circulating in bat populations for decades, which also means that SARS-CoV-2 evolved in bats and not in any intermediate host. The direct bat-to-human spill-over theory is not entirely improbably as it had happened with prior bat coronaviruses. For example, a serological surveillance study found that 2.7% of 218 villagers in Yunnan Province of China, a region where RaTG13 sarbecovirus was first isolated, carried antibodies specific for SARS-related coronavirus (SARSr-CoVs) of bats (Wang et al., 2018). This further hints at the possibility that SARS-CoV-2, whose closest known relative in RaTG13, could have jumped to humans from bats without the need for an intermediate host. Besides, SARS-CoV-2 does not seem to replicate efficiently in 13 different bat-derived cell lines, indicating that SARS-CoV-2 may not have evolved in bats (Lau et al., 2020; Chu et al., 2020).

At the same time, SARS-CoV-2 could bind to the human ACE2 receptor with a remarkable affinity at 10-20-times higher than that of SARS-CoV-1 (Davidson, Wysocki, & Batlle, 2020).

5.3 The role of intermediate hosts (pangolin?)

SARS-CoV-2/bat coronavirus genomic divergence is an important piece of evidence that suggests the virus could have passed to humans through an intermediate species. Indeed, although the bat's RaTG13 coronavirus is closer to SARS-CoV-2 throughout the genome, it is also true that the pangolin's coronavirus S1 protein is much more closely related to SARS-Cov2 than the bat's RaTG13 coronavirus. Currently, the evidence indicates that pangolins (*Manis javanica*) may serve as an intermediate host for SARS-CoV-2 due to the high sequence identity with a CoV isolated from them (Lam et al., 2020). The genome of pangolin-CoV has a 90.55% and 91.02% sequence identity to the genome of RaTG13 and SARS-CoV-2, respectively. Furthermore, the RBD of pangolin-CoV and SARS-CoV-2 only differ by one amino acid change, and this amino acid is not one of the five critical residues involved in the human ACE2 receptor binding (Zhang, WU, & Zang, 2020). But the confirmation of an intermediate host is not yet possible due to sequence diversity on the S protein between pangolin-CoV and SARS-CoV-2. Moreover, a recent study found no evidence of coronavirus infection in wild pangolin entering the upstream portion of the wildlife trade in Malaysia. This supports the hypothesis that infection originated from anthropogenic smuggling (Lee et al., 2020). Besides, cells expressing the pangolin ACE2 receptor are not permissive to infection by the RaTG13 bat sarbecovirus (Li et al., 2020). Since RaTG13 could not infect a cell through the pangolin ACE2 receptor, it is unlikely that pangolin is the intermediate host of SARS-CoV-2. As we can see in the role of the pangolin in the outbreak, there are conflicting opinions among scientists. Some of them support the evidence of the first study (Lam et al., 2020), while others questioned the direct jump of the

virus from pangolins to humans, noting differences between the pangolin and human viruses and the unclear path established from bats to pangolins (Li et al., 2020). Indeed both bats and pangolins have viruses similar to the new coronavirus, so it is possible that they form the raw material for the SARS-CoV-2 eventually transformed in humans or animals. Scientists at the University of Cambridge extended the phylogenetic network analyses from 160 complete virus genomes to determine the age and origin of SARS-CoV-2 (Forster et al., 2020). They suspected that the new coronavirus virus might have been spreading quietly in animals and humans for years, and did not come from the animal market in Hubei province. Then it mutated (a coronavirus typically acquires one mutation a month), and gradually evolved into a highly adaptive and final 'human-efficient' form months ago, but stayed inside a bat or other animal or even human for several months without infecting other individuals. The first outbreak could be a recent event that resulted from the last few mutations that completed the leap from harmless strain to the deadly pathogen.

5.4 Animal origin and route of transmission of SARS-CoV-2 to humans

Epidemiological investigations, including monitoring of coronavirus, aim to confirm the animal reservoir and establish their potential for COVID-19. As discussed above, the current evidence provided by the phylogenetic analysis of SARS-CoV-2 and genomic comparisons with other coronaviruses present in animals, led to speculation that SARS-CoV-2 originates from an animal reservoir (bats), and that it made the leap to humans through an intermediate host (e.g., the pangolin). However, to date, there is insufficient scientific evidence to safely identify a particular reservoir, as occurred with the SARS and MERS viruses, or to explain the route of transmission to humans. Further studies are therefore needed to understand the diversity and distribution of coronavirus in bats and their role in the current COVID-19 pandemic.

6 Animals SARS-CoV-2 infects

The first global documented case of non-domestic animals infected with SARS-CoV-2 were the four tigers and three lions residing in the Bronx zoo in New York, following exposure to caretakers who carried SARS-CoV-2 (OIE, 2020a). Although the tigers developed respiratory symptoms, they later recovered (https://www.nationalgeographic.com/animals/2020/04/tiger-coronavirus-covid19-positive-test-bronx-zoo). Notably, infectious SARS-CoV-2 particles could be identified in the carnivores' respiratory and faecal samples. Detailed genomic analyses further revealed two distinct SARS-CoV-2 genomes in the carnivores that are identical to the SARS-CoV-2 genomes isolated from the caretakers, indicating that at least two separate events of human-to-animal transmission occurred (McAloose et al., 2020). Soon, reports of pets contracting SARS-CoV-2 from their owners emerged, although they were mostly asymptomatic. The first three such cases happened in Hong Kong in April and May 2020, where two dogs (OIE, 2020b) and a cat (The Government of the Hong Kong, 2020) owned by hospitalized COVID-19 patients were tested positive for SARS-CoV-2. Notably, infected cats are also not necessarily asymptomatic. There have been cases of SARS-CoV-2-positive cats with gastrointestinal and respiratory symptoms reminiscent of COVID-19, such as in Belgium (Promed, 2020) and Spain (Segalés et al., 2020). Presently, at least 40 to 50 other cases of SARS-CoV-2-positive cats, dogs, and tigers have occurred, with the most likely transmission source coming from humans. Direct infection experiments further support the susceptibility of certain animals to SARS-CoV-2 (**Table 1**). Such studies have found that the novel coronavirus could efficiently replicate in cats and ferrets, but not in dogs, pigs, chickens, and ducks. Cats, in particular, shed high amounts of SARS-CoV-2 orally and nasally for several days and could transmit the virus to other cats via aerosols or direct contact, despite showing no clinical symptoms of COVID-19-like disease

(**Table 1**). Deliberately infecting macaques with SARS-CoV-2 also recapitulated clinical disease reminiscent of COVID-19. Indeed, ferrets, hamsters, and macaques are the standard animal models used in the study of the pathogenesis and transmission of SARS-CoV-2 (Johansen et al., 2020). In contrast, SARS-CoV-2 could replicate in certain animals without producing clinical signs of COVID-19-like disease, such as tree shew, New Zealand white rabbit, and cattle (**Table 1**). Nevertheless, the chances of animals contracting SARS-CoV-2 from humans in nature should be minimal. A serological study, for instance, did not find any SARS-CoV-2 antibodies in hundreds of sera samples from cats and dogs, as well as from pigs, monkeys, rabbits, rodents, ducks, cows, and horses in Harbin, China (Deng et al., 2020). The absence of antibodies indicates that an organism has not been exposed to the virus. However, analyzing 15 dogs belonging to owners with COVID-19 detected positive antibody responses against SARS-CoV-2 in two dogs (Sit et al., 2020). Similarly, a preprint study noted that 15 out of 102 cat sera sampled in Wuhan, China shortly after the outbreak had antibodies specific for SARS-CoV-2 (Zhang et al., 2020). Another preprint analyzed 817 companion animals in Northern Italy, where they identified SARS-CoV-2 antibodies in 3.4% of dogs and 3.9% of cats, but none were tested positive on RT-PCT (Patterson et al., 2020). In the Netherlands antibodies to SARS-CoV-2 have been detected in cats (Oreshkova et al., 2020). Regardless, while anthroponosis of SARS-CoV-2 has occurred from humans to animals, the reverse has not happened, except for minks. A recent experimental study demonstrated the susceptibility of raccoon dogs for SARS-CoV-2 infection after intranasal inoculation and transmission to direct contact animals (Conrad et al. 2020). Notably, the high level virus shedding, combined to minor clinical signs, and seroconversion, led to speculation that raccoon dogs whether free-living or held in captivity might have a role as intermediate host. This is also confirmed by the lack of mutations during

480 replication indicating that the virus is well adapted to this potential intermediate host. This
481 evidence is consistent with the demonstrated potential of several carnivore species to become
482 infected by SARS-CoV-2 as a result of reverse zoonosis, possibly leading to reinfections of
483 humans. There are no reports of natural infection of raccoon dogs in the context of the SARS-
484 CoV-2 pandemic.

485

486

487 **7 Anthroponotic risks of SARS-CoV-2**

488 Among recent zoonotic pandemic, there are viruses that did not transmitted back to wildlife or
489 domestic animal populations after establishment in people (e.g., human immunodeficiency virus,
490 which causes acquired immunodeficiency syndrome). Others have repeatedly crossed species
491 boundaries (e.g., pandemic H1N1 influenza A virus) (Schrenzel et al., 2011). However, as
492 different types of coronaviruses can circulate among animals, there have been concerns
493 substantaited by recent evidence of risks of "reverse zoonotic" or anthroponotic transmission,
494 where humans transmit coronaviruses to other animals in nature (Messenger, Barnes & Gray,
495 2020; Edwards & Santini 2020; Munir et al., 2020), including naïve wildlife and other animal
496 populations (Olival et al., 2020). This the case of a documented events of natural transmission
497 of SARS-CoV-2 from humans to tigers, lions, cats, dogs, and minks during the COVID-19
498 pandemic (Abdel-Moneim & Abdelwhab, 2020; Tazerji et al., 2020). As follows, the potential
499 host range and reservoir species of SARS-CoV-2 can be broad (Santini & Edwards, 2020). The
500 possibility of zoonotic transfer occurring again after anthroponosis, where animals pass SARS-
501 CoV-2 back to humans, cannot be discounted. While rarely used, the term 'reverse

anthroponosis' is useful in this context, and the mink outbreak incident of mink-to-human transmission, as described later, is a stark example and warning of this.

7.1 Human-mink-human transmission

There is evidence of virus transmission from human to minks who have tested positive with COVID-19. Six countries, namely Denmark, the Netherlands, Spain, Sweden, Italy and the United States of America have reported SARS-CoV-2 in farmed minks to the World Organisation for Animal Health (WHO, 2020b). The culprit of the mink outbreaks are the husbandry conditions, with the overcrowding of thousands of individuals in cages made of wire netting, allowing free airflow and contact between animals. Therefore it is not surprising that rapid animal-to-animal transmission accelerated the evolution of SARS-CoV-2. In most countries, the first infections on mink farms were identified through contact tracing following confirmation of COVID-19 in symptomatic humans (Koopmans, 2020). In the Netherlands, with about 125 mink farms, an outbreak of COVID-19 occurred in two of the farms in mid-April of 2020 and later in more than forty mink farms (Oreshkova et al., 2020). The minks (*Neovison vison*) showed increased incidents of respiratory distress and mortality, and consequently diagnosed positive for pneumonia and SARS-CoV-2 RNA on various tissues, including the lungs, throat, and conchae. The interstitial pneumonia was the most relevant striking post-mortem finding in nearly all examined mink that died at the peak of the outbreaks (Molenaar et al., 2020). Evidence suggests that such outbreaks originated from farm owners who were infected with SARS-CoV-2. Research since has shown that mink have transmitted the virus to each other. Concerningly, these farms were maintained separately, which indicates that multiple anthroponotic events had happened. 11 out of 99 cats on the infected mink farms tested positive without clinical signs, and the virus genome sequence was similar to that in the minks.

Moreover, serological surveillance of stray cats around the mink farms detected positive antibody responses against SARS-CoV-2 in seven out of 24 cats, suggesting that interspecies animal transmission may have happened. Infection was also found in one dog in the farms. Consequently, the risk of SARS-CoV-2 transmission between farmed and domestic animals on infected mink farms is high for cats and dogs (OIE, 2020c). Even if in these settings, the risk of cats or dogs transmitting SARS-CoV-2 to humans is considered low, it is important to further investigate the potential virus transmission of SARS-CoV-2. In the Netherlands, 97 individuals among owners and employees of the 16 SARS-CoV-2 positive mink farms were tested according to national protocol by either serological assays and/or Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). In total, 66 of 97 (68%) of the persons tested had evidence for SARS-CoV-2 infection. On one of the infected mink farms, five of seven individuals working or living on the farm initially negative, when rested were found positive for SARS-CoV-2 RNA after developing COVID-19 related symptoms. Whole genome sequencing were obtained and the clustering of these sequences with the sequences derived from minks indicated a 'genomic signature' that the employees were infected with SARS-CoV-2 after mink on the farm became infected. Similarly, transmission in another mink farms led to zoonotic transmission events from mink to human. This are undoubtedly the first reverse anthroponotic events of SARS-CoV-2 acknowledged so far. However, since the first mink farm outbreak was identified in April 2020, no spill-over to people living in close proximity to mink farms had occurred (Munnink et al. 2020; ECDC, 2020b). Since June 2020, Denmark have experienced an extensive of SARS-CoV-2 outbreaks in mink farms. Due to a mutated SARS-CoV-2 found circulating in over 200 mink farms, on 5 November 2020, the Ministry of Environment and Food of Denmark announced the culling of all mink in

549 the country, estimated to approximately 17 million animals, including the breeding stock,
550 and the long-term shutdown of the Danish mink industry (Ministry of Environment and Food of
551 Denmark, 2020). This policy change follows an alert from the Danish National Institute of
552 Public Health, related to the spill-back of SARS-CoV-2 from mink farms into the
553 community, and accumulated mutations in the S protein gene (Lassaunière et al., 2020).
554 Indeed, Denmark reported 214 human cases of COVID-19 infected with SARS-CoV-2 virus
555 variants related to mink. Some of these cases have no known link to mink farms. The SARS-
556 CoV-2 variants detected in these cases were part of at least five closely-related clusters; each
557 cluster was characterised by a specific mink-related variant, identified in humans and animals
558 from infected mink farms. Twelve cases among humans living in the surrounding area,
559 presented a unique mink-associated variant, referred to as the "cluster 5", which was reported as
560 circulating in August and September 2020, and presented a combination of mutations never
561 observed before (WHO, 2020b; ECDC, 2020b). The genetic changes consist of three
562 substitutions and one deletion, in the S protein. Since the S protein contains the RBD (receptor-
563 binding domain), and is a major target for immune response, such mutations could, in theory,
564 have implications for viral fitness (ability to infect humans and animals), transmissibility, and
565 antigenicity. But scientific and laboratory-based studies are needed to clarify the extent of the
566 possible implications of these new variant in terms of SARS-CoV-2 treatment, diagnostic tests
567 and virus antigenicity. It could also have an impact on the effectiveness of developed vaccine
568 candidates, and possibly require them to be updated. Preliminary findings indicate that the
569 mutations in the S protein might lead to moderately decreased sensitivity to human neutralizing
570 antibodies. Indeed, as vaccines mainly target the S protein, there's a risk of future vaccines not
571 working against mink-coronavirus if it becomes widespread in humans. Why this mutation? All

572 replicating viruses, including coronaviruses, continuously accumulate genomic mutations that
573 persist due to natural selections and contribute to the enhancement of viral replication and
574 infection, as well as to quelling the host immune attack. SARS-CoV-2 has accumulated
575 mutations since its emergence in the human population in 2019, with a typical rate of only two
576 single-letter mutations per month in its genome. It is known that when a virus switches host
577 species un increased mutation rate can occur due to the virus adapting to its new host. It is likely
578 that the rapid circulation of SARS-CoV-2 in the mink population, triggered a strong antibody
579 reaction among the infected minks, which might have exerted a selective pressure on the virus,
580 which began to mutate to quell the antibodies. This made possible the adaptation of SARS-CoV-
581 2 to the ACE 2 receptor of the minks and the selection of a variant of virus with mutated S
582 protein. This mechanism of antigenic drift is well known with human flu: there are variations
583 every year and we already know that next year's flu virus will probably have a new antigenic
584 structure. Minks belong to the *Mustelidae* family, that includes ferrets. This animal
585 species is the standard model for assessing the potential of airborne transmission of influenza
586 viruses, particularly those viruses with pandemic potential, and have been used as a model to
587 test transmissibility of SARS-CoV-2. Based on actual evidence from several groups in
588 experimental infections, ferrets akin to minks are susceptible to SARS-CoV-2 through direct
589 contact but also through indirect airborne spread (Richard et al., 2020). As is demonstrated
590 that infected humans are considered the primary source of infection to minks, the same would
591 happen with ferrets. While is being theorized that there is a possibility that a new coronavirus
592 strain might emerge (Callaway, 2020), of further concern are: the reverse anthroponotic events,
593 similar to human-mink-human transmission, that could happen with other animals within the
594 host range of SARS-CoV-2, such as companion animals in constant close contact with humans,

595 and the potential formation of a non-human reservoir of SARS-CoV-2, which would extend
596 to escaped and wild mustelids or other wildlife, from where the virus could spill-back to
597 humans (Koopmans, 2020). ECDC has recently published a rapid risk assessment of the human
598 health risk posed by SARS-CoV-2 mink related variant (ECDC, 2020b). **Table 2** summarizes
599 the main findings. In terms of response strategies, to decrease the risk posed to public health,
600 national health authorities should consider implementing the following measures aimed at mink
601 farms, mink farm workers and communities in contact with mink farms: - human testing with
602 sequencing and characterisation of antigenic properties and virus infectivity; - infection
603 prevention and control measures for mink farm workers and visitors; - animal testing and
604 prevention of spread from animals; - development of 'One Health' preparedness and response
605 strategies, with enhanced coordination between the agricultural, animal health, and human
606 health (including occupational health and safety) sectors.

608 7.2 Potential host range (or reservoirs) of SARS-CoV-2: the *in vivo* and in *vitro* studies
609

610 However, only so much can be done with direct experimental infections *in vivo* or real animals.
611 To better grasp the possible host range of SARS-CoV-2, research has relied on *in silico* and *in*
612 *vitro* studies where large-scale screening is more feasible, although it is a caveat that such
613 models may not necessarily translate *in vivo*. Viral transmission or replication efficiency in
614 biological systems, for example, cannot be measured *in silico* or *in vivo*. The chief
615 determinant of SARS-CoV-2 cell entry is its compatibility with the ACE2 receptor on the host
616 cell surface (Shang et al., 2020; Zhang et al., 2020). Once viral cell infection is established, the
617 downstream canonical innate (Romo et al., 2016) and adaptive (Litman et al., 2010) immune
618 responses and pathways should be similar among vertebrates. For this reason, *in silico* and *in*

619 *vitro* research have focused on deciphering the possible interactions between the RBD of SARS-
620 CoV-2 S-protein with the ACE2 receptor on animal host cells. Some of the earliest *in silico*
621 studies analyzed the five amino acid residues that participate in SARS-CoV-2 RBD binding to
622 the ACE2 receptor of different mammals (**Table 3**). Later studies used different computational
623 approaches to make predictions based on a broader range of amino acids involved in the
624 interactions between SARS-CoV-2 S-protein and animal ACE2 receptor (**Table 3**). While some
625 discrepancies exist, results have been mostly consistent with predicted binding in certain
626 mammals such as primates, ferrets, dogs, cats, lions, tigers, hamsters, pigs, racoon dogs, and
627 others. In contrast, SARS-CoV-2 binding is unlikely with the ACE2 receptors of mice, rats, and
628 most birds, fishes, amphibians, and reptiles (Table 2). As *in silico* modelling narrowed down the
629 probable host range of SARS-CoV-2 to certain animals, *in vitro* models further provide
630 confirmations. For example, studies have shown that pseudotype SARS-CoV-2 could infect
631 cell lines that express the ACE2 receptor of other animals, such as horseshoe bat, rabbit,
632 pangolin, camel, cattle, horse, goat, sheep, ferret, monkey, cat, and dog (**Table 4**). However,
633 there is a disconnect in the results following other methods. For example, cell lines derived
634 from horseshoe bat were not conducive to SARS-CoV-2 replication for reasons that may involve
635 the downstream cellular responses following ACE2 receptor entry (**Table 4**). Another notable
636 case is with pigs where *in silico* and *in vitro* studies supported SARS-CoV-2 infection, but not *in*
637 *vivo* where the pigs produced no evident clinical symptoms or antibody responses following
638 experimental infection (Meekins et al., 2020). Despite that, most animals predicted as
639 susceptible *in silico* and *in vitro*, such as cats, primates, hamsters, or ferrets, can indeed
640 contract SARS-CoV-2 in experimental settings (**section 4.2; Table 1**). Besides, animals not

predicted as susceptible *in silico* or *in vitro*, such as rats and mice, are often not susceptible *in vivo* as well.

8 SARS-CoV-2 risk assessment in susceptible farmed animal populations

In light of recent mink outbreaks, which suggest genetic/antigenic drift of SARS-CoV-2, following introduction from humans, the OIE has published a draft guidance on reducing the risk of spill-over from humans to domestic animals (OIE, 2020c). This guidance is aimed to support public health, and other partners in reducing the risk of introduction of SARSCoV-2 to susceptible farmed animal populations. The One Health approach is essential to address the risks and related pathways associated with different farming systems, and for timely and effective measures to adopt in case of SARS-CoV-2 introduction to a farm. The OIE risk assessment outcomes is concisely illustrated in **Table 5**. In a previous paper, OIE assessed the likelihood of exposure of humans or animals to SARS-CoV-2 in COVID-19 affected areas at a global level, through contact with wild animals, livestock, companion animals, aquatic animals, and handling or consumption of animal carcasses, meat/organs, body fluids and excretions (OIE, 2020d). It has to be noted that this document does not include the assessment of the likelihood of post-exposure human or animal infection. Specifically, in regards to contact with livestock, the risk is considered negligible, i.e. extremely unlikely to occur, for live pigs and poultry in general. Whilst the likelihood of exposure cannot currently be assessed for live ovine, caprine, bovine, camelid, rabbit and equine species, as the information available is limited to their ACE2 binding affinity to SARS-CoV-2 RBD. The contact with live companion animals is considered: - moderate (i.e. potentially occurring) for cats, ferrets and hamsters, and susceptible uncommon exotic pets such as monkeys and bats owned by or in contact with COVID-19 patient(s), infected animals or environments known to be contaminated with

SARS-CoV-2; - low (i.e. unlikely to occur), for companion dogs owned by or in contact with COVID-19 patient(s), infected animals or environments known to be contaminated with SARS-CoV-2. The likelihood of exposure cannot currently be assessed for exotic pets and other mammals or reptiles due limited information of ACE2 binding affinity to SARS-CoV-2 RBD. A necessary caveat for the OIE assessment is that the name of some animal species does not imply a role in SARS-CoV-2 zoonotic spill-over, considering that: a direct precursor virus has not been detected in any wild animal species to date; - it is unknown if the precursor virus is still circulating in the original reservoir or intermediate host. Having said that, the millions of human cases of COVID-19 with their shedding of high levels of virus in new contaminated environments other than the original natural reservoir, require this kind of assessment together with evidenced susceptibility of different animal species.

9 SARS-CoV-2 risk animal prevention

Considering the potential risk of SARS-CoV-2 zoonosis, precautions are advised when handling farm animals, pets or companion animals, especially those that may have been exposed to other animals or persons carrying SARS-CoV-2. For pets, the Royal Veterinary College (**RVC, 2020**) and OIE (OIE, 2020d) have pointed out the following basic hygiene measures that should be adopted to limit the spread of SARS-CoV-2:

- limit the contact of animals with persons sick or undergoing medical treatment for COVID-19; for instance, seeking help from another family member to take care of the animals.
- use a mask and wash hands after touching and walking the animals.

- animals belonging to owners with COVID-19 infection should be kept indoors as much as possible to avoid contact with other animals.

Moreover, in light of recent surveillance findings in mink, which suggest genetic/antigenic drift of SARS-CoV-2, following introduction from humans, the OIE has publish a draft guidance on reducing the risk of spill-over from humans to domestic animals (OIE, 2020c). However, as new information emerges, these guidelines may change. The CDC (CDC, 2020) Federation of Veterinarians of Europe (FVE, 2020) and other organizations have also made the same recommendations, at least until more information is known about the virus and the role of pets. The World Small Animal Veterinary Association (WSAVA) issued a document, which provides more detailed information on pets and COVID-19 and addresses both Veterinarians and pet owners; for example, the basic hygiene rules for bacterial infections (e.g., *E. coli* and *Salmonella*), such as frequent hands washing with soap and clean water after touching the animals, are highlighted (WSAVA, 2020).

10. CoVs surveillance

As predicted *in silico* and *in vitro*, the potential host range of SARS-CoV-2 is broad. Although such research models may or may not translate *in vivo*, it nevertheless warrants further caution and animal surveillance. Neglecting the potential of SARS-CoV-2 to infect a variety of animals may provide opportunities for additional evolution and new reservoirs and transmission chains of coronaviruses in the wild or wet markets, which may bring consequences to public health in the future. Considering the millions of people already infected and the many reported cases of human-to-animal transmissions of SARS-CoV-2, it is not unreasonable to assume that silent or hidden SARS-CoV-2 infection in wildlife or domestic animals have occurred (Gryseels et al.,

709 2020). The role of veterinary virologists, with their research on coronavirus evolution,
710 circulation, and pathogenesis in domestic and wild animals, is essential for coordinating
711 integrated surveillance studies on the aetiology of viral zoonoses and their impact on the health
712 of animals, humans, and the ecosystem. Indeed, through the years, veterinary epidemiologists
713 and virologists have been dealing with the circulation of pathogenic viruses among wild animals
714 (epidemiological surveillance) and their impact on the health of animals, humans, and the
715 ecosystem. Evolutionary studies and phylogenetic analyses of coronavirus (as well as other
716 viruses) with the next-generation sequencing technology have clarified the origin, diversity, and
717 distribution of coronaviruses among animal species and humans, and provided the backbone for
718 the surveillance and epidemic intelligence activities aimed to understand and predict the spill-
719 over and to prevent or limit future pandemics. Coronaviruses appear to have origins in a variety
720 of bat species. Since bats naturally infected or experimentally infected do not demonstrate
721 clinical signs of disease, researchers came to speculate that they are the potential reservoirs or
722 ancestral hosts for several coronaviruses. For instance, bats and rats are thought to be the
723 natural hosts for human coronaviruses (e.g., HCoV-NL63 and HCoV-229E). To date, over 200
724 novel coronaviruses have been identified in bats, and approximately 35% of the bat virome
725 sequenced to date is composed of coronaviruses (Banerjee et al., 2019). But they need at least an
726 intermediate host (see **Figure 2**) to complete the jump to humans. Indeed, coronaviruses are
727 very keen to jump species barriers (inter-species transmission) to evolve and find new ecological
728 niches. This spill-over was confirmed for SARS-CoV and MERS-CoV, with masked palm
729 civets and dromedary camels acting as intermediate hosts, respectively, and now for SARS-
730 CoV-2, which is highly genetically related (>96% homology) to the beta-coronavirus RaTG13 of
731 *Rhinolophus affinis*. However, the 4% genomic differences reflect decades of evolution gap and

this lineage is not sufficiently high to implicate it as the immediate ancestor. While this his indicates that an intermediate host facilitating coronavirus evolution is involved, the zoonotic source of the virus remains unidentified to date (Dorpa et al., 2020). Bats also play a key role as a reservoir of other viruses lethal for humans (e.g., Ebola, Nipah, Hendra, Marburg, Rabies), which pose a serious threat to human and animal health, in particular when human activities (e.g., deforestation, hunting, urbanization) by disrupting their ecosystem create conditions for repeated spill-over to humans (Letko et al., 2020). The surveillance of coronaviruses in wildlife to understand the their origin, diversity, and distribution should be conducted rigorously, particularly around hot spots marked as risky, such as Myanmar, Laos, Vietnam, and south and southwest China (Morens, et al., 2020). Integrating such wildlife surveillance with human epidemiological data, and advancing interdisciplinary research studies (e.g., reservoir/vector genetics, ecology, patterns of zoonoses transmission), would help understand and predict potential spill-over phenomena and prevent or limit future human pandemics. These studies will provide essential data to explain their role as emerging and zoonotic pathogens, streamline the viral surveillance in bat populations, and address the drivers of disease emergence. To date, seven of the 15 virus species currently assigned to the alpha- and beta-coronavirus genera, which primarily affect mammals, have only been isolated in bats. Similarly, coronaviruses that are genetically related to human coronaviruses 229E and NL63 were detected (Hu et al., 2015). Recent veterinary studies have focused on the surveillance of coronavirus in bats living in the area in close proximity to other animal hosts and highlighted the potential of a spill-over to humans. A three-year Italian monitoring study of coronavirus and paramyxovirus (PMV), helped to characterise the viral diversity in the bat population of the Northwest regions of Italy (Rizzo et al., 2017). The investigation focused on coronavirus and PMV due to their proven

ability to switch hosts and their zoonotic potential. Using the PCR method, 20 new coronaviruses and 3 PMV strains were identified and phylogenetically characterised. The study helped identify alpha and beta-coronavirus in new species of bats and in Italian regions that have never previously been monitored. A more recent study conducted in Italy, detected CoVs RNA in faecal samples of three different bat species. Phylogenetic analyses based on RNA-dependent RNA polymerase (RdRp) sequences revealed a SARS-like β-coronavirus in three bat species. The SarBatCoV1 virus identified belonged to clade 2b, which includes most of the SARS-like CoVs found in bats, as well as human SARS-Cov (Lecis et al., 2019). There is a consistent evidence that sewage contaminated by SARS-CoV-2 by human faecal, goes into natural aquatic environment. The events recognize the transmission from human faeces, through municipal wastewater treatment plants (WWTP) effluents (given it can survive the wastewater treatment process) (Geller et al., 2012) and ultimately the introduction into the natural aquatic environment. Although SARS-CoV-2 is inactivated significantly faster than non-enveloped human enteric viruses with the known waterborne transmission (such as adenoviruses, norovirus, rotavirus, and hepatitis A), as for others HCoV (SARS and MERS) can survive for extended periods in the aqueous environment. HCoV infective virus can be still detected in the effluent from these plants (Wigginton et al., 2015), and based on metagenomics, 80% of the samples from effluent class B sewage sludge from 5 WWTP in the US were found to contain coronaviruses. Similarly, SARS-CoV-2 was detected for the first time in untreated wastewater in Australia and in sewage from WWTPs servicing 6 cities and an airport in the Netherlands (Medema et al.2020). Another implication of the possible circulation of HCoV in the natural environment is the up-taking of the virus by the wildlife host, that can drink contaminated water and could subsequently serve as novel reservoir hosts for the virus (spillback into the human

778 population). These findings clearly signal the need to conduct wastewater surveillance of
779 COVID-19 in the community in the framework of epidemiological monitoring of COVID-19
780 with the aim to reduce virus circulation in the environment (Franklin & Bevins, 2020).
781 Moreover, the wildlife surveillance near WWTP is particularly important in this kind of spots to
782 elucidate whether SARS-CoV-2 has spilled over into wildlife including bats that might become a
783 permanent reservoir of this coronavirus and COVID-19 infection to humans. These studies
784 demonstrate how surveillance activities carried out within a specific geographical area might
785 contribute to the knowledge of the extent of the viral circulation in bats that live in close
786 proximity to other animal hosts with direct implications for preventing diseases in humans.
787 Phylogenetic analyses also have the advantage of identifying viruses with genetic prerequisites
788 for human infection, especially at hot spots with ecological conditions facilitating the spill-over,
789 and informing the most appropriate prevention and control strategies to manage potential threats
790 to public health. Indeed, this pandemic as with previous ones, tells us that due to the
791 intensification of human-animal interactions in recent decades, the effective mitigation of future
792 pandemics that could threaten humans, the economy and society, requires a fully integrated One
793 Health approach. Under the One Health umbrella, many global collaborative projects thrived in
794 recent years, such as the PREEMPT (https://www.preemptproject.org/) and PREDICT
795 (https://ohi.vetmed.ucdavis.edu/programs-projects/predict-project.). The latter is a multi-
796 partnered project with more than 60 laboratories around the world. Using the One Health
797 approach, the project builds viral surveillance platforms for identifying and monitoring zoonotic
798 pathogens, notably those (e.g., influenza viruses, coronaviruses, paramyxoviruses, filoviruses,
799 flaviviruses), that can spill-over from animal hosts to people, and for investigating the
800 behaviours, practices, and ecological and biological factors driving disease emergence,

transmission, and spread. Data from field and laboratories studies is fed into computer models that predict virus spill-over potential. The main aims are developing recommendations and countermeasures to minimize the pandemic risk and identifying the optimal timing for delivery of a vaccine targeting virus elimination.

11. Final remarks

SARS-CoV-2 is rapidly transmitting across the globe and causing unprecedented disruption in the social life and the world economy market. More and better science is needed to address the many sources of uncertainties around the COVID-19 infection, e.g., its original wildlife animal reservoir, the intermediate host, the route of virus transmission to humans, the mutations impacting the pathogenicity of SARS-CoV-2, the potential transmission from wild animals and domestic animals, notably the role of the most susceptible animal hosts (e.g., mustelids) in spreading the disease in human communities. New scientific evidence proves that pigs, cats, ferrets, and minks, have similar or identical SARS cellular receptors found in humans and support viral replication. This highlights the possibility that SARS-CoV-2 makes a further jump into new animal hosts, without the need for significant genetic modifications. Moreover, the random genetic mutations incurred by the virus during replication could increase the potential for endemic development in some animal species, including domestic species, and the subsequent panzootic potential. This epidemiological landscape signals the need to strengthen both the regional and global surveillance projects in hotspots with ecological conditions conducive to cross-species viral transmission, and a renewed 'SARS-Cov-2 animal surveillance', that includes livestock and pets. The veterinary services have a prominent duty to check if farming biosafety and biosecurity measures are properly implemented to limit the risk of zoonotic events associated with SARS-CoV-2, including infection prevention for animal workers, farm visitors and those who may be involved

in animal husbandry or culling. The Covid-19 pandemic requires scientific and collaborative field missions among OIE-WHO partners to identify the zoonotic source of the virus and the route of introduction to the human population, including the possible role of intermediate hosts. It is welcomed the recent WHO announcement of a study into the origins of SARS-CoV-2, which considering the evidence of easy adaptation and mutation of the virus to new susceptible hosts, should preferably look at the role of other animal species kept for food, fur, or other products. Considering the limited funds allocated by the Governments to manage the health threats, the hope is that both at the EU and global level, decision-makers and regulators will come to scaling up public health capacity at all levels, and prioritizing funding for pandemic early warning systems and epidemic intelligence gathering. Surveillance integrated platforms, such as PREEMPT and Eco Health Alliance, proved to be the key tool for the spotting and monitoring of emerging zoonotic pathogens and the understanding of the underlying factors contributing to their pandemic spread. The Covid-19 pandemic, like SARS and MERS, tells us that to predict and prevent future pandemics, it is therefore necessary to work collaboratively in a One Health perspective across borders and disciplines and to bolster and target wildlife surveillance, including bats, in hot spots characterised by risky human-animal interfaces. In this regard, the task of curbing the spread of SARS-CoV-2 requires a nationwide coordinated approach with an effective and centralised multi-disciplinary task to fully operationalise the One Health concept.

Acknowledgements

Funding statement

No funding was received for this work**.**

Declaration of Competing Interest

None.

References

1. Abdel-Moneim, A. S., & Abdelwhab, E. M. (2020). Evidence for SARS-CoV-2 Infection of Animal Hosts. Pathogens 2020, 9, 529. https://doi.org/10.3390/pathogens9070529.
2. American Society for Microbiology (ASM). COVID-19 Transmission Dynamics April 20, 2020. https://www.asm.org/Articles/2020/April/COVID-19-Transmission-Dynamics.
3. Andersen, K.G., Rambaut, A., Lipkin, W.I., Edward C. Holmes, E.C., & Robert F. Garry, r.f. (2020). The proximal origin of SARS-CoV-2. *Nat Med* 26, 450–452 (2020). https://doi.org/10.1038/s41591-020-0820-9.
4. Anne M. Davidson, A.M., Wysocki, J., & Batlle, D. (2020). Interaction of SARS-CoV-2 and Other Coronavirus With ACE (Angiotensin-Converting Enzyme)-2 as Their Main Receptor. Therapeutic Implications. *Hyperthension*. Volume 76, Issue 5, November 2020, Pages 1339-1349. https://doi.org/10.1161/HYPERTENSIONAHA.120.15256.
5. Banerjee, A., Kulcsar, K., Misra, V., Frieman, M., & Mossman, K. (2019). Bats and Coronaviruses. *Viruses*, 11, 41. https://doi.org/10.3390/v11010041.
6. Banerjee, A., Kulcsar, K., Misra, V., Frieman, M., and Mossman, K. (2019). Bats and Coronaviruses, *Viruses*, 11(1): 41. https://doi.org/10.3390/v11010041.
7. Boni, M.F., Lemey, P., Jiang, X., Lam, T.T.Y., Perry, B.W., Castoe, T.A…Robertson, D.L. (2020). Evolutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19 pandemic. *Nat Microbiol* 5, 1408–1417 (2020). https://doi.org/10.1038/s41564-020-0771-4.
8. Brook, C.E, Boots, M., Chandran, K., Dobson, A.P., Drosten, C., Graham, A.L., van Leeuwen, A. (2020). Accelerated viral dynamics in bat cell lines, with implications for zoonotic emergence. *Ecology Epidemiology and Global Health.* eLife 2020;9:e48401 doi: 10.7554/eLife.48401.
9. Callaway, E. (2020). Making sense of coronavirus mutations. *Nature*, Vol 585. https://media.nature.com/original/magazine-assets/d41586-020-02544-6/d41586-020-02544-6.pdf.
10. Callaway, E. (2020). The coronavirus is mutating - does it matter? Different SARS-CoV-2 strains haven't yet had a major impact on the course of the pandemic, but they might in future. *Nature*. 08. https://www.nature.com/articles/d41586-020-02544-6#correction-0.
11. Centre for Disease Control and Prevention (CDC, 2019). Coronavirus Disease 2019 (COVID-19), Frequently asked questions, https://www.cdc.gov/coronavirus/2019-ncov/faq.html#2019-nCoV-and-animals. Accessed on November 23 2020.
12. Chan, J.F.W., Kok, K.H., Zhu, Z., Chu, H., To, K.K., Yuan, S., & Yuen, K.Y. (2020). Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with

atypical pneumonia after visiting Wuhan. *Emerg Microbes Infect.* 28;9(1):221-236, https://www.ncbi.nlm.nih.gov/pubmed/31987001.

13. Chaw, S. M., Tai, J. H., Chen, S. L., Hsieh, C. H., Chang, S. Y., Yeh, S. H.,... Wang, H. Y. (2020). The origin and underlying driving forces of the SARS-CoV-2 outbreak. *J Biomed Sci* 27, 73 (2020). https://doi.org/10.1186/s12929-020-00665-8.
14. Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y… Zhang. L. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *LanceT,* 395, 507–513. https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(20)30211-7.pdf.
15. Chu, H., Chan, J. F., Yuen, T. T., Shuai, H., Yuan, S., Wang, Y…Yuen, K.Y. (2020). Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. *The Lancet*. Microbe, 1(1), e14–e23. https://doi.org/10.1016/S2666-5247(20)30004-5.
16. Coen, J. (2020). Wuhan seafood market may not be source of novel virus spreading globally. Science. https://www.sciencemag.org/news/2020/01/wuhan-seafood-market-may-not-be-source-novel-virus-spreading-globally.
17. Conrad M.F., Breithaupt, A., Müller, T., Sehl J., Buschmann, A.B., Rissmann, M... Mettenleiter, T.C. (2020). Susceptibility of raccoon dogs for SARS-CoV-2. bioRxiv preprint doi: https://doi.org/10.1101/2020.08.19.256800.
18. Corman, V.M, Muth, D., Niemeyer D., Drosten, C. (2018). Hosts and Sources of Endemic Human Coronaviruses. *Adv Virus Res*. 2018;100:163-188. doi: 10.1016/bs.aivir.2018.01.001.
19. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5:536–544.
20. Crowley, D., Becker, D., Washburn, A., and Plowright; R. (2020). Identifying Suspect Bat Reservoirs of Emerging Infections. *Vaccines*, 2020,8, 228. doi:10.3390/vaccines8020228.
21. Davy, C.M., Donaldson, M.E., Subudhi, S., Rapin N., Warnecke L., Turner, JM... Kyle, C.J. (2018). White-nose syndrome is associated with increased replication of a naturally persisting coronaviruses in bats. *Scientific Reports*. 2018; 8(15508). https://doi.org/10.1002/ece3.3234.
22. De Groot, R.J., Baker, S.C., Baric, R., Enjuanes, L., Gorbalenya, A.E., & Holmes, K.V.(2012). Coronavirdae. In A.A.Q. King, M.J. Adams, E.B., Carstens, &E. J. Lefkowitz (Eds). Ninth Report of the international committee on taxonomy of viruses (pp.806-828). San Diego, CA: Elsevier Academic Press.
23. Deng, J., Jin, Y., Liu, Y., Sun, J., Hao, L., Jingjing Bai, J… Tian, K. (2020). Serological survey of SARS-CoV-2 for experimental, domestic, companion and wild animals excludes intermediate hosts of 35 different species of animals. *Transboundary and emerging diseases*, 67(4), 1745–1749. https://doi.org/10.1111/tbed.13577.
24. Edwards, S.J.L. & Santini, JM. Anthroponotic risk of SARS-CoV-2, precautionary mitigation, and outbreak management. *Lancet Microbe*. 2020;1(5):e187-e188. https://doi.org/10.1016/S2666-5247(20)30086-0.
25. European Centre for Disease Control (ECDC, 2020a). Rapid Risk Assessment: Resurgence of reported cases of COVID 19 in the EU/EEA, the UK and EU candidate and potential candidate countries. Retrieved from https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-resurgence-reported-cases-covid-19
26. European Centre for Disease Control (ECDC, 2020b). Detection of new SARS-CoV-2 variants related to mink 12 November 2020. Rapid risk assessment. https://www.ecdc.europa.eu/sites/default/files/documents/RRA-SARS-CoV-2-in-mink-12-nov-2020.pdf.

27. European Food Safety Authority (EFSA, 2020a). Coronavirus: no evidence that food is a source or transmission route. Retrieved from https://www.efsa.europa.eu/en/news/coronavirus-no-evidence-food-source-or-transmission-route.
28. Forni, D., Cagliani, R., Clerici, M., & Sironi, M. (2017). Molecular Evolution of Human Coronavirus Genomes. Trends in Microbiology, Vol. 25, No.1. https://doi.org/10.1016/j.tim.2016.09.001.
29. Forni, D., Cagliani, R., Clerici, M., and Manuela, S. (2017). Molecular Evolution of Human Coronavirus Genomes. *Trends in Microbiology*, Vol.25, No.1. https://doi.org/10.1016/j.tim.2016.09.001.
30. Forster, P., Forster, L., Renfrew, C., & Forster, M. (2020). Phylogenetic network analysis of SARS-CoV-2 genomes. Proceedings of the National Academy of Sciences of the United States of America, 117(17), 9241–9243. https://doi.org/10.1073/pnas.2004999117.
31. Franklin, A.B., Bevins. S.N. (2020). Spillover of SARS-CoV-2 into novel wild hosts in North America: A conceptual model for perpetuation of the pathogen. *Science of The Total Environment.* Volume 733. 139358. https://doi.org/10.1016/j.scitotenv.2020.139358.
32. FVE (2020). COVID-19 & the veterinary profession. https://www.fve.org/covid-19-faqs/. Accessed on November 23 2020.
33. Geller, C.; Varbanov, M.., & Duval, R.E. (2012). Human Coronaviruses: Insights into Environmental Resistance and Its Influence on the Development of New Antiseptic Strategies. Viruses 2012, 4, 3044-3068. https://www.mdpi.com/1999-4915/4/11/3044.
34. Gorbalenya, A.E., Enjuanes, L., Ziebuhr, J., & Snijder, E. J. (20026). Nidovirales: Evolving the largest RNA virus genome. *Virus Research* 117 (2006) 17–37. DOI: 10.1016/j.virusres.2006.01.017.
35. Gorse, G.J., O'Connor, T.Z., Hall, S.L., Vitale, J.N., & Nichol, K.L. (2009). Human coronavirus and acute respiratory illness in older adults with chronic obstructive pulmonary disease. *J Infect Dis.*;199(6):847-57. https://www.ncbi.nlm.nih.gov/pubmed/19239338.
36. Graham, R.L. & Baric, R.S. (2010). Recombination, Reservoirs, and the Modular Spike: Mechanisms of Coronavirus Cross-Species Transmission. *Journal of Virology* 84, 3134-3146. https://jvi.asm.org/content/84/7/3134.
37. Gralinski, L.E. and Menachery, V.D. (2020). Return of the Coronavirus: 2019-nCoV. *Viruses* 12(2), 135; https://doi.org/10.3390/v12020135.
38. Gryseels, S., De Bruyn, L., Gyselings, R., Calvignac−Spencer, S., Leendertz, F.H. & Leirs, H. (2020), Risk of human−to−wildlife transmission of SARS−CoV−2. *Mam. Rev.* https://doi.org/10.1111/mam.12225.
39. Hangping, Y., Xiangyun, L., Qiong C., Kaijin X., Yu C., Linfang C., Lanjuan L.. (2020). Patient-derived mutations impact pathogenicity of SARS-CoV-2. Preprint, medRxiv: doi: https://doi.org/10.1101/2020.04.14.20060160.
40. Harding, A. (2020). The 12 deadliest viruses on Earth . Live Science. https://www.livescience.com/61848-scientists-hunt-unknown-viruses.html.
41. Honigsbaum, M., & Krishnan, L. (2020). Taking pandemic sequelae seriously: from the Russian influenza to COVID-19 long-haulers. *The Lancet*. Perspectives. Volume 396, Issue 10260, P1389-1391. DOI: https://doi.org/10.1016/S0140-6736(20)32134-6.
42. Horton, R. (2020). Offline: COVID-19 is not a pandemic. *The Lancet*. Comment. Volume 396, Issue 10255, P874. DOI: https://doi.org/10.1016/S0140-6736(20)32000-6.
43. https://www.globaltimes.cn/content/1196713.shtml. Accessed on November 23, 2020.
44. https://www.linkedin.com/pulse/covid-19-one-health-issue-two-perspectives-katharina-st%C3%A4rk/, Accessed on May 12 2020.

45. https://www.nationalgeographic.com/animals/2020/04/tiger-coronavirus-covid19-positive-test-bronx-zoo/. Accessed on November 23, 2020.
46. Hu, B., Ge, X., Wang, L.F. & Shi, Z. (2015). Bat origin of human coronaviruses. *Virol J 12, 221 (2015*). https://doi.org/10.1186/s12985-015-0422-1.
47. Huang, C., Wang, Y., Li, X., Ren, L., Zao, J., Hu, Y….. Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet,* 395, Issue 10223, 497–506. DOI: https://doi.org/10.1016/S0140-6736(20)30183-5.
48. Hulswit, R.J., de Haan, C.A., and Bosch, B.J. (2016). Coronavirus Spike Protein and Tropism Changes. Adv. Virus Res. 96, 29–57. DOI: 10.1016/bs.aivir.2016.08.004.
49. Ji, W., Wang, W., Zhao, X., Zai J. & Li, X. (2020). Cross−species transmission of the newly identified coronavirus 2019−nCoV. Research Article. *Journal of Medical Virology*. https://onlinelibrary.wiley.com/doi/full/10.1002/jmv.25682.
50. Johansen, M.D., Irving, A., Montagutelli, X. Tate, M. D.,. Rudloff, I., Nold, M.F… Hansbro, P. M. (2020). Animal and translational models of SARS-CoV-2 infection and COVID-19. *Mucosal Immunol* 13, 877–891. https://doi.org/10.1038/s41385-020-00340-z.
51. Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, John L., Gittleman, J.L., Daszakc, P. (2008). Global trends in emerging infectious diseases. *Nature*. 2008; 451(7181): 990–993. https://doi.org/10.1038/nature06536doi.
52. Ju,B., Zhang, Q., Ge, X., Wang, R.,Yu, J., Shan, S., . Zhang, L.. (2020). Potent human neutralizing antibodies elicited by SARS-CoV-2 infection. https://www.biorxiv.org/content/10.1101/2020.03.21.990770v2.
53. Koopmans, M. (2020). SARS-CoV-2 and the human-animal interface: outbreaks on mink farms. *The Lancet*. https://doi.org/10.1016/ S1473-3099(20)30912.
54. Korber, B., Fischer, W.M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W……Montefiori, D.C. (**2020a**). Spike mutation pipeline reveals the emergence of a more transmissible form of SARS-CoV-2. bioRxiv preprint doi: https://doi.org/10.1101/2020.04.29.069054.
55. Korber, B., Fischer, W.M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W… De Silva, T.I (2020). Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus. *Cell*, Volume 182, Issue 4, pages 794-795. https://doi.org/10.1016/j.cell.2020.06.043.
56. Korber, B., Fischer, W.M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., Hengartner, N., Giorgi, E.E., Bhattacharya, T., Foley, B., et al. (2020). Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell. Published online July 3, 2020. https://doi.org/10. 1016/j.cell.2020.06.043.
57. Lainé, N., & Morand, S. (2020, September 22). Linking humans, their animals, and the environment again: A decolonized and more-than-human approach to 'One Health'. https://doi.org/10.31235/osf.io/fxzbk.
58. Lam, T.T.Y, Jia, N., Zhang, Y.W., Shum, M.H.H, Jiang, Zhu, H.C…Cao, W.C. (2020). Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins. *Nature* 583, 282–285. https://doi.org/10.1038/s41586-020-2169-0.
59. Lam, T.T.Y., Jia,, N., Zhang, Y.W., Shum, M.H.H., Jiang, J.F., Zhu, H.C….Cao, W.C. (2020). Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins. *Nature* 583, 282-285 (2020), https://doi.org/10.1038/s41586-020-2169-0.
60. Lassaunière, R., Fonager, J., Massaunière R, Fonager, J., Rasmussen, M., Frische, A… Fomsgaard, A. (2020). Working paper on SARS-CoV-2 spike mutations arising in Danish mink, their spread to humans and neutralization data. SARS-CoV-2 spike mutations arising in Danish mink and their spread to humans. https://files.ssi.dk/Mink-cluster-5-short-report. (accessed Nov 23, 2020).

61. Lau, S.K.P., Wong, A.C.P., Luk, H.K.H., Li, K.S.M., Fung, J., He, Z… Woo, P.C.Y. (2020). Differential Tropism of SARS-CoV and SARS-CoV-2 in Bat Cells. *Emerg Infect Dis*. 2020 Dec;26(12):2961-2965. doi: 10.3201/eid2612.202308. Epub 2020 Jul 30.
62. Lauring, A.S., & Andino, R. (2010). Quasispecies theory and the behavior of RNA viruses. *PLoS Pathogens*. 6, e1001005. https://doi.org/10.1371/journal.ppat.1001005.
63. Lecis, M., Mucedda, E., Pidinchedda, Pittau, M., & A. Alberti. (2019). Molecular identification of Betacoronavirus in bats from Sardinia (Italy): first detection and phylogeny, *Virus Genes* 55, 60–67 (2019). DOI: 10.1007/s11262-018-1614-8.
64. Ledford, H. (2020). Coronavirus reinfections: three questions scientists are asking: second infections raise questions about long-term immunity to COVID-19 and the prospects for a vaccine. *Nature* | Vol 585 | 10 September 2020. https://www.nature.com/articles/d41586-020-02506-y.
65. Lee, J., Hughes, T., Lee, M-H., Field, H., Rovie-Ryan, J.J...Daszak, P. (2020). No evidence of coronaviruses or other potentially zoonotic viruses in Sunda pangolins (*Manis javanica*) entering the wildlife trade via Malaysia. bioRxiv preprint doi: https://doi.org/10.1101/2020.06.19.158717.
66. Letko, M., Seifert, S.N., Olival, K.J., Plowright, R.K., and Munster, V.J. (2020). Bat-borne virus diversity, spillover and emergence. *Nat Rev Microbiol* 18, 461–471 (2020). https://doi.org/10.1038/s41579-020-0394-z.
67. Li, Q., Wu, J., Nie, J., Zhang, L., Hao, H., Liu, S.… Wang, Y. (2020). The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. *Cell* 182, 1284–1294, https://doi.org/10.1016/j.cell.2020.07.012.
68. Li, X., Giorgi, E.E., Marichann, M.H., Foley, B., Xiao, C., Kong, X.P……Chen, Y. (2020). Emergence of SARS-CoV-2 through Recombination and Strong Purifying Selection. *Science Advances* doi: 10.1126/sciadv.abb9153.
69. Li, X., Zai, J., Zao, Q., Nie, Q., Foley, B.T. and Chaillon, A., Evolutionary history, potential intermediate animal host, and cross−species analyses of SARS−CoV−2. *J Med Virol*. 2020;92:602–611. https://doi.org/10.1002/jmv.25731.
70. Li, Y., Wang, H., Tang, X., Fang, S., Ma, D., Du, C…Zhong, Z. (2020). SARS-CoV-2 and three related coronaviruses utilize multiple ACE2 orthologs and are potently blocked by an improved ACE2-Ig. J Virol 94:e01283-20. https://jvi.asm.org/content/jvi/94/22/e01283-20.full.pdf.
71. Litman, G. W., Rast, J. P., & Fugmann, S. D. (2010). The origins of vertebrate adaptive immunity. Nature reviews. Immunology, 10(8), 543–553. https://doi.org/10.1038/nri2807.
72. Longdon, B., Brockhurst, M. A., Russell, C. A., Welch, J. J., & Jiggins, F. M. (2014). The evolution and genetics of virus host shifts. PLoS Pathog 10(11): e1004395. doi:10. 1371/journal.ppat.1004395.
73. Lorusso, A., Calistri, P., Petrini, A., Savini, G., & Decaro, N. (2020). Novel coronavirus (SARS-CoV-2) epidemic: a veterinary perspective. *Veterinaria Italiana* 2020, xx (x), xxx-xxx, http://www.izs.it/vet_italiana/pdf_4/VetIt_2173_11599_1.pdf.
74. Mansbach, R.A., Chakraborty, S., Nguyen, K., Montefiori, D.C., Bette Korber, B. and Gnanakaran, S. (2020). The SARS-CoV-2 Spike Variant D614G Favors an Open Conformational State. Preprint at bioRxiv. https://doi.org/10.1101/2020.07.26.219741.
75. McAloose, D., Laverack, M., Wang, L., Killian, M. L., Caserta, L. C., Yuan, F…Diel, D.G. (2020). From people to Panthera: natural SARS-CoV-2 infection in tigers and lions at the Bronx Zoo. mBio 11:e02220-20. https://doi.org/10.1128/mBio.02220-20.
76. Medema, G., Heijnen, L., Elsinga, G., Italiaander, R. & Brouwer, A. (2020). Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. *Environ. Sci. Technol. Lett.* 7, 7, 511–516. https://doi.org/10.1021/acs.estlett.0c00357.

77. Meekins, D. A., Morozov, I., Trujillo, J. D., Gaudreault, N. N., Bold, D….. Richt J.A. (2020). Susceptibility of swine cells and domestic pigs to SARS-CoV-2, *Emerging Microbes & Infections*, 9:1, 2278-2288, DOI: 10.1080/22221751.2020.1831405.
78. Messenger, A.M., Barnes, A.N. and Gray, G.C. (2020). Reverse zoonotic disease transmission (zooanthroponosis): a systematic review of seldom-documented human biological threats to animals. PLoS One. 2014 Feb 28;9(2):e89055. doi: 10.1371/journal.pone.0089055. PMID: 24586500; PMCID: PMC3938448.
79. Ministry of Environment and Food of Denmark. COVID-19: all mink in Denmark must be culled. 2020. https://en.mfvm.dk/news/news/nyhed/covid-19-all-mink-in-denmark-must-be-culled/ (accessed Nov 23, 2020).
80. Molenaar, R.J., Vreman, S., van der Honing, R.W.H, Zwart, R., de Rond, J… van der Poel, W.H.M. (2020). Clinical and Pathological Findings in SARS-CoV-2 Disease Outbreaks in Farmed Mink (Neovison vison). *Veterinary Pathology* 1-5. Brief communication.. DOI: 10.1177/0300985820943535.
81. Morens, D. M., Breman, J. G., Calisher, C. H., Doherty, P. C., Hahn, B. H.…Taubenberger, J. K. (2020). The Origin of COVID-19 and Why It Matters. *The American journal of tropical medicine and hygiene*, 103(3), 955–959. https://doi.org/10.4269/ajtmh.20-0849.
82. Munir, K., Ashraf, S., Munir, I., Khalid, H., Muneer, M. A., Mukhtar, N… Zaho, X. (2020). Zoonotic and Reverse Zoonotic Events of SARS-CoV-2 and their Impact on Global Health. *Emerging Microbes & Infections*, 9:1, 2222-2235, DOI: 10.1080/22221751.2020.1827984.
83. Munnink, B.B.O., Sikkema, R.S., Nieuwenhuijse, D.F., Molenaar, R.J., Munger, E... Koopmans, M.P.G.(2020). Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science*. eabe5901. doi: 10.1126/science.abe5901. Epub ahead of print. PMID: 33172935.
84. Murakami, S., Kitamura, T., Suzuki, J., Sato, R., Aoi, T., Fujii, M....Horimoto, T. (2020). Detection and Characterization of Bat Sarbecovirus Phylogenetically Related to SARS-CoV-2, Japan. *Emerging Infectious Diseases*, 26(12), 3025-3029. https://dx.doi.org/10.3201/eid2612.203386.
85. Muth, D., Corman, V.M., Roth, H., Binger, T., Dijkman, R., Gottula, L.T.…Dorosten, C. (2018). Attenuation of replication by a 29 nucleotide deletion in SARS-coronavirus acquired during the early stages of human-to-human transmission. *Scientific Reports*, 8:15177. https://doi.org/10.1038/s41598-018-33487-8.
86. OIE (2020a). Follow-up report, https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=33546.
87. OIE (2020b). https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=33885.
88. OIE (2020c). Guidance on working with farmed animals of species susceptible to infection with SARS-CoV-2. Draft published 05/11/2020. https://www.oie.int/fileadmin/Home/MM/Draft_OIE_Guidance_farmed_animals_cleanMS05.11.pdf.
89. OIE (2020d). Exposure of humans or animals to SARS-COV-2 from wild, livestock, companion and aquatic animals. Qualitative exposure assessment. FAO Animal Production and Health/paper 181. http://www.fao.org/3/ca9959en/CA9959EN.pdf.
90. Olival, K.J., Cryan, P.M., Amman, B.R., Barik, R.S., Blehert, D.S., Brook, C.E…Wang, L.F. (2020). Possibility for reverse zoonotic transmission of SARS-CoV-2 to free-ranging wildlife: A case study of bats. Article in PLoS Pathogens. https://doi.org/10.1371/journal.ppat.1008758.
91. Oreshkova, N., Molenaar, R.J., Vreman, S., Harders, F., Munnink, B.B.O., der Honing, R.W.H.…Stegeman, A. (2020). SARS-CoV-2 infection in farmed minks, the Netherlands, April

and May 2020. Euro Surveill. 25(23):pii=2001005. https://doi.org/10.2807/1560-7917.ES.2020.25.23.2001005.

92. O'Shea, T.J., Cryan, P.M., Cunningham, A.A., Fooks, A.R., Hayman, D.T.S., Luis, A.D.…Wood, J.L.N. (2014). Bat flight and zoonotic viruses. *Emerging Infectious Diseases*, 20(5). https://doi.org/10.3201/eid2005.130539.
93. Pachetti, M., Marini, B., Benedetti, F., Giudici, F., Mauro, E., Storici, P……Ippodrino, R. (2020). Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. *Journal of Translational Medicin*e, 18, 179. https://doi.org/10.1186/s12967-020-02344-6.
94. Patterson, E. I., Elia, G., Grassi, A., Giordano, A., Desario, C., Medardo, M.…Decaro, N. (2020). Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. BioRxiv. Doi: https://doi.org/10.1101/2020.07.21.214346.
95. Pene, F., Merlat, A., Vabret, A., Rozenberg, F., Buzyn, A., Dreyfus, F., Lebon, P. (2003). Coronavirus 229E-related pneumonia in immunocompromised patients. *Clin Infect Dis.* 1;37(7):929-32. doi: 10.1086/377612. https://www.ncbi.nlm.nih.gov/pubmed/13130404.
96. Plante, J.A., Liu, Y., Liu, J., Xia, H., Johnson, B.A., Lokugamage, K.G.…Shi, P.Y. (2020). Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* (2020). https://doi.org/10.1038/s41586-020-2895-3.
97. Plowright, R.K., Eby, P., Hudson, P.J., Smith , I.L., Westcott , D., Bryden, W.L… McCallum, H. (2015). Ecological dynamics of emerging bat virus spillover. *Proc. R. Soc*. B 282: 20142124. http://dx.doi.org/10.1098/rspb.2014.2124.
98. Plowright, R.K., Field, H.E., Smith, C., Divljan, A., Palmer, C., Tabor, G…Foley, J.E. (2008). Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (Pteropus scapulatus). *Proc Biol Sci*. 2008 Apr 7; 275(1636): 861–869. doi: 10.1098/rspb.2007.1260.
99. PREDICT. https://ohi.vetmed.ucdavis.edu/programs-projects/predict-project. Accessed on November 23 2020.
100. PREEMPT. https://www.preemptproject.org/. Accessed on November 23 2020.
101. Promed (2020). Subject: PRO/AH/EDR> COVID-19 update (58): Belgium, animal, cat, clinical case, RFI Archive Number: 20200327.7151215. https://promedmail.org/promed-post/?id=20200327.7151215.
102. Rausch, J.W., Capoferri, A.A., Katusiime, M.G, Patro, S.C., & Kearne. M.F. (2020). Low genetic diversity may be an Achilles heel of SARS-CoV-2. PNAS | October 6, 2020 | vol. 117 | no. 40. www.pnas.org/cgi/doi/10.1073/pnas.2017726117.
103. Rehman, S., Sharique, L., Ihsan, A., & Liu, Q. (2020). Evolutionary Trajectory for the Emergence of Novel Coronavirus SARS-CoV-2. *Pathogens* 9, 240. doi: 10.3390/pathogens9030240.
104. Richard, M., Kok, A., de Meulder, D., Bestebroer, T.M., Lamers, M.M…Herfst, S. (2020). SARS-CoV-2 is transmitted via contact and via the air between ferrets. Nat Commun 11, 3496 (2020). https://doi.org/10.1038/s41467-020-17367-2.
105. Rizzo, F. Edenborough, K.M., Toffoli, R., Culasso, P., Zoppi, S.,…Mandola, M.C. (2017). Coronavirus and paramyxovirus in bats from Northwest Italy. *BMC Vet Res* **13,** 396 (2017). https://doi.org/10.1186/s12917-017-1307-x.
106. Romo, M.R., M., Pérez-Martínez, D., & Castillo Ferrer, C. (2016). Innate immunity in vertebrates: an overview. Immunology, 148(2), 125–139. https://doi.org/10.1111/imm.12597.
107. Royal Veterinary College (RVC) (2020). Companion animals and COVID-19. https://www.rvc.ac.uk/news-and-events/rvc-news/companion-animals-and-covid-19, Accessed on November 23 2020.
108. Santini, J. M., & Edwards, S. (2020). Host range of SARS-CoV-2 and implications for public health. *The Lancet*. Microbe. Volume 1, Issue 4, e141-e142. DOI:https://doi.org/10.1016/S2666-5247(20)30069-0. https://doi.org/10.1016/S2666-5247(20)30069-0.

109. Schrenzel, M.D., Tucker, T.A., Stalis, I.H., Kagan, R.A., Burns, R.P., Denison, A.M… Rideout, B.A. (2020). Pandemic (H1N1) 2009 virus in 3 wildlife species, San Diego, California, USA. *Emerging Infectious Diseases*. 2011; 17 (4):747–9. doi: 10.3201/eid1704.101355.
110. Segalés, J., Puig, M., Rodon, J., Avila-Nieto, C., Carrillo, J., et al. (2020). Detection of SARS-CoV-2 in a cat owned by a COVID-19-affected patient in Spain. Proceedings of the National Academy of Sciences of the United States of America. Research article. https://www.pnas.org/content/pnas/117/40/24790.full.pdf.
111. Shang, J., Wan, Y., Luo, C., Ye, G., Geng, Q…..Li, F.(2020). Cell entry mechanisms of SARS-CoV-2. Proceedings of the National Academy of Sciences of the United States of America, 117(21), 11727–11734. https://doi.org/10.1073/pnas.2003138117
112. Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H…Li, F. (2020). Structural basis of receptor recognition by SARS-CoV-2. *Nature* 581, 221–224 (2020). https://doi.org/10.1038/s41586-020-2179-y.
113. Shi, J., Wen, Z.,. Zong, G., Yang, H., Wang, C., Liu, R… Bu, Z. (2020). Susceptibility of ferrets, cats, dogs, and different domestic animals to SARS-coronavirus-2. https://www.biorxiv.org/content/10.1101/2020.03.30.015347v1.full.pdf.
114. Simmons, N.B. and A.L. Cirranello. (2020). Bat Species of the World: A taxonomic and geographic database. https://batnames.org/. Accessed on 23/11/2020.
115. Sit, T.H.C., Brackman, C.J., Ip, S.M., Tam, K.W.S., Law, P.Y.T.,..Peiris, M. (2020). Infection of dogs with SARS-CoV-2. Nature 586, 776–778 (2020). https://doi.org/10.1038/s41586-020-2334-5.
116. Suwannarong, K. & Schuler, S. (2016). Bat consumption in Thailand. *Infect Ecol Epidemiol*. Volume 6, Issue 1. https://doi.org/10.3402/iee.v6.29941.
117. Tazerji, S.S., Phelipe Duarte, M., Rahimi, P., Shahabinejad, F., Dhakal, S…Singh, Y. Malik. (2020). Transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to animals: an updated review. *J Transl Med* 18, 358 (2020). https://doi.org/10.1186/s12967-020-02534-2.
118. The Government of the Hong Kong (2020). Special administrative region, press release, Pet cat tests positive for COVID-19, virus, https://www.info.gov.hk/gia/general/202003/31/P2020033100717.htm. Accessed on November 23, 2020.
119. Tobaiqy, M., Qashqary, M., Al-Dahery,S., Mujallad, A., Hershan, A.A., Kamal, M.A., Helmi, N. (2020). Therapeutic Management of COVID-19 Patients: A systematic review. *Infection Prevention in Practice*. Volume 2, Issue 3, September 2020. https://doi.org/10.1016/j.infpip.2020.100061.
120. Troyer, E.A., Kohn, J.N., and Hong, S. (2020). Are we facing a crashing wave of neuropsychiatric sequelae of COVID-19? Neuropsychiatric symptoms and potential immunologic mechanisms. *Brain Behav Immun.* Apr 13. https://doi.org/10.1016/j.bbi.2020.04.027.
121. van Dorp, L., Acman, M., Richard, D., Shaw, L.P., Ford, C.E., Ormond, L. Balloux, F. (2020). Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. *Infect Genet Evol*. 83: 10435. doi: 10.1016/j.meegid.2020.104351.
122. Van Dorp, L., Acmana, M., Richard, D., Shawda, LP., Forda, C.E……Balloux, F. (2020). Emergence of genomic diversity and recurrent mutations in SARS-CoV-2, *Infection, Genetics and Evolution*. Volume 83, September 2020, 104351. https://doi.org/10.1016/j.meegid.2020.104351.
123. Vijaykrishna, D, Smith, G.J.D., Zhang, J.X., Peiris, J.S.M., Chen, H. & Guan, Y. (2007). Evolutionary insights into the ecology of coronaviruses. *Journal of Virology*. 2007; 81(8):4012–20. DOI: 10.1128/JVI.02605-06.

124. Volz, E.M., Hill, V., McCrone, J.T., Price, A., Jorgensen, D., O'Toole, A…Rambaut, A. (2020). Evaluating the effects of SARS-CoV-2 Spike mutation D614G on transmissibility and pathogenicity. Preprint at bioRxiv. https://doi.org/10.1101/2020.07.31.20166082.
125. Wan, Y., Shang, J., Graham, R., Baric, R.S.&, Li, F. (2020). Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J Virol* 94:e00127-20. https://doi.org/10.1128/JVI.00127-20.
126. Wang, L. F. & Anderson, D. E. (2019). Viruses in bats and potential spillover to animals and humans. *Current Opinion in Virology*. Volume 34, February 2019, Pages 79-89. https://doi.org/10.1016/j.coviro.2018.12.007.
127. Wang, N., Li, S. Y., Yang, X. L., Huang, H.M., Zhang, Y.J….Shi, Z.L. (2018). Serological Evidence of Bat SARS-Related Coronavirus Infection in Humans, China. Virologica Sinica, 33(1), 104–107. https://doi.org/10.1007/s12250-018-0012-7.
128. WHO (2020b). SARS-CoV-2 mink-associated variant strain- Denmark. https://www.who.int/csr/don/06-november-2020-mink-associated-sars-cov2-denmark/en/.
129. Wigginton, K.R., Yea, Y., &. Ellenberga, R. M. (2015). Emerging investigators series: the source and fate of pandemic viruses in the urban water cycle. *Environmental Science: Water Research & Technology*. Issue 6, 2015. https://doi.org/10.1039/C5EW00125K.
130. World Health Organization. (WHO, 2020a). Novel Coronavirus-Japan (ex-China). https://www.who.int/csr/don/16-january-2020-novel-coronavirus-japan-ex-china/en/. Retrieved November 23, 2020.
131. Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O……McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367, 1260–1263. DOI: 10.1126/science.abb2507.
132. WSAVA (2020). Scientific and One Health Committees, Advisory document: updated as of March 20, 2020. The New Coronavirus and Companion Animals- Advice for WSAVA. https://wsava.org/wp-content/uploads/2020/03/COVID-19_WSAVA-Advisory-Document-Mar-19-2020.pdf. Accessed on November 23 2020. .
133. Wu, F., Zhao, S., Yu, B., Chan, Y.M., Wen, W., Song, Z.G…Yong-Zhen Zhang, Y.Z. 2020). A new coronavirus associated with human respiratory disease in China. Nature 579, 265–269 (2020). https://doi.org/10.1038/s41586-020-2008-3.
134. Wu, F., Zhao, S., Yu, B., Chen, Y.M., Wang, W., Song, Z.G., Hu, Y., Tao, Z.W., Tian,J.H., Pei, Y.Y. and Yuan, M.L., 2020. A new coronavirus associated with human respiratory disease in China. Nature, 579(7798), pp.265-269.https://doi.org/10.1038/s41586-020-2008-3.
135. Xie, J., Li,Y., Shen, X., Goh, G., Zhu, Y., Cui, J… Zhou, P. (2018). Dampened STING-Dependent Interferon Activation in Bats. *Cell Host & Microbe* 23, 297–301. https://doi.org/10.1016/j.chom.2018.01.006.
136. Yuen, K. S., Ye, Z. W., Fung, S. Y., Chan, C. P., & Jin, D. Y. (2020). SARS-CoV-2 and COVID-19: The most important research questions. *Cell Biosci* 10, 40 (2020). https://doi.org/10.1186/s13578-020-00404-4.
137. Yurkovetskiy, l., Wang, X., Pascal, K.E., Tomkins-Tinch, C., Nyalile, T., Wang, Y…Luban, J. (2020). Structural and Functional Analysis of the D614G SARS-CoV-2 Spike Protein Variant. Preprint at bioRxiv https://doi.org/10.1101/2020.07.04.187757.
138. Zhang, G.J., Cowled, C., Shi, Z., Huang, Z., Bishop-Lilly, K.A., Fang, X….Wang, J. (2013). Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science*, Vol. 339, Issue 6118, pp. 456-460. DOI: 10.1126/science.1230835.
139. Zhang, L., Jackson, C., Mou, H., Ojha, A., Rangarajan, E., Izard, T, Choe. H. (2020). The D614G mutation in the SARS-CoV-2 spike protein reduces S1 shedding and increases infectivity. https://www.biorxiv.org/content/10.1101/2020.06.12.148726v1.

140. Zhang, Q, Zhang, H, Huang, K, Yang, Y., Hui, X., Gao, J…Jin, M. (20202). SARS-CoV-2 neutralizing serum antibodies in cats: a serological investigation. bioRxiv;. **doi:** https://doi.org/10.1101/2020.04.01.021196.
141. Zhang, T., Wu, Q., Zang Z. (2020). Probable Pangolin Origin of 2019-nCoV Associated with Outbreak of COVID-19. current-biology-D-20-00299. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3542586..
142. Zhang, T., WU, Q., Zang, Z. (2020). Probable Pangolin Origin of 2019-nCoV Associated with Outbreak of COVID-19. Available at SSRN: https://ssrn.com/abstract=3542586 or http://dx.doi.org/10.2139/ssrn.3542586.
143. Zhang, Y. Z., & Holmes, E. C. (2020). A Genomic Perspective on the Origin and Emergence of SARS-CoV-2. *Cell*, 181(2), 223–227. https://doi.org/10.1016/j.cell.2020.03.035.
144. Zhang, Y., Geng, X., Tan, Y., Li, Q., Xu, C…..Wang, H. (2020). New understanding of the damage of SARS-CoV-2 infection outside the respiratory system. *Biomedicine & pharmacotherapy*, Vol 127, 110195. https://doi.org/10.1016/j.biopha.2020.110195.
145. Zhou, P., Fan, H., Lan, T., Yang, X.L., Shi, W.F., Zhang, W. Y…Ma, J.Y. (2018). Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature.* ; 556(7700):255-258. https://www.ncbi.nlm.nih.gov/pubmed/29618817.
146. Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, W., Zhang, L…Shi, Z.L.,(2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273. https://doi.org/10.1038/s41586-020-2012-7.